A Study of the Molecular Conformations and the Electronic Structures of Peptide Nanorings and Nanotubes

ペプチドナノリング及びナノチューブの分子構造並びに電子構造に関する研究

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Chapter 1

Introduction

1.1 Introduction

Progress in modern nano-technology has been able to lead the physical components of machines and devices to dramatic miniaturization. Researchers in this field have continued to devise nano-scale materials and discover new chemical and physical properties. Fullerenes[1] and carbon nanotubes[2] are of especial interest and their applications in molecular electronics are continuously researched. Attention to the nano-constructs has not been limited to these carbon-based molecules but has also been expanded into biomolecules. A fusion of nano-technology and bioscience has given rise to a new interesting field termed “nano-bio”. The opening of this interdiscipline now lets biochemists and bioengineers attempt to manufacture various biomediated materials that involve the spontaneous arrangement of atoms and molecules. Based on the self-assembling process, many functional biomaterials have a chance to be fabricated.

Since the 1980s, several new types of biomolecules have been synthesized. Of immediate concern is the nano-tubular protein structure termed “peptide nanotube” (Figure 1.1), which was first synthesized by Ghadiri et al. in 1993[3]. The peptide nanotube (PNT) is formed by the spontaneous stacking of cyclic peptides (or peptide nanorings; PNRs) consisting of an alternate sequence of D- and L-amino acid residues (D,L-peptide). Because of its peculiar periodic structure with an open-ended hollow core, PNTs have attracted the interest of many researchers. PNT also has an excellent advantage in molecular modeling. Because the number and kind of component amino acids can be artificially adjusted, both the internal diameter and surface properties can be controlled. Therefore, the PNT is expected to have a wide range of potential applications in medicine, biology, chemistry, physics, materials science, and so on.
1.2 Previous Studies

Theoretical prediction of the hollow tubular structure based on the d,l-peptide nanorings was first provided by De Santis et al. in 1974[4]. They applied the mathematical conformation analysis derived by Shimanouchi and Mizushima[5] and also Miyazawa[6] to regular enantiomeric amino acid sequences (DLDL····) and indicated that an even number of alternating d- and l-amino acids would form closed rings capable of stacking through backbone-backbone hydrogen bonding.

Early attempts to experimentally verify this prediction met with limited success, partially due to extreme insolubility of the targeted peptides. Lorenzi et al. synthesized cyclo[-(d-Phe-l-Phe)₃] and cyclo[-(d-Val-l-Val)₃] PNRs and tried to show the formation of the PNT structures in 1989[7]. However, an X-ray crystallographic study revealed that the expected backbone-backbone hydrogen bonding was absent, but instead each peptide was found to be tightly packed with solvent molecules.

The first confirmation of the peptide nanotube (PNT) was reported by Ghadiri et al. in 1993[3]. They chose the sequence of cyclo[-(d-Ala-l-Gln-d-Ala-l-Glu)]ₙ (ₙ = 2) and demonstrated the formation of the micro-bundles of the PNTs based on electron microscopy and electron diffraction. The resulting infrared spectra also revealed that the individual PNT having a van der Waals internal diameter of about 7Å included the backbone-backbone (inter-ring) hydrogen bonding being analogous to that in the antiparallel β-sheet. His group also focused on the adjustability of the number of component amino acids and reported the larger PNT of cyclo[-(d-Ala-l-Gln-d-Ala-l-Glu)]ₙ (ₙ = 3)[8]. The electron microscopy and electron diffraction displayed the micro-bundle form of this PNT, which had the larger van der Waals internal diameter.
of about 13Å.

In addition to the above mentioned size adjustability, the external surface properties of PNTs can be controlled by a choice of amino acid side chains[9]-[30], i.e., an appropriate choice of component amino acid residues allows matching of the surface characteristics to the properties of the surrounding media. One example was given by Ghadiri group in 1994 as the design of artificial transmembrane ion channels using the open-ended hollow tubular structure[9]. His group reported that the octapeptide nanotube consisting of appropriate hydrophobic side chains (cyclo[-d-Leu-1-Gln-(d-Leu-1-Trp)]n; n = 3) was embedded into nonpolar lipid bilayers and displayed the proton transport activity. Since then, several other octapeptide nanotubes have been examined by fluorescence proton-transport assays and single-channel conductance measurements, and those PNTs displayed highly efficient transport activities for K⁺ and Na⁺ (greater than 10⁷ ions s⁻¹), rivaling the activity of the related natural gramicidin A product[27].

The possibility of controlling the internal diameter even allows the transport of small hydrophilic molecules across lipid bilayers through the hollow pores of the PNTs. Ghadiri group successively reported that the large PNT of cyclo[-d-Leu-1-Gln-(d-Leu-1-Trp)]₄ (n = 4), which possesses a van der Waals internal diameter of about 10Å, displayed efficient glucose and/or l-glutamic acid transport activity, whilst its smaller octapeptide counterpart (n = 3) lacked such transport activity for those molecules[10,
A computational study has also been carried out for the transmembrane channel model of the PNT. A molecular dynamics simulation for the PNT of \( \text{cyclo}[-(\text{D}-\text{Ala-L-Gln-D-Ala-L-Glu})_n] \) \((n = 2)\) suggested that a particular ordering of water molecules inside the hollow pore is responsible for the observed high rates of transport[11]. The theoretical as well as experimental studies have indicated potential biological applications of the PNTs as size-selective ion channels, molecular transport, drug delivery vehicles, etc.

Apart from their biological uses, the application of PNTs in nano-electronics has also been the focus of several other researchers. From this viewpoint, several theoretical studies have been performed to explore the physical properties of PNTs: Lewis et al. investigated the stable molecular structures and the energetic stability of \( \text{cyclo}[-(\text{D}-\text{Ala-L-Gln-D-Ala-L-Glu})_n] \) \((n = 1 - 4)\) PNTs on the basis of first-principles density functional calculations[31]. Fukasaku et al. investigated the electronic structures of \( \text{cyclo}[-(\text{Gly})_8]\) PNR and PNT using the density functional method and suggested that a migrated proton or hydroxyl ion into a hollow core may cause the thermal acceptor-donor-levels in the energy gap[32, 33]. Carloni et al. studied the electronic structures of the aggregated \( \text{cyclo}[-(\text{D}-\text{Ala-L-Gln-D-Ala-L-Glu})_n] \) \((n = 2)\) PNTs[34], and Jishi et al. discussed the influence of some guest atoms in the \( \text{cyclo}[-(\text{D}-\text{Ala-Gly})_4]\) PNT[35]. Moreover, the author of this thesis has theoretically studied how the skeletal folding of the polypeptide open chains, head-to-tail cyclization, and inter-ring interaction changed the electronic structure in his master’s thesis[36].

### 1.3 Purpose

A series of previous studies has provided the basic information about the properties of the PNTs. However, it seems that the obtained results are still within the targeted amino acid sequences only. In order to expand the application of PNTs, one should deeply comprehend how the differences in the number and kind of amino acids affects the stable molecular structures, the energetic stability, and the electronic structures of the PNRs and PNTs. If these relationships are systematically understood, one will be able to produce various PNRs and PNTs whose conformations and functions are artificially tailor-made.

One aim of the present study is to provide the guiding principle of molecular modeling for the peptide nanorings and nanotubes. For this purpose, the possible molecular conformations of the PNRs and PNTs are investigated and a novel type of backbone
structure is simultaneously explored by a mathematical analysis and also by \textit{ab initio} energy calculations. Moreover, the effect of the amino acid substitution is studied for all 20 encoded residues, and the electronic characteristics of the individual side chains are theoretically systematized.

Although the above approaches are aimed at the actual application of PNRs and PNTs, these studies would also provide us with a deep understanding of the “electronic roles and functions” of peptides or amino acids themselves. For many years, quantum biologists in the protein science field have tried to elucidate the electronic roles of the individual amino acids as well as the functions of their integrated protein forms. However, the vastness, complexity, and randomness of proteins have confounded and prevented us from performing detailed quantum mechanical calculations. But now, the simple and curious periodic forms of the PNRs and PNTs allow the straightforward use of the traditional quantum calculations. From some of the obtained results, one will be able to deduce several conclusions which would be true for the proteins of living matter.

Besides the theoretical studies, the actual synthesis of several PNTs is performed in order to investigate their intimate morphology. Although a few micro-order images of the PNTs have already been reported by optical microscopy, cryoelectron microscopy, and so on\cite{8}, it is necessary to investigate the intimate morphology of the PNTs at a nano-order level so as to manipulate the PNT molecules and apply them in nanoelectronics. Especially, the discussion on how the difference in the number ($n$) of component residues changes the morphology of the PNTs is an important subject of the present thesis. Thus, two D,L-peptide nanotubes of $n = 6$ and $n = 8$ are synthesized and their morphology is compared using atomic force microscopy.

The author makes an attempt to find not only the usual D,L-peptide nanotubes but also a new type of PNT which consists of an unusual but “natural” all L-amino acid sequence. Following the theoretical consideration based on the conformation analysis and \textit{ab initio} energy calculations, the author endeavors to synthesize and observe the novel all L-amino acid PNT.

1.4 Overview

This thesis is composed of six chapters as shown in Figure 1.3.

In Chapter 2, we discusses the possible backbone conformation of general periodic polymers which have a homo unit sequence (LLLL\cdots) or a regular enantiomeric unit sequence (DLDL\cdots) based on the mathematical analysis derived by Shimanouchi and
Mizushima[5] and also by Miyazawa[6]. First, the mathematical analysis is applied to the simplest polymer whose unit cell consists of a single atom \((m = 1, \text{ referred to as the M1 polymer})\). The conformation analysis is then expanded to higher polymers which include two \((m = 2)\) and three \((m = 3)\) atoms in the unit cell \((\text{referred to as the M2 and M3 polymers, respectively})\). The mathematical treatment for the M3 polymer leads to the conformation analysis of the periodic polypeptide, because the peptide backbone consists of three atoms per unit cell \((C^\alpha, C, \text{ and } N)\). Assuming some internal parameters of the polypeptide, the possible backbone conformations of the homo-polypeptide having a homo amino acid sequence \((LLLL\cdots)\) and also of the hetero-polypeptide having an alternate D- and L-amino acid sequence \((DLDL\cdots)\) are examined. Based on the mathematical analysis, one can find the possible (helical) pitch number \(n\) and helical translation \(d\) as a function of the two internal rotation angles \(\phi\) and \(\psi\).

The conformation analysis for the hetero-polypeptide makes it possible to determine the D.L-peptide nanoring (nanotube) backbones. Based on the mathematical analysis, two nanoring backbones of the “Extended-type” and the “Bound-type” are introduced in Chapter 3. \textit{Ab initio} Hartree-Fock molecular orbital calculations are successively carried out for those peptide nanorings (PNRs), and the energetically stable backbone conformations \((cyclo[-(Gly)_n], \text{ where } n = 6, 8, 10, \text{ and } 12)\) are investigated. The effect of the amino acid substitution is, moreover, examined for all 20 encoded residues, and the energetic stability and the electronic structures of these homo-residue PNRs \((cyclo[-(\text{D-AA-L-AA})_3], \text{ where AA is an abbreviation for Amino Acid})\) are discussed. Finally, the geometry optimizations of the peptide nanotubes (PNTs) are carried out, and the stable molecular conformations and the band structures of the PNTs are investigated using the Hartree-Fock crystalline orbital method.

In Chapter 4, the author describes the synthesis of the following two kinds of D.L-peptide nanotubes: the hexapeptide nanotube (6PNT) and the octapeptide nanotube (8PNT). To compare their morphology in terms of the number of component residues, the same type of amino acid sequence should be employed for both 6PNT and 8PNT. Therefore, taking into account the easiness of the synthesis, solubility, and the intertube interaction, the alternating D-Ala and L-Gln sequences are chosen for the targeted PNTs; i.e., \(cyclo[-(\text{D-Ala-L-Gln})_3]\) and \(cyclo[-(\text{D-Ala-L-Gln})_4]\). The synthesized peptides are identified by mass spectrometry, and then the morphology is investigated by atomic force microscopy. The resulting images are well analyzed and discussed using the results of the theoretical calculations.

In Chapter 5, the mathematical conformation analysis of homo-L-amino acid sequence is first described, and then unusual pentapeptide nanoring and nanotube back-
Chapter 1: Introduction

<general polymer>

Chapter 2: regular homo sequence; m=1,2,3
⇒ Conformation Analysis
regular enantiomeric sequence; m=1,2,3
⇒ Conformation Analysis

<D,L-peptide nanorings and nanotubes>

Chapter 3: ring backbone; cyclo[-(Gly)ₙ] (n=6,8,10,12)
⇒ Energetics
⇒ Electronic Structures
amino acid substitution; cyclo[-(D-AA-L-AA)₃]
⇒ Energetics
⇒ Electronic Structures

Chapter 4: cyclo[-(D-Ala-L-Gln)ₙ] (n=3,4)
⇒ Synthesis
⇒ Atomic Force Microscopy

<homo-L-peptide nanoring and nanotube>

Chapter 5: ring backbone; cyclo[-(Gly)₅]
⇒ Conformation Analysis
⇒ Energetics

<superscript>5</sup>cyclo[-(L-Gln)₅]
⇒ Synthesis
⇒ Atomic Force Microscopy

Chapter 6: Summary

Figure 1.3: Overview of the present thesis.
bones are theoretically predicted. Consequently, the energetics of the peptide nanoring and nanotube \((cyclo[-(\text{Gly})_5])\) is studied based on \textit{ab initio} Hartree-Fock calculations. In the last half of this chapter, the synthesis and the atomic force microscopy results are reported, and the morphology of the homo \textit{l}-amino acid pentapeptide nanotube is discussed.

In Chapter 6, the conclusions based on the research described in this thesis are summarized, and future prospects are provided.
Bibliography


Chapter 2

Conformation Analysis of Regular Periodic Polymers

2.1 Introduction

Polymorphy in stereo-structures of polymeric backbones is one of the most characteristic and important features found in macromolecules and high-polymers. The control and utilization of the backbone configurations are today’s desire and target for molecular modeling of organic, inorganic, and bio-polymers. Particularly, a design of novel peptides (or proteins) is a current hot topic and also a focus in the present thesis.

The first step of molecular designing and polymorphy controlling is the understanding and classification of possible skeletal backbones of periodic polymers. It is achieved by a conformation analysis for regular polymers derived by Shimanouchi and Mizushima in 1950s[1]. They gave general mathematical expressions for the helical parameters in terms of the bond lengths, the bond angles, and the internal rotation angles. The mathematical expressions can be applied to any helical polymers, i.e., $[-(M_1M_2\ldots M_m)]_\infty$ (where $M$ represents an atom on the backbone). Using this mathematical approach, Shimanouchi, Mizushina, and also Miyazawa predicted several backbone conformations of polyoxymethylene, polyolefin, polypeptide, cellulose, etc.[1, 2]

Following their conformation analysis for polypeptide (homo-l-amino acid sequence), De Santis et al. applied the mathematical treatment to a regular enantiomeric amino acid sequence (an alternate sequence of d- and l-amino acids) and forecasted the peptide nanotube (PNT) structure formed by the stacking of cyclic peptides in 1974[3]. About 30 years after their theoretical prediction, Ghadiri et al. reported the first synthesis of the PNT structure[4].
Although the conformation analysis derived by Shimanouchi and Mizushima is simply based on a mathematical technique of linear transformation, it is powerfully efficient for the building of novel periodic polymers. Nevertheless, few investigations based on this analysis have been carried out for the polymorphy in the stereo-structures of polymeric backbones since the reports by Miyazawa and De Santis.

In this chapter, the author reinvestigates the possible backbone conformation of regular periodic polymers \([- (M_1 M_2 \ldots M_m)]_{\infty}\) (homo sequences of the \((M_1 M_2 \ldots M_m)\) unit) and also applies the analysis to regular enantiomeric sequences of the unit, i.e., \([-D(M_1 M_2 \ldots M_m)]_{\infty} \quad \text{and} \quad [-L(M_1 M_2 \ldots M_m)]_{\infty}\), for the sake of the comprehension and design of the periodic polymers’ conformations. First, a basic concept of the mathematical analysis is described, and then the conformation analysis is practically applied to the regular homo sequences and enantimeric sequences of the unit which consists of one \((m = 1)\), two \((m = 2)\) or three \((m = 3)\) atom(s). The mathematical treatment for the \(m = 3\) polymers also leads to the conformation analysis of polypeptides, because the polypeptide backbone consists of three atoms per unit cell \((\text{C}^\alpha, \text{C}, \text{and} \text{N})\). Therefore, we can explore the possible backbone conformations of the peptide nanorings (PNRs) and peptide nanotubes (PNTs).

### 2.2 Unitary transformation

#### 2.2.1 Internal parameters and cylindrical parameters

Let us consider an infinitely periodic high polymer \([- (M_1 M_2 \ldots M_i \ldots M_m)]_{\infty}\) whose \((s)\)th unit cell consists of \(m\) skeletal atoms \((M_1^s \ldots M_m^s)\) as in Figure 2.1). How can we describe the backbone conformation of this periodic polymer mathematically? One way to describe the backbone conformation is to express all the atomic configurations as a function of the internal parameters relating to the \(i\)th atom, i.e., the bond lengths \((r_i)\), the bond angles \((\alpha_i)\), and the internal rotation angles \((\tau_i)\) [Figure 2.2 (a)]. On the other hand, the same backbone conformation can also be expressed as a function of the external cylindrical parameters, i.e., the radius \(\rho_i\), the helical pitch angle \(\theta_i\) \((0 \leq \theta \leq \pi)\), and the helical translation \(d_i\) as in Figure 2.3 (a).

Here, one should be noted that the internal parameters [Figure 2.2 (a)] and the cylindrical parameters [Figure 2.3 (a)] are connected by performing the unitary transformation between those two coordinate systems. Based on the unitary transformation, we can find the cylindrical parameters of the radius, pitch angle, and the helical translation as a function of the bond lengths, the bond angles, and the internal rotation angles.
Figure 2.1: Illustration of the backbone structure of an infinitely periodic high polymer $\left[-\left(M_{1}M_{2}\ldots M_{i}\ldots M_{m}\right)\right]_{\infty}$ whose periodic unit consists of $m$ skeletal atoms $M_{1}^{s}\ldots M_{m}^{s}$. The right-handed rectangular coordinate systems are shown in the figure.


**Internal coordinate systems**

(a) internal parameters  (b) rectangular coordinates

![Diagram](image)

Figure 2.2: Definition of the internal parameters (a) and the right-handed rectangular coordinates (b) in the internal coordinate systems.

2.2.2 Internal coordinate systems

In order to perform the unitary transformation and obtain a relationship between the internal parameters and cylindrical parameters, atomic configurations of the polymeric backbone should be expressed using the rectangular coordinates. First, let us introduce the internal rectangular coordinates relating to the $i$th atom [Figure 2.2 (b)]. Here, the right-handed rectangular coordinate systems are chosen and the sets of the individual coordinates in the $s$th unit cell ($M_s^i$) are determined as follows: The origin of the coordinate system $i$ is set to be consistent with a position of the $i$th atom with its $x_i$ axis on the bond connecting the $i$th atom to the $(i+1)$th. The $y_i$ axis lies on the plane determined by the two bonds $r_i$ and $r_{i-1}$ in such a way that the angle between $r_{i-1}$ and the positive direction of $y_i$ is acute. The positive direction of the $z_i$ axis is so taken as to make the coordinate system right-handed.

In the $s$th unit cell, a transformation of the coordinates $X_s^i = (x_i, y_i, z_i)$ into $X_{s-1}^i = (x_{i-1}, y_{i-1}, z_{i-1})$ is expressed as

$$X_{s-1}^i = A_i X_s^i + \vec{r}_{i-1},$$  \hspace{1cm} (2.1)

where an orthogonal matrix $A_i$ is a transformation matrix from the coordinates $X_s^i$ into $X_{s-1}^i$ being composed of the following two operations of rotations. The first is a
rotation $\Delta_i$ around the axis $z_i$ by the bond angle $\alpha_i$ and expressed as
\[
\Delta_i = \begin{pmatrix}
\cos(\pi - \alpha_i) & -\sin(\pi - \alpha_i) & 0 \\
\sin(\pi - \alpha_i) & \cos(\pi - \alpha_i) & 0 \\
0 & 0 & 1
\end{pmatrix}.
\] (2.2)

The second is a rotation $\Gamma_i$ around the axis $x_{i-1}$ by the internal rotation angle $\tau_i$ as
\[
\Gamma_i = \begin{pmatrix}
1 & 0 & 0 \\
0 & \cos \tau_i & -\sin \tau_i \\
0 & \sin \tau_i & \cos \tau_i
\end{pmatrix}.
\] (2.3)

Thus, the transformation matrix $A_i$ is given as
\[
A_i = \Gamma_i \Delta_i
\]
\[
= \begin{pmatrix}
1 & 0 & 0 \\
0 & \cos \tau_i & -\sin \tau_i \\
0 & \sin \tau_i & \cos \tau_i
\end{pmatrix}
\begin{pmatrix}
-\cos \alpha_i & -\sin \alpha_i & 0 \\
\sin \alpha_i & -\cos \alpha_i & 0 \\
0 & 0 & 1
\end{pmatrix}
\]
\[
= \begin{pmatrix}
-\cos \alpha_i & -\sin \alpha_i & 0 \\
\sin \alpha_i \cos \tau_i & -\cos \alpha_i \cos \tau_i & -\sin \tau_i \\
\sin \alpha_i \sin \tau_i & -\cos \alpha_i \sin \tau_i & \cos \tau_i
\end{pmatrix}.
\] (2.4)

Symbol $\vec{r_i}$ is the translation vector from the atom $M_i^s$ to $M_i^{s+1}$ and given using the bond length $r_i$ as
\[
\vec{r_i} = \begin{pmatrix}
 r_i \\
0 \\
0
\end{pmatrix}.
\] (2.5)

One should remember that this polymer maintains the periodicity among the corresponding atoms of the adjacent unit cells, $M_i^{s-1}$, $M_i^s$, and $M_i^{s+1}$. Taking into account the periodicity of units, a transformation of the coordinates $X_i^s$ in the $s$th unit cell into $X_i^{s-1}$ in the $(s-1)$th is given as
\[
X_i^{s-1} = A \cdot X_i^s + \vec{B}
\]
\[
= (A^{\text{unit}} \cdot R) \cdot X_i^s + \vec{B},
\] (2.6)

where symbols $A^{\text{unit}}$ and $\vec{B}$ are an orthogonal matrix and a translation vector for one period, respectively. $A^{\text{unit}}$ is given by the $m$ times multi-product form of the two rotation-operations around the axis $z_i$ and $x_{i-1}$ of the composing $i$th skeletal atoms in the $s$th cell,
\[
A^{\text{unit}} = \Gamma_1 \Delta_1 \ldots \Gamma_m \Delta_m,
\] (2.7)
and $\vec{B}$ is given by
\[
\vec{B} = r_m + \sum_{i=1}^{m-1} (\Gamma_1 \Delta_1 \ldots \Gamma_i \Delta_i) \vec{r_i}.
\] (2.8)
External coordinate systems

(a) external parameters   (b) rectangular coordinates

Figure 2.3: Definition of the cylindrical parameters (a) and the right-handed rectangular coordinates (b) in the external coordinate systems.
Here, as mentioned in the former section, two types of unit sequences are considered in this thesis, i.e., a regular homo unit sequence \([-\{M_1M_2\ldots M_m\}\}_{\infty}\) and a regular enantiomeric unit sequence \([-\eta(M_1M_2\ldots M_m)-\xi(M_1M_2\ldots M_m)]_{\infty}\). The former is a “homo-rotatory” catenation, in which adjacent unit cells have the same coordinate system of the right-handed (or left-handed). The latter is a “hetero-rotatory” catenation, in which adjacent unit cells have the different coordinate system. The difference between the homo-rotatory and hetero-rotatory catenation is distinguished by matrix \(R\): \(R^{homo}\) for the homo-rotatory and \(R^{hete}\) for the hetero-rotatory, where \(R^{homo}\) is a unit matrix of
\[
R^{homo} = \begin{pmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{pmatrix},
\]
and \(R^{hete}\) is
\[
R^{hete} = \begin{pmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & -1
\end{pmatrix},
\]
which changes the coordinate system from the right-handed (left-handed) to the left-handed (right-handed).

2.2.3 External coordinate systems

Next, let us introduce the external rectangular coordinates [Figure 2.3 (b)]. Here, the sets of right-handed rectangular coordinates \(\Xi_i = (\xi_i, \eta_i, \zeta_i)\) are chosen as follows: The origin of the \(i\)th coordinates coincides with the foot of the perpendicular to the helical axis drawn from the \(i\)th atom, and the \(\xi_i\) axis being on this perpendicular with its positive direction pointing towards the \(i\)th atom. The \(\zeta_i\) axis lies on the helical axis and the \(\eta_i\) axis is defined at the right-handed system.

Since the periodicity of the units is conserved among the corresponding atoms in the adjacent unit cells, a transformation of the coordinates \(\Xi_i^{s} = (\xi_i, \eta_i, \zeta_i)\) in the \(s\)th unit cell into \(\Xi_i^{s-1} = (\xi_i, \eta_i, \zeta_i)\) in the \((s-1)\)th is provided as
\[
\Xi_i^{s-1} = N \cdot \Xi_i^{s} + \vec{D} = (N^{unit} \cdot R) \cdot \Xi_i^{s} + \vec{D},
\]
where matrix \(N\) means a rotation operator using the counterclockwise rotation around the \(\zeta_i\) axis:
\[
N^{unit} = \begin{pmatrix}
\cos \theta^{unit} & -\sin \theta^{unit} & 0 \\
\sin \theta^{unit} & \cos \theta^{unit} & 0 \\
0 & 0 & 1
\end{pmatrix},
\]
(2.12)
where \( \theta_{\text{unit}} = \sum_{i=1}^{m} \theta_i \). The translation vector \( \vec{D} \) is given as

\[
\vec{D} = \begin{pmatrix} 0 \\ 0 \\ d_{\text{unit}} \end{pmatrix},
\]

(2.13)

where \( d_{\text{unit}} = \sum_{i=1}^{m} d_i \). Hereafter, \( \theta_{\text{unit}} \) and \( d_{\text{unit}} \) are referred to just as \( \theta \) and \( d \), respectively.

### 2.2.4 Conformation analysis

#### \( \theta \) analysis

Let us consider the relation between the internal coordinate system and the external coordinate system. \( X_i \) in the internal coordinate system [Figure 2.2 (b)] and \( \Xi_i \) in the external coordinate system [Figure 2.3 (b)] have the following relation:

\[
\Xi_i = TX_i + \vec{\rho},
\]

(2.14)

where \( T \) is an orthogonal matrix,

\[
T = \begin{pmatrix} t_{11} & t_{12} & t_{13} \\ t_{21} & t_{22} & t_{23} \\ t_{31} & t_{32} & t_{33} \end{pmatrix},
\]

(2.15)

and vector \( \vec{\rho} \) is a translation of \( \vec{\xi}_i \) along the radius \( \rho \) [Figure 2.3 (a)] toward the \( M_i \) atom,

\[
\vec{\rho} = \begin{pmatrix} \rho \\ 0 \\ 0 \end{pmatrix}.
\]

(2.16)

From eq. 2.14,

\[
X_i = T^{-1}(\Xi_i - \vec{\rho}).
\]

(2.17)

By substituting eq. 2.17 to eq. 2.1,

\[
T^{-1}(\Xi_{i-1} - \vec{\rho}) = A(T^{-1}(\Xi_i - \vec{\rho}) + \vec{B}).
\]

(2.18)

The above equation is rewritten as

\[
\Xi_{i-1} = TAT^{-1}\Xi_i - (TAT^{-1} + E)\vec{\rho} + T\vec{B},
\]

(2.19)

where \( E \) is a unit matrix. By comparing eq. 2.19 with eq. 2.11, we have

\[
N = TAT^{-1}.
\]

(2.20)
Since eq. 2.20 verifies that transformation matrices $N$ and $A$ have a relation of “similarity”, traces of the two matrices should be the same. Therefore, the following important equation is obtained:

$$Tr[N] = Tr[A]. \quad (2.21)$$

By eq. 2.21, one can find a relation between the pitch angle $\theta$ and the internal parameters as

$$\cos \theta = \frac{1}{2}(a_{11} + a_{22} + a_{33} - 1), \quad (2.22)$$

where $a_{ij}$ means the $ij$th element of the transformation matrix $A$.

**d analysis**

The helical translation $d$ is obtained by defining vector $\vec{s} = \frac{\vec{z}}{|\vec{z}|}$, which corresponds to the unit vector of the core axial direction. Because $\vec{s}$ is conserved by the transformation $N$, we have the following eigenvalue equation:

$$N \vec{s} = 1 \vec{s}. \quad (2.23)$$

From eq. 2.20, the above equation is rewritten as

$$A \vec{s} = 1 \vec{s}. \quad (2.24)$$

Here, one should notice that the transform matrix $A$ is an orthogonal matrix. Therefore,

$$A^t A = E, \quad (2.25)$$

where $A^t$ represents the transpose of the matrix $A$. By eq. 2.25, we have

$$A^t \vec{s} = 1 \vec{s}. \quad (2.26)$$

From eqs. 2.24 and 2.26, the following equation is obtained:

$$(A - A^t) \vec{s} = 0 \vec{s}. \quad (2.27)$$

Thus, $\vec{s}$ is given by

$$\vec{s} = \frac{1}{C} \begin{pmatrix} a_{32} - a_{23} \\ a_{13} - a_{31} \\ a_{21} - a_{12} \end{pmatrix}, \quad (2.28)$$
where $C$ is a normalized factor given as

$$
C = \sqrt{(a_{32} - a_{23})^2 + (a_{13} - a_{31})^2 + (a_{21} - a_{12})^2}.
$$

(2.29)

Because an inner product of $\vec{B}$ and $\vec{s}$ provides a helical translation along the core axial direction, the helical translation $d$ is given as

$$
d = \vec{B} \cdot \vec{s}.
$$

(2.30)

$\rho$ analysis

From Figure 2.4, the cylindrical radius $\rho$ is easily obtained using $\vec{B}$, $d$, and $\theta$ as

$$
2\rho \sin(\theta/2) = \sqrt{|\vec{B}|^2 - d^2}.
$$

(2.31)
In the following sections, possible backbone conformations of the periodic polymers of \( m = 1, 2, 3 \) are investigated theoretically through \( \theta \) and \( d \) analyses (eqs. 2.22 and 2.30). Note that the helical pitch angle \( \theta \) depends on the bond angles \( \alpha_i \) and the internal rotation angles \( \tau_i \) only, while the helical translation \( d \) is a function of \( \alpha_i, \tau_i \), and also the bond lengths \( r_i \).

### 2.3 Stereo-structure of a polymeric backbone; \( m=1 \)

#### 2.3.1 Homo-rotatory polymer

\( \theta \) analysis

Let us apply the aforementioned analysis to the periodic backbone whose unit cell consists of a single atom \((m = 1, \) referred to as the M1 polymer as shown in Figure 2.5). Assuming that all the backbone atoms have a covalent linkage, it is reasonable that the values of the bond length \( r \) and the bond angle \( \alpha \) are fixed and the stereo-structure of the polymeric backbone is determined only by the internal rotation angle \( \tau \). Moreover, we consider that all skeletal atoms are catenated homo sequentially while maintaining the same internal rotation angle \( \tau \) (homo-rotatory M1 polymer, referred to as \( \text{homoM}_1 \)). The transformation matrix \( A \) for the \( \text{homoM}_1 \) polymer is given using \( A^\text{unit} \) and \( R^\text{homo} \) as

\[
A^\text{homoM}_1 = A^\text{unit} \cdot R^\text{homo} = \begin{pmatrix} -\cos \alpha & -\sin \alpha & 0 \\ \sin \alpha \cos \tau & -\cos \alpha \cos \tau & -\sin \tau \\ \sin \alpha \sin \tau & -\cos \alpha \sin \tau & \cos \tau \end{pmatrix}.
\] (2.32)

Since matrix \( A^\text{homoM}_1 \) should be similar to matrix \( N^\text{homoM}_1 = N^\text{unit} \cdot R^\text{homo} = N^\text{unit} \) (eq. 2.12), traces of the two matrices should be coincident \((Tr[A^\text{homoM}_1] = Tr[N^\text{homoM}_1])\) as in eq. 2.21). Thus, the helical pitch angle \( \theta^\text{homoM}_1 \) is given as a function of both the bond angle \( \alpha \) and the internal rotation angle \( \tau \) as

\[
\cos \theta^\text{homoM}_1 = \frac{1}{2}[(1 - \cos \alpha) \cos \tau - \cos \alpha - 1].
\] (2.33)

The three-dimensional plot of the possible helical rotation angles \( \theta^\text{homoM}_1 \) (values of \( \cos \theta^\text{homoM}_1 \)) is shown in Figure 2.6 (a) with changing the bond angle \( \alpha \) and the internal rotation angle \( \tau \). The characteristic feature is that the minimum value of \( \cos \theta^\text{homoM}_1 = -1 \) is found at \( \tau = \pm \pi \), independent of the change in \( \alpha \). On the contrary,
$\tau = 0$ gives the maximum value of $\theta^{homoM1} = \pi - \alpha$, which varies in accordance with the given $\alpha$ value. This feature is well understood by drawing a cross section of Figure 2.6 (a), e.g., the cross section at $\alpha = 2\pi/3$ [Figure 2.6 (b)]. In the figure, a cosinodal dependence of the helical pitch angle ($\cos \theta$) on the internal rotation angle $\tau$ is found. It gives the maximum value of $\cos \theta^{homoM1} = 0.5 (\theta = \pi/3)$ at $\tau = 0$ (point C), because the value of $\alpha = 2\pi/3$ rewrites eq. 2.33 as

$$\cos \theta^{homoM1} = \frac{3}{4} \cos \tau - \frac{1}{4}. \quad (2.34)$$

By considering the other cross sections, we can estimate the possible helical pitch angle $\theta$ and also the helical pitch number $n = 2\pi/\theta$ for the $homoM1$ polymer. Since the maximum and minimum $\cos \theta^{homoM1}$ appear at $\tau = 0$ and $\tau = \pm \pi$, respectively, the possible $\theta^{homoM1}$ and $n^{homoM1}$ are limited within

$$\pi - \alpha \leq \theta^{homoM1} \leq \pi, \quad (2.35)$$

and

$$2 \leq n^{homoM1} \leq \frac{2\pi}{\pi - \alpha}. \quad (2.36)$$

Several backbone conformations of the $homoM1$ polymer are illustrated by Figure 2.7. The $homoM1$ polymer basically forms a helical coil, and the sign of the internal rotation angles $\tau$ distinguishes a direction of the helical rotation of the right-handed or the left-handed. This feature can be found clearly by focusing on the conformations at point B ($\tau = +71.5^\circ$) and point D ($\tau = -71.5^\circ$) in Figure 2.7, where both of the $\tau$ values provide $\cos \theta = 0 (\theta = \pi/2)$ as in Figure 2.6 (b). The former (+71.5°) produces
Figure 2.6: Three-dimensional plot of the calculated possible $\cos \theta$ for $homoM1$ and $heteM1$ polymers with varying the bond angle $\alpha$ and the internal rotation angle $\tau$ (a), and its cross section at $\alpha = 2\pi/3$ (b). For the bond angle, the value of $\cos \theta_{homoM1}$ changes from the minimum -1 at $\tau = \pm \pi$ to the maximum 0.5 at $\tau = 0$, while that of $\cos \theta_{heteM1}$ goes from the minimum 0.5 at $\tau = 0$ to the maximum 1 at $\tau = \pm \pi$. 
the right-handed helix while the latter \((-71.5^\circ)\) does the left-handed helix with the same helical pitch \(n\), i.e., backbones at points B and D have an enantiomeric relation (Figure 2.7). Similarly, the other \(\tau\) values on the \(\cos \theta\) line (red) in Figure 2.6 provide the helical structures with the different pitch \(n\), and if any two points have the same \(\cos \theta\) value those two conformations are enantiomeric.

However, one can find exceptions when \(\tau = +\pi\) (point A), \(\tau = -\pi\) (point E), and \(\tau = 0\) (point C), which provide non-helical backbones. Because \(\tau = +\pi\) and \(\tau = -\pi\) give an exactly same rotation \(\pi\), points A and E have the same backbone of a trans-planar zigzag chain (Figure 2.7). On the other hand, the internal rotation angle \(\tau = 0\) (point C) provides a flat disk. Since we here adopt \(\alpha = 2\pi/3\), the conformation at point C becomes a “closed” flat disk (Figure 2.7).

\(d\) analysis

In the case of the \(homoM1\) polymer, the helical translation \(d\) is given as

\[
d^{homoM1} = \vec{B}^{homoM1} \cdot \vec{s},
\]

(2.37)

where

\[
\vec{B}^{homoM1} = r_1^\gamma,
\]

(2.38)
and \( s \) is given by eq. 2.28. Based on the helical translation \( d \) analysis (eq. 2.37), we can clearly understand the relation between the helical direction and internal rotation angle \( \tau \). Focusing on the sign of the resulting \( d \) values, one can distinguish the helical direction of the \( \text{homoM1} \) polymer (Figure 2.8), i.e., \( d > 0 \) gives a right-handed helix, while \( d < 0 \) gives a left-handed helix.

Here, let us focus on the boundary between the right-handed and left-handed helices. At point C in Figure 2.8, a helicity of the backbone disappears because of the zero helical translation toward the helical axis (\( d = 0 \)). Considering that the \( \text{homoM1} \) polymer includes an only atom in a unit cell, \( d = 0 \) requires to set all backbone atoms on the same plane. Therefore, the backbone at point C becomes a flat disk (Figure 2.7). One should also notice that the flat disk can close if \( n_{\text{homoM1}} \) is an integer, but not a decimal (rational or irrational number). A decimal pitch provides an “open” flat disk, which includes an overt steric hindrance in its own backbone. Therefore, this type of backbone conformation seems to be unrealistic.

### 2.3.2 Hetero-rotatory polymer

\( \theta \) analysis

Next, let us discuss possible backbone conformations in the hetero-rotatory M1 polymer (hereafter, \( \text{heteM1} \)). The \( \text{heteM1} \) backbone is formed by alternating the sign of the
internal rotation angle \( \tau \) (\( \cdots, -\tau, \tau, -\tau, \cdots \)), i.e., by varying the coordinate system from the right-handed to the left-handed alternately. Since the change of the coordinate system is expressed by matrix \( \mathbf{R}^{\text{hete}} \) (eq. 2.10), the transformation matrix \( \mathbf{A}^{\text{heteM1}} \) is obtained as

\[
\mathbf{A}^{\text{heteM1}} = \mathbf{A}^{\text{unit}} \cdot \mathbf{R}^{\text{hete}}
\]

\[
= \begin{pmatrix}
-\cos \alpha & -\sin \alpha & 0 \\
\sin \alpha \cos \tau & -\cos \alpha \cos \tau & \sin \tau \\
\sin \alpha \sin \tau & -\cos \alpha \sin \tau & -\cos \tau
\end{pmatrix}
\]

(2.39)

The transformation matrix \( \mathbf{N} \) for the \( \text{heteM1} \) polymer is rewritten using \( \mathbf{R}^{\text{hete}} \) as

\[
\mathbf{N}^{\text{heteM1}} = \mathbf{N}^{\text{unit}} \cdot \mathbf{R}^{\text{hete}}
\]

\[
= \begin{pmatrix}
\cos \theta & -\sin \theta & 0 \\
\sin \theta & \cos \theta & 0 \\
0 & 0 & -1
\end{pmatrix}
\]

(2.40)

Because the similarity between \( \mathbf{A}^{\text{heteM1}} \) and \( \mathbf{N}^{\text{heteM1}} \) provides \( \text{Tr}[\mathbf{A}^{\text{heteM1}}] = \text{Tr}[\mathbf{N}^{\text{heteM1}}] \), the helical pitch angle \( \theta \) in the \( \text{heteM1} \) polymer is given as a function of \( \alpha \) and \( \tau \) as

\[
\cos \theta^{\text{heteM1}} = -\frac{1}{2}(1 + \cos \alpha) \cos \tau + \cos \alpha - 1.
\]

(2.41)

In Figure 2.6 (a), the resulting 3D plot of the \( \cos \theta^{\text{heteM1}} \) values is shown with varying \( \alpha \) and \( \tau \). The \( \text{heteM1} \) polymer produces the minimum \( \cos \theta^{\text{heteM1}} \) at \( \tau = 0 \) and the maximum \( \cos \theta^{\text{heteM1}} \) (\( \cos \theta^{\text{heteM1}} = 1 \)) at \( \tau = \pm \pi \). This feature is opposite to that found in the \( \text{homoM1} \) polymer and understood by the cross section at \( \alpha = 2\pi/3 \) [Figure 2.6 (b)]. It displays the minimum value of \( \cos \theta^{\text{heteM1}} = 0.5 \) (\( \theta = \pi/3 \)) at \( \tau = 0 \) (point I), because \( \alpha = 2\pi/3 \) rewrites eq. 2.41 as

\[
\cos \theta^{\text{heteM1}} = -\frac{1}{4} \cos \tau + \frac{3}{4}.
\]

(2.42)

Considering the other cross sections, we can estimate the possible helical pitch angle \( \theta^{\text{heteM1}} \) and the helical pitch number \( n^{\text{heteM1}} \) (\( n^{\text{heteM1}} = 2\pi/\theta^{\text{heteM1}} \)). Since the maximum and minimum \( \cos \theta^{\text{heteM1}} \) appear at \( \tau = \pm \pi \) and \( \tau = 0 \), respectively, the possible \( \theta^{\text{heteM1}} \) value is limited within

\[
0 \leq \theta^{\text{heteM1}} \leq \pi - \alpha,
\]

(2.43)

and the pitch number \( n^{\text{heteM1}} \) is within

\[
\frac{2\pi}{\pi - \alpha} \leq n^{\text{heteM1}} \leq \infty.
\]

(2.44)
In addition to the above \( \theta \) analysis, the helical translation \( d \) analysis provides important information about the possible backbone conformations of the \( \text{hete}M1 \) polymer. Similar to the \( \text{homo}M1 \) polymer, the helical translation \( d \) in the \( \text{hete}M1 \) polymer is given as

\[
d^{\text{hete}M1} = \vec{B}^{\text{hete}M1} \cdot \vec{s},
\]

where

\[
\vec{B}^{\text{hete}M1} = \vec{r}_1.
\]

The resulting \( d \) values in the \( s \)th unit cell are plotted in Figure 2.8 (blue line). Here, one should notice the following characteristic in the \( \text{hete}M1 \) polymer. Because the coordinate system is changed from the right-handed to the left-handed alternately, the sign of the helical translation \( d \) is changed alternately among adjacent unit cells, i.e., \( \cdots, d^{s-2}, -d^{s-1}, d^s, -d^{s+1}, \cdots \), where \( d^s \) represents the helical translation \( d \) from the \( s \)th unit cell to the \((s + 1)\)th. Since the infinite sum of \( \sum_{s=1}^{\infty} d^s \) diverges, the \( \text{hete}M1 \) polymer basically forms a “coronal ring” of \( d \neq 0 \), in which one cannot distinguish the helicity of the right-handed or left-handed. This feature is recognized, for instance, at points G and K of \( \theta = \pi/4.5 \) [Figure 2.6 (b)]. Both the corresponding internal rotation angles \( \tau = +93.7^\circ \) and \( \tau = -93.7^\circ \) provide the same “open” ring structure of \( n = 9 \) as shown in Figure 2.7. This characteristic is distinctly different from that found in the \( \text{homo}M1 \) polymer, which produces both the right-handed and left-handed helices with the same pitch \( n \) (for example, points B and D).

Although the open ring structure is allowed mathematically, this conformation is considered to be forbidden chemically, because the skeletal atoms cause the significant steric hindrance in its own backbone. However, one should be noted that if the pitch number \( n \) is even the backbone does not include an overt steric hindrance. An even pitch number \( (n = 2m, \text{where } m \text{ is a natural number}) \) lets the \( \text{hete}M1 \) backbone be a “closed” coronal ring, because the sum of the helical translation \( d \) per helical turn \( (\sum_{s=1}^{n} d^s) \) does not diverge but converges to \( \sum_{s=1}^{n} d^s = 0 \), i.e., the position of the \( M_i^s \) atom coincides with the position of the \( M_i^{s+n} \) atom exactly. An example can be found at points H and J (\( \theta = \pi/4 \)) in Figure 2.6 (b), where the corresponding internal rotation angles \( \tau = +81.7^\circ \) and \( \tau = -81.7^\circ \) provide the same closed ring structure of \( n = 8 \) as in Figure 2.7. This is the general characteristics in the \( \text{hete}M1 \) polymer, i.e., the \( \text{hete}M1 \) basically produces the closed rings, while the \( \text{homo}M1 \) forms the open helical structures[5].

Here, one should focus the following exceptions in the \( \text{hete}M1 \) polymer. If \( n = \infty \) (points F and L), the coronal ring backbone is closed by \( \infty \) time of unit rotation around
the helical axis, i.e., the heteM1 backbone is not closed but open to be a trans-planar chain (Figure 2.7). This conformation is consistent with the trans-planar open chain at points A and E in the homoM1 polymer[6].

The other exception is \( d = 0 \) at point I (Figure 2.8). Since the heteM1 polymer includes an only atom in a unit cell, \( d = 0 \) provides a flat disk, in which all backbone atoms are on the same plane (Figure 2.7). This flat disk conformation occurs at \( \tau = 0 \) (point I) being equal to the case of the homoM1 polymer (point C), because the two \( \cos \theta \) lines of homoM1 and heteM1 come in contact with each other at \( \tau = 0 \). Therefore, the resulting flat disk of the heteM1 is exactly coincident with that of the homoM1 polymer. Since we here adopt \( \alpha = 2\pi/3 \), the “closed” flat disk is produced when \( n = 6 \) \( (\theta = \pi/3) \).

Another \( \alpha \) value also has a chance to provide a closed flat disk as long as the \( \alpha \) value requests both \( d = 0 \) and \( n \) being an integer, i.e., an odd pitch number of the closed ring has a potential to be formed in the heteM1 polymer. For example, the heteM1 polymer with \( \alpha = 108^\circ \) produces a closed flat disk of \( n = 5 \) at \( \tau = 0 \).

### 2.4 Stereo-structure of a polymeric backbone; \( m=2 \)

#### 2.4.1 Homo-rotatory polymer

\( \theta \) analysis

Next, let us apply the mathematical treatment to the higher polymer whose periodic unit consists of two backbone atoms \( (m = 2; \text{ the M2 polymer as in Figure 2.9}) \). First, we discuss the homo-rotatory M2 polymer (hereafter, homoM2), in which all unit cells catenate regularly while maintaining the individual internal rotation angles \( \tau_1 \) and \( \tau_2 \) \((\cdots, \tau_1, \tau_2, \tau_1, \tau_2, \cdots)\).

In the case of the homoM2 polymer, the transformation matrix \( A \) is given by

\[
A^{\text{homoM2}} = (A_1 \cdot A_2) \cdot R^{\text{homo}}, \tag{2.47}
\]

where

\[
A_1 = \begin{pmatrix}
-\cos \alpha_1 & -\sin \alpha_1 & 0 \\
\sin \alpha_1 \cos \tau_1 & -\cos \alpha_1 \cos \tau_1 & -\sin \tau_1 \\
\sin \alpha_1 \sin \tau_1 & -\cos \alpha_1 \sin \tau_1 & \cos \tau_1
\end{pmatrix}, \tag{2.48}
\]

\[
A_2 = \begin{pmatrix}
-\cos \alpha_2 & -\sin \alpha_2 & 0 \\
\sin \alpha_2 \cos \tau_2 & -\cos \alpha_2 \cos \tau_2 & -\sin \tau_2 \\
\sin \alpha_2 \sin \tau_2 & -\cos \alpha_2 \sin \tau_2 & \cos \tau_2
\end{pmatrix}. \tag{2.49}
\]
Figure 2.9: Definition of the backbone conformational parameters in the M2 polymer.

Since the matrix $A^{homoM2}$ should be similar to the matrix $N^{homoM2} = N^{unit} \cdot R^{homo} = N^{unit}$ (eq. 2.12), traces of the two matrices should be coincident ($Tr[A^{homoM2}] = Tr[N^{homoM2}]$). Thus, the helical pitch angle $\theta^{homoM2}$ is given as

$$
\cos \theta^{homoM2} = \frac{1}{2} (-1 + \cos \alpha_1 \cos \alpha_2 + \cos \tau_1 \cos \tau_2 + \cos \alpha_1 \cos \alpha_2 \cos \tau_1 \cos \tau_2 - \sin \alpha_1 \sin \alpha_2 \cos \tau_1 \cos \tau_2 - \sin \alpha_1 \sin \alpha_2 \cos \tau_2 - \cos \alpha_1 \sin \tau_1 \sin \tau_2 - \cos \alpha_2 \sin \tau_1 \sin \tau_2),
$$

(2.50)

How does the pitch angle $\theta^{homoM2}$ change with varying two internal rotation angles $\tau_1$ and $\tau_2$? To obtain the $\theta^{homoM2}$ values as a function of $\tau_1$ and $\tau_2$ and to visualize the possible backbone conformations, the remaining two bond angles $\alpha_1$ and $\alpha_2$ should be assumed. Setting our sights on the conformation analysis of polypeptide chains discussed in the next section ($m = 3$), let us assume $\alpha_1 = 111^\circ$ and $\alpha_2 = 116^\circ$[7]. The assumption of these values gives the possible $\cos \theta^{homoM2}$ values as a function of $\tau_1$ and $\tau_2$ (Figure 2.10). By drawing the contour map of Figure 2.10, we can also obtain the equi-$\cos \theta$ lines in the $homoM2$ polymer. In Figure 2.11, equi-$\cos \theta^{homoM2}$ lines of $\theta = 2\pi/5, \pi/2, 5\pi/9, 2\pi/3$, and $\pi$ are shown. It is characteristic that the resulting contour map has an inversion center at the origin of $(\tau_1, \tau_2) = (0, 0)$.

Let us focus on the extrema of the calculated $\cos \theta^{homoM2}$ values. The maximum $\cos \theta^{homoM2}$ is found at the four corners of $(\tau_1, \tau_2) = (\pm \pi, \pm \pi)$ in Figure 2.11. From eq. 2.50, one can find that the values $(\tau_1, \tau_2) = (\pm \pi, \pm \pi)$ gives the maximum $\cos \theta^{homoM2} = \cos |\alpha_1 - \alpha_2|$, i.e., the maximum $\cos \theta^{homoM2}$ is determined only by the difference between the two bond angles $\alpha_1$ and $\alpha_2$. On the other hand, the minimum value of $\cos \theta^{homoM2} = \ldots$
Figure 2.10: 3D plot of $\cos \theta$ values for $homoM2$ and $heteM2$ polymers with varying internal rotation angles $\tau_1$ and $\tau_2$. 
Figure 2.11: Equi-$n$ lines in the homoM1 polymer obtained by Figure 2.10. Equi-$n$ lines corresponding to $n = 2, 3, 4, 5, 6, 8,$ and $9$ are shown while superposing $d = 0$ lines.
−1 is found at four points of \((\tau_1, \tau_2) = (0, \pm \pi)\) and \((\pm \pi, 0)\). Thus, possible \(\theta_{\text{homo}M^2}\) and \(n_{\text{homo}M^2}\) values are limited within

\[
|\alpha_1 - \alpha_2| \leq \theta_{\text{homo}M^2} \leq \pi, \quad (2.51)
\]

and

\[
2 \leq n_{\text{homo}M^2} \leq \frac{2\pi}{|\alpha_1 - \alpha_2|}. \quad (2.52)
\]

**d analysis**

As mentioned in the former section, a helical direction of the polymeric backbone is distinguished by a sign of the helical translation \(d\). In the M2 polymer, however, the degrees of freedom in the internal rotation angles are increased comparing to that in the M1 polymer. How does the increase change the helicity?

In the case of the \(\text{homo}M^2\) polymer, the helical translation \(d\) is given by

\[
d_{\text{homo}M^2} = \vec{B}_{\text{homo}M^2} \cdot \vec{s}, \quad (2.53)
\]

where

\[
\vec{B}_{\text{homo}M^2} = A_1 \vec{r}_1 + \vec{r}_2. \quad (2.54)
\]

Here, two different \(d\) are defined for one conformation: one is \(d_{1,1}\) corresponding to the helical translation \(d\) from \(M_1^\circ\) atom to \(M_1^{s+1}\) atom, and the other is \(d_{2,2}\) corresponding to that from \(M_2^\circ\) atom to \(M_2^{s+1}\) atom. However, \(d_{1,1}\) is equivalent to \(d_{2,2}\) in the homorotatory sequence, because \(d_{1,1} = d_{1,2} + d_{2,1} = d_{2,1} + d_{1,2} = d_{2,2}\) (Figure 2.12). Therefore, a unique \(d\) map is obtained with varying \(\tau_1\) and \(\tau_2\) values (Figure 2.13).

Let us focus on the \(d = 0\) line in Figure 2.13. In the case of the \(\text{homo}M^2\) polymer, the \(d = 0\) line appears on the line of \(\tau_2 \simeq -\tau_1\) (\(\text{AGA}\); solid line). The helical direction of the \(\text{homo}M^2\) polymer is changed beyond this \(\tau_2 \simeq -\tau_1\) line, and \(d > 0\) provides the right-handed helix while \(d < 0\) gives the left-handed helix. Besides the \(d = 0\) line, the helical direction of the \(\text{homo}M^2\) polymer is also distinguished by the other type of \(d\) lines (broken lines of \(\text{EF}\) or \(\text{EF}'\) in Figure 2.13). Because the \(d\) surface shows the discontinuity on these broken lines and the sign of the \(d\) value is changed beyond them, the “discontinuous” \(d\) lines are a turning point from the right-handed to the left-handed, and vice versa. According to the sign of the \(d\) values, the area of the right-handed helix \((d > 0)\) and that of the left-handed helix \((d < 0)\) appear alternately with the change in \(\tau_1\) and \(\tau_2\) values (Figure 2.13).

Based on the above \(\theta\) and \(d\) analyses, possible backbone conformations of the \(\text{homo}M^2\) polymer are examined. The resulting backbone conformations at points A~I are
Figure 2.12: Illustration of the helical translation $d_{1,1}$ and $d_{2,2}$ in the $homoM2$ polymer. $d_{1,1}$ is given by $d_{1,2} + d_{2,1}$ and $d_{2,2}$ is given by $d_{2,1} + d_{1,2}$. 
Figure 2.13: 3D plot of the helical translation $d = d_{1,1} = d_{2,2}$ in the homoM2 polymer with varying the internal rotation angles $\tau_1$ and $\tau_2$. 
Figure 2.14: Illustration of the resulting backbone conformations at points A~I (Figure 2.11) in the homoM2 polymer.

illustrated by Figure 2.14. The set of the internal rotation angles \((\tau_1, \tau_2) = (\pm \pi, \pm \pi)\) (point A in Figure 2.11) places all backbone atoms on the same plane \((d = 0, \text{ Figure 2.13})\). If two bond angles were equal \((\alpha_1 = \alpha_2)\), the resulting backbone would become a straight trans-planar zigzag because \(n = \infty\) by eq. 2.52. However, because we here adopt \(\alpha_1 = 111^\circ\) and \(\alpha_2 = 116^\circ\) \((\alpha_1 \neq \alpha_2)\), the resulting backbone draws a certain arc while maintaining the planarity of \(d = 0\) (point A in Figure 2.14). In order to close this arched planar backbone without an overt steric hindrance, \(\alpha_1\) and \(\alpha_2\) should be limited to the values which request \(n\) being an integer. Fortunately, the present \(\alpha\) values close the arched planar backbone with \(n = 72\), because \(n\) is given as \(n = \frac{2\pi}{|\alpha_1 - \alpha_2|}\).

The \(|d|\) value is significantly increased when the internal rotation angles \((\tau_1, \tau_2)\) deviate from \((\tau_1, \tau_2) = (\pm \pi, \pm \pi)\) (point A in Figure 2.13), except for \(\tau_2 \simeq -\tau_1\) line (AGA; solid line). This is because the backbone begins to spiral along the helical axis (at point B), while at point A the molecular plane is perpendicular to the helical axis (Figure 2.14). Since point B fulfills both \(d > 0\) and \(n = 6\) (Figures 2.13 and 2.11), the resulting backbone has the right-handed helicity with the pitch six. A right-handed
helix \((d > 0)\) is also formed at point C, but the resulting backbone has the smaller helical pitch of \(n = 5\) (Figure 2.14).

On the other hand, the conformations at points D, E, and F have the same pitch number \((n = 2)\) but the different structures. According to our definition of eq. 2.30, these points have the minus \(d\) values (Figure 2.13)\[9\]. Therefore, the conformation at point D becomes the left-handed helix with \(n = 2\) (Figure 2.14). On the contrary, the conformations at points E and F become the \textit{cis-planar} chains (Figure 2.14) because \((\tau_1, \tau_2) = (0, \pm \pi)\) and \((\pm \pi, 0)\). If the two bond lengths were equal \((r_1 = r_2)\), the two \textit{cis-planar} backbones would become equivalent. But, since we here adopt \(r_1 = 1.52\text{Å}\) and \(r_2 = 1.33\text{Å}\) \((r_1 \neq r_2)\), these two conformations are different. From these results, one can find that the M2 polymer causes more than one conformation having the same pitch and the same helical direction (right-handed or left-handed). This characteristic is contrastive to the M1 polymer, caused by an increase in the degrees of freedom in the internal rotation angles \((\tau_1 \text{ and } \tau_2)\).

How about the conformations on the \(d = 0\) line (\(\tilde{\text{AGA}}\); solid line)? Let us focus on the conformation at point G. The backbone conformation at point G becomes a flat disk, in which all the backbone atoms are set on the same plane (Figure 2.14). This is because the two internal rotation angles of \((\tau_1, \tau_2) = (0, 0)\) provide \(d = 0\) (Figure 2.13), i.e., \(d_{1,1} = d_{2,2} = d_{1,2} = d_{2,1} = 0\) (Figure 2.12). Since the corresponding \(n\) value is not an integer \((n = 2.7)\), the flat disk is not closed but open as in Figure 2.14.

The other \((\tau_1, \tau_2)\) sets on the \(d = 0\) line \((\tau_2 \simeq -\tau_1)\) produce not a flat disk but a coronal ring, because \(d\) satisfies \(d_{1,2} = -d_{2,1} \neq 0\) to be \(d_{1,1} = d_{2,2} = 0\) (Figure 2.12). Although a decimal pitch number cannot close the coronal ring, an integer pitch allows the formation of a closed coronal ring on the \(d = 0\) line. For example, the closed ring with an even pitch of \(n = 6\) and an odd pitch of \(n = 5\) is produced at points H and I, respectively (Figure 2.14). The closed ring formation in the \textit{homoM2} polymer is caused by the increase in the degrees of freedom in the M2 polymer.

### 2.4.2 Hetero-rotatory polymer

\(\theta\) analysis

Next, let us consider the hetero-rotatory M2 polymer \((\text{heteM2})\), in which the sign of two internal rotation angles \((\tau_1 \text{ and } \tau_2)\) is changed alternately among the adjacent unit cells as \(\cdots, \tau_1, \tau_2, -\tau_1, -\tau_2, \tau_1, \tau_2, \cdots\).

In the case of the \textit{heteM2} polymer, the transformation matrix \(A\) is rewritten using
The helical pitch angle $\theta_{\text{heteM2}}$ is given (by $Tr[A_{\text{heteM2}}] = Tr[N_{\text{heteM2}}]$) as

$$
cos \theta_{\text{heteM2}} = \frac{1}{2} \left( 1 + \cos \alpha_1 \cos \alpha_2 - \cos \tau_1 \cos \tau_2 \right) + \cos \alpha_1 \cos \alpha_2 \cos \tau_1 \cos \tau_2 - \sin \alpha_1 \sin \alpha_2 \cos \tau_1 \\
- \sin \alpha_1 \sin \alpha_2 \cos \tau_2 + \cos \alpha_1 \sin \tau_1 \sin \tau_2 \\
- \cos \alpha_2 \sin \tau_1 \sin \tau_2.
$$

The calculated $\cos \theta_{\text{heteM2}}$ values are plotted in Figure 2.10 with varying $\tau_1$ and $\tau_2$. The resulting $\cos \theta_{\text{heteM2}}$ surface osculates with the $\cos \theta_{\text{homoM2}}$ surface at the four corners ($\tau_1, \tau_2$) = ($\pm \pi, \pm \pi$) and also at the center ($\tau_1, \tau_2$) = (0, 0), because those points provide $\cos \theta_{\text{heteM2}} = \cos |\alpha_1 - \alpha_2| = \cos \theta_{\text{homoM2}}$ and $\cos \theta_{\text{heteM2}} = \cos (\alpha_1 + \alpha_2) = \cos \theta_{\text{homoM2}}$, respectively. The latter ($\tau_1, \tau_2$) = (0, 0) provides the minimum $\cos \theta$ for the $\text{heteM2}$ polymer, while these $\tau$ values give the saddle point in the $\text{homoM2}$ polymer. On the other hand, the maximum value of $\cos \theta_{\text{heteM2}} = 1$ is found at four points of ($\tau_1, \tau_2$) = (0, $\pm \pi$) and ($\pm \pi, 0$). Thus, the possible helical pitch angle $\theta_{\text{heteM2}}$ and the pitch number $n_{\text{heteM2}}$ are found within

$$
0 \leq \theta_{\text{heteM2}} \leq 2\pi - (\alpha_1 + \alpha_2),
$$

and

$$
\frac{2\pi}{2\pi - (\alpha_1 + \alpha_2)} \leq n_{\text{heteM2}} \leq \infty.
$$

The helical translation ($d$) analysis can also be performed for the $\text{heteM2}$ polymer. In the case of the $\text{heteM2}$ polymer, the helical translation $d$ is given by

$$
d_{\text{heteM2}} = \vec{B}_{\text{heteM2}} \cdot \vec{s},
$$
Figure 2.15: Illustration of the helical translation $d_{1,1}$ and $d_{2,2}$ in the heteroM2 polymer. $d_{1,1}$ is given by $d_{1,2} + d_{2,1}$ and $d_{2,2}$ is given by $d_{2,1} - d_{1,2}$.

where

$$B^{\text{heteroM2}} = A_1 r_1^2 + r_2^2. \quad (2.61)$$

Analogous to the homoM2 polymer, two different $d$ can be defined for one conformation of the heteroM2 polymer; $d_{1,1}$ corresponding to the helical translation $d$ from $M_s^1$ atom to $M_{s+1}^1$ atom and $d_{2,2}$ corresponding to that from $M_s^2$ atom to $M_{s+1}^2$ atom (Figure 2.15). In the hetero-rotatory sequence, $d_{1,1}$ is not equal to $d_{2,2}$, because $d_{1,1}$ is given by $d_{1,1} = d_{1,2} + d_{2,1}$ while $d_{2,2}$ is given by $d_{2,2} = d_{2,1} - d_{1,2}$. Therefore, two $d$ maps of $d_{1,1}$ and $d_{2,2}$ can be obtained for the heteroM2 polymer.

In Figures 2.16 (a) and 2.17 (a), $d_{1,1}$ values and $d_{2,2}$ values are plotted respectively with varying $\tau_1$ and $\tau_2$ values. The equi-$d$ lines of $d = 0$ (solid) and “discontinuous” $d$ (broken) are also shown in Figures 2.16 (b) and 2.17 (b). By comparing these two equi-$d$ maps, one can find that the discontinuous $d$ lines (broken lines) of $d_{1,1}$ coincide with those of $d_{2,2}$, while $d_{1,1} = 0$ line does not coincide with $d_{2,2} = 0$ line. $d_{1,1} = 0$ line provides a relation of $\tau_2 \simeq \tau_1$, but $d_{2,2} = 0$ line provides a relation of $\tau_2 \simeq -\tau_1$.

Here, we should also consider the following characteristic. $d = 0$ requires $d_{1,2} = -d_{2,1}$ or $d_{1,2} = d_{2,1}$ (Figure 2.15). Therefore, if $d_{1,2} = -d_{2,1} \neq 0$ or $d_{1,2} = d_{2,1} \neq 0$, a coronal ring (open or closed) is produced. Moreover, if $d_{1,2} = d_{2,1} = 0$, all backbone atoms put on the same plane so that a flat disk (open or closed) is produced. Those backbones are closed if the corresponding pitch $n$ is an integer, but in the other cases the resulting backbone does not close per turn (open). If $d \neq 0$, only an even pitch
Figure 2.16: 3D plot of $d_{1,1}$ values in the heteM2 polymer with varying the internal rotation angles $\tau_1$ and $\tau_2$ (a). Equi-$d$ lines of $d = 0$ and “discontinuous” $d$ are shown in figure (b).
Figure 2.17: 3D plot of $d_{2,2}$ values in the $heteM2$ polymer with varying the internal rotation angles $\tau_1$ and $\tau_2$ (a). Equi-$d$ lines of $d = 0$ and “discontinuous” $d$ are shown in figure (b).
number can close the backbone.

Taking into account the above $d$ characteristic and also the calculated $\cos \theta$ ($n$) values, one can find the possible backbone conformations in the $heteM2$ polymer. In Figure 2.18, the resulting $equi-n^{heteM2}$ lines are illustrated while superimposing the lines of $d = 0$, i.e., $d_{1,1} = 0$ and $d_{2,2} = 0$ (solid lines) and “discontinuous” $d$ lines (broken lines). The several resulting backbone conformations (at points J-Q in Figure 2.18) are also illustrated by Figure 2.19.

First, let us focus on the conformations at several special points. At the four corners [points J; $(\tau_1, \tau_2) = (\pm \pi, \pm \pi)$] and the center [point M; $(\tau_1, \tau_2) = (0, 0)$], the resulting backbones are exactly equivalent to those corresponding to the $homoM2$ polymer (J and M in Figure 2.19), because those two $\cos \theta$ surfaces osculate mutually at these points. Since both points J and M satisfy $d_{1,1} = d_{2,2} = d_{1,2} = d_{2,1} = 0$, the resulting conformations have a unique molecular plane. According to the $\tau$ values, the arched $trans$-planar backbone and the open flat disk are resulted, respectively (Figure 2.19). On the contrary, the resulting backbone at point P (Q) is a $cis$-planar being coincident with that for point E (F), although two $equi-\cos \theta$ surfaces do not osculate. This is because the corresponding internal rotation angles $(\tau_1, \tau_2) = (\pm \pi, 0)$ [or $(0, \pm \pi)$] are equivalent at this point P (or Q).

The above open planar chains are, however, rather exceptions for the $heteM2$ polymer, and the other sets of $(\tau_1, \tau_2)$ produces a coronal ring (open or closed) due to its “hetero-rotatory” catenation. Because points K and O are intersections of $n = 9$ and $d = 0$, conformations at those points become closed coronal rings with pitch nine (Figure 2.18). However, the resulting closed ring conformations are different, between the two because point K provides $d_{2,2} = 0$ but point O gives $d_{1,1} = 0$. Therefore, the conformation at point K puts all $M_2$ atoms (red) on the same plane, while the conformation at point O puts all $M_1$ atoms (blue) on the same plane (Figure 2.19).

If $n$ is an even number, the resulting backbone is always closed per turn due to the hetero-rotatory sequence. Therefore, $\phi$ and $\psi$ values on the $n = 6$ line produces a closed ring with pitch six, for instance. If the corresponding $d$ satisfies $d = 0$, either $M_1$ atoms or $M_2$ atoms are put on the same plane, i.e., all $M_2$ (red) atoms are put on the same plane at point L because $d_{2,2} = 0$, while all $M_1$ (blue) atoms are put on the same plane at point N because $d_{1,1} = 0$ (Figure 2.19).
Figure 2.18: *Equi-n* lines in the *heteM1* polymer obtained by Figure 2.10. *Equi-n* lines corresponding to $n = 3, 6, 9, 12, 15, 18, \text{ and } \infty$ are shown while superposing $d = 0$ lines in the figure.
**Figure 2.19:** Illustration of the resulting backbone conformations at points J~Q (Figure 2.18) in the *heteM2* polymer.
In this section, we discuss a periodic polymer whose unit cell consists of three skeletal atoms \( (m = 3, \text{ the M3 polymer}) \). First, the mathematical treatment is expanded to the M3 polymer and relations between the internal parameters and the external cylindrical parameters are formulated.

By further assuming some internal parameters, possible backbone conformations of periodic polypeptides can be understood, because a peptide backbone consists of three skeletal atoms per unit cell \((C^\alpha, C, \text{ and } N)\). Therefore, we can study stereo-structures of a homo-polypeptide \((\text{homoPP})\) having a homo-\(L\)-amino acid sequence \((\text{LLLL} \cdots)\) and a hetero-polypeptide \((\text{hetePP})\) having an alternating \(D\)– and \(L\)-amino acid sequence \((\text{DLDL} \cdots)\) on the basis of the analysis of homo- and hetero-rotatory M3 polymers.

### 2.5.1 Homo-rotatory polymer

First, let us consider a general homo-rotatory M3 polymer \((\text{homoM3})\), in which all unit cells catenate regularly while maintaining individual internal rotation angles \(\tau_1, \tau_2, \text{ and } \tau_3\) in the same direction, i.e., \(\cdots, \tau_1, \tau_2, \tau_3, \tau_1, \tau_2, \tau_3, \cdots\) (Figure 2.20).

#### \(\theta\) analysis

In the case of the \(\text{homoM3}\), a transformation matrix \(A\) is given as

\[
A^{\text{homoM3}} = (A_1 \cdot A_2 \cdot A_3) \cdot R^{\text{homo}},
\]  

(2.62)
where

\[
A_1 = \begin{pmatrix}
-\cos \alpha_1 & -\sin \alpha_1 & 0 \\
\sin \alpha_1 \cos \tau_1 & -\cos \alpha_1 \cos \tau_1 & -\sin \tau_1 \\
\sin \alpha_1 \sin \tau_1 & -\cos \alpha_1 \sin \tau_1 & \cos \tau_1
\end{pmatrix}, 
\]

(2.63)

\[
A_2 = \begin{pmatrix}
-\cos \alpha_2 & -\sin \alpha_2 & 0 \\
\sin \alpha_2 \cos \tau_2 & -\cos \alpha_2 \cos \tau_2 & -\sin \tau_2 \\
\sin \alpha_2 \sin \tau_2 & -\cos \alpha_2 \sin \tau_2 & \cos \tau_2
\end{pmatrix},
\]

(2.64)

\[
A_3 = \begin{pmatrix}
-\cos \alpha_3 & -\sin \alpha_3 & 0 \\
\sin \alpha_3 \cos \tau_3 & -\cos \alpha_3 \cos \tau_3 & -\sin \tau_3 \\
\sin \alpha_3 \sin \tau_3 & -\cos \alpha_3 \sin \tau_3 & \cos \tau_3
\end{pmatrix}.
\]

(2.65)

Since a matrix \(A_{homoM3}\) is similar to a matrix \(N_{homoM3} = N_{unit} \cdot R_{homo} = N_{unit}\) (eq. 2.12), traces of the two matrices should be coincident \((Tr[A_{homoM3}] = Tr[N_{homoM3}]\)). Thus, a helical pitch angle \(\theta_{homoM3}\) is given as

\[
\cos \theta_{homoM3} = \frac{1}{2}(a_{11}^{homoM3} + a_{22}^{homoM3} + a_{33}^{homoM3} - 1),
\]

(2.66)

where symbols \(a_{ii}^{homoM3}\) mean a diagonal element of the matrix \(A_{homoM3}\) given by

\[
a_{11}^{homoM3} = \cos \alpha_3(\sin \alpha_1 \sin \alpha_2 \cos \tau_2 - \cos \alpha_1 \cos \alpha_2) \\
+ \sin \alpha_1 \cos \alpha_3, \\
+ \sin \alpha_1 \sin \alpha_3 \sin \tau_2 \sin \tau_3,
\]

(2.67)

\[
a_{22}^{homoM3} = \sin \alpha_3(\sin \alpha_1 \cos \alpha_2 \cos \tau_1 \\
+ \sin \alpha_1 \sin \tau_1 \sin \tau_2 + \cos \alpha_1 \sin \alpha_2 \cos \tau_1 \cos \tau_2) \\
+ \cos \alpha_3 \sin \tau_3(\sin \tau_1 \cos \tau_2 - \cos \alpha_1 \cos \tau_1 \sin \tau_2) \\
+ \cos \alpha_3 \cos \tau_3(\sin \alpha_1 \sin \alpha_2 \cos \tau_1 \\
- \cos \alpha_2 \sin \tau_1 \sin \tau_2 \\
- \cos \alpha_1 \cos \alpha_2 \cos \tau_1 \cos \tau_2),
\]

(2.68)

\[
a_{33}^{homoM3} = \cos \tau_3(\cos \tau_1 \cos \tau_2 + \cos \alpha_1 \sin \tau_1 \sin \tau_2) \\
- \sin \tau_3(\cos \alpha_1 \cos \alpha_2 \sin \tau_1 \cos \tau_2 \\
- \sin \alpha_1 \sin \alpha_2 \sin \tau_1 - \cos \alpha_2 \cos \tau_1 \sin \tau_2). 
\]

(2.69)
**d analysis**

A helical translation ($d$) analysis also gives us important information about the backbone conformation. In the case of the \textit{homoM3} polymer, the $d$ value is given as

$$d^{\text{homoM3}} = \vec{B}^{\text{homoM3}} \cdot \vec{s},$$  \hspace{1cm} (2.70)

where

$$\vec{B}^{\text{homoM3}} = A_1 A_2 \vec{r}_2 + A_1 \vec{r}_1 + \vec{r}_3.$$  \hspace{1cm} (2.71)

Here, three different $d$ can be defined for one conformation; $d_{1,1}$ corresponding to the helical translation $d$ from $M_s^1$ atom to $M_s^{1+1}$ atom, $d_{2,2}$ corresponding to that from $M_s^2$ atom to $M_s^{2+1}$ atom, and $d_{3,4}$ corresponding to the helical translation $d$ from $M_s^3$ atom to $M_s^{3+1}$ atom (Figure 2.20). However, these three $d$ are equivalent in the homo-rotatory sequence. Therefore, unique $d$ map can be obtained for the \textit{homoM3} polymer.

Based on eqs. 2.66 and 2.70, we can analyze the backbone conformations of a general \textit{homoM3} polymer as a function of the internal parameters. In the following, we specify our discussion on the backbone conformations of a homo-rotatory polypeptide (\textit{homoPP}) in order to understand and explore possible molecular structures of the homo L-amino acid sequence (homo-L-peptide).

**2.5.2 Homo-L-polypeptide**

\(\theta\) analysis

Here, let us focus on the bond nature of polypeptides (Figure 2.21). A skeletal conformation of polypeptide is said to be determined dominantly by internal rotation angles $\phi$, $\psi$, and $\omega$ rather than bond lengths $r_i$ and bond angles $\alpha_i$[10], because $r_i$ and $\alpha_i$ are hardly changed owing to the polypeptides’ covalent nature. Taking into account this characteristic, following experimental values are quoted for $r_i$ and $\alpha_i$: $r_1 = 1.52\text{Å}$, $r_2 = 1.33\text{Å}$, $r_3 = 1.45\text{Å}$, $\alpha_1 = 111^\circ$, $\alpha_2 = 116^\circ$, and $\alpha_3 = 122^\circ$[8]. Moreover, we assume a flat amide plane of $\omega = \pi$ between C$^\alpha$ atoms, which is commonly found in polypeptides unless they include any peculiar hydrogen bonds or steric hindrances in their own backbones. Under these assumptions, three transformation matrices $A_1$, $A_2$, and $A_3$ are rewritten as

$$A_1 = \begin{pmatrix} -\cos 111^\circ & -\sin 111^\circ & 0 \\ \sin 111^\circ \cos \phi & -\cos 111^\circ \cos \phi & -\sin \phi \\ \sin 111^\circ \sin \phi & -\cos 111^\circ \sin \phi & \cos \phi \end{pmatrix}$$  \hspace{1cm} (2.72)

47
Figure 2.21: Illustration of a polypeptide chain and definition of the internal conformation parameters of bond lengths \((r_1, r_2, r_3)\), bond angles \((\alpha_1, \alpha_2, \alpha_3)\) and internal rotation angles \((\phi, \psi, \omega)\). When \(\omega = \pi\), \(\vec{z}_2\) on the C atom coincides with a normal vector of the amide plane.

Here, we follow a standard notation of the internal rotation angles of polypeptides, i.e., \(\tau_1 = \phi\) and \(\tau_2 = \psi\).

Based on eqs. 2.72-2.74, one can obtain a relation between a helical pitch angle \((\cos \theta)\) and internal rotation angles \(\phi\) and \(\psi\) in the homoPP. Calculated \(\cos \theta^{\text{homoPP}}\) values reveal that the \(\cos \theta\) surface has a “butterfly” shape [Figure 2.22 (a)], and this feature is originated from a crossing composition of two \(\cos \theta\) plots of the homoM1 polymer [Figure 2.6 (b)]. By drawing \(\text{equi-cos} \theta\) lines (\(\text{equi-n}\) lines), we can find a characteristic that the \(\phi\)-\(\psi\) map has an inversion center at the origin of \((\phi, \psi) = (0, 0)\). The
inverse symmetry \( \cos \theta(\phi, \psi) = \cos \theta(-\phi, -\psi) \) causes an enantiomeric relation between two backbones having internal rotation angles \((\phi, \psi)\) and \((-\phi, -\psi)\). However, those two backbones lose their reflected image if side chains are actually substituted.

Equi-\(\cos \theta\) lines plotted in Figure 2.22 (b) \(\theta = \pi, 5\pi/6, 2\pi/3, 5\pi/9, \pi/2\) and \(2\pi/5\) correspond to the equi-\(n\) lines of \(n = 2, 2.4, 3, 3.6, 4\) and \(5\), respectively. \(\phi\) and \(\psi\) values on those lines provide helical structures having the corresponding pitch number, i.e., not only an integer pitch but also a decimal pitch number is available in the homoPP. However, the \(\phi-\psi\) map indicates an important result that a possible \(\cos \theta^{\text{homoPP}}\) value is limited within

\[
-1 \leq \cos \theta^{\text{homoPP}} \leq 0.454. \tag{2.75}
\]

Therefore, an available helical pitch number in the homoPP is restricted as

\[
2 \leq n^{\text{homoPP}} \leq 5.7. \tag{2.76}
\]

\(d\) analysis

By further assuming three bond lengths as \(r_1 = 1.52\text{Å}, r_2 = 1.33\text{Å}, r_3 = 1.45\text{Å}\) [7], one can obtain possible \(d\) values based on eq. 2.70. The resulting \(d\) values show that the homoPP basically forms a right-handed helix or left-handed helix in a region of \(d > 0\) or \(d < 0\), respectively (Figure 2.23). The right-handed region and the left-handed region appear alternately in Figure 2.23.

Regions of the right-handed helix and the left-handed helix are separated by \(d = 0\) lines (solid lines) or a “discontinuous” \(d\) line (broken line) (Figure 2.23). The \(d = 0\) lines draw curves between \((\phi, \psi) = (0, -\pi)\) and \((-\pi, 0)\) or \((\phi, \psi) = (0, \pi)\) and \((\pi, 0)\), and on these lines a “zero-transfer” helix is formed. This zero-transfer helix corresponds to the middle structure between right-handed and left-handed helices and we cannot distinguish the helical direction anymore. In contrast, the helical direction of right-handed or left-handed is changed discontinuously beyond the broken line (discontinuous line) along \(\psi \simeq -\phi\) \((\tau_2 \simeq -\tau_1)\).

Inclination angle (\(\Theta\)) analysis

In order to discuss details in the stereo-structures of the homoPP (the M3 polymer), let us introduce an inclination angle \(\Theta\) of the skeletal (amide) plane. This angle is defined by an angle between the amide plane and the helical axis and represented by an inner product between an unit vector of the helical axial \(\vec{t}\) and a normal vector of the amide plane [Figure 2.24 (a)]. The latter vector is equivalent to \(\vec{z}_2\) of the internal
Figure 2.22: 3D plot of $\cos \theta^{homoPP}$ values (a) and equi-$\cos \theta$ lines in the $homoPP$ (b). Equi-$\cos \theta$ lines of $n = 2, 2.4, 3, 3.6, 4$, and 5 are shown in figure (b).
Figure 2.23: 3D plot of $d$ values in the homoPP with varying $\phi$ and $\psi$ angles. The $d = 0$ lines (solid lines) and the “discontinuous” $d$ line (broken line) are shown in the figure. Along the broken line, $d$ values are discontinuously changed from minus to plus, and vice versa. The employed internal parameters in the calculation are the same as those used for Figure 2.22.
orthogonal coordinate system when $\omega = \pi$ (Figure 2.21). Thus, the resulting inner product is given as

$$|\cos \Theta| = |\vec{z}_2 \cdot \vec{t}| = \frac{1}{C} \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \begin{pmatrix} a_{32} - a_{23} \\ a_{13} - a_{31} \\ a_{21} - a_{12} \end{pmatrix}. \quad (2.77)$$

Here, symbol $C$ is a normalized factor given by

$$C = \sqrt{(a_{32} - a_{23})^2 + (a_{13} - a_{31})^2 + (a_{21} - a_{12})^2}. \quad (2.78)$$

Equi-$\Theta$ lines resulted from eq. 2.77 are shown in Figure 2.24 (b). This equi-$\Theta$ map is useful to characterize the backbone conformation of polypeptide (the M3 polymer). In the following, we discuss the several characteristic conformations of the homoPP based on the equi-$\cos \theta$ map [Figure 2.22 (b)], the $d$ values (Figure 2.23), and the equi-$\Theta$ map [Figure 2.24 (b)].

**Extended-type and Bound-type backbones**

First, let us focus on the intersections of $\Theta = \pi/2$ and $n = 2$ lines. The intersections appear at four corners (point A) and the center (point B) of Figure 2.25 (a), and both of the corresponding internal rotation angles $(\phi, \psi) = (\pm \pi, \pm \pi)$ and $(\phi, \psi) = (0^\circ, 0^\circ)$ provide the planar chains. However, the different internal rotation angles cause a difference in backbone conformations between the two points. At point A, the most “extended” planar chain (EP) is produced, while at point B, the most “bound” planar chain (BP) is produced [Figure 2.25 (b)].

EP and BP structures are typical backbone conformations of the Extended-type and the Bound-type, respectively. The other $(\phi, \psi)$ sets on the $\phi$ and $\psi$ map provide not the planar chains but the general helical structures. According to the $\phi$ and $\psi$ values, the helical backbones are classified into two types; the Extended-type (E-type) backbone with $|\phi|, |\psi| \geq \pi/2$ and the Bound-type (B-type) backbone with $|\phi|, |\psi| \leq \pi/2$ [Figure 2.25 (a)].

**$\alpha$-structure**

The other points on the $\Theta = \pi/2$ line produce an “$\alpha$-structure” being allied to a typical secondary structure of the $\alpha$-helix [Figure 2.24 (b)], because $\Theta = \pi/2$ lets the amide plane be parallel to the helical axis. Structural characteristic of the $\alpha$-structure is well understood by focusing on the intersections of the $n = 3$ lines and the $\Theta = \pi/2$ line [Figure 2.26 (a)]. The $\Theta = \pi/2$ line crosses four $n = 3$ lines at four points (points C,
Figure 2.24: Illustration of the inclination angle $\Theta$ (a) and the calculated equi-$\Theta$ lines in the homoPP. The lines of $\Theta = \pi/2, \pi/3, \pi/4$ and $0^\circ$ are shown in figure (b).
Figure 2.25: Multi-contour map of the homoPP by superimposing the $\Theta = \pi/2$ line on the $n = 2$ line (a). Stereo-structures at points A and B are illustrated by figure (b).
Figure 2.26: Multi-contour map of the homoPP in which the \( d = 0 \) lines (and the discontinuous \( d \) line; dotted) and \( \Theta = \pi/2 \) line are superimposed on the \( n = 3 \) lines (a). Geometrical points of typical helical forms, i.e., the right- and left-handed \( \alpha \)-helices (\( \alpha^R \) and \( \alpha^L \)), the right- and left-handed \( \pi \)-helices (\( \pi^R \) and \( \pi^L \)), and the right- and left-handed \( 3_{10} \)-helices (\( 3_{10}^R \) and \( 3_{10}^L \)), are also plotted in figure (a). In figure (b), the side-view and top-view of the backbone conformations at points A~D are illustrated.
D, E and F). Although these four points provide the same pitch number of \( n = 3 \), the resulting helices have different conformations.

The difference is first distinguished by a direction of the helical translation. While the right-handed helices are produced at points C and E \((d > 0)\), the left-handed helices are produced at points D and F \((d < 0)\). Note that the backbone conformation at point C (E) is an enantiomer of the conformation at point F (D) [Figure 2.26 (b)].

Here, one should also notice the difference between the conformations at point C (F) and point E (D). Although these two conformations have the same helical direction of the right-handed (left-handed), they have different helical translations. The conformation at points C and F have the more elongated (extended) helical backbones, while the conformations at points E and D have the more shrunk (bound) backbones [Figure 2.26 (b)]. The former correspond to the Extended-type (E-type) backbones \((|\phi|, |\psi| \geq \pi/2)\) and the latter correspond to the Bound-type (B-type) backbones \((|\phi|, |\psi| \leq \pi/2)\).

Geometrical points of the well-known \( \alpha^-\), \( \pi^-\), and \( 3_{10}^-\)-helices are also plotted in Figure 2.26 (a). By this figure, we can find structural characteristics of these typical helices. An enantiomeric relation is found in the corresponding helices between the right-handed (superscript R) and left-handed (superscript L), i.e., \( \alpha^R \) and \( \alpha^L \), \( \pi^R \) and \( \pi^L \), \( 3_{10}^R \) and \( 3_{10}^L \). All the three helices have the Bound-type (B-type) conformation due to the \( \phi \) and \( \psi \) values of \(|\phi|, |\psi| \leq \pi/2\). However, only \( \alpha^-\)-helices with \( n = 3.6 \) and \( \pi^-\)-helices with \( n = 4.4 \) appear almost on the \( \Theta = \pi/2 \) (\( \alpha^-\)-structure) line [Figure 2.26 (a)], which provides the amide planes being parallel to the helical axis.

\( \beta^-\)-structure

In contrast to the \( \alpha^-\)-structure on the \( \Theta = \pi/2 \) line, \( \phi \) and \( \psi \) values on the \( n = 2 \) line provide a “\( \beta^-\)-structure” whose backbone is pleated like the \( \beta^-\)-strand chain found in the \( \beta^-\)-sheets. Geometrical points of the parallel \( \beta^-\)-sheet (\( \beta^p \)) and the anti-parallel \( \beta^-\)-sheet (\( \beta_a^- \)) appear just on the \( n = 2 \) line [Figure 2.27 (a)].

By superimposing the \( \text{equi-} \Theta \) line \((\Theta = \pi/3)\) on the \( n = 2 \) line, one can understand a structural characteristic of the \( \beta^-\)-structure very well [Figure 2.27 (a)]. The \( n = 2 \) line crosses \( \Theta = \pi/3 \) lines at four points (G, H, I, and J). At points G and J, the Extended-type (E-type) \( \beta^-\)-structure is produced, while at points H and I the Bound-type (B-type) \( \beta^-\)-structure is formed [Figure 2.27 (b)]. Since a helicity of the right-handed or the left-handed disappears when \( n = 2 \), the backbone conformation at point J (I) coincides with the conformation at point G (H). It indicates that two backbone conformations of the E-type and the B-type are available with the same inclination angle in the \( \beta^-\)-structure.

Here, one should notice that both the parallel and anti-parallel \( \beta^-\)-sheets (\( \beta^p \) and
Figure 2.27: Multi-contour map of the homoPP with superimposing the $\Theta = \pi/3$ lines on the $n = 2$ line (a). The top-view and side-view of the backbone conformations at points G (J) and H (I) are shown in figure (b).
βα) have the E-type conformations with the internal rotation angles of \(|φ|, |ψ| ≥ \pi/2\) [Figure 2.27 (a)]. This feature is different from the α-, π-, and 310-helices, which all have the B-type conformations [Figure 2.26 (a)]. From Figure 2.27 (a), one can also find that the \(β_p\) has an inclination angle of \(Θ = \pi/3\), while the \(β_a\) has the larger inclination angle (more elongated backbone).

Closed ring

As mentioned above, the homo-L-amino acid sequence (homoPP) basically produces helical forms including α- and β-structures. This result seems to be reasonable and agrees on a commonly accepted view. But here, one can find an interesting point that not only the helical structures but also a closed ring structure is formed by the homoPP. This characteristic is very unusual, considering that a closed ring is said to be produced using the alternating D- and L-amino acid sequence[3].

To search a closed ring structure, let us focus on the \(d = 0\) lines (KML and K′M′L′) [Figure 2.28 (a)]. As discussed before, \(φ\) and \(ψ\) values on the \(d = 0\) lines provide a “zero-transfer” helix, which corresponds to the middle structure between right-handed and left-handed helices. An available pitch number of the zero-transfer helix is limited between 4.8 (point K (K′)) and 5.7 (point L (L′)), and those minimum and maximum values provide flat disks in which all backbone atoms are on the same skeletal plane because \(Θ = 0°\). The backbones of those flat disks do not close per helical turn due to the decimal pitch numbers. Therefore, the flat disks become “open” disks as shown in Figure 2.28 (b).

In order to close the zero-transfer helix and obtain a nanoring structure, an integer pitch number is further required in addition to \(d = 0\). An interesting result is that the \(d = 0\) line crosses the \(n = 5\) line (an odd pitch number) at point M (M′). This result indicates that a novel pentapeptide nanoring can be produced by the homo-L- (homo-D-)amino acids sequence. Since the point M (M′) appears on the \(Θ = \pi/2\) line accidentally, amide planes in the pentapeptide nanoring are parallel to the ring (helical) axis like the α-structure. Therefore, the pentapeptide nanoring has a potential to form the peptide nanotube by the ring-stacking through inter-ring hydrogen bonding.

2.5.3 Hetero-rotatory polymer

How does the possible backbone conformation change when the amino acid sequence is altered from the homo-rotatory to the hetero-rotatory as D,L,D,L,····? To discuss the backbone conformation of the hetero-D,L-polypeptide (hetePP), let us consider the gen-
Figure 2.28: Multi-contour map of the homoPP with superimposing $\Theta = 0, \pi/2$ and $d = 0$ lines on $n = 3, 4,$ and $5$ lines (a). The top-view and side-view of the backbone conformations at points K (K'), L (L') and M (M') are shown in figure (b).
eral hetero-rotatory M3 polymer (heteM3), in which a sign of three internal rotation angles (τ₁, τ₂, and τ₃) is changed alternately among the adjacent unit cells such as ⋮, τ₁, τ₂, τ₃, −τ₁, −τ₂, −τ₃, τ₁, τ₂, τ₃, ⋮.

θ analysis

In the case of the heteM3 polymer, the transformation matrix A is rewritten using \( R^{hete} \) as

\[
A^{heteM3} = A^{unit} \cdot R^{hete} = (A_1 \cdot A_2 \cdot A_3) \cdot R^{hete}. \tag{2.79}
\]

Because the transformation matrix \( N^{heteM3} \) is also given as

\[
N^{heteM3} = N^{unit} \cdot R^{hete} = \begin{pmatrix}
\cos \theta & -\sin \theta & 0 \\
\sin \theta & \cos \theta & 0 \\
0 & 0 & -1
\end{pmatrix}, \tag{2.80}
\]

a helical pitch angle \( \theta^{heteM3} \) is given (by \( Tr[A^{heteM3}] = Tr[N^{heteM3}] \)) as

\[
\cos \theta^{heteM3} = \frac{1}{2}(a_{11}^{heteM3} + a_{22}^{heteM3} + a_{33}^{heteM3} + 1), \tag{2.81}
\]

where

\[
a_{11}^{heteM3} = \cos \alpha_3 (\sin \alpha_1 \sin \alpha_2 \cos \tau_2 \\
- \cos \alpha_1 \cos \alpha_2) + \sin \alpha_1 \sin \alpha_3 \sin \tau_2 \sin \tau_3 \\
+ \sin \alpha_3 \cos \tau_3 (\cos \alpha_1 \sin \alpha_2 \\
+ \sin \alpha_1 \cos \alpha_2 \cos \tau_2), \tag{2.82}
\]

\[
a_{22}^{heteM3} = \sin \alpha_3 (\sin \alpha_1 \cos \alpha_2 \cos \tau_1 \\
+ \sin \alpha_2 \sin \tau_1 \sin \tau_2 + \cos \alpha_1 \sin \alpha_2 \cos \tau_1 \cos \tau_2) \\
+ \cos \alpha_3 \sin \tau_3 (\sin \tau_1 \cos \tau_2 - \cos \alpha_1 \cos \tau_1 \sin \tau_2) \\
+ \cos \alpha_3 \cos \tau_3 (\sin \alpha_1 \sin \alpha_2 \cos \tau_1 \\
- \cos \alpha_2 \sin \tau_1 \sin \tau_2 \\
- \cos \alpha_1 \cos \alpha_2 \cos \tau_1 \cos \tau_2), \tag{2.83}
\]

\[
a_{33}^{heteM3} = \sin \tau_3 (\cos \alpha_1 \cos \alpha_2 \sin \tau_1 \cos \tau_2 \\
- \sin \alpha_1 \sin \alpha_2 \sin \tau_1 - \cos \alpha_2 \cos \tau_1 \sin \tau_2) \\
- \cos \tau_3 (\cos \tau_1 \cos \tau_2) \\
+ \cos \alpha_1 \sin \tau_1 \sin \tau_2). \tag{2.84}
\]
A helical translation \( (d) \) in the \( \text{heteM3} \) polymer is given by the following equation;

\[
d^\text{heteM3} = \vec{B}^\text{heteM3} \cdot \vec{s},
\]

where

\[
\vec{B}^\text{heteM3} = A_1 A_2 \vec{r}_2 + A_1 \vec{r}_1 + \vec{r}_3.
\]

Note that there are three different \( d \) values for one conformation; \( d_{1,1} \) corresponding to the helical translation \( d \) from \( M_1^s \) atom to \( M_1^{s+1} \) atom, \( d_{2,2} \) corresponding to that from \( M_2^s \) atom to \( M_2^{s+1} \) atom, and \( d_{3,3} \) corresponding to the helical translation \( d \) from \( M_3^s \) atom to \( M_3^{s+1} \) atom (Figure 2.20). Thus, three \( d \) maps of \( d_{1,1} \), \( d_{2,2} \), and \( d_{3,3} \) can be obtained for the \( \text{heteM3} \) polymer.

Based on eqs. 2.81 and 2.85, one can analyze backbone conformations of the \( \text{heteM3} \) polymer as a function of the internal parameters. In the following, we discuss the conformations of the hetero-D,L-polypeptide (\( \text{hetePP} \)) by quoting the experimental values for \( r_i \) and \( \alpha_i \); \( r_1 = 1.52 \) Å, \( r_2 = 1.33 \) Å, \( r_3 = 1.45 \) Å, \( \alpha_1 = 111^\circ \), \( \alpha_2 = 116^\circ \), \( \alpha_3 = 122^\circ \), and \( \omega = 180^\circ \) as in the case of the \( \text{homoPP} \) (eqs. 2.72-2.74).

### 2.5.4 hetero-D,L-polypeptide

\( \theta \) analysis

Let us investigate possible \( \cos \theta \) values in the \( \text{hetePP} \) while varying two internal rotation angles \( \phi \) and \( \psi \). The resulting 3D plot of the \( \cos \theta \) shows a “ten-gallon hat” shape [Figure 2.29 (a)], which corresponds to the form obtained by rotating the “butterfly” shape of the \( \cos \theta \) surface in the \( \text{homoPP} \) [Figure 2.22 (a)] about the center \((\phi, \psi) = (0, 0)\) by \( \pi/2 \) and then overturning it. Figure 2.29 (a) reveals that the possible \( \cos \theta \) value in the \( \text{hetePP} \) is drastically changed from that in the \( \text{homoPP} \) and limited within

\[
0.259 \leq \cos \theta^\text{hetePP} \leq 1.
\]

Since \( \theta = 2\pi/n \), a possible helical pitch number of the \( \text{hetePP} \) is given as

\[
4.8 \leq n^\text{hetePP} \leq \infty.
\]

In Figure 2.29 (b), \( \text{equi-}n \) lines (\( \text{equi-} \cos \theta \) lines) of \( n = 5, 6, 8, 10, 12, 20 \), and \( \infty \) are shown. By this \( \phi \) and \( \psi \) map, we can clearly find a relation between the pitch number \( n \) and internal rotation angles \( \phi \) and \( \psi \).
Figure 2.29: The 3D plot of the $\cos^{\hetePP} \theta$ values (a) and equi-$\cos \theta$ lines in the $\hetePP$ (b). Equi-$\cos \theta$ lines of $n = 5, 6, 8, 10, 12, 20, \text{ and } \infty$ are shown in figure (b).
By further assuming three bond lengths as $r_1 = 1.52\text{Å}$, $r_2 = 1.33\text{Å}$, $r_3 = 1.45\text{Å}$[7], one can obtain possible $d$ values in the $hetePP$ based on eq. 2.85. In the case of the $hetePP$, three backbone atoms of $C^\alpha$, C, and N (Figure 2.21) correspond to $M_1$, $M_2$, and $M_3$ (Figure 2.20), respectively. Therefore, $d_{1,1}$, $d_{2,2}$, and $d_{3,3}$ are denoted by $d_{C^\alpha,C^\alpha}$, $d_{C,C}$, and $d_{N,N}$. 3D plots of the calculated $d_{C^\alpha,C^\alpha}$, $d_{C,C}$, and $d_{N,N}$ values are shown in Figures 2.30 (a), 2.31 (a), and 2.32 (a). The equi-$d$ lines of $d = 0$, i.e., $d_{C^\alpha,C^\alpha}$, $d_{C,C}$, and $d_{N,N}$ (solid lines), and “discontinuous” $d$ (broken line) are also shown in Figures 2.30 (b), 2.31 (b), and 2.32 (b).

$d > 0$ and $d < 0$ regions are separated by $d = 0$ lines (solid lines) and the “discontinuous” $d$ line (broken line). $d = 0$ lines require a “zero” helical translation per unit, while the discontinuous $d$ line changes a sign of $d$ value discontinuously from plus to minus, and vice versa. The same discontinuous $d$ lines are found along $\psi \simeq \phi$ among $d_{C^\alpha,C^\alpha}$, $d_{C,C}$, and $d_{N,N}$. On the contrary, the resulting $d = 0$ lines are different among those three [Figures 2.30 (b), 2.31 (b), and 2.32 (b)].

**Inclination angle ($\Theta$) analysis**

An inclination angle ($\Theta$) analysis is also helpful to discuss the backbone conformation of the $hetePP$. Possible $\Theta$ values in the $hetePP$ can be obtained by eq. 2.77, and the 3D plot of the $d$ values are shown in Figure 2.33 (a). Let us focus on the $\Theta = \pi/2$ line ($\psi \simeq -\phi$). In the case of the $hetePP$, $\Theta = \pi/2$ provides the $\beta$-structure as illustrated by Figure 2.33 (b). This feature is contrast to that in the $homoPP$, which forms the $\beta$-structure along the $n = 2$ line [Figure 2.27 (a)]. However, the $n = 2$ line in the $homoPP$ coincides with the $\Theta = \pi/2$ line in the $hetePP$, because the direction of the helical axis is rotated by $\pi/2$ in the $hetePP$ [Figure 2.33 (b)]. Therefore, $\psi \simeq -\phi$ values provide the $\beta$-strand open chain in the $homoPP$, while the same values provide the annular $\beta$-structure in the $hetePP$ [Figure 2.33 (b)].

If $\Theta \neq \pi/2$, amide planes are inclined against the helical (ring) axis with the corresponding $\Theta$ value [Figure 2.33 (c)]. The smallest inclination angle of $\Theta = 0^\circ$ provides flat disks at $(\phi, \psi) = (\pi, 0)$, $(-\pi, 0)$, $(0, \pi)$, and $(0, -\pi)$, which coincide with the flat (open) disks found in the $homoPP$ [Figure 2.28 (b)].

**Closed ring**

Based on the $\theta$, $d$, and $\Theta$ analyses, let us discuss several characteristic conformations of the $hetePP$. In the $hetePP$, an even pitch number $n$ always provides a closed ring struc-
Figure 2.30: 3D plot of $d_{C\alpha,C\alpha}$ values in the hetePP with varying $\phi$ and $\psi$ angles (a). Equi-$d$ lines of $d_{C\alpha,C\alpha} = 0$ (solid lines) and “discontinuous” $d$ (broken line) are shown in figure (b). Along the broken line, $d$ values are discontinuously changed from minus to plus, and vice versa. The employed internal parameters in the calculation are the same as those used for Figure 2.29.
Figure 2.31: 3D plot of $d_{c,c}$ values in the *hetePP* with varying $\phi$ and $\psi$ angles (a). *Equi-d* lines of $d_{c,c} = 0$ (solid lines) and “discontinuous” $d$ (broken line) are shown in figure (b). Along the broken line, $d$ values are discontinuously changed from minus to plus, and vice versa. The employed internal parameters in the calculation are the same as those used for Figure 2.29.
Figure 2.32: 3D plot of $d_{N,N}$ values in the $hetePP$ with varying $\phi$ and $\psi$ angles (a). Equi-$d$ lines of $d_{N,N}=0$ (solid lines) and “discontinuous” $d$ (broken line) are shown in figure (b). Along the broken line, $d$ values are discontinuously changed from minus to plus, and vice versa. The employed internal parameters in the calculation are the same as those used for Figure 2.29.
Figure 2.33: Equi-Θ lines in the hetePP (a). The lines of Θ = π/2, π/3, π/4 and 0 are shown. Examples of the backbones with Θ = π/2 and π/3 are also shown in figures (b) and (c), respectively.
ture. Therefore, \(\phi\) and \(\psi\) values on even \(n\) lines, e.g., \(n = 6, 8, 10, 12, \cdots\) [Figure 2.29 (a)], provide peptide nanorings consisting of the even number of amino acid residues. A notable point is that a closed ring of \(n = 4\) cannot be produced by the present internal parameters used for the peptide backbone. This is because the minimum pitch number of the \(hetePP\) is given as 4.8 (eq. 2.88).

If \(n\) is an odd number and \(d \neq 0\) (\(d_{C^o,C^o} \neq 0, d_{C,C} \neq 0,\) or \(d_{N,N} \neq 0\)), the backbone does not close per helical turn, because a sum of \(d^s\) (\(\sum_{s=1}^{n} d^s\)) does not converge per helical turn due to a sequence of \(\cdots, d, -d, d, -d, \cdots\). Therefore, an “open” coronal ring is formed. The open ring structure is unrealistic because it must include a significant steric hindrance in its backbone. On the contrary, if \(n\) is an odd number and \(d = 0\) (\(d_{C^o,C^o} = 0, d_{C,C} = 0,\) or \(d_{N,N} = 0\)), the backbone is closed per turn without an overt steric hindrance in the backbone. If \(n\) is a decimal number, the backbone never closes per turn, irrespective of the \(d\) value. Therefore, a decimal pitch number causes an unrealistic open coronal ring. These are general characteristics found in the \(hetePP\) backbones.

Next, we discuss details in the conformation of the \(hetePP\) based on a multi-contour map (Figure 2.34), in which the \(\Theta = \pi/2\) and \(d = 0\) lines (\(d_{C^o,C^o} = 0, d_{C,C} = 0,\) and \(d_{N,N} = 0\)) are superimposed on the equi-\(n\) lines of \(n = 5, 6, 7\) and 9. Intersecting points of the \(\Theta = \pi/2\) line and the equi-\(n\) lines provide the \(\beta\)-structure of the \(hetePP\), in which a normal vector of the amide plane is perpendicular to the helical (ring) axis as in Figure 2.33 (b). Figure 2.34 reveals that the \(hetePP\) can form the \(\beta\)-structure with \(n = 6, 7\) and 9 but not \(n = 5\), as long as the present internal parameters are employed[7].

The \(\Theta = \pi/2\) line crosses four \(n = 6\) lines at points A, B, A’, and B’ (Figure 2.34). Since the even pitch number requires a closed backbone, hexapeptide (six-residue) nanorings of the \(\beta\)-structure are formed at these four points (Figure 2.35). Here, the backbone conformation at point A’ (B’) coincides with the backbone conformation at point A (B), because the helical direction of right-handed or left-handed cannot be distinguished in the ring conformation.

Although point A (A’) and point B (B’) both provide closed ring structures with \(n = 6\), the resulting diameters are different, i.e., the former has the larger diameter and the latter has the smaller diameter (Figure 2.35). The larger ring at point A (A’) corresponds to the Extended-type (E-type) conformation and the smaller ring at point B (B’) corresponds to the Bound-type (B-type) conformation, because the former has the internal rotation angles of \(|\phi|, |\psi| \geq \pi/2\) while the latter has those of \(|\phi|, |\psi| \leq \pi/2\). Thus, two nanoring structures are available even with the same
Figure 2.34: Multi-contour map of the *hetePP* with superimposing $\Theta = \pi/2$ and $d = 0$ lines (and discontinuous $d$ line; dotted) on the lines of $n = 5, 6,$ and $9$. 
Figure 2.35: $\beta$-structures of the hetePP at points A (A')~D (D').
Figure 2.36: Stereo-structures of the hetePP at points E (E’)~G (G’).
point C (C’) and point D (D’) also provide the E-type and the B-type backbone, respectively (Figure 2.34). However, the resulting backbones become “open” rings (Figure 2.35) since these points provide an odd pitch number (n = 9). These open rings are considered to be unrealistic because they include overt steric hindrance in their own backbones. Therefore, the β-structure of peptide nanorings limits its pitch number to be even (n = 6, 8, 10, 12, ⋯).

The formation of closed rings is also common in other φ and ψ sets on the even n lines, although amide planes in those closed rings are inclined against the ring axis (Θ ≠ π/2) (Figure 2.33). On the other hand, φ and ψ values on the odd n lines do not close the backbone basically. These are essential features found in the hetePP. However, one can find an exception at intersections of d = 0 lines and odd n lines. As mentioned before, those points can provide closed rings irrespective of n is an even or odd because of the zero helical translation. Since there are three d = 0 lines of \(d_{C^\alpha,C^\alpha} = 0\), \(d_{C,C} = 0\), and \(d_{N,N} = 0\), different closed ring forms can be produced with the same pitch. For example, the intersection of \(d_{C^\alpha,C^\alpha} = 0\) and \(n = 5\) (points E and E’) provides a five-residue nanoring, in which all C\(^\alpha\) atoms are on the same plane (Figure 2.36). The intersection of \(d_{N,N} = 0\) and \(n = 7\) (points F and F’) provide a seven-residue nanoring, in which all N atoms are on the same plane (Figure 2.36). Points G and G’ provide a nine-residue nanoring, in which all C atoms are on the same plane (Figure 2.36).

### 2.6 Summary

To examine the possible backbone conformations of peptide nanorings and nanotubes, a mathematical conformation analysis, derived by Shimanouchi and Mizushima, was performed for a periodic polypeptide system consisting of three backbone atoms per unit (\(m = 3\)). Before analyzing the M3 (\(m = 3\)) polymer (polypeptide), we first discussed possible conformations of the simplest M1 (\(m = 1\)) polymers of both the homo-rotatory unit sequence and the hetero-rotatory (alternate) unit sequence. The mathematical treatment was also applied to the higher M2 (\(m = 2\)) polymer and the M3 (\(m = 3\)) polymer. Finally, possible polypeptide conformations of the homo-L-amino
acid sequence (homo-polypeptide) and the alternate D- and L-amino acid sequence (hetero-polypeptide) were investigated. The results of these studies are summarized below.

**M1 polymer**

- Basically, the homo-rotatory M1 polymer \((homoM1)\) produces a “helical coil”, while the hetero-rotatory M1 polymer \((heteM1)\) forms a “coronal ring”. The possible helical pitch angle \(\theta\) and the pitch number \(n\) are determined by eqs. (2.35) and (2.36) for the \(homoM1\), while those for the \(heteM1\) are given by eqs. (2.43) and (2.44).

- A helical direction of the \(homoM1\) is distinguished by a sign of the helical translation \(d\); the right-handed helix \((d > 0)\), the left-handed helix \((d < 0)\), or the flat disk \((d = 0)\).

- In order to close the \(heteM1\) backbone, it is required that \(n\) is an even number, or \(n\) is an integer and \(d = 0\).

**M2 polymer**

- Analogous to the M1 polymer, the homo-rotatory M2 polymer \((homoM2)\) basically produces a helical coil, while the hetero-rotatory M2 polymer \((heteM2)\) forms a coronal ring. The possible helical pitch angle \(\theta\) and the pitch number \(n\) are determined by eqs. (2.51) and (2.52), while those for the \(heteM2\) polymer are given by eqs. (2.58) and (2.59).

- An increase in the degrees of freedom due to the two internal rotation angles \(\tau_1\) and \(\tau_2\) causes a polymorphy in both the \(homoM2\) and the \(heteM2\) polymers. There are two backbone conformations having the same \(\theta\) but different internal rotation angles \((\tau_1, \tau_2)\).

- This increase in the degrees of freedom causes a possibility that both the \(homoM2\) and \(heteM2\) polymers produce a coronal ring having an odd pitch number.

**M3 polymer (polypeptide)**

- Calculated 3D plots of the possible \(\cos \theta\) values in the homo-rotatory polypeptide \((homoPP)\) and the hetero-rotatory polypeptide \((hetePP)\) are given in Figure 2.37.
Figure 2.37: Resulting 3D plot of $\cos \theta$ for the homoPP and hetePP with varying two internal rotation angles $\phi$ and $\psi$. 
Figure 2.38: Multi-contour map of the *homoPP*. This map gives us an information of the backbone stereo-structures such as the helical pitch number $n$, helical translation $d$, and the inclination angles of the amide plane $\Theta$. It also reveals the characteristics of the backbones such as the E-type or the B-type conformation, and the helical direction of the right-handed or left-handed. Symbols EP at $(\phi, \psi) = (\pm\pi, \pm\pi)$ and BP at $(\phi, \psi) = (0, 0)$ mean the E-type and B-type planar backbone, respectively, and also disk at $(\phi, \psi) = (0, \pm\pi)$ or $(\pm\pi, 0)$ means the resulting backbone becomes a flat disk.
Figure 2.39: Multi-contour map of the *hetePP*. This map gives us an information of the backbone stereo-structures such as the helical pitch number $n$, helical translation $d$, and the inclination angles of the amide plane $\Theta$. It also reveals the characteristics of the backbones such as the E-type or the B-type conformation, and the helical direction of the right-handed or left-handed. Symbols EP at $(\phi, \psi) = (\pm \pi, \pm \pi)$ and BP at $(\phi, \psi) = (0, 0)$ mean the E-type and B-type planar backbone, respectively, and also disk at $(\phi, \psi) = (0, \pm \pi)$ or $= (\pm \pi, 0)$ means the resulting backbone becomes a flat disk.
The \( \cos \theta \) surface of the \textit{homoPP} contacts that of the \textit{hetePP} at \((\phi, \psi) = (\pi, 0), (-\pi, 0), (0, \pi), \) and \((0, -\pi)\). At those points, the flat disk structures are produced.

- By superimposing several contour maps of the conformation parameters such as the helical translation \( d \) and the inclination angle \( \Theta \) on that of the helical pitch angle \( \theta \) (pitch number, \( n \)), the stereo-structures of the \textit{homoPP} and \textit{hetePP} are specified (Figures 2.38 and 2.39).

- The possible pitch number of the \textit{homoPP} is limited between 2 and 5.7, while that of the \textit{hetePP} is limited between 4.8 and \( \infty \).

- The \textit{homoPP} basically forms a right-handed or left-handed helix having an even, odd, or a decimal pitch number. On the other hand, the \textit{hetePP} basically forms a closed ring having an even pitch number.

- Any two polypeptides whose two internal rotation angles \((\phi, \psi)\) values are in an inverse symmetric position about the origin \((\phi, \psi) = (0, 0)\) are mutually enantiomeric.

- \(|\phi|, |\psi| \geq \pi/2\) cause the Extended-type (E-type) backbone, while \(|\phi|, |\psi| \leq \pi/2\) provide the Bound-type (B-type) backbone.

- Exceptionally, both the \textit{homoPP} and \textit{hetePP} can produce unusual peptide nanorings having an odd number of residues (five residues).
Bibliography


[5] The exceptions are a flat disk at $\tau = 0$ and a trans-planar zig-zag chain at $\tau = \pm \pi$.

[6] Both the homoM1 and heteM1 polymers produce the same trans-planar backbone when $\tau = \pm \pi$. However, the homoM1 polymer produces this form when $n = 2$, while the heteM1 polymer does when $n = \infty$.

[7] The bond angles and the bond lengths in the general polypeptide are listed in Table in Chemistry[8]: $\alpha_1 = 111^\circ$, $\alpha_2 = 116^\circ$, $\alpha_3 = 122^\circ$, $r_1 = 1.52\,\text{Å}$, $r_2 = 1.33\,\text{Å}$, $r_3 = 1.45\,\text{Å}$.

[8] Table in Chemistry (Kagaku Binran in Japanese), Ed. by The Chemical Society of Japan (Maruzen, Tokyo, 1984).

[9] Points E and F provide $d < 0$ while points E’ and F’ give $d > 0$.

[10] A standard deviation of the bond length is less than 0.02 Å and that of the bond angles is about 2$^\circ$[11]. However, one should be noted that some ideal values for identical bonds differ by more than 0.1 Å and 10$^\circ$[11].


[12] The $d$ value is determined for the individual backbone atom, i.e., in the case of polypeptide, three $d$ values ($d_{C^\alpha}$, $d_C$, and $d_N$) are given. In Figure 2.34, we only show the $d_{C^\alpha} = 0$ line for the D,L sequence. The other $d$ lines of $d_N = 0$ and $d_C = 0$ also provide the intersections with $n = 5$ at $(\phi, \psi) = (\pm 153^\circ, 50^\circ)$ and $(\phi, \psi) = (\pm 150^\circ, 53^\circ)$, respectively. On the other hand, in the case of the homo L-amino acid sequence, $d_{C^\alpha} = d_C = d_N$. 

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For the d,l-polypeptide, the formation of a flat disk requires the internal rotation angles being $(\phi, \psi) = (0, \pm \pi)$ and $(\pm \pi, 0)$, in addition to an even pitch number $n$. According to Figure 2.34, the conformation of $(\phi, \psi) = (\pm \pi, 0)$ or $(0, \pm \pi)$ gives the non-integer helical pitch number of $n = 4.8$ and $n = 5.7$, respectively. Thus, the d,l alternate amino acids sequence cannot produce a flat closed disk, as far as the peptide maintains the present standard geometrical parameters.
Chapter 3

Ab initio Studies of the Electronic and Molecular Structures of D,L-Peptide Nanorings and Nanotubes

3.1 Introduction

In the former chapter, we theoretically examined the possible backbone conformations of the periodic polymers whose unit cells consist of one \((m = 1)\), two \((m = 2)\), and three \((m = 3)\) atom(s). This mathematical analysis was also applied to the polypeptide system, and we found that an alternate sequence of D- and L-amino acids (D,L-peptide) generally produces the peptide nanorings (PNRs). The next subject in this chapter is to investigate the energetic stability of those mathematically predicted PNRs and/or to explore the energetically stable molecular forms of the PNRs and the self-stacking peptide nanotube (PNTs).

For this purpose, we first review the conformation analysis of the D,L-peptide (heteropolypeptide; hetePP) briefly and discussed two kinds of the PNR backbones; the Extended-type and the Bound-type. Successively, ab initio Hartree-Fock calculations are performed and energetically stable conformations of those PNR backbones are examined. The effect of the amino acid substitution was, moreover, investigated for all 20 encoded residues, and the electronic structures of those homo-residue PNRs are discussed. Finally, the geometry optimization of the PNTs is carried out and the stable molecular conformations and their band structures are studied.
3.2 Extended-type and Bound-type peptide nanorings

First, we briefly review the conformation analysis of the hetero-polypeptide (hetePP) in which D- and L-amino acid residues are catenated alternately. As found in Chapter 2 (eqs. 2.81~2.84), the pitch angle \( \theta \) in the homoPP (D,L-peptide) is expressed as a functional form of the internal parameters as

\[
\cos \theta_{\text{hetePP}} = \frac{1}{2} (a_{11}^{\text{hetePP}} + a_{22}^{\text{hetePP}} + a_{33}^{\text{hetePP}} + 1), \tag{3.1}
\]

where

\[
a_{11}^{\text{hetePP}} = \cos \alpha_3 (\sin \alpha_1 \sin \alpha_2 \cos \psi - \cos \alpha_1 \cos \alpha_2) + \sin \alpha_1 \sin \alpha_3 \sin \psi \sin \omega \\
+ \sin \alpha_3 \cos \omega (\cos \alpha_1 \sin \alpha_2 + \sin \alpha_1 \cos \alpha_2 \cos \psi), \tag{3.2}
\]

\[
a_{22}^{\text{hetePP}} = \sin \alpha_3 (\sin \alpha_1 \cos \alpha_2 \cos \phi + \sin \alpha_2 \sin \phi \sin \psi + \cos \alpha_1 \sin \alpha_2 \cos \phi \cos \psi) \\
+ \cos \alpha_3 \sin \omega (\sin \phi \cos \psi - \cos \alpha_1 \cos \phi \sin \psi) \\
+ \cos \alpha_3 \cos \omega (\sin \alpha_1 \sin \alpha_2 \cos \phi \\
- \cos \alpha_2 \sin \phi \sin \psi - \cos \alpha_1 \cos \alpha_2 \cos \phi \cos \psi), \tag{3.3}
\]

\[
a_{33}^{\text{hetePP}} = \sin \omega (\cos \alpha_1 \cos \alpha_2 \sin \phi \cos \psi - \sin \alpha_1 \sin \alpha_2 \sin \phi - \cos \alpha_2 \cos \phi \sin \psi) \\
- \cos \omega (\cos \phi \cos \psi + \cos \alpha_1 \sin \phi \sin \psi). \tag{3.4}
\]

Here, by quoting the following experimental values for \( \alpha_i \); \( \alpha_1 = 111^\circ \), \( \alpha_2 = 116^\circ \), and \( \alpha_3 = 122^\circ \)[13, 14] and also assuming the flat amide plane of \( \omega = 180^\circ \), we can obtain the possible \( \theta \) values (or \( n \) values, where \( n = 2\pi/\theta \)) in the D,L-peptide with respect to two internal rotation angles \( \phi \) and \( \psi \) (Figure 2.37).

The periodic D,L-peptide basically produces the coronal ring (zero-transfer helix) on the surface of \( \cos \theta_{\text{hetePP}} \) in Figure 2.37. The resulting minimum value of \( \cos \theta = 0.259 \) reveals that the available pitch number \( n \) is limited to more than 4.8. However, one should note that the possible \( n \) is further restricted to an even number so as to form the closed rings. Therefore, the minimum ring (residue) number is given as \( n = 6 \), i.e., an even number of \( n = 6, 8, 10, 12, \ldots \) is available in the PNRs while maintaining the \( S_n \) symmetry.

The possible PNR conformations are well understood by drawing the 2D contour map of \( \cos \theta_{\text{hetePP}} \) (Figure 3.1). The numerical values on the solid lines in Figure
Figure 3.1: The *equi-cos* θ map of the D,L-peptide by varying the internal rotation angles φ and ψ. The pitch number *n* is given by integers on the solid lines. On the dotted line of ψ ≃ −φ, the β-strand type backbones are formed. In the calculation, the following experimental values are quoted for the bond lengths and bond angles; \( r_1 = 1.52 \ \text{Å}, \ r_2 = 1.33 \ \text{Å}, \ r_3 = 1.45 \ \text{Å}, \ \alpha_1 = 111^\circ, \ \alpha_2 = 116^\circ, \ \text{and} \ \alpha_3 = 122^\circ[16] \). Moreover, the flat amide plane of \( \omega = 180^\circ \) is assumed. If the three bond angles are the same (\( \alpha_1 = \alpha_2 = \alpha_3 \)), these lines show a complete linearity. The actual polypeptide, however, causes a difference in these bond angles, and the non-linear relation between ψ and φ is generated in the individual lines.
3.1 correspond to the resulting pitch number \( n \). The cosinodal dependence of \( \phi \) and \( \psi \) included in eqs. (3.2)-(3.4) causes four independent equi-\( n \) lines having the same \( n \) number. Those lines are respectively approximated by \( \psi \simeq \phi + \pi \pm c \) and \( \psi \simeq \phi - \pi \mp c \), where \( c \) is a constant defined by \( 0 < c < \pi \). Because those four lines have an inverse symmetry around the origin of \( (\phi, \psi) = (0, 0) \), any two sets of \( (\phi, \psi) \) having the above inverse symmetry produce an exactly equivalent backbone. Therefore, only in the meaning of determining the backbone conformation, can one reduce the variation region to half \( (\psi > \phi \) or \( \psi < \phi \)).

An important result is that, even in the half region of \( \psi > \phi \) or \( \psi < \phi \), two equi-\( n \) lines appear for the individual \( n \) number. This result indicates that two different backbone conformations are possible in the same \( n \)-residue PNR. Those conformations are grouped into the Bound-type (B-type) due to the internal rotation angles of \(-\pi < \psi \pm \phi < \pi\), or the Extended-type (E-type) in the other region (Figure 3.1). The characteristic of these two conformations is that E-type PNRs have the conventional trans zigzag backbones, while the B-type PNRs have the new conformations whose skeletons are rather shrunk (Figure 3.2). These two types of the PNRs, thus, have a different internal diameter, i.e., the smaller B-type PNRs and the larger E-type PNRs.

Here, for the purpose of specifying the PNR conformation, we further narrow our discussion on such PNRs having their internal rotation angles \( (\phi, \psi) \) of the crossing points between the individual solid line and the dotted line of \( \psi \simeq -\phi \) (Figure 3.1). The internal rotation angles on this dotted line provide the backbones whose skeletal planes are parallel to the ring axis. Therefore, the values of \( \phi \) and \( \psi \) at these crossing points provide the PNR backbones of the \( \beta \)-strand type. Hereafter, we call these PNRs the \( \beta \)-ring (BR) forms. We show those mathematical E-type and the B-type BRs of \( n = 6 \) in Figures 3.3 (a) and (b), respectively. Because of the amide (O=C-N-H) being parallel to the ring axis, those BRs are supposed to construct tubular structures through inter-ring hydrogen bonds analogous to the \( \beta \)-sheets. The smaller diameter of the B-type BRs further excites our interest in the smaller B-type PNTs in addition to the conventional E-type PNTs.

### 3.3 Energetics of the peptide nanorings

The next subject is to investigate whether the above \( \text{(mathematical)} \) BR structures are energetically stable. For a quantitative discussion of the stable PNRs, we should investigate the energetics using parameter-free methods. Therefore, Restricted Hartree-Fock molecular orbital (MO) calculations were carried out by employing a Gaussian98
Figure 3.2: Illustration of the extended-type (E-type) peptide backbone and the bound-type (B-type) one[15].
Figure 3.3: Side view and top view of the E-type BR (a) and B-type BR (b) consisting of six Gly residues. We assume that both backbones have amide planes of $\omega = 180^\circ$. The intra-ring hydrogen bonds (HBs) cause an opposite role in E-type and B-type BRs; an attractive force occurs via HBs in the former, but a repulsive force exists in the latter due to the steric hindrance.

program[16]. The normal vibrational analysis was also performed for the geometry optimization of the $n$-residue PNRs while maintaining the $S_n$ symmetry. All of the calculations were carried out using the 6-31G** basis set for an intimate discussion of the hydrogen bonds.

### 3.3.1 $\beta$-ring backbone

Let us start from homo-Gly BRs having E-type and B-type backbones. The resulting relationship between the total energy (per residue) and the residue (pitch) number $n$ is shown as the broken lines in Figure 3.4 (a). An important result is that the E-type BRs are more stable than the B-type BRs, independent of any $n$ number. It is also characteristic that E-type BRs hardly change their own total energy[17], although a significant energetic destabilization occurs in the B-type BRs with increasing
Figure 3.4: Relationship between the calculated total energy (per residue) and the residue (pitch) number \( n \) (a), and that between the inter-atomic H···O distance of the intra-ring N-H···O and \( n \) in the E-type and B-type PNRs (b). In both figures, we show these relations for the four PNRs of the BRs (broken lines) and the optimized NRs (solid lines), respectively. We also depict the region of the steric hindrance by the gray tone.

This destabilization in the B-type BRs is caused by the steric hindrance between hydrogen (H) and oxygen (O) atoms located on the adjacent amide planes [Figure 3.3 (b)]. This feature is confirmed by the corresponding inter-atomic distance between H···O [Figure 3.4 (b)], which reveals that the B-type BRs allow these two atoms to approach unrealistically, while the corresponding inter-atomic distance in the E-type BRs is still large enough to avoid steric hindrance.

### 3.3.2 Optimized nanoring backbone

The steric conformations of E-type and B-type BRs are changed by the geometry optimizations [Figures 3.5 (a) and (b)]. The resulting internal parameters of the bond lengths, the bond angles, and the internal rotation angles are listed in Tables 3.1, 3.2, and 3.3, respectively. The resulting internal rotation angles clearly reveal that both E-type and B-type PNRs do not conserve the complete amide plane of \( \omega = 180^\circ \) (Table...
Figure 3.5: Optimized molecular structures of the E-type PNRs (a) and B-type PNRs (b) consisting of 12, 10, 8, and 6 Gly residues.

3.3), and we cannot find the complete BR conformation anymore. Thus, we hereafter term these optimized PNRs as the novel ring (NR) forms.

The characteristic feature found in the E-type NRs is that all three internal rotation angles deviate from those of the BR structure ($\psi \approx \phi$ and $\omega=180^\circ$) with decreasing $n$ number, i.e., the $\omega$ value deviates from (-)180° and the remaining two angles deviate from $\psi \simeq -\phi$ (Table 3.3). This characteristic is caused by the intra-ring hydrogen bond (HB) of N-H···O induced by the cyclization of the peptide chain [Figure 3.6 (a)]. This feature is also confirmed by the reduction of the inter-atomic H···O distance from the E-type BR to the E-type NR [Figure 3.4 (b)] and by an increase in the overlap population between H···O (by 55%).

In the B-type PNRs, on the other hand, the H···O distance is so shortened that significant steric hindrance occurs with increasing $n$ number if the backbones maintain the BR forms [Figure 3.4 (b)]. The H···O distance is therefore elongated to about 2.0 Å, independent of the $n$ number [Figure 3.4 (b)]. When $n$ is small, only a small
### Table 3.1: Bond lengths in the optimized E-type and B-type homo-Gly PNRs of \(n=6-12\) (RHF/6-31G**).

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<th>Bound-type</th>
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<td>(C-N) [Å]</td>
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### Table 3.2: Bond angles in the optimized Extended-type and Bound-type homo-Gly PNRs of \(n=6-12\) (RHF/6-31G**).

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<th>Bound-type</th>
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<td>(n)</td>
<td>(\angle N-C^\alpha-C)</td>
<td>(\angle C^\alpha-C-N)</td>
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</tr>
<tr>
<td>12</td>
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</table>

### Table 3.3: Internal rotation angles in the optimized Extended-type and Bound-type homo-Gly PNRs of \(n=6-12\) (RHF/6-31G**). Although Gly has no chirality, we show the values corresponding to the L-amino acid residues of the *equatorial* substitution [Figure 3.7 (a)].

<table>
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<th>Bound-type</th>
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<td>(n)</td>
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<td>12</td>
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Figure 3.6: Side view and top view of the E-type (optimized) NR (a) and the B-type (optimized) NR (b) consisting of six Gly residues.

structural change is required so as to relax the steric hindrance. Thus, we can find only a slight $\omega$ deviation in the B-type NR of $n = 6$ (Table 3.3). On the contrary, the relaxation of the steric hindrance further requires a large $\omega$ deviation when $n \geq 8$. Therefore, the amide plane is gradually distorted with the increasing $n$ number.

A noticeable point is that the relaxation energy in the B-type PNRs overcomes the stabilization energy in the E-type PNRs [Figure 3.4 (a)]. As a result, the total energies of E-type NRs and B-type NRs become comparable. By focusing on the resulting energy difference, we can find that the smaller rings prefer the novel B-type backbone, while the larger rings prefer the conventional E-type backbone: When $n \geq 10$, the E-type NRs are still more stable than the B-type NRs. However, at a small $n$ of $n \leq 8$, the B-type NRs become more stable than the E-type NRs.
3.3.3 Substitution of amino acids

The next subject is to investigate the effects of the amino acid substitution on the stable molecular structures and the energetics of the PNRs. In this section, we discuss the optimized molecular conformations of the PNRs whose amino acid residues are replaced homogeneously by one of 20 encoded amino acids (homo-residue PNRs).

Here, one should notice that two types of substitution means are presumable; one is the equatorial substitution [Figure 3.7 (a)] and the other is the axial substitution [Figure 3.7 (a)]. For E-type PNRs, the equatorial substitution appears in the region of \(\psi > \phi + \pi\), and the axial substitution appears in the region of \(\psi < \phi - \pi\) [Figure 3.1 (b)]. For B-type PNRs, on the other hand, the equatorial substitution appears in the region of \(\phi - \pi < \psi < \phi\), and the axial substitution appears in the region of \(\phi < \psi < \phi + \pi\).

However, in the present thesis, we only focus on the equatorial substitution, because the axial substitution is considered to prevent its own stacking due to the repulsion between the side chains of the adjacent PNRs. Besides, we limit our discussion to the smallest six-residue PNRs because of the limited computer resource. Therefore, the geometry optimization was carried out for the homo-residue PNRs based on Restricted Hartree-Fock molecular orbital method using the 6-31G** basis set while maintaining the \(S_6\) symmetry.

One of the important results given by the present calculations is that all kinds of
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<td>1.360</td>
</tr>
<tr>
<td>Ser</td>
<td>1.535</td>
<td>1.361</td>
</tr>
</tbody>
</table>
Table 3.5: Bond angles in the 20 kinds of homo-residue E-type PNRs and B-type PNRs (RHF/6-31G**).

<table>
<thead>
<tr>
<th>residue</th>
<th>Extended-type</th>
<th>Bound-type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\angle$N-C$^{\alpha}$-C</td>
<td>$\angle$C$^{\alpha}$-C-N</td>
</tr>
<tr>
<td>Pro</td>
<td>112.2° 118.5° 118.9°</td>
<td>120.6° 120.4° 115.5°</td>
</tr>
<tr>
<td>Trp</td>
<td>107.0° 116.3° 123.3°</td>
<td>112.7° 116.9° 126.7°</td>
</tr>
<tr>
<td>Asp</td>
<td>106.0° 116.3° 122.2°</td>
<td>113.0° 117.1° 126.2°</td>
</tr>
<tr>
<td>Asn</td>
<td>106.0° 116.1° 122.2°</td>
<td>112.8° 116.8° 126.0°</td>
</tr>
<tr>
<td>His</td>
<td>106.6° 116.5° 122.8°</td>
<td>112.9° 116.9° 126.0°</td>
</tr>
<tr>
<td>Met</td>
<td>106.9° 116.2° 122.4°</td>
<td>113.2° 117.1° 126.4°</td>
</tr>
<tr>
<td>Glu</td>
<td>106.7° 116.3° 122.2°</td>
<td>113.2° 117.1° 126.4°</td>
</tr>
<tr>
<td>Lys</td>
<td>106.0° 116.5° 123.0°</td>
<td>112.8° 116.9° 126.6°</td>
</tr>
<tr>
<td>Arg</td>
<td>106.2° 116.5° 122.9°</td>
<td>112.8° 116.9° 126.4°</td>
</tr>
<tr>
<td>Val</td>
<td>104.8° 116.7° 123.3°</td>
<td>113.0° 117.0° 127.2°</td>
</tr>
<tr>
<td>Ile</td>
<td>104.9° 116.6° 123.8°</td>
<td>112.8° 117.6° 127.3°</td>
</tr>
<tr>
<td>Leu</td>
<td>106.1° 116.5° 123.1°</td>
<td>112.9° 117.0° 126.9°</td>
</tr>
<tr>
<td>Ala</td>
<td>106.9° 116.2° 122.6°</td>
<td>113.1° 116.9° 126.4°</td>
</tr>
<tr>
<td>Gln</td>
<td>106.6° 116.3° 123.0°</td>
<td>113.0° 116.8° 126.4°</td>
</tr>
<tr>
<td>Cys</td>
<td>108.4° 115.9° 122.2°</td>
<td>113.0° 117.1° 125.8°</td>
</tr>
<tr>
<td>Tyr</td>
<td>107.6° 116.2° 122.6°</td>
<td>112.9° 116.9° 126.6°</td>
</tr>
<tr>
<td>Phe</td>
<td>107.7° 116.2° 122.6°</td>
<td>112.9° 116.9° 126.5°</td>
</tr>
<tr>
<td>Gly</td>
<td>109.3° 116.1° 121.1°</td>
<td>113.7° 115.9° 122.3°</td>
</tr>
<tr>
<td>Thr</td>
<td>108.5° 115.5° 119.6°</td>
<td>113.6° 118.2° 127.4°</td>
</tr>
<tr>
<td>Ser</td>
<td>109.1° 115.5° 119.3°</td>
<td>113.3° 117.7° 126.8°</td>
</tr>
</tbody>
</table>
Table 3.6: Internal rotation angles in 20 kinds of homo-residue PNRs having E-type conformation (RHF/6-31G**). $\chi_1$ is a dihedral angle of N-C$_\alpha$-C$_{3\beta}$-C$_{\gamma}$ in the side chain. The rotamer types of the side chains are classified into the following three groups: gauche(+) defined by $\chi_1 = -60^\circ \pm 60^\circ$, gauche(-) defined by $\chi_1 = +60^\circ \pm 60^\circ$, and trans defined by $\chi_1 = 180^\circ \pm 60^\circ$ (Figure 3.8).

<table>
<thead>
<tr>
<th>residue</th>
<th>$\phi$</th>
<th>$\psi$</th>
<th>$\omega$</th>
<th>$\chi_1$</th>
<th>rotamer type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro</td>
<td>-75.3°</td>
<td>128.9°</td>
<td>171.8°</td>
<td>31.8°</td>
<td>gauche(-)</td>
</tr>
<tr>
<td>Trp</td>
<td>-118.0°</td>
<td>134.3°</td>
<td>-173.8°</td>
<td>-69.1°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Asp</td>
<td>-143.8°</td>
<td>108.2°</td>
<td>180.0°</td>
<td>-177.0°</td>
<td>trans</td>
</tr>
<tr>
<td>Asn</td>
<td>-145.1°</td>
<td>106.1°</td>
<td>179.4°</td>
<td>-176.5°</td>
<td>trans</td>
</tr>
<tr>
<td>His</td>
<td>-131.6°</td>
<td>120.6°</td>
<td>-177.0°</td>
<td>-176.5°</td>
<td>trans</td>
</tr>
<tr>
<td>Met</td>
<td>-119.6°</td>
<td>136.7°</td>
<td>-171.1°</td>
<td>-64.5°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Glu</td>
<td>-119.7°</td>
<td>138.3°</td>
<td>-169.7°</td>
<td>-63.2°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Lys</td>
<td>-129.3°</td>
<td>126.8°</td>
<td>-174.1°</td>
<td>-176.8°</td>
<td>trans</td>
</tr>
<tr>
<td>Arg</td>
<td>-128.4°</td>
<td>126.3°</td>
<td>-174.8°</td>
<td>-177.0°</td>
<td>trans</td>
</tr>
<tr>
<td>Val</td>
<td>-129.3°</td>
<td>134.1°</td>
<td>-168.3°</td>
<td>-177.1°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Ile</td>
<td>-127.4°</td>
<td>134.2°</td>
<td>-169.5°</td>
<td>-66.4°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Leu</td>
<td>-128.2°</td>
<td>126.4°</td>
<td>-175.0°</td>
<td>-179.1°</td>
<td>trans</td>
</tr>
<tr>
<td>Ala$^2$</td>
<td>-121.5°</td>
<td>133.1°</td>
<td>-172.9°</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Gln</td>
<td>-124.9°</td>
<td>127.0°</td>
<td>-176.1°</td>
<td>-179.1°</td>
<td>trans</td>
</tr>
<tr>
<td>Cys</td>
<td>-110.0°</td>
<td>134.2°</td>
<td>-176.8°</td>
<td>-61.6°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Tyr</td>
<td>-117.2°</td>
<td>130.8°</td>
<td>-176.5°</td>
<td>-60.7°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Phe</td>
<td>-116.2°</td>
<td>131.1°</td>
<td>-176.6°</td>
<td>-61.2°</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Gly$^2$</td>
<td>-109.0°</td>
<td>143.1°</td>
<td>-170.1°</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Thr</td>
<td>-97.2°</td>
<td>162.1°</td>
<td>-161.0°</td>
<td>-62.6°</td>
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</tr>
<tr>
<td>Ser</td>
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<td>163.7°</td>
<td>-162.4°</td>
<td>60.8°</td>
<td>gauche(-)</td>
</tr>
</tbody>
</table>
Table 3.7: Internal rotation angles in 20 kinds of homo-residue PNRs having B-type conformation (RHF/6-31G**). $\chi_1$ is a dihedral angle of N-C$^\alpha$-C$^\beta$-C$^\gamma$ in the side chain. The rotamer types of the side chains are classified into the following three groups; gauche(+) defined by $\chi_1 = -60^\circ \pm 60^\circ$, gauche(-) defined by $\chi_1 = +60^\circ \pm 60^\circ$, and trans defined by $\chi_1 = 180^\circ \pm 60^\circ$ (Figure 3.8).

<table>
<thead>
<tr>
<th>residue</th>
<th>Bound-type</th>
<th>rotamer type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro</td>
<td>$\phi$=49.2$^\circ$, $\psi$=-0.8$^\circ$, $\omega$=-137.9$^\circ$, $\chi_1$=-22.5$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Trp</td>
<td>$\phi$=75.3$^\circ$, $\psi$=-58.5$^\circ$, $\omega$=-177.4$^\circ$, $\chi_1$=-64.3$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Asp</td>
<td>$\phi$=75.6$^\circ$, $\psi$=-59.8$^\circ$, $\omega$=-177.5$^\circ$, $\chi_1$=-66.1$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Asn</td>
<td>$\phi$=78.6$^\circ$, $\psi$=-60.1$^\circ$, $\omega$=-176.2$^\circ$, $\chi_1$=-55.2$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>His</td>
<td>$\phi$=75.0$^\circ$, $\psi$=-64.8$^\circ$, $\omega$=-176.7$^\circ$, $\chi_1$=-178.2$^\circ$</td>
<td>trans</td>
</tr>
<tr>
<td>Met</td>
<td>$\phi$=73.7$^\circ$, $\psi$=-60.5$^\circ$, $\omega$=-178.2$^\circ$, $\chi_1$=-61.4$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Glu</td>
<td>$\phi$=73.8$^\circ$, $\psi$=-60.5$^\circ$, $\omega$=-178.2$^\circ$, $\chi_1$=-60.7$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Lys</td>
<td>$\phi$=74.5$^\circ$, $\psi$=-61.3$^\circ$, $\omega$=-177.4$^\circ$, $\chi_1$=-170.7$^\circ$</td>
<td>trans</td>
</tr>
<tr>
<td>Arg</td>
<td>$\phi$=74.6$^\circ$, $\psi$=-61.0$^\circ$, $\omega$=-177.2$^\circ$, $\chi_1$=-170.2$^\circ$</td>
<td>trans</td>
</tr>
<tr>
<td>Val</td>
<td>$\phi$=71.8$^\circ$, $\psi$=-54.4$^\circ$, $\omega$=-180.0$^\circ$, $\chi_1$=-177.5$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Ile</td>
<td>$\phi$=72.1$^\circ$, $\psi$=-55.1$^\circ$, $\omega$=-179.4$^\circ$, $\chi_1$=-59.9$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Leu</td>
<td>$\phi$=73.7$^\circ$, $\psi$=-60.7$^\circ$, $\omega$=-177.8$^\circ$, $\chi_1$=-175.7$^\circ$</td>
<td>trans</td>
</tr>
<tr>
<td>Ala$^2$</td>
<td>$\phi$=74.7$^\circ$, $\psi$=-60.6$^\circ$, $\omega$=-177.7$^\circ$</td>
<td>——</td>
</tr>
<tr>
<td>Gln</td>
<td>$\phi$=74.9$^\circ$, $\psi$=-61.2$^\circ$, $\omega$=-177.4$^\circ$, $\chi_1$=-58.5$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Cys</td>
<td>$\phi$=75.5$^\circ$, $\psi$=-61.9$^\circ$, $\omega$=-177.1$^\circ$, $\chi_1$=-58.6$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Tyr</td>
<td>$\phi$=76.3$^\circ$, $\psi$=-59.2$^\circ$, $\omega$=-177.2$^\circ$, $\chi_1$=-57.4$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Phe</td>
<td>$\phi$=76.2$^\circ$, $\psi$=-59.4$^\circ$, $\omega$=-177.2$^\circ$, $\chi_1$=-57.7$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Gly$^2$</td>
<td>$\phi$=83.8$^\circ$, $\psi$=-64.5$^\circ$, $\omega$=-175.3$^\circ$</td>
<td>——</td>
</tr>
<tr>
<td>Thr</td>
<td>$\phi$=76.5$^\circ$, $\psi$=-45.0$^\circ$, $\omega$=-179.2$^\circ$, $\chi_1$=-47.1$^\circ$</td>
<td>gauche(+)</td>
</tr>
<tr>
<td>Ser</td>
<td>$\phi$=80.3$^\circ$, $\psi$=-47.4$^\circ$, $\omega$=-178.5$^\circ$, $\chi_1$=85.0$^\circ$</td>
<td>gauche(-)</td>
</tr>
</tbody>
</table>
Figure 3.8: Three types of the side-chain rotamer; gauche(+) (a), gauche(-) (b), and trans (c).
homo-residue PNRs have both the Extended-type (E-type) and the Bound-type (B-type) backbones as their local minimum forms. Moreover, the substitution of the amino acid residues hardly changes the bond lengths and the bond angles of the PNR backbones; The resulting bond lengths are changed at most by ±1% (Table 3.4), and the bond angles are within ±4% (Table 3.5). The detailed structural difference, therefore, appears through the internal rotation angles $\phi$, $\psi$, and $\omega$ (Tables 3.6 and 3.7)[18].

The energetically stabler backbone type in the individual homo-residue PNR is determined by an energy comparison between the E-type and B-type PNRs. The present result reveals that the preferable backbone type is not unique but depends on the individual amino acid side chains even with the same number ($n$) of residues (Figure 3.9): The resulting energy difference indicates that seven residues of Gln, Cys, Tyr, Phe, Gly, Thr, and Ser prefer the B-type backbone, while the remainder prefer the E-type backbone.

Among all of the 20 encoded amino acid residues, five residues of Pro, Trp, Asp, Asn, and His appreciably prefer the E-type backbone by more than 1.5 kcal/mol per residue (Figure 3.9)[19]. Therefore, those residues are expected to produce E-type PNRs predominantly (E-forming residues; in Figure 3.10). On the contrary, Gly, Thr, and Ser rather prefer the B-type backbone by more than 2 kcal/mol per residue. Thus, those three residues are supposed to be B-forming residues (Figure 3.11). An interesting point is that the remaining homo-residue PNRs of Met, Glu, Lys, Arg, Val, Ile, Leu, Ala, Gln, Cys, Tyr, and Phe show a small and indistinguishable energy difference.

Figure 3.9: Total energy differences between E-type and B-type PNRs with respect to the replaced amino acid residues. A positive value means that the E-type PNR is more stable than the B-type PNR, while a negative value means the opposite.
Figure 3.10: Optimized molecular structures of E-forming homo-residue PNRs, in which the amino acid side chains locate the \textit{equatorial} position. E-type backbones are shown in the figure.

Figure 3.11: Optimized molecular structures of B-forming homo-residue PNRs, in which the amino acid side chains locate the \textit{equatorial} position. B-type backbones are shown in the figure.
Figure 3.12: Optimized molecular structures of E/B-forming homo-residue PNRs, in which the amino acid side chains locate the *equatorial* position. Energetically preferable backbone type (E-type or B-type) is shown in the figure.
(within 1 kcal/mol per residue). Therefore, those residues have a potential to produce both E-type and B-type PNRs (E/B-forming residues; in Figure 3.12).

The resulting energetics is basically understood by the presence or absence of the hydrogen bonding interaction between the side chains and the backbone. One of the examples is the replacement by Asp and Asn, which belong to E-forming residues. The replacement by those residues induces the hydrogen bond (HB) of O···H-N between the side-chain O atom and the backbone H-N bond (Figure 3.13). This HB functions effectively in the E-type PNRs but not in the B-type PNRs. The reason is that the B-type backbone has an undesirable N-H bond inclined against the ring axis, and therefore, the O···H distance is more elongated than that in the E-type PNRs by 13%. Because of this characteristic, those residues appreciably prefer the E-type backbone to the B-type backbone[20] (Figure 3.9).

On the contrary, homo-residue PNRs of Thr and Ser include the HB only in the B-type conformation but not in the E-type conformation (Figure 3.13), in contrast to the PNRs of Asp and Asn. This opposite feature is caused by the difference in the HB nature. HBs in the homo-residue PNRs of Thr and Ser are those O-H···O between the side-chain O-H bond and the backbone O atom. Therefore, in the B-type conformation, the backbone C=O bond is so oriented toward the side chain that the HB of O-H···O is induced and points the side-chain O-H bond toward the backbone. In the E-type conformation, on the contrary, the backbone C=O bond is not oriented toward the side chains but toward the ring axis. Therefore, it is hard to produce the HB (O-H···O) between the side-chain O-H bond and the backbone O atom (Figure 3.13). Because of this characteristic, Thr and Ser prefer the B-type backbone to the E-type backbone (Figure 3.9).

The replacement by the remaining E/B-forming residues does not produce any plain HBs between the side chains and the backbone (Figure 3.13). Therefore, homo-residue PNRs of those residues do not show a prominent energetic stability in either the E-type or the B-type. However here, one should remember that the PNR backbone itself (homo-Gly PNR) rather prefers the B-type conformation without HBs between the side chains and the backbone (when \( n = 6 \)). Thus, we can also conclude that the substitution of the amino acid residues tends to enhance the energetic stability of the E-type PNRs compared with that of the B-type PNRs (Figure 3.9).
Figure 3.13: Three groups of the molecular structures in homo-residue PNRs. The replacement by Asp and Asn, which belong to E-forming residues, induces the hydrogen bond (HB) of O···H-N between the side-chain O atom and the backbone H-N bond in the E-type conformation, but not in the B-type one. On the contrary, the replacement by Thr and Ser, which belong to B-forming residues, produces the HB of O-H···O between the side-chain O-H bond and the backbone O atom in the B-type PNR, but not in the E-type PNR. The replacement by the E/B-forming residues does not cause any HBs between the side chains and the backbone in neither the E-type nor the B-type. We illustrate the homo-residue PNRs of Asn, Ala, and Ser on behalf of those three groups of molecular structures, respectively.
3.4 Electronic structures of the peptide nanorings

3.4.1 Peptide nanoring backbones

In this section, we discuss the electronic structures of homo-residue PNRs. Let us first focus on the simplest homo-Gly PNRs (PNR backbones). The resulting electronic structure of the E-type backbone (Figure 3.14) is similar to that of the B-type backbone (Figure 3.15). In both types of homo-Gly PNRs, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are formed by the backbone $\pi$ states whose orbital lobes are in the ring plane (in-plane) as shown in those figures.

The substitution of the amino acid residues inserts additional energy levels into the electronic structure of the PNR backbone. A noticeable point is that the intruded levels around the HOMO or LUMO are well localized at the replaced side chains, i.e., no overt orbital mixing occurs between the side chains and the backbone. Moreover, the HB interactions between the side chains and the backbone (O···H-N and O-H···O) do not hybridize with the intruded states and also with the frontier $\pi$ states due to the orthogonality between the $\pi$ states and H 1s orbitals. Thus, the electronic structures of homo-residue PNRs cannot be classified based on the energetics but be classified based on the substituent groups.

3.4.2 Substitution of amino acids

The resulting electronic structures of homo-residue PNRs are classified into the following three groups: In the first group (Group I), the energy levels of the side chains are buried in the valence states or the conduction states (Figure 3.16). Those intruded levels are nearly six-fold degenerated, because the neighboring side chains are so apart that those side chains do not interact mutually. This type of electronic structure is found in the homo-residue PNRs of Pro and Asn of the E-forming residues and of Val, Ile, Leu, Ala, and Gln of the E/B-forming residues.

Another type of the electronic structures is classified as Group II, in which the localized state originating from side-chain oxygen (O), nitrogen (N), or sulfur (S) appears at the top of the valence state (Figure 3.17)[21]. This type of electronic structure is found in homo-residue PNRs of Asp (E-forming residue), of Met, Glu, Lys, Arg, and Cys (E/B-forming residues) and of Thr and Ser (B-forming residues). In the homo-residue PNRs of Asp, Glu, Thr, and Ser, the on-site energy of the O 2p state appears just below the HOMO being nearly six-fold degenerated (Figure 3.17). However, when...
Figure 3.14: Electronic structure of the E-type PNR backbone (homo-Gly) (RHF/6-31G**). The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are also illustrated.
Figure 3.15: Electronic structure of the B-type PNR backbone (homo-Gly) (RHF/6-31G**). The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are also illustrated.
Figure 3.16: The electronic structure of Group I, in which the energy levels of the side chains are buried in the valence states or the conduction states. Homo-residue PNRs of Pro and Asn (E-forming residues) and of Val, Ile, Leu, Ala, and Gln (E/B-forming residues) have this type of electronic structure. All the intruded levels are nearly six-fold degenerated, respectively. The electronic structure of the E-type homo-Leu PNR is shown on behalf of this type of electronic structures.
Figure 3.17: The electronic structure of Group II, in which the on-site energy of oxygen (O), nitrogen (N), or sulfur (S) appears at the top of the valence state. This type is found in the homo-residue PNRs of Asp (E-forming residue), of Met, Glu, Lys, Arg, and Cys (E/B-forming residues) and of Thr and Ser (B-forming residues). All the intruded levels are nearly six-fold degenerated, respectively. The electronic structure of the E-type homo-Met PNR is shown on behalf of this type of electronic structures. The change in the on-site energy is also shown in the figure.
Figure 3.18: The electronic structure of Group III, in which the side-chain \( \pi \) electrons modify the upper valence state and the lower conduction state. Homo-residue PNRs of Trp, His, Tyr, and Phe have this type of electronic structure. All the intruded levels are nearly six-fold degenerated, respectively. The electronic structure of the B-type homo-Phe PNR is shown on behalf of this type of electronic structures.
the component residues are replaced by Lys or Arg, the on-site levels are lifted and intruded into the energy gap due to the higher energy of the N 2p state (Figure 3.17). Those fully occupied levels are furthermore pulled upward in the PNRs of Met or Cys, which includes a sulfur atom having a much higher on-site energy (Figure 3.17).

The replacement by the remaining aromatic residues (Trp, His, Tyr, and Phe) provides the other type of electronic structure (Group III), in which both the upper valence state and the lower conduction state are modified by the side-chain $\pi$ electrons (Figure 3.18). The number of the intruded $\pi$ states is uniquely determined by the atomicity of the membered ring in the replaced side chains. When the side chains include the 6-membered ring (in Phe), six $\pi$ states appear in the electronic structure while maintaining the individual $\pi$ states nearly six-fold degenerated (Figure 3.18). Among those $\pi$ states, quasi-degenerated $\pi$ and $\pi^*$ levels[22] are intruded into the energy gap and form the highest occupied state and the lowest unoccupied state, respectively. Moreover, the resolution of these quasi-degenerated energy is enhanced in the homo-Tyr PNR because of the symmetry lowering by an additional oxygen atom [Figure 3.19 (a)].

In homo-His PNR, on the other hand, five $\pi$ states appear in the electronic structure due to the 5-membered ring of the side chain (Figure 3.20). Because the membered ring includes nitrogen atoms, the resolution of the quasi-degenerated energy is further enhanced. However, two occupied $\pi$ levels are still within the energy gap while maintaining the individual $\pi$ states being six-fold degenerated (Figure 3.20). On the contrary, two unoccupied $\pi^*$ levels are both crowded out of the energy gap and buried in the conduction states. In consequence, the lowest unoccupied state is formed by the backbone state, while the highest occupied state is still formed by the side-chain $\pi$ orbital[23].

If the component residues are replaced by Trp having a 5&6-membered ring, nine $\pi$ states are embedded in the electronic structure [Figure 3.19 (b)]. Among those $\pi$ states, three $\pi$ levels and one $\pi^*$ level appear in the energy gap, and therefore, the frontier orbitals of the homo-Trp PNRs are both formed by the side-chain $\pi$ states in the present calculation.

All of the above results indicate that the membered ring forms in the side chains are expected to be a certain reaction site in the PNRs and also in the other peptide systems.
Figure 3.19: Electronic structures of the E-type PNR of Tyr (a) and Trp (b), which belong to Group III. All the intruded $\pi$ states are nearly six-fold degenerated.
Figure 3.20: The electronic structure of the E-type homo-His PNR, which belongs to Group III. All the intruded \( \pi \) states are nearly six-fold degenerated.
3.5 Peptide nanotubes

How do the peptide nanorings (PNRs) stack to form the peptide nanotubes (PNTs)? In this section, we discuss the electronic and molecular structures of the PNT backbone. Because one can prospect two kinds of the PNR stacking means of parallel and antiparallel, we should consider the following four types of PNTs: One is the PNT in which E-type PNRs are stacked parallel (E-type parallel PNT). The remainders are those PNTs of E-type antiparallel, B-type parallel, and B-type antiparallel. For these four types of the PNTs, Restricted Hartree-Fock molecular orbital calculations were carried out by employing Gaussian98 program[16] using 6-31G** basis set.

The geometry optimization and total energy calculations were carried out as follows: We start to obtain an optimal inter-ring distance using the model tube constructed by stacking three NRs parallel or antiparallel. After the inter-ring optimization, the molecular structure of three rings is fully optimized by freezing the above inter-ring distance. Next, the center ring is picked out and the initial PNT model is constructed by stacking these rings. After re-calculating the optimal inter-ring distance, the molecular structure of the PNT (three rings) is fully optimized. Finally, by picking out the center ring, we determine the “optimized” PNT unit. The change in the resulting total energy is calculated by varying the inter-ring distance in these “optimized” PNTs. The geometry optimization is performed for both E-type and B-type backbones of the PNTs, which are formed by the stacking of the smallest D,L-peptide nanorings consisting of six Gly residues.

3.5.1 Optimized molecular structures

Extended-type backbone

First, let us discuss the molecular structures of the E-type PNTs. The optimized backbone conformations of the E-type parallel and E-type antiparallel PNT are shown in Figures 3.21 (a) and (b). The resulting internal parameters reveal that the PNR unit of the parallel PNT is similar to that of the antiparallel PNT (Tables 3.8, 3.9, and 3.10). In both PNTs, the $\omega$ angle approaches (-)180°, and the remaining two angles are close to $\psi \simeq -\phi$ (Table 3.10). This feature makes the backbone conformation revert to the (initial mathematical) BR form. The present calculations also provide the similar inter-ring distances of 4.90 Å for the E-type parallel PNT and 4.95 Å for the E-type antiparallel PNT.

The conformation change from the isolated PNR to the PNT is caused by the inter-ring hydrogen bonds (HBs) of N-H···O and Cα-H···O [Figures 3.21 (a) and (b)]. In
Table 3.8: Bond lengths in the unit of E-type parallel, E-type antiparallel, B-type parallel, and B-type antiparallel PNTs (RHF/6-31G**).

<table>
<thead>
<tr>
<th>Stacking mean</th>
<th>Extended-type</th>
<th>Bound-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>parallel</td>
<td>1.523</td>
<td>1.334</td>
</tr>
<tr>
<td>antiparallel</td>
<td>1.523</td>
<td>1.333</td>
</tr>
</tbody>
</table>

Table 3.9: Bond angles in the unit of E-type parallel, E-type antiparallel, B-type parallel, and B-type antiparallel PNTs (RHF/6-31G**).

<table>
<thead>
<tr>
<th>Stacking mean</th>
<th>Extended-type</th>
<th>Bound-type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΘN-Cα-C</td>
<td>ΘCα-C-N</td>
</tr>
<tr>
<td>parallel</td>
<td>109.7°</td>
<td>116.8°</td>
</tr>
<tr>
<td>antiparallel</td>
<td>109.4°</td>
<td>116.8°</td>
</tr>
</tbody>
</table>

Table 3.10: Internal rotation angles in the PNR units of E-type parallel, E-type antiparallel, B-type parallel, and B-type antiparallel PNTs (RHF/6-31G**).

<table>
<thead>
<tr>
<th>Stacking mean</th>
<th>Extended-type</th>
<th>Bound-type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>φ</td>
<td>ψ</td>
</tr>
<tr>
<td>parallel</td>
<td>-119.2°</td>
<td>124.1°</td>
</tr>
<tr>
<td>antiparallel</td>
<td>-119.5°</td>
<td>125.0°</td>
</tr>
</tbody>
</table>
the E-type parallel PNT, the H···O distance of N-H···O is reduced to 2.0 Å and that of Cα-H···O is 2.5 Å. As a result, the overlap populations of those inter-ring HBs increase to overcome the intra-ring HB. This is the reason why the optimized backbone reverts to the BR form. However, the complete amide plane of ω = 180° is not reproduced because the intra-ring HB is still alive in the PNT (Table 3.10). The same feature is also found in the antiparallel PNT.

The structural difference between the parallel and the antiparallel PNT is that the former has a monotonous stacking form [Figure 3.21 (a)], while the latter provides a twisted PNT form, i.e., the adjacent PNRs are rotated alternately [Figure 3.21 (b)]. This feature is caused by the competition between the two inter-ring HBs of N-H···O and Cα-H···O. The parallel stacking gives the minimum H···O distance when the twist angle is around 0° [Figure 3.22 (a)]. On the contrary, in the antiparallel stacking, the minimum H···O distance is found when the twist angle is +18° [Figure 3.22 (a)]. Thus, the PNRs are rotated in the E-type antiparallel PNT by 10.4° [Figure 3.22 (b)].

**Bound-type backbone**

B-type PNTs also show a similar twisted nature in the antiparallel stacking [Figure 3.23 (b)], although the parallel stacking of the B-type PNRs provides a monotonous stacking form [Figure 3.23 (a)]. The corresponding twist angle of 22.0° [Figure 3.24 (b)] is well understood by the fact that the antiparallel stacking of the B-type PNRs gives the minimum H···O distance at -24° [Figure 3.24 (a)]. One should also notice that the negative value of the twist angle indicates that the B-type PNRs are rotated in the opposite direction compared with the E-type PNRs.

The optimal inter-ring distances in the B-type PNTs are rather different between parallel and antiparallel stacking. While the B-type parallel PNT has a value of 5.31 Å, the antiparallel PNT provides a larger inter-ring distance of 5.75 Å (Figure 3.25). This feature is caused by the inter-ring repulsion of Cα-H···H-Cα. Although the parallel PNT has no overt repulsion, the B-type antiparallel PNT includes a slight Cα-H···H-Cα repulsion between the adjacent PNRs [Figure 3.23 (b)]. Therefore, the inter-ring distance in the B-type antiparallel PNT is more elongated than that in the B-type parallel PNT. This feature does not appear in E-type PNTs, because the H···H distance in the E-type antiparallel PNT is increased by 5% compared with that in the B-type antiparallel PNT [Figure 3.21 (b)].

Finally, it is appended that the individual PNR units in both E-type and B-type PNTs have the coincident ring (tube) axis while maintaining the S6 symmetry. Therefore, the resulting PNTs have a straight rod shape irrespective of the stacking means
E-type PNT backbone

(a) parallel stacking

(b) antiparallel stacking

Figure 3.21: Top view and side view of the optimized molecular structure of the E-type parallel PNT (a) and E-type antiparallel PNT (b). In the antiparallel stacking, the individual PNRs are twisted alternately by 10.4°.
Figure 3.22: The averaged H⋯O distance of N-H⋯O and C$\alpha$-H⋯O (a), and the potential barrier (b) by varying the twist angle between the adjacent PNRs. The vertical line in figure (b) shows the energy difference from 0$^\circ$. 
Figure 3.23: Top view and side view of the optimized molecular structure of the B-type parallel PNT (a) and the B-type antiparallel PNT (b). In the antiparallel stacking, the individual PNRs are twisted alternately by 22.0°.
Figure 3.24: The averaged H⋯O distance of N-H⋯O and Cα-H⋯O (a), and the potential barrier (d) by varying the twist angle between the adjacent PNRs. The vertical line in figure (b) shows the energy difference from 0°.
of parallel and antiparallel. This straight rod shape is a common feature found in the D,L-peptide nanotubes.

### 3.5.2 Energetics

#### Condensation energy

Let us discuss the energetics of these four PNTs. We first compare the condensation energies with respect to the different stacking means of parallel and antiparallel. In the E-type conformation (Figure 3.25), the parallel stacking and the antiparallel stacking provide almost the same condensation energies of 5.0 kcal/mol per residue; the resulting energy difference is at most 0.03 kcal/mol per residue. On the contrary, in the B-type conformation, the parallel stacking causes a larger condensation than that of the antiparallel stacking by 0.54 kcal/mol per residue (Figure 3.25). This is because the B-type antiparallel PNT includes the C\(\alpha\)-H···H-C\(\alpha\) repulsion between the adjacent PNRs as discussed in the former section [Figure 3.23 (b)].

#### Total energy difference

How does the energy difference occur between the E-type and B-type conformations? The present energetics (Figure 3.25) reveal that the stacking of the E-type PNRs causes a larger condensation than that of the B-type PNRs, irrespective of the stacking means of parallel and antiparallel. As a result, the energetic preferability of the isolated single B-type PNR (energetically more stable than an E-type PNR by 2.32 kcal/mol per residue) is compensated, and the total energies of E-type and B-type (parallel) PNTs become comparable. The resulting energy difference between E-type and B-type parallel PNTs is as small as 0.14 kcal/mol per residue (Figure 3.25). The present result indicates the existence of both backbone conformations of the E-type PNT having a larger diameter and the B-type PNT having a smaller diameter. To sum up, we can also suggest that the B-type PNT backbone prefers the parallel stacking form, while the E-type PNT backbone has a chance to have both stacking forms of parallel and antiparallel.

### 3.5.3 Band structures

Finally, the band structures of the PNTs were investigated based on the Hartree-Fock crystalline orbital (CO) method using the Crystal98 program[25]. The calculated band structures and the density of states (DOS) of the E-type and B-type parallel PNTs are shown in Figures 3.26 and 3.27. Several previous calculations based on
Figure 3.25: Relationship between the total energy (per residue) and the inter-ring distances in the PNTs.
Figure 3.26: Calculated band structures and the density of states (DOS) of the E-type parallel PNT (RHF/6-31G**).
Figure 3.27: Calculated band structures and the density of states (DOS) of the B-type parallel PNT (RHF/6-31G**).
the DFT approach have revealed a wide-gap semiconducting nature or an insulating electronic feature in some E-type PNTs[9, 26, 27, 28, 29]. The present Hartree-Fock CO calculations also show an equivalent electronic structure and similar characteristic band-edge states of the E-type PNT (Figure 3.26)[30]. Both the highest occupied valence band (HOVB) and the lowest unoccupied conduction band (LUCB) are formed by the in-plane $\pi$ states analogous to the PNR backbone (Figure 3.14).

The calculated effective masses of electrons and holes in the geometrically optimized PNTs are considerably large toward the tube axis. This less electronic delocalization is caused by the in-plane $\pi$ orbital nature of the band-edge states. For the delocalization of the band-edge states toward the tube axis, these in-plane $\pi$ states should hybridize with the horn-like H 1s orbitals of the adjacent PNRs. However, the orthogonality between the in-plane $\pi$ and H 1s states prohibits such orbital mixing in the optimized PNT geometry.

The in-plane $\pi$ orbital nature of the HOVB and LUCB states is also conserved when the PNT is formed by the B-type PNRs. Therefore, a similar band structure is obtained in the B-type parallel PNT (Figure 3.27). This feature is also irrespective of the stacking means, and furthermore, the twist of the adjacent PNRs hardly changes the frontier electronic states. Thus, electrons or holes are localized even in the twisted antiparallel PNTs.

### 3.6 Summary

Following the mathematical conformation analysis, the possible molecular conformations in peptide nanorings and nanotubes were investigated by *ab initio* Hartree-Fock calculations. The results are summarized as follows:

- The theoretical study reveals that an even number of the alternating D- and L-amino acid sequences produces the peptide nanorings (PNRs) of $n \geq 6$. Moreover, even with the same $n$ number, two types of the backbone conformation are predicted; i.e., the conventional Extended-type (E-type) and the novel Bound-type (B-type).

- The energetically preferable backbone type changes in accordance with its ring size, i.e., the smaller rings ($n \leq 8$) prefer the B-type backbone, while the larger rings ($n \geq 10$) prefer the E-type backbone.

- *Ab initio* calculations for the amino acid substitution reveal that all 20 encoded residues can form both E-type and B-type PNRs, while either type is provided
as the energetically stabler form in accordance with the kind of the replaced side chains.

- Electronically, the HOMO and LUMO states of the PNR backbones are formed by the in-plane $\pi$ states. The replacement by the appropriate residues, furthermore, intrudes additional levels in the energy gap and forms the frontier orbitals localized at the side chains.

- E-type and B-type PNRs can both stack to form the peptide nanotubes (PNTs). While the parallel stacking of the PNRs provides a monotonous stacking form, the antiparallel stacking causes a twisted PNT form.

- The B-type PNT energetically prefers the parallel stacking form, while the E-type PNT has a chance to have both stacking means of parallel and antiparallel.
Appendix: Alternate stacking of the B-type and E-type peptide nanorings

As mentioned in the former section, the resulting energy difference between the E-type and B-type PNTs is very small (Figure 3.25). Therefore, we should also consider the alternate stacking of B-type and E-type PNRs. Those E-type and B-type PNRs have different internal diameters even with the same number of residues \( n \). Thus, the difference might break the orbital orthogonality found between the in-plane \( \pi \) band-edge states and the horn-like H 1s states. In this additional section, we discuss this “B-E alternate” PNT (Figure 3.28).

The total energy calculation of the B-E alternate PNT gives a well-distinguished potential valley at the inter-ring distance of 5.20 Å, which is the value between the inter-ring distance of the E-type and the B-type PNTs. The present calculation also indicates that the B-E alternate PNT is less stable than the B-type parallel PNT but at most only by 1.80 kcal/mol per residue.

The resulting band structure and DOS reveal that both band-edge states still remain to be localized (Figure 3.28). Those band-edge states are formed by the in-plane \( \pi \) states, but those \( \pi \) states are swept away into the B-type PNR because of the supermolecular form due to the alternate stacking. Therefore, the orbital mixing via the inter-ring HBs hardly occurs even in this B-E alternate PNT. In order to induce a certain amount of the band-edge electronic delocalization, it would be necessary to produce some other designs, e.g., an inter-ring bridge via amino acid side chains.
Figure 3.28: Calculated band structures and the density of states (DOS) of the B-E alternate PNT (RHF/6-31G**).
Bibliography


In the section 3.3, we also use those values as the initial values for the geometry optimization.


With increasing n number, the skeletal backbones of E-type BRs approach that of the fully extended trans-planar structure, which is the most energetically stable peptide skeleton. Therefore, the total energy of the E-type BR slightly decreases with increasing n number.

While the side chains have a large number of degrees of freedom, the initial side-chain conformation was determined to be \( \chi_1 = -120^\circ \) and \( \chi_i = 180^\circ \) in the present calculations. This initial structure lets the normal vector of the side-chain plane be perpendicular to the \( S_6 \) axis. The PNR geometry was then optimized while maintaining the \( S_6 \) symmetry. As a result, one of three rotamer types (gauche(+), gauche(-), or trans) was obtained for the individual homo-residue PNR as the local minimum structure (Figure 3.8). The resulting \( \chi_1 \) angles and rotamer type are also listed in Tables 3.6 and 3.7. The PNRs might also have the other rotamer types for the local minimum forms. However, the other rotamer types are out of consideration in the present thesis, because of the large number of degrees of freedom in the calculations.

Only the homo-Pro PNR shows a considerably large energetic stability in the E-type conformation (by 20.6 kcal/mol per residue). This is because the peculiar atomic loop included in the Pro residue generates the large backbone distortion especially in the B-type conformation as shown in Table 3.7.

Homo-residue PNRs of Trp and His also show the large energetic stability in the E-type conformation, despite the fact that they include no HB between the side chains and the backbone.

These \( \pi \) states do not degenerate completely because the point group symmetry of Phe is reduced from the \( D_{6h} \) symmetry of benzene due to the connection of a \( C^\beta \) atom. However, the individual \( \pi \) levels still maintain the six-fold degeneracy.

This characteristic is well understood using the Hückel approach. If the membered ring of \((\text{CH})_m\) consists of six atoms \((m = 6)\), the energy value of the degenerated \( \pi \) and \( \pi^* \) levels is given by \( E_{\pi} = \alpha + \beta \) and \( E_{\pi^*} = \alpha - \beta \), respectively[24]. However, those levels are lifted to be \( E_{\pi} = \alpha + 0.62\beta \) and \( E_{\pi^*} = \alpha - 1.62\beta \) when the atomicity
of the membered ring is changed to $m = 5$ as shown in Figure 3.20. Therefore, the
$\pi^*$ levels are put into the conduction band, while the $\pi$ levels are still within the
energy gap.

[24] The energy of the $\pi$ electrons in the membered rings of $(\text{CH})_m$ is given using the
coulomb integral $\alpha$ and the resonance integral $\beta$ by $E_j = \alpha + 2\beta \cos \frac{2\pi j}{m}$, where
$j = 0, 1, 2, ..., m/2$ if $j$ is an even number, or $j = 0, 1, 2, ...,(m - 1)/2$ if $j$ is an odd
number.


[30] The resulting HF energy gap of the E-type parallel PNT is 14.92 eV, which is
considerably larger than our previous DFT result of 4.29 eV. This is because $ab initio$
Hartree-Fock calculations overestimate the energy gap due to the neglect of
the inter-electron correlation, while the first-principles DFT calculations underesti-
mate the corresponding value. Therefore, these values should be calibrated.
Chapter 4

Syntheses and Atomic Force Microscopy Observations of the D,L-Peptide Nanotubes

4.1 Introduction

In the former chapters, possible molecular conformations of the D,L-peptide nanorings and nanotubes were examined based on the mathematical conformation analysis, and also their stable forms as well as the electronic structures were investigated by *ab initio* calculations. In this chapter, following to those theoretical studies, the author challenges to the synthesis and the observation of a couple of peptide nanotubes (PNTs) with the aim of the molecular manipulation and application.

In order to manipulate the PNT molecules and apply them in nano-electronics, it is necessary to investigate their intimate morphology at the nano-order level. Especially, to observe the single PNT forms is the crux of the matter. Thus, in this chapter, the atomic force microscopy (AFM) observations are performed and the morphology of the PNTs is discussed. In parallel, we also focus on the other important subject, that is, how the difference in the number of the component residues changes the morphology of the PNTs. In this respect, the following two kinds of the D,L-peptide nanotubes are synthesized and observed [Figures 4.1 (a) and (b)]; the hexapeptide nanotube (6PNT) and the octapeptide nanotube (8PNT). Focusing attention on these two PNTs is based on our mathematical conformation analysis (in Chapter 2), which indicates that the former corresponds to the smallest D,L-peptide nanotube while the latter corresponds to the second smallest one. Therefore, by carrying out the synthesis and AFM observations, we are able to study how small a D,L-peptide nanotube we could fabricate and observe.
The same type of amino acid sequence should be employed for both 6PNT and 8PNT to compare their morphology in terms of the number of the component amino acids. Therefore, alternating D-Ala and L-Gln sequence are chosen for the targeted PNTs, i.e., \( \text{cyclo}[-(D-\text{Ala}-L-\text{Gln})_3] \) [6PNR; Figure 4.1 (a)] and \( \text{cyclo}[-(D-\text{Ala}-L-\text{Gln})_4] \) [8PNR; Figure 4.1 (b)]\textsuperscript{9}. One of the reasons why these sequences are chosen is that the peptides are easily cyclized on the resin if the target PNRs include the Gln residue as described later. The other reason is that this alternate catenation of Gln and Ala has the potential to assemble these PNTs through the Gln-Gln hydrogen bonds and/or Ala-Ala hydrophobic interaction beyond the inter-tubes. Therefore, we can discuss the difference in the inter-tube interaction among those 6PNTs and 8PNTs.

Before synthesizing and observing these PNTs, we examine \textit{ab initio} energetics and investigate whether the targeted 6PNRs and 8PNRs could stack themselves to form PNT structures. These theoretical studies also provide important information about the molecular structures of the PNTs. The calculated diameters and the inter-ring distance in the PNTs will help in analyzing the AFM images of the synthesized PNTs.

### 4.2 \textit{Ab initio} Energetics

First, the energetically stable molecular forms of the 6PNT and 8PNT are investigated by Restricted Hartree-Fock calculations\textsuperscript{10}. Here, the following two kinds of PNR
stacking should be considered for each PNT, i.e., parallel and antiparallel stacking. Therefore, the geometry optimization was performed for both stacking means of the 6PNRs and 8PNRs.

The geometry optimization and total energy calculations were carried out as follows: First, we fully optimized the isolated 6PNR and 8PNR forms, respectively. Next, we stacked four optimized 6PNRs or 8PNRs both parallel and antiparallel and then optimized the inter-ring distances while freezing the geometry of the individual PNR. The molecular structures of four PNRs were then re-optimized with no restriction. We selected two of the resulting central PNRs and re-calculated the optimal inter-ring distance. The resulting final structures were regarded as the “optimized” parallel and antiparallel PNT units. Although it was preferable to use the higher basis sets such as the split-valence basis with polarization functions, the large number of component atoms prevents that approach to this PNT system. Therefore, the stable molecular forms of these PNTs were investigated using the minimal basis set. It is sufficient for us to qualitatively discuss the AFM morphology using this broad estimation.

The present calculations indicate an important result that both 6PNRs and 8PNRs can condense to form PNT structures [Figures 4.2 (a) and (b)]. Furthermore, both parallel and antiparallel stacking are possible with distinguishable potential valleys [Figure 4.3]. The resulting optimal inter-ring distances are almost same in all PNTs (4.75-4.80Å), and the condensation energies are within 2.6~3.5 kcal/mol per residue. Judging from the resulting values only, both 6PNRs and 8PNRs tend to prefer antiparallel stacking to parallel. The resulting energy difference between parallel and antiparallel stacking is 0.28 kcal/mol in 6PNTs and 0.19 kcal/mol in 8PNTs [Figure 4.3]. However, one should be noted that such small values do not have meaning under the current calculation level. Therefore, we cannot determine the relative merits between parallel and antiparallel stacking by the present calculations.

The ring stacking is caused by two kinds of inter-ring N-H···O hydrogen bonds; One is the backbone-backbone hydrogen bond and the other is the sidechain-sidechain (Gln-Gln) hydrogen bond. While the former provides the PNT's cylindrical skeleton, the latter produces the inter-ring packing in the PNTs. With respect to the competition of these two hydrogen bonds, the above small energy differences come from the cancellation of these two hydrogen bonds between parallel and antiparallel stacking. One should, however, pay attention to the fact that high-accuracy calculations need to be carried out with polarization functions in order to discuss the intimate energy difference between parallel and antiparallel stacking.

Still, the present calculations provide important information about the diameter of
Figure 4.2: Optimized molecular structure of the antiparallel 6PNT of cyclo[-(D-Ala-L-Gln)-₃] (a) and of the antiparallel 8PNT of cyclo[-(D-Ala-L-Gln)₄] (b).
Figure 4.3: The relation between the inter-ring distance and the total energy (per residue) in the 6PNTs and 8PNTs obtained by Restricted Hartree-Fock calculations with the STO-3G basis set.
Table 4.1: Resulting target mass of the 6PNR and the 8PNR measured by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOFMS). We also show the calculated values in the column.

<table>
<thead>
<tr>
<th>substance</th>
<th>6PNR(exp./calcd.)</th>
<th>8PNR(exp./calcd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H⁺]</td>
<td>598.5/598.3</td>
<td>796.9/797.4</td>
</tr>
<tr>
<td>[M+Na⁺]</td>
<td>620.5/620.3</td>
<td>818.7/819.4</td>
</tr>
<tr>
<td>[M+K⁺]</td>
<td>636.3/636.3</td>
<td>834.7/835.4</td>
</tr>
</tbody>
</table>

the 6PNTs and 8PNTs. The calculated diameter of the antiparallel 6PNT is 1.27 nm to Ala and 1.95 nm to Gln [Figure 4.2 (a)]. The diameter of the antiparallel 8PNT is slightly larger than that of the 6PNT; 1.42 nm to Ala and 2.12 nm to Gln [Figure 4.2 (b)]. The parallel PNTs also provide the same diameters as those of the antiparallel PNTs. Thus, if the rod-shaped structures of 1~2 nm in height are observed, we can recognize them as the single 6PNTs or 8PNTs, although we cannot distinguish the stacking manner of parallel or antiparallel.

4.3 Synthesis and mass spectrometry

Both cyclo[-(d-Ala-L-Gln)₃] and cyclo[-(d-Ala-L-Gln)₄] were synthesized based on the solid phase method using Fmoc chemistry[16]. In this method, linear peptide precursors are initially assembled on a support of Rink amide resin, and head-to-tail cyclization is performed while the peptides are still bound to the support. Using this method, we can easily cyclize the peptides on the resin and obtain the targeted PNRs. The outline of the synthesis is given as follows.

First, Fmoc-protected Rink amide resin was prepared [Figure 4.4 (a)]. Next, N-Fmoc-protected L-glutamic acid α-allyl ester (Fmoc-L-Glu-OAll) was attached to a deprotected Rink amide resin via the dehydrated carboxyl group on the L-Glu side-chain [Figure 4.4 (b)]. The remaining linear peptide synthesis was performed with five (seven) additional alternating cycles of deprotection and connection of d-Ala and L-Gln to afford the linear precursors [Figure 4.4 (c)]. Pd(PPh₃)₄ and PPD were then used to remove both the C-terminal and N-terminal protections [Figure 4.4 (d)]. Cyclization was accomplished by activation of the deprotected support-bound peptide with BOP/HOBt/DIEA [Figure 4.4 (e)], and then TFA/TIS/H₂O was used for the cleavage of the cyclic peptides from the resin support [Figure 4.4 (f)]. The reaction time of the cyclization was 53 h for the 6PNR, while that of the 8PNR was about 26 h.

After careful purification[2], the synthesized peptides were identified by matrix as-
Figure 4.4: Strategy of the present Fmoc solid-phase peptide synthesis.
Figure 4.5: Mass spectra of the synthesized \textit{cyclo}[-(\textit{d}-\textit{Ala}-\textit{l}-\textit{Gln})_3] measured by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOFMS). The measured mass of the ionized cyclic peptide ([M+Na]^+) well corresponds to the calculated value of the individual peptide. The calculated stable molecular forms of those isolated nanorings are also inserted in the figures (RHF/6-31G**).
Figure 4.6: Mass spectra of the synthesized cyclo\(\text{-}(\text{d-Ala-L-Gln})_4\) measured by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOFMS). The measured mass of the ionized cyclic peptide ([M+Na]+) well corresponds to the calculated value of the individual peptide. The calculated stable molecular forms of those isolated nanorings are also inserted in the figures (RHF/6-31G**).
sisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). The resulting mass spectra of the 6PNR and 8PNR are shown in Figures 4.5 and 4.6, respectively. In Table 4.3, we compare the experimental mass values ionized by $H^+$, $Na^+$ and $K^+$ with those calculated values. The resulting mass-deviation was at most 0.03% for the 6PNR and 0.08% for the 8PNR. These good agreements prove the presence of both $\text{cyclo}[-(d-\text{Ala}-\text{L-Gln})_3]$ and $\text{cyclo}[-(d-\text{Ala}-\text{L-Gln})_4]$. The ultimate yield is 18% for the 6PNR and 20% for the 8PNR.

4.4 Atomic force microscopy observations

The morphology of the synthesized PNTs was investigated with the tapping mode AFM. The actual PNT morphology will depend on the experimental conditions, especially the employed solvent and substrate. Thus, two kinds of solvents and substrates were prepared respectively to compare the morphology between the 6PNT and the 8PNT. Here, the common organic solvents of DMSO[17] and NMP[17] were employed because these solvents dissolved both of the synthesized peptides[13]. An Au substrate and an SiH-SiO$_2$ substrate were also prepared for comparison. On the former substrate, the evaporated gold particles cover the mica sheet and form the Au(111) surface. By using this substrate, we can discuss the affinity of the PNTs for the metal surface. In the latter substrate, the hydrophobic SiH part and the hydrophilic SiO$_2$ part are patterned on Si(111) using photo lithography. Because the synthesized peptides include the hydrophobic Ala residues and the hydrophilic Gln residues, the interaction of the side chains with either the SiH part or SiO$_2$ part is expected.

4.4.1 Peptide nanotubes and nano-bundles

Peptide/DMSO solution on Au substrate

First, both of the synthesized 6PNR and 8PNR products were dissolved in DMSO at a concentration of 0.05 mM at 50°C. Then, the Au substrates were incubated in the 6PNR/DMSO and 8PNR/DMSO solution for 72 h and 2 h, respectively. After removal, they were dried with blowing argon gas, and then the AFM observations were carried out under ambient conditions. Hereafter, these two samples are denoted by 6PNR/DMSO/Au and 8PNR/DMSO/Au.

The resulting AFM image of 6PNR/DMSO/Au shows many rod-shaped structures of 100~150 nm long lying sporadically on the substrate [Figure 4.7 (a)]. The observed heights are 1.5~2.0 nm, which correspond well with the calculated diameter (1.27~1.95
Figure 4.7: AFM image of 6PNR/DMSO/Au (a) and of 8PNR/DMSO/Au (b). We can find many rod-shaped structures in (a), while only the projections are visible in (b).

nm) of cyclo-[d-Ala-L-Gln]$_3$ [Figure 4.2 (a)]. The observed widths of about 20 nm seem to be rather large. However, we should also take into account the resolving power of the present AFM tip. While the observed height is clearly distinguishable, the finite tip size of 20∼30 nm in diameter tends to overestimate the value of the width; e.g., when the real diameter of the construct is about 1 nm, the Si tip shows the width to be 10∼15 nm. Judging from the measured heights and widths, it is considered that the synthesized 6PNTs are lying singly on the substrate. Based on the calculated optimal inter-ring distance (4.75∼4.80˚A), the number of the component 6PNRs is estimated as 200∼300.

A notable result is that the other sample of 8PNR/DMSO/Au provides a completely different AFM image [Figure 4.7 (b)]. On this substrate, we could not find any rod-shaped structures. Instead, many protruded structures were observed with a height of 2∼6 nm. Although we cannot identify these projections only by the resulting image, it is considered that the 8PNTs themselves are not there but clumps of the peptides (or solvent) are adhered to the substrate[14]. This feature verifies that even a slight difference in the component residue number (six or eight) causes a different affinity for the employed solvent.

**Peptide/DMSO solution on SiH-SiO$_2$ substrate**

How does the different substrate change their morphology? Next, the other samples were prepared by immersing the SiH-SiO$_2$ substrate in the 6PNR/DMSO and
8PNR/DMSO solutions for one hour. These substrates (denoted by 6PNR/DMSO/SiH-SiO$_2$ and 8PNR/DMSO/SiH-SiO$_2$) were dried with blowing argon gas and then observed with the AFM under ambient conditions.

The AFM observations also provided completely different images between 6PNR and 8PNR. In the case of 8PNR/DMSO/SiH-SiO$_2$, we can find no product, that is, neither rods nor projections are observed on the substrate. In the case of 6PNR/DMSO/SiH-SiO$_2$, on the contrary, we can find many rod-shaped structures of 150~450 nm long [Figures 4.8 (a)-(c) and 4.9 (d) and (e)]. An interesting point is that these rods are packed together like “sardines”. They are gathering at the depression on the SiH surface [Figures 4.8 (b) and (c)] and also at the step between SiH and SiO$_2$ [Figure 4.9 (d)], where the SiO$_2$ part has a flat plateau of about 5 nm in height while the SiH part has a rough surface. These results lead us to the conclusion that the interaction between the synthesized peptides and the substrate is rather weak, so that the peptides drifted up and were captured by the rough parts of the surface during the blowing of the argon gas.

The measured heights and widths of these rods are 10~15 nm and 30~60 nm, respectively [along line A in Figure 4.8 (c)]. These values are considerably larger than the calculated values for the single 6PNT [Figure 4.2 (a)]. Therefore, the individual rods are regarded as nano-bundles of the 6PNTs. Attentive observations also enable us to find some non-assembled finer rods of 150~200 nm long on the same substrate [Figure 4.9 (e)]. The measured heights of about 2 nm and the widths of about 20 nm [along the lines B and C in Figure 4.9 (e)] indicate that these non-assembled rods are single 6PNTs (or bundles of only a few PNTs).

**Peptide/NMP solution on Au substrate**

In the preceding section, we discussed how a different substrate changed the morphology of the PNTs. In this section, we investigate how a different solvent, i.e., NMP, changes their morphology. The AFM samples were prepared as follows: First, the synthesized 6PNR and 8PNR products were dissolved in NMP at a concentration of 0.05 mM at room temperature. Next, the Au substrates were incubated in the 6PNR/NMP and 8PNR/NMP solutions for one hour, respectively. After removal, they were dried with blowing argon gas, and then the AFM observations were carried out under ambient conditions. Hereafter, these two samples are denoted by 6PNR/NMP/Au and 8PNR/NMP/Au.

The resulting AFM image of 6PNR/NMP/Au shows many protruding structures on the substrate [Figure 4.10 (a)]. The heights of the projections are 3~6 nm, which
Figure 4.8: AFM images of 6PNR/DMSO/SiH-SiO\textsubscript{2} (a)-(c). Figures (b) and (c) are magnified images of figure (a). The measured height along line A is also shown. We can find both nano-bundles and single nanotubes on the same substrate.
Figure 4.9: AFM images of 6PNR/DMSO/SiH-SiO$_2$ (d) and (e). Figure (d) is a magnified images of Figure 4.8 (a) and figure (e) is that of Figure 4.8 (b). The measured heights along lines B and C are also shown.
Figure 4.10: AFM image of 6PNR/NMP/Au (a) and of 8PNR/NMP/Au (b). While many protruding structures are found in figure (a), a straight single nanotube is found in figure (b) along the Au step. The measured height and width of the single nanotube along the lines A and B are also shown.
are similar to those of the aforementioned 8PNR/DMSO/Au [Figure 4.7 (b)]. The projections in Figure 4.10 (a) are therefore considered to be clumps of the peptides, not nanotubes[15]. This AFM image is in strong contrast to the image of 6PNR/DMSO/Au [Figure 4.7 (a)], which shows many 6PNTs on the Au substrate. The present results indicate that even the same PNT provides a different morphology depending on the employed solvent and substrate.

In contrast to 6PNR/NMP/Au, the AFM image of 8PNR/NMP/Au clearly shows the “straight” form of the 8PNT [Figure 4.10 (b)]. The measured height of 1.0~1.8 nm (Figure 4.10) corresponds well to the calculated diameter of cyclo[(D-Ala-L-Gln)₄] [Figure 4.2 (b)]. The width of about 20 nm also implies the single tubular form of the 8PNT, taking into account the resolving power of the present AFM tip. The observed length of 700~800 nm, which is longer than the 6PNT nanotube, indicates that the number of the component 8PNRs to be 1,500~1,700. Here, one should note that the 8PNT was observed along the Au step [Figure 4.10 (b)] due to the weak interaction between the side chains and the gold particles. This result indicates that we have a chance to control the orientation of the PNT by designing the step on the substrate.

This straight nature of the D,L-peptide nanotubes is well understood by the aforementioned ab initio calculations, which revealed that the D,L-peptide nanorings stack themselves to form the straight nanotube while maintaining the Sₙ symmetry of the nanoring backbone (Chapter 3).

4.4.2 Peptide micro-bundles and aggregated-bundles

In addition to the nanotubes and nano-bundles, the present AFM observations also indicate the existence of many micro-order assemblies of the 6PNTs and 8PNTs. The resulting assembled forms are, however, rather different between the 6PNTs and 8PNTs. In the following, we compare the morphology of these assemblies.

Let us analyze the resulting AFM images of the micro-order assemblies of the 6PNTs first. The 6PNTs can form not only nano-bundles but also many micro-bundle structures [Figure 4.11 (a)] on the same substrate of the sample 6PNR/DMSO/SiH-SiO₂ (in Figure 4.8). We can find both micro-bundles and nano-bundles even in the same image [Figure 4.11 (b)]. The measured heights of these micro-bundles are 100~200 nm and the widths are 200~300 nm [along line A in Figure 4.11 (a)]. Because these values are much larger than those of the 6PNT nano-bundles, these micro-bundles are considered to be assemblies of a multitude of 6PNTs. The lengths of these micro-bundles are not uniform but have a range of 3~5 μm. Taking into account the calculated inter-ring distance (Figure 4.3), we can approximate the number of component 6PNRs
Figure 4.11: AFM images of 6PNR/DMSO/SiH-SiO$_2$ (a) and (b). The measured height along line A is also shown. The 6PNTs form many micro-bundles as well as nano-bundles on the same substrate.
Figure 4.12: AFM images of 8PNR/NMP/Au (a)-(c). (b) and (c) are magnified images of (a). The measured height along line A is also shown. The 8PNTs produce the “rice-shaped” aggregated-bundles. Those aggregated-bundles are observed on the same substrate as Figure 4.10 (b).
to be 6,000∼10,000. The existence of the micro-bundle forms based on peptides is of great interest from the viewpoint of both medical and industrial applications; e.g., their exploitation as a long-distance drag delivery vehicle and/or as micro-electronic components will be expected.

Compared with the micro-bundle forms of the 6PNTs, the 8PNTs produce interesting “rice-shaped” structures [Figure 4.12 (a)]. These micro-order constructs of 4∼5 µm in length are observed on the same substrate of 8PNR/NMP/Au, which gave us the image of the straight 8PNTs [Figure 4.10 (b)]. The measured heights of 700∼900 nm and the widths of 1.0∼1.5 µm [along line A in Figure 4.12 (a)] are larger than the micro-bundles of the 6PNTs (in Figure 4.11). These large values and the magnified image [Figure 4.12 (b)] reveal that the rice-shaped structures correspond to the aggregated-bundles assembled by some micro-bundles of 300∼400 nm in width. By further magnifying the image [Figure 4.12 (c)], we can also notice that the individual micro-bundles are the assembled forms of the 8PNTs, in which many nanotubes are tightly packed together.

4.4.3 Inter-tube interacting manner

What causes the difference in the assembled forms between 6PNTs and 8PNTs? We finally discuss the inter-tube interaction of the 6PNTs and 8PNTs in terms of the difference in the number of component amino acid residues. Because the targeted PNTs consist of Gln and Ala residues, the following three types of the inter-sidechain interaction are considered, i.e., Gln-Gln, Ala-Ala, and Gln-Ala.

For the consideration of the difference of the bundle forms between the 6PNT and the 8PNT, let us focus on the topology of their crystalline systems. In order to construct the space-filling crystalline models, the AQ6 nanotubes should form a hexagonal system while the AQ8 nanotubes should form a tetragonal system. Taking into account these crystalline systems and also the alternate sequence of d-Ala and l-Gln in the nanoring unit, two types of bundle models can be supposed for the 6PNT and the 8PNT, respectively: One is the 6PNT(QQ) model [Figure 4.13 (a)] or the 8PNT(QQ) model [Figure 4.13 (c)] consisting of the side-chain interaction between the same kinds of residues, i.e., Gln-Gln and Ala-Ala. The other is the 6PNT(QA) model [Figure 4.13 (b)] or the 8PNT(QA) model [Figure 4.13 (d)] including the interaction between the different kinds of residues, i.e., Gln-Ala. For these four types of bundle models, ab initio calculations were employed and their energetics and the optimal inter-tube distances were investigate[16].

The resulting total energy difference from the isolated system (nanoring units) is
Figure 4.13: Four types of the inter-tube interaction models in the peptide nanotube bundles. Figures (a) and (b) are of the AQ6 nanotube bundle, and figures (c) and (d) are of the AQ8 nanotube bundles. Models of figures (a) and (c) include preferable inter-tube interactions through Gln-Gln hydrogen bonding and Ala-Ala hydrophobic interaction, while those of Figures (b) and (d) have undesirable interactions through Gln-Ala.
Figure 4.14: The relation between the inter-tube distance (from center to center) and the condensation energy in the 6PNT and 8PNT bundles (RHF/STO-3G). The present result indicates that both of the nanotubes prefer the inter-tube interaction through Gln-Gln hydrogen bonding (6PNT Q-Q and 8PNT Q-Q). The optimal nearest neighbor inter-tube distance is 1.82 nm for the 6PNTs and 1.75 nm for the 8PNTs, respectively.
shown in Figure 4.14. *Ab initio* calculations reveal that both the 6PNT and the 8PNT prefer the (QQ) model in which only the interactions between the same kinds of residues are included, i.e., Gln-Gln and Ala-Ala. The (QA) models including the interaction between the different residues does not stabilize the energy in the 8PNT, or provides only a small condensation energy in the 6PNT (about a third of the condensation in the 6PNT(QA)), because the number of the hydrogen bonds between Gln and Gln side chains (N-H⋯O) is smaller than that in the former models. Therefore, the 6PNTs and the 8PNTs are considered to pack and form the bundle models of the 6PNT(QQ) and the 8PNT(QQ), respectively[17].

Taking into account the optimal distances of 1.82 nm for the 6PNT(QQ) model and 1.75 nm for the 8PNT(QQ) model, we can construct their three-dimensional bundle models [Figures 4.15 (a) and (b)]. Comparing the two bundle models, one can notice that the 8PNTs densely pack themselves to form nanotube bundles [Figure 4.15 (b)], but the hexagonal bundle of the 6PNTs has larger spatial gaps between adjacent nanotubes [Figure 4.15 (a)], in which the solvent can easily enter. Therefore, the 8PNTs cause the tight and large aggregated bundles as shown in Figure 4.12 (c), whilst the 6PNTs are too tight to form the large aggregated bundles and so provide the thinner bundles [Figures 4.11 (a) and (d)].

Based on the optimal bundle models in Figures 4.15 (a) and (b), we can calculate the number of the assembling nanotubes in the nano-bundles and micro-bundles in Figures 4.9 (e), 4.11 (a), and 4.12 (a). From the gross estimate, one can find that the individual nano-bundle of the 6PNTs consists of several hundred nanotubes (~300 nanotubes) [Figure 4.9 (e)], while the micro-bundle includes thousands of 6PNTs (6,000~17,000 nanotubes) [Figure 4.11 (a)]. The aggregated 8PNT bundle in Figure 4.12 (a) includes more nanotubes than the 6PNT micro-bundles (as many as 700,000 or more nanotubes).

### 4.5 Summary

Both the hexapeptide cyclo[-(D-Ala-L-Gln)₃] nanotube and the octapeptide cyclo[-(D-Ala-L-Gln-)₄] nanotube were synthesized and their morphology was investigated by AFM observations. *Ab initio* calculations were also performed for those models to support the analysis of the observed morphology. The results are summarized as follows:

- *Ab initio* calculations indicate that both cyclo[-(D-Ala-L-Gln-)₃] and cyclo[-(D-Ala-L-Gln-)₄] nanorings can stack themselves to form nanotube structures.
- The cyclo[-(D-Ala-L-Gln-)₃] and cyclo[-(D-Ala-L-Gln-)₄] were synthesized and iden-
Figure 4.15: Resulting crystal models of the 6PNTs (a) and the 8PNTs (b). The calculated inter-tube distances (length and breadth) are also shown in the figures.
Figure 4.16: Hierarchy diagram of the PNT system. The size of the nanotubes and the bundles of the 6PNTs and 8PNTs is summarized.

- AFM images of many nano-bundles of the hexapeptide nanotubes were obtained. Their micro-bundle forms were also observed on the same substrate.

- The straight single octapeptide nanotubes were observed along the Au step. The octapeptide nanotubes also formed micro-order aggregated-bundles.

- The difference in the assembled forms between the hexapeptide and octapeptide nanotubes is caused by the different space filling manner of these nanotubes through inter-tube Gln-Gln hydrogen bonds and Ala-Ala hydrophobic interaction.

- The size of the observed peptide nanotubes and bundles is summarized in Figure 4.16.
Bibliography


[9] The cyclo[-(d-Ala-L-Gln)$_4$] nanotube (8PNT) was also synthesized by the Ghadiri group[2]. However, the synthesis of the smaller cyclo[-(d-Ala-L-Gln)$_3$] nanotube (6PNT) has not been reported yet. Therefore, both cyclo[-(d-Ala-L-Gln)$_3$] and cyclo[-(d-Ala-L-Gln)$_4$] are synthesized and compared their morphology in the present thesis.


Abbreviations of chemical substances in the text denote the several reagents used in the present synthesis; Fmoc = 9-fluorenylmethoxycarbonyl, Pd(PPh\textsubscript{3})\textsubscript{4} = tetrakis (triphenylphosphine) palladium, PPD = piperidine, HBTU = 2-(1H-benzotriazole-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole hydrate, DIEA = N,N-diisopropylethylamine, BOP = benzotriazol-1-yloxy tris (dimethylamino) phosphonium hexafluorophosphate, TFA = trifluoroacetic acid, TIS = triisopropylsilane, DMSO = dimethyl sulphoxide, and NMP = N-methylpyrrolidone.

The synthesized peptides can also be dissolved in TFA. The solvent, however, does damage the substrate. Therefore, we tried to use only two organic solvents, DMSO and NMP, for the AFM observations.

Our AFM observations for neat DMSO on the Au substrate under the same conditions also show the projections with heights of 1.5~3.0 nm. However, the observed heights of the projections in 8PNR/DMSO/Au are larger than those.

It is confirmed that neat NMP on the Au substrate does not form any projections. Therefore, the observed projections in 6PNR/NMP/Au are considered to be clumps of the peptides.

Restricted Hartree-Fock molecular orbital calculations were employed for the four types of bundle models using the STO-3G basis set. In the calculations, four nanoring units of the stable peptide nanotube are allocated on the same plane first. Then, the total energy of these four nanoring models was calculated with varying the inter-ring (inter-tube) distance while freezing the internal parameters of the nanoring units.

The 6PNT(QQ) model and the 8PNT(QQ) model would also include the inter-tube hydrophobic interaction through Ala and Ala side chains. However, this hydrophobic interaction is out of consideration under the present Hartree-Fock molecular orbital calculations.
Chapter 5

Theoretical Prediction and Atomic Force Microscopy Observations of the Peptide Nanotube Consisting of Homo-L-Amino Acid Pentapeptide Nanorings

5.1 Introduction

In Chapter 3, we discussed the electronic and molecular structures of D,L-peptide nanorings and nanotubes based on \textit{ab initio} calculations. Those calculations revealed that the D,L-peptide nanorings having an even number \((n)\) of amino acids stacked to form a straight nanotube while maintaining \(S_n\) symmetry. Following to the theoretical prediction, in Chapter 4, the synthesis of the hexapeptide \((n = 6)\) and octapeptide \((n = 8)\) nanotubes was performed and the straight nature of those D,L-peptide nanotubes was confirmed by atomic force microscopy.

Although the nanoring and nanotube structures based on the D,L-peptide (heteropolypeptide; \textit{hetePP}) are common ones, we found in Chapter 2 that even the homo-L-amino acid sequence (homo-polypeptide; \textit{homoPP}) could form the closed ring with the pitch number \(n = 5\). This pentapeptide nanoring is fascinating because of the small internal diameter, the “natural” all L-amino acid sequence, and the odd number of residues. Therefore, if the novel pentapeptide nanotube can be produced by the stacking of the homo-L-peptide nanoring, new kinds of properties have a chance to be produced.

In this chapter, we first review the conformation analysis of the periodic homo-
polypeptide \((\text{homoPP})\) briefly and focus our attention on the backbone structure of the pentapeptide nanoring. Next, the stable molecular forms and the energetic stability of the pentapeptide nanorings and nanotubes are discussed based on \textit{ab initio} Hartree-Fock calculations. Following the theoretical predictions, the synthesis and the AFM observations of the homo-\textit{L}-peptide nanotube of \textit{cyclo}[-(Gln\textsubscript{5})] are reported and the morphology is discussed based on \textit{ab initio} calculated predictions.

5.2 Conformation analysis

First, let us briefly review the mathematical analysis of the \textit{homoPP}. The possible backbone conformation of the \textit{homoPP} can be systematically determined by the mathematical linear transformation between the internal and external coordinates. As mentioned in section 2.5 in Chapter 2, the helical pitch angle \(\theta (\theta = 2\pi/n)\) in the periodic \textit{homoPP} is given as a function of the internal parameters of bond lengths \(r_i\), bond angles \(\alpha_i (i = 1, 2, 3)\) and internal rotation angles \(\phi, \psi, \omega\) as

\[
\cos \theta^{\text{homoPP}} = \frac{1}{2}(a_{11}^{\text{homoPP}} + a_{22}^{\text{homoPP}} + a_{33}^{\text{homoPP}} - 1),
\]

where symbols \(a_{11}, a_{22},\) and \(a_{33}\) are the diagonal elements of the orthogonal transfer matrix of the coordinates \((A)\) and are given as,

\[
a_{11}^{\text{homoPP}} = \cos \alpha_3 (\sin \alpha_1 \sin \alpha_2 \cos \psi - \cos \alpha_1 \cos \alpha_2) \\
+ \sin \alpha_3 \cos \omega (\cos \alpha_1 \sin \alpha_2 + \sin \alpha_1 \cos \alpha_2 \cos \psi) \\
+ \sin \alpha_1 \sin \alpha_3 \sin \psi \sin \omega,
\]

\[
a_{22}^{\text{homoPP}} = \sin \alpha_3 (\sin \alpha_1 \cos \alpha_2 \cos \phi) \\
+ \sin \alpha_2 \sin \phi \sin \psi + \cos \alpha_1 \sin \alpha_2 \cos \phi \cos \psi) \\
+ \cos \alpha_3 \sin \omega (\sin \phi \cos \psi - \cos \alpha_1 \cos \phi \sin \psi) \\
+ \cos \alpha_3 \cos \omega (\sin \alpha_1 \sin \alpha_2 \cos \phi - \cos \alpha_2 \sin \phi \sin \psi \\
- \cos \alpha_1 \cos \alpha_2 \cos \phi \cos \psi),
\]

\[
a_{33}^{\text{homoPP}} = \cos \omega (\cos \phi \cos \psi + \cos \alpha_1 \sin \phi \sin \psi) \\
- \sin \omega (\cos \alpha_1 \cos \alpha_2 \sin \phi \cos \psi) \\
- \sin \alpha_1 \sin \alpha_2 \sin \phi - \cos \alpha_2 \cos \phi \sin \psi).
\]

Here, by quoting the following experimental values, \(r_1 = 1.52\text{Å}, r_2 = 1.33\text{Å}, r_3 = 1.45\text{Å},\) and \(\alpha_1 = 111^\circ, \alpha_2 = 116^\circ, \alpha_3 = 122^\circ[12],\) and also by postulating the flat amide
Figure 5.1: Definition of the internal parameters of a polypeptide chain (a) and the calculated possible internal rotation angles $\phi$ and $\psi$ in the homoPP having the helical pitch number $n$ (solid lines) (b). Internal rotation angles $\phi$ and $\psi$ correspond to those rotations about the N-C$^\alpha$ and C$^\alpha$-C bonds, respectively. For the calculation of $\phi$ and $\psi$, we assume the internal parameters of the polypeptide backbone to be $r_1 = 1.52$ Å, $r_2 = 1.33$ Å, $r_3 = 1.45$ Å, and $\alpha_1 = 111^\circ$, $\alpha_2 = 116^\circ$, $\alpha_3 = 122^\circ$, and $\omega = 180^\circ$, and give the corresponding helical pitch number $n$ on the solid lines.
Figure 5.2: Top view and side view of the mathematically predicted pentapeptide nanoring. This pentapeptide nanoring form has the $C_5$ symmetry.

plane of $\omega = 180^\circ$ [Figure 5.1 (a)], we can find the possible $\theta$ (or $n$) as a function of the remaining two rotation angles $\phi$ and $\psi$.

In Figure 5.1 (b), the calculated equi-$n$ lines ($n = 2\pi/\theta$) in the homoPP are plotted by varying angles $\phi$ and $\psi$. If $n$ amino acid residues are included per helical turn, the available rotation angles $\phi$ and $\psi$ are limited by those along the corresponding solid line having the pitch number $n$. Figure 5.1 (b) reveals that the inherent internal parameter of the present homoPP limits the largest pitch number to 5.7.

The helical direction in the homoPP changes in accordance with the internal rotation angles $\phi$ and $\psi$. The right-handed turn is formed in regions I and III in Figure 5.1 (b), while the left-handed turn forms in regions II and IV. The broken lines dividing the regions cause the critical “helix” conformations having a zero-helical-transfer (per turn)[13]. Our mathematical treatment thus predicts that zero-transfer helices certainly occur even in the homoPP. Figure 5.1 (b) also reveals that the available number $n$ in these zero-transfer helices is between 4.8 and 5.7. A fractional number is, however, meaningless and only an integer pitch is required to close the zero-transfer helix thus limiting $n$ to 5. This means that an unusual cyclic but completely closed pentapeptide nanoring can be mathematically formed by the homoPP (Figure 5.2). This result is highly unexpected and is opposed to the commonly accepted result that the homoPP does form an “open helix” while the D,L-peptide produces a “closed ring”.

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5.3 Ab initio calculations

5.3.1 Pentapeptide nanoring

Next, the energetically stable molecular form of the pentapeptide nanoring skeleton \((\text{cyclo[-(Gly)₅]})\) was investigated based on Restricted Hartree-Fock calculations with the 6-31G** basis set[14]. Although the mathematically predicted nanoring (type 1 in Figure 5.2) does not have a significant steric hindrance deceptively, its inherent repulsion between adjacent amide planes modifies the molecular structure. A geometry optimization with the \(C_5\) symmetry constraint relaxes the repulsion with \(A\) mode deformation by increasing the \(\angle \text{NC}^\alpha \text{C}\) bond angle by 2.9 %. As a result, the geometry is deformed from type 1 to type 2 with the energy reduction by 61.2 kcal/mol per ring (Figure 5.3).

The normal vibrational analysis, however, indicates that the nanoring of type 2 is not a local minimum structure but a form with high order saddle point. The form of type 2 has imaginary frequencies at the degenerate irreducible representations \(E_1\) and \(E_2\) as in Figure 5.3. Lifting the \(C_5\) symmetry constraints causes the degenerate mode deformations and uniquely gives a local minimum (LM) structure of type 3, in which three of the five amide planes have the same direction while the other two turn away. By this deformation, the energy is further decreased by 29.1 kcal/mol. An interesting result is that these two amide planes can easily rotate and the other local minimum (LM) structure of type 4 is formed. The energy barrier from type 3 to the transition state (TS) of type 5 is 2.35 kcal/mol, while that from type 4 to type 5 is as small as 0.87 kcal/mol (Figure 5.3).

5.3.2 Pentapeptide nanotube

\(Ab\ initio\) calculations also reveal that the amide groups being perpendicular to the ring plane cause ring stacking through inter-ring hydrogen bonds. As a result, a pentapeptide nanotube is formed with a small hollow core of 4.5 Å (an outside diameter is 7.6 Å) (Figure 5.4). The present calculations estimate the average inter-ring distance and the ring’s condensation energy to be 5.1 Å and 37.6 kcal/mol, respectively[15]. Here, we also found the interesting result that the optimized pentapeptide nanotube has a characteristically meandering form in contrast to the straight rod shape of the \(D,L\)-peptide nanotubes. This meandering nature is caused by the aforementioned symmetry breaking of the pentapeptide nanoring: The optimized nanoring has five amide groups orienting in the sequence of up-down-down-up-down (Figure 5.4), compared
Figure 5.3: Energe diagram of $\text{cyclo}[-(\text{Gly})_5]$ nanorings. A geometry optimization with the $C_5$ symmetry constraint relaxes the repulsion with A mode deformation and reduces the geometry from type 1 to type 2. The nanoring of type 2 is not a local minimum structure but a form with high order saddle point. The normal vibrational analysis reveals that the pentapeptide nanoring has two local minimum (LM) structures of type 3 and type 4. The deformation from type 3 to type 4 is caused by the rotation of two amide planes. Normal modes of the rotation are shown as vectors in the ring model of the transition state (TS) of type 5.
with the mathematically predicted form having the sequence of all up (Figure 5.2). Therefore, the component nanoring has a $C_1$ symmetry instead of a $C_5$ symmetry. Because the core axis is not distinctly determined for the individual nanorings, those rings stack not straight but tortuously.

Here, one should be noted that the value of the curvature radius might be changed in accordance with a kind of component amino acids and/or a number of the stacking nanorings: An average curvature radius of 14.8 nm was obtained for the backbone model (homo-Gly) of the five-ring stacking nanotube. But, an important point is that the meandering nature is a general aspect inhered in the backbone structure of this all l-amino acid nanotube.

### 5.4 Synthesis and mass spectrometry

Inspired by the above theoretical proposition, pentapeptide nanotubes were synthesized by a solid-phase method using Fmoc chemistry\[16\]. Here, we choose all l-Gln sequence ($\text{cyclo}[-(\text{l-Gln})_5]$) because it is easy to cyclize the peptide on a resin as in the following experimental outline. First, the N-Fmoc-protected l-Glu $\alpha$-allyl ester is attached to a Rink amide resin via a dehydrated carboxyl group on the l-Glu side chain with HBTU/HOBt/DIEA\[17\]. The remaining peptide synthesis is performed with four further alternating cycles of Fmoc deprotection with PPD\[17\] and connection of l-Gln with HBTU/HOBt/DIEA to afford the linear precursors of the pentapeptide sequences. Pd(PPh$_3$)$_4$\[17\] and PPD are then used to remove both terminal protections. The peptide cyclization is accomplished by the activation of the deprotected support-bound peptide with HATU/HOAt/DIEA\[17\], and then TFA/TIS/H$_2$O\[17\] is used for cleavage of the cyclic peptide from the resin support. The synthesized peptides are purified by reversed-phase high performance liquid chromatography, and then identified by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) (Figure 5.5). The resulting value of 663.8 $m/z$ precisely agrees with the expected one of 663.7 $m/z$ ([M+Na]$^+$, M=$\text{cyclo}[-(\text{l-Gln})_5]$).

### 5.5 AFM observations

The morphology of the synthesized peptides was observed with the tapping mode AFM. The AFM sample was prepared as follows: The synthesized peptides were dissolved in EtOH at a concentration of 0.1 mM. Next, a mica substrate was immersed in the EtOH solution. After evaporating all of the solution at room temperature, the AFM
Figure 5.4: Optimized molecular forms of \textit{cyclo\{-(Gly)\}_5\} nanotube. The nanorings stack themselves to form a meandering nanotube. The calculated average inter-ring distance and the curvature radius are also shown.
Figure 5.5: Mass spectra of the synthesized cyclo[(-L-Gln)$_5$] measured by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOFMS). The measured mass of the ionized cyclic peptide ([M+Na]$^+$) well corresponds to the calculated value of the individual peptide.

Observations were carried out under ambient conditions.

The resulting AFM images show many meandering tubular structures on the substrate[18]. The lengths of these constructs vary from nano-order [about 700 nm; Figure 5.6 (a)] to micro-order [1~2µm; Figure 5.6 (b)] in the same sample. Their measured heights of 0.8~1.8 nm are considered to be of the effective external diameter of the synthesized cyclo[(-L-Gln)$_5$], taking into account the bulk of the Gln side chains connected to the ring backbone (cyclo[(-Gly)$_5$]) of 7.6 Å in outside diameter (Figure 5.4).

The observed width of 10~15 nm in the nano-order tube [Figure 5.6 (a)] is also understood to be of the apparent diameter of a single nanotube. The reason is that the present AFM tip has a finite size of 20~30 nm in diameter, so that it overestimates the value of the width as 10~15 nm if the diameter of the construct is about 1 nm. Therefore, it is considered that the nano-order construct in Figure 5.6 (a) corresponds to the single pentapeptide nanotube lying on the substrate. In Figure 5.6 (b), on the other hand, we can find both the thinner tubes with a 10~15 nm width and the thicker ones of about 50 nm. These results lead us to the conclusion that some of the synthesized nanotubes are not isolated but twining themselves. This characteristic assembled form might also be caused by the lower (C$_1$) symmetry of the individual nanorings.

The resulting images of the cyclo[(-L-Gln)$_5$] nanotube also show an interesting fact that the observed curvature radius is not uniform but varies in the range of 200 nm~2 µm [Figure 5.6 (b)], i.e., the synthesized nanotube has a flexible meandering form.
Figure 5.6: AFM images of the homo-L-peptide nanotubes and d,L-peptide nanotubes. Figures (a) and (b) show the meandering tubular form of cyclo-[L-Gln]₅ nanotube on mica. We observed both nano-order tube (a) and the micro-order tubes (b) in the same sample.

This meandering tubular form is very contrastive to the rigid straight form observed for the d,L-peptide nanotube (Figure 4.10 (b) in Chapter 4). Although the value of the observed curvature radius does not coincide with the calculated value completely, we can qualitatively understand the meandering nature based on ab initio prediction mentioned in the former section. The observed flexible form also suggests that the component nanorings do not have the same structure but some variant ring forms in the pentapeptide nanotube. This feature is supported based on ab initio calculations which indicate the existence of two local minimum ring forms and also a transition form (Figure 5.3).

## 5.6 Summary

The existence of unusual pentapeptide nanotubes was demonstrated by both theoretical and experimental approaches and the following results were obtained.

- Although the homo-L-amino acid sequence basically forms right-handed or left-handed helices, the homo-L-peptide has a chance to produce a “closed ring” when the pitch (residue) number is five.
- A geometry optimization and the normal vibrational analysis indicates that the
pentapeptide nanoring stabilizes by breaking the C$_5$ symmetry.

- The stable pentapeptide nanorings stack themselves to form a meandering nanotube through the inter-ring hydrogen bonds.

- The cyclo[-(L-Gln)$_5$] nanotube was synthesized by a solid-phase method using Fmoc chemistry. The resulting mass spectrum of 663.8 $m/z$ precisely agrees with the expected value of 663.7 $m/z$ ([M+Na]$^+$, M=cyclo[-(L-Gln)$_5$]).

- The morphology of the synthesized nanotube was investigated with the tapping mode AFM and its meandering tubular form was observed. The lengths of the nanotube are from nano-order (about 700 nm) to micro-order (1-2 $\mu$m).

Several characteristic features of the homo-L-pentapeptide nanotube, such as the small diameter, the natural all L-amino acid sequence, the odd number of residues, and the flexible meandering form are expected to extend the potential applications in not only biology and medicine but also in nano-electronics.
Bibliography


[8] Ghadiri et al. succeeded in synthesizing cyclo[-β3-HLeu]4-. However, it consisted of β-amino acids and provides not a strict square but an octagon[9].


[13] The angles φ and ψ along the boundary between regions II and III give the β-strand structure of n = 2, instead of the closed ring structure.

The geometry optimization of the nanotube is carried out by stacking five nanorings. We pick up the center ring of the optimized nanotube and estimate the condensation energy. In this calculation, we use the 3-21G basis set because of the large number of component atoms.


Abbreviations of chemical substances denote the following reagents; Fmoc = 9-fluorenylmethoxycarbonyl, Pd(Ph₃)₄ = tetrakis (triphenylphosphine) palladium, PPD = piperidine, HBTU = 2-(1H-benzotriazole-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole hydrate, DIEA = N,N-diisopropylethylamine, HATU = O-(7-azabenzotriazole-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxy-7-azabenzotriazole, TFA = trifluoroacetic acid, and TIS = triisopropylsilane.

We carried out the blank test using neat EtOH without synthesized peptides. The resulting AFM image showed no objects on the mica substrate, and only the flat surface of mica was observed.
Chapter 6

Summary

6.1 Conclusions

The aim of the present thesis was to provide the guiding principle of molecular modeling of peptide nanorings (PNRs) and peptide nanotubes (PNTs). For this purpose, the possible molecular conformations of PNRs and PNTs were investigated and novel backbone structures were explored by a mathematical conformation analysis. Various energetically stable backbone forms of the PNRs and PNTs were also investigated and their electronic structures were discussed based on \textit{ab initio} molecular orbital calculations. The effect of the amino acid substitution was studied and the electronic characteristics of the individual side chains were systematically understood.

Not only the theoretical studies based on the mathematical analysis and the computational calculations, but also the experimental synthesis and the atomic force microscopy were carried out for the D,L-peptide nanotubes. The PNTs consisting six and eight amino acid residues were synthesized and their self-assembling morphologies were compared in terms of the difference in the number of component amino acid residues.

In addition to the conventional D,L-peptide nanotubes, an unusual peptide nanotube made up of all L-amino acid residues was newly synthesized. Following the theoretical prediction and synthesis, an atomic force microscopy study of the homo-L-pentapeptide nanotube was carried out and the self-assembling morphology was investigated. The results of these studies are summarized below.

Conformation analysis of regular periodic polymers

The mathematical conformation analysis indicates that the alternate sequence of D- and L-amino acid residues (D,L-peptide) basically forms a peptide nanoring having an even number of residues. Moreover, even with the same number of residues, two types
of nanoring backbones can be formed, i.e., the larger Extended-type (E-type) and the smaller Bound-type (B-type). While the former is the conventional backbone type, the latter B-type backbone is a novel ring conformation first predicted in this thesis.

On the other hand, the homo-L-amino acid sequence basically forms the right-handed or left-handed helix. However, the conformation analysis indicates that the homo-L-peptide can form an unexpected peptide nanoring at the boundary between the right-handed and the left-handed helices. Interestingly, the number of residues is limited to five in order to close the peptide backbone of the homo-L-amino acid sequence. This result suggests the possibility of an all-new peptide nanotube (nanoring) having an odd number of residues.

**Ab initio** studies of the electronic and molecular structures of D,L-peptide nanorings and nanotubes

*Ab initio* calculations reveal that the energetically preferable backbone type of the D,L-peptide nanorings changes in accordance with the ring size, i.e., the smaller rings \((n \leq 8)\) prefer the B-type backbone, while the larger rings \((n \geq 10)\) prefer the E-type backbone. The energy calculations for the amino acid substitution reveal that all 20 encoded residues can form both E-type and B-type peptide nanorings as local minimum structures, while either type is provided as the energetically stabler form in accordance with the kind of replaced side chains.

Electronically, both the highest occupied molecular orbital and the lowest unoccupied molecular orbital of the nanoring backbones are formed by the in-plane \(\pi\) states. The replacement by the appropriate residues provides additional energy levels in the energy gap and forms the frontier orbitals localized at the replaced side chains.

The energy calculations for the self-assembling peptide nanotubes indicate that both the E-type and B-type nanorings can stack to form the peptide nanotubes. Because the resulting total energies become comparable, both the larger E-type nanotube and the smaller B-type nanotube are theoretically predicted. The present *ab initio* calculations also indicate that the parallel stacking of the peptide nanorings provides a monotonous stacking form, while the antiparallel stacking causes a twisted nanotube.

**Syntheses and atomic force microscopy observations of the D,L-peptide nanotubes**

The *cyclo-*\((\text{D-Ala-L-Gln})_3\)* and *cyclo-*\((\text{D-Ala-L-Gln})_4\)* nanotubes were synthesized by the solid-phase method and identified by mass spectrometry. The morphology of the synthesized peptides was investigated by atomic force microscopy, and the single peptide
nanotubes was first reported in this thesis. The observed D,L-peptide nanotubes have a straight form and this characteristic is well understood based on \textit{ab initio} calculations.

The microscopic study revealed that the synthesized peptides form not only single nanotubes but also self-assembling bundles. The observed bundle forms are quite different between the hexapeptide and octapeptide nanotubes. While the hexapeptide nanotubes show thinner nano-bundles and also micro-bundles, the octapeptide nanotubes produce larger aggregated-bundles, which are formed by the assembly of several micro-bundles. \textit{Ab initio} calculations indicate that the formation of these bundles is mainly caused by the inter-tube Gln-Gln hydrogen bonding and the different assembled forms are derived from the difference in the space filling manner between the hexapeptide and octapeptide nanotubes.

**Theoretical prediction and atomic force microscopy observations of the peptide nanotube consisting of homo-L-amino acid pentapeptide nanorings**

In this thesis, the first synthesis of an unusual pentapeptide nanotube was also reported. This new peptide nanotube consists of five L-amino acid residues in the component nanoring ($cyclo[-(\text{L-Gln})_5]$) being different from the already-known D,L-peptide nanotubes having an even number of residues. The morphology of the synthesized nanotube was investigated by atomic force microscopy and the meandering tubular structures were observed on the substrate. This meandering characteristic is in stark contrast to the straight nature of the D,L-peptide nanotubes. This result is consistent with \textit{ab initio} calculations which show that the homo-L-pentapeptide nanorings stabilize by breaking the C$_5$ symmetry and therefore stack themselves to form a meandering nanotube through the inter-ring hydrogen bonds.

### 6.2 Potential applications and perspectives

Because of the designing flexibility, the potential applications of the peptide nanorings and nanotubes seem to be vast. Some examples are introduced below.

**6.2.1 Ion receptors**

The isolated peptide nanorings are expected to be exciting novel amphi-ionophores which show strong affinities for both cations and anions. Kim et al. have theoretically investigated the interactions of D,L-peptide nanorings with cations (Li$^+$ and Na$^+$) and anions (F$^-$ and Cl$^-$). They revealed that, in the presence of cations, the C=O group
tends to fold inward to capture a cation, whereas in the presence of anion, the N-H group tends to fold inward to capture an anion[1]-[3]. Nakanishi et al. have also investigated the host-guest complexation of the hexapeptide nanorings based on \textit{ab initio} calculations and reported the similar binding structures for cations and anions (Figure 6.1)[4]. To apply the peptide nanorings as ion receptors, more experimental studies should be carried out.

### 6.2.2 Drug delivery systems

The peptide nanotubes (PNTs) may be applied as drug delivery systems. Using the hollow tubular structure as a nano-capsule, molecules or drugs have a potential to be transported to the target [Figure 6.2 (a)]. The Ghadiri group reported that not only ions but also an amino acid and glucose can enter the hollow core of the PNT[5, 6]. Future tasks are how to build up a process of delivery, e.g., inclusion of drugs, attachment to the target, and release of the drugs [Figure 6.2 (a)].

### 6.2.3 Antibacterial agents

Recently, Ghadiri’s group proposed an interesting application of the PNTs as selective antibacterial agents[7]. His group found that some PNTs are preferentially active against bacterial cells compared with mammalian cells; increase membrane permeability, collapse transmembrane ion potentials, and cause rapid cell death [Figure 6.2 (b)].
Figure 6.2: Illustration of the drug delivery system (a) and the antibacterial agent (b) based on the peptide nanotube.
The effectiveness of the PNTs as selective antibacterial agents is highlighted by the high efficacy observed against lethal methicillin-resistant *Staphylococcus aureus* infections in mice. Their experimental result has made a strong impact on recent medicine and biotechnology.

### 6.2.4 Molecular devices

Not only for biological and medical uses, but also the potential applications as molecular devices are the focus of interest.

#### Photo-switching

Ghadiri’s group reported the potential application of the D,L-peptide nanorings (nanotubes) as a photoswitchable system[8]. They have examined a covalent dimeric system in which two peptide nanorings are connected through an azobenzene [Figure 6.3 (a)]. The peptides were further modified by the N-methylation of alternate amides, restricting the hydrogen bonding capability to one face of each peptide nanoring. The study revealed that only the *cis* conformation of the azobenzene would permit intra-tubular hydrogen bonding between the two linked peptides, whereas the *trans* conformation would form the inter-tubular bridging as in Figure 6.3 (a). The *trans* → *cis* and *cis* → *trans* isomerizations were induced by irradiation at 366 nm (ultraviolet) and visible light, respectively.

#### Molecular switching

The conformation change between two types of nanotube (nanoring) backbones may also be utilized for molecular switching [Figure 6.3 (c)]. In this thesis, both the larger Extended-type (E-type) backbone (Figure 3.21) and the smaller Bound-type (B-type) backbone (Figure 3.23) were theoretically predicted. If the two conformations are switchable, the peptide nanotubes can be used as molecular devices.

#### Molecular wiring

The peptide nanotubes will also be used as molecular wiring if a metal wire is inserted into the hollow core [Figure 6.3 (c)]. The theoretical calculations provided by Jishi et al. indicated that the octapeptide nanotubes behave as insulators with wide band gaps and the metal atoms inserted into the hollow core hardly interact with the tube wall[9]. This result suggests that the conductive metal wire in the hollow core be coated by the insulating integument of the nanotube skeleton.
Figure 6.3: Illustration of several potential applications using peptide nanotubes. The photo-switching, the molecular switching, and the molecular wiring using the peptide nanotubes are illustrated by figures (a), (b), and (c).
6.2.5 Peptide nanotubes made up of amino acid derivatives

The peptide nanotubes focused on in this thesis are made up of all \( \alpha \)-amino acids, which are commonly found in organisms [Figure 6.4 (a)]. Although the \( \alpha \)-amino acid includes only one carbon atom between the backbone’s N-H and C=O groups, the amino acid derivatives, which include two or more carbon atoms between these groups, can also be artificially synthesized, e.g., \( \beta \)- and \( \gamma \)-amino acids [Figures 6.4 (b) and (c)]. Based on these amino acid derivatives, several new peptide nanotubes have been reported.

\( \beta \)-amino acid

Seebach and coworkers reported that the sequence of four \( \beta \)-substituted \( \beta \)-amino acids (cf. \( R_1^1=H \), \( R_2^{\neq}=H \) in Figure 6.4 (b)) could form the peptide nanoring and also the self-assembling peptide nanotube based on X-ray diffraction data[10]. Ghadiri group has successively reported that the \( \beta \)-tetrapeptide nanotube indicated ion channel activities similar to those of cyclic \( \text{D,L-} \alpha \)-peptides, with the \( K^+ \) transport rates of \( 1.9 \times 10^7 \) ions per second. Because the catenation of \( \beta \)-amino acids induces a uniform alignment of amide groups in the component nanorings, the \( \beta \)-amino acid nanotubes will have a macrodipole moment toward the tube axis. This dipole is expected to exert interesting effects on the channel conductance, the interaction with electric fields, and so on.

\( \gamma \)-amino acid

The peptide nanotube including \( \gamma \)-amino acids [Figure 6.4 (c)] has also been recently reported by Granja et al.[12] They chose the alternate sequence of the \( \text{d-} \alpha \)-amino acid and \( \gamma \)-Acc \([(1R,3S)-3\text{-aminocyclohexanecarboxylic acid}] \) and succeeded in obtaining not only the peptide nanotube but also the peptide nanoring dimers by N-methylation. In contrast to a hydrophilic pore in the \( \alpha \)- or \( \beta \)-peptide nanorings, the peptide nanoring containing \( \gamma \)-Acc has a hydrophobic central cavity due to the three carbon atoms in

![Chemical structures of \( \alpha \), \( \beta \), and \( \gamma \)-amino acids (a)-(c).](image-url)
Figure 6.5: Illustration of the disulfide bridging peptide nanotube (a) and nano-sheet (b).

The γ-amino acid backbone. Therefore, the α-γ-peptide nanoring dimer (or nanotube) has an ability to occupy a chloroform molecule in the hydrophobic cavity[12].

**The other amino acid derivatives**

The peptide nanotubes made up of δ-amino acid, ε-amino acid, etc. also have a chance to be produced[13, 14]. Based on the mathematical conformation analysis discussed in this thesis, we will be able to explore the possible molecular conformations of those various peptide nanotubes built up of the amino acid derivatives.

**6.2.6 Inter-ring bridging through disulfide bonding**

The inter-ring bridging through sulfur atoms (disulfide bond) may change the dimension of the assembling form. Based on *ab initio* calculations, Kasahara et al. proposed that not only the one-dimensional peptide nanotube [Figure 6.5 (a)] but also the two-dimensional nano-sheet may be formed by the inter-ring disulfide bridging [Figure 6.5 (b)][15]. Using this type of inter-ring bridging, the self-assembled monolayer of the peptide nanorings has a chance to be fabricated.

As introduced above, peptide nanorings and nanotubes have a wide range of potential applications. By modifying the backbone and side chains of the nanoring (nanotube), many useful and functional molecules are expected to be produced.
Bibliography


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