Spontaneous Assembly Formation of Cyclic Dimer of β-Amino Acid in Water

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(Received March 30, 2004; CL-040341)

A novel cyclic dimer composed of chiral L-β-homoalanine was synthesized, and the formation of molecular assembly in water was investigated. The cyclic dimer was found to spontaneously form a spherical assembly with diameter of 40–100 nm in water. The cyclic dimer takes a boat conformation with *cis*-amide configuration, and forms intermolecular hydrogen bondings in the assembly. The assembling property of the cyclic dimer of β-amino acid is distinct from that of α-amino acid.

A molecular assembly composed of cyclic peptides[1] is an interesting subject in view of supramolecular chemistry. There are several advantages in using cyclic peptides as components for molecular assemblies: i) cyclic peptide has a regular conformation, ii) hydrogen bondings, hydrophobic interaction and π–π interaction, which are driving forces for self assembling, can be integrated in the molecule under suitable molecular designing, iii) cyclic peptide has several variables such as ring size, chirality, and constituent amino acids which should characterize morphology of the molecular assembly. For example, a cyclic octapeptide with alternating sequence of D- and L-α-amino acids has been reported to form a tubular assembly in CH_3CN by Ghadiri and co-workers.[1] The molecular assembly functions as an ion or a biomolecule channel in a bilayer membrane.[2,3]

Recently, β-amino acid has attracted much attention as a new building block of polypeptides with a specific conformation.[4] When cyclic peptides are prepared from β-amino acids, the orientation of amide bonds against the cyclic skeleton may be arranged in a quite different manner from that in cyclic peptides from α-amino acids. Novel molecular assemblies therefore may be prepared from cyclic peptides of β-amino acid, those cannot be prepared by the conventional cyclic peptides of α-amino acid. In the present study, several kinds of cyclic dimers composed of α-amino acid or β-amino acid were synthesized and compared in terms of the formation of molecular assembly in water. A distinct difference of a cyclic dimer of β-amino acid from cyclic dimers of α-amino acid is clarified.

Figure 1 shows molecular structures of cyclic dimers of α-amino acid, *cyclo*(L-Val)$_2$, *cyclo*(L-Leu)$_2$, and *cyclo*(L-Phe)$_2$, and cyclic dimer of L-β-homoalanine, *cyclo*(HA)$_2$. We chose the α-amino acids with a bulky side chain to make the structure being distorted from a planar structure, which may render the cyclic peptide dispersible in water due to a difficulty in intermolecular stacking. The cyclic dimers of α-amino acids were synthesized according to the literature.[5] *cyclo*(HA)$_2$ was synthesized by the conventional liquid-phase synthesis and the cyclization reaction was carried out by the method of active ester using *N*-hydroxysuccimide, and confirmed by ^{1}H NMR spectroscopy and mass spectroscopy.[6]

The aqueous suspensions of the peptides were treated by a probe-type sonicator to try to obtain aqueous dispersion. However, all the three kinds of cyclic dimers composed of α-amino acid could not be dispersed in water. This should be due to the strong intermolecular hydrogen bondings in a solid state. The tight molecular packing of cyclic dimers of α-amino acids probably arises from a planar conformation, which enables formation of 2-D network of hydrogen bondings in a solid state.[7] In addition to hydrogen bondings, hydrophobic interaction among stacked cyclic skeletons in a solid state makes the cyclic dimers being dispersed difficult in water. On the other hand, cyclo(HA)$_2$ was successfully dispersed in water without yielding any precipitate. To gain further information, the dispersion of cyclo(HA)$_2$ was subjected to dynamic light scattering measurement at room temperature. The measurement revealed that cyclo(HA)$_2$ forms a molecular assembly in water with hydrodynamic diameter of ca. 150 nm. The hydrodynamic diameter scarcely changed even after a few days, suggesting that this assembly is stable.

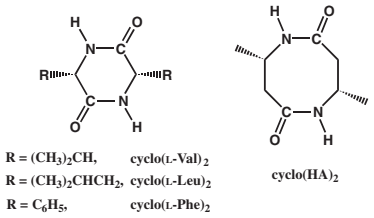

Figure 1. Molecular structures of cyclic dimers composed of α- or β-amino acid.

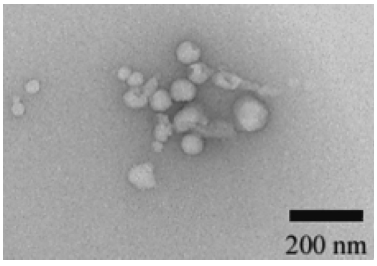

Figure 2. TEM image of the aqueous dispersion of *cyclo*(HA)$_2$ with negative staining by uranyl acetate.

Morphology of the assembly was investigated by transmission electron microscopy (TEM), and the TEM image is shown in Figure 2. Spherical assembly with diameter of 40–100 nm was observed, which is smaller than the hydrodynamic diameter. There is no evidence for vesicle formation from *cyclo*(HA)$_2$ in the TEM image. Further, the assembly did not encapsulate a hydrophilic fluorescent probe, 5(6)-carboxyfluorescein. Taken together, the spherical assembly should adopt a stuffed structure.

The main driving force to form the molecular assembly of

cyclo$(HA)_2$ should be intermolecular hydrogen bondings. To figure out this point, configuration of amide groups and formation of hydrogen bondings were investigated by Fourier transform infrared (FT-IR) spectroscopy. Figure 3A shows the FT-IR spectrum of *cyclo*$(HA)_2$ in a solid state. Amide I absorption (mainly C=O stretching mode) was observed at around 1620 cm^{-1}, whereas amide II absorption (mainly N–H bending mode) was not observed, indicating that the two amide groups in *cyclo* $(HA)_2$ are *cis*-form. The amide groups retain the *cis*-form in methanol as well. Absorption of N–H stretching was observed at lower wave number (ca. 3250 cm^{-1}), which supports formation of intermolecular hydrogen bondings.

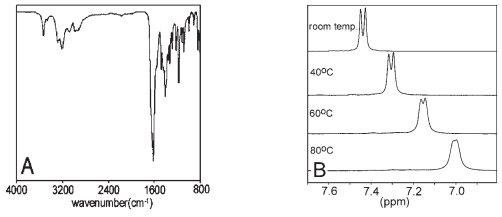

Figure 3. FT-IR spectrum of *cyclo*$(HA)_2$ in a solid state(A), and ^1H NMR spectra in the amide proton region of *cyclo*$(HA)_2$ in DMSO-d_6 at various temperatures (B).

^1H NMR spectroscopy of *cyclo*$(HA)_2$ in DMSO-d_6 was performed at various temperatures (from room temperature to 80 °C). As the temperature was elevated, chemical shift of amide proton shifted to lower magnetic field significantly (Figure 3B). The temperature dependence of the chemical shift was calculated to be 6.63×10^{-3} ppm/°C, indicating that two amide protons in *cyclo*$(HA)_2$ are exposed to solvent, not accommodated inside the ring.[9] Two dihedral angles in *cyclo*$(HA)_2$, $\theta_1(H\text{–}N\text{–}C^{\beta}\text{–}H^{\beta})$ and $\theta_2(H^{\beta}\text{–}C^{\beta}\text{–}C^{\alpha}\text{–}H^{\alpha})$, were evaluated on the basis of spin coupling constants (J) according to the Karplus equations.[8,9] The spin coupling constant between amide proton and H^{β} was 7.9 Hz, whereas the constant between H^{β} and H^{α} had two values, 6.5 and 10.5 Hz. According to these values, $\theta_1(H\text{–}N\text{–}C^{\beta}\text{–}H^{\beta})$ and $\theta_2(H^{\beta}\text{–}C^{\beta}\text{–}C^{\alpha}\text{–}H^{\alpha})$ were calculated to be 154°, and 36°, and 154°, respectively. Under constraint of the dihedral angles as obtained, the geometry optimization of *cyclo*$(HA)_2$ was carried out by using the MM2 method. The calculation showed that a C_2 symmetric boat conformation with *cis*-amide groups is energetically stable (Figure 4). *cyclo*$(HA)_2$ should take the similar conformation in an aqueous dispersion, because the conformation of such a small cyclic peptide is mainly determined by internal factors and less affected by external interactions such as solvation. Notably, this conformation is quite similar to that of *cyclo*(β-alanyl)$_2$ in a solid state, which has been reported to form a flexed one-dimensional molecular chain of alternating cyclic skeleton and hydrogen bonding.[10] The flexed structure is different from a planar structure, which has been reported for the crystalline structure of cyclic dimers of α-amino acid.[9] It is considered that *cyclo*$(HA)_2$ takes the similar structure in the molecular

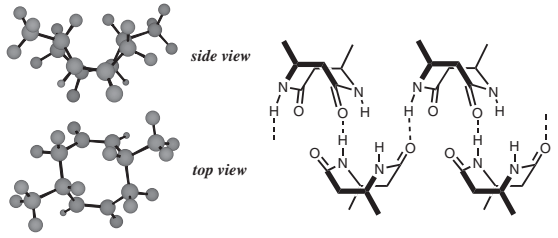

Figure 4. Geometrically optimized conformation of *cyclo*-$(HA)_2$ under constraint of NMR results (left). Tape structure formed by *cyclo*$(HA)_2$ (right).

assembly because of the conformation similar to *cyclo*(β-alanyl)$_2$ (Figure 4). Hydrophobic interaction in the assembly should become moderate between the flexed structures compared with that between the planar structures of cyclic dimers of α-amino acid. The flexed structures may be like flexible tapes to be folded and assembled into a spherical morphology in water.

In conclusion, we demonstrated that a cyclic dimer of β-amino acid, *cyclo*$(HA)_2$, forms a spherical molecular assembly in water, which could not be prepared from cyclic dimers of α-amino acid. Assembly formation of *cyclo*$(HA)_2$ in water is probably due to a boat conformation with *cis*-amide groups to form a tape-like structure. It should be noted that the addition of just two methylene groups to the cyclic skeleton of α-amino acid dimmer makes a drastic change in the assembly property in water. Further investigation to clarify the formation mechanism of the molecular assembly is now under way.

References and Notes

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Published on the web (Advance View) June 7, 2004; DOI 10.1246/cl.2004.810