Spherical Self-Assembly of a Synthetic α-Helical Peptide in Water

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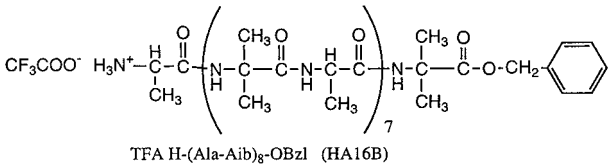
A self-assembly of a helical peptide in an aqueous medium was investigated in an attempt to compose a supramolecular structure. A peptide, HA16B, having a hydrophobic α-helical body and a hydrophilic polar head was synthesized and hydrated in water by sonication to obtain a transparent dispersion. Circular dichroism measurement showed that the peptide took an α-helical conformation in the dispersion. Transmission electron microscopy and dynamic light scattering measurement revealed that spherical assemblies were formed with an extremely narrow distribution of size (diameter in the range of 30−40 nm), which is much larger than the molecular length of the peptide. These observations strongly suggest that the peptide assembly took a liposome-like vesicular structure.

Introduction

Chemical synthesis of an artificially designed protein is one of the craving dreams for organic chemists.[1] The major difficulties in realizing the dream exist not only in the synthesis but also in the prediction of the steric structure of the protein from the amino acid sequence.[2,3] A possible way to avoid the difficulties is putting small peptides taking a specific secondary structure together into a well-ordered molecular assembly.[4−6] A helix bundle structure formed by an association of helix segments is one of the common structures found in naturally occurring proteins.[7] It provides a rigid frame for a sophisticated arrangement of functional groups in proteins. The inter-helix interaction is considered to be the major factor for formation and stability of the helix bundle. It is considered that helical peptides may form a regular structure by self-assembly such as vesicles, tubules, and bilayers. For the purpose of constructing such a regular assembly of helical peptides, the peptide should take a stable secondary structure with a defined molecular shape. α-Aminoisobutyric acid (Aib) containing peptides consisting of more than eight residues has been reported to take a stable helical conformation.[8] A crystalline structure of Boc-(Ala-Aib)$_8$-OCH_3 (BA16M) has been investigated by X-ray diffraction.[9]



TFA H-(Ala-Aib)$_8$-OBzl (HA16B)

Figure 1. Molecular structure of HA16B.

It was found that the peptide possesses a unit cell of 9.3 × 9.3 × 25.7 Å, which is in good agreement with the dimensions calculated for a complete α-helical structure. Interestingly, this peptide forms a solid monolayer with a well-ordered molecular arrangement, when deposited on the air/water interface at a high surface pressure,[10,11] indicating that BA16M has a stable conformation and can be packed in a regular array. Amphiphilicity is another requirement for peptides to construct a self-assembly in an aqueous medium. The balance between hydrophilicity and hydrophobicity in a lipid-like molecule has been pointed out as the key factor for the formation and the shape of lipid self-assemblies.[12−15]

The trifluoroacetic acid salt of an amphiphilic helical peptide, HA16B (Figure 1), was used in the present study. HA16B possesses the same helix part as the above-mentioned BA16M and forms an interfacial monolayer at the air/water interface.[10]

Material and Methods

HA16B was prepared as previously reported.[10] The peptide assembly was made up according to the preparation method of

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phospholipid liposomes[16] without any sizing procedure such as centrifugation or gel filtration. A chloroform solution of HA16B (10 mg/2 mL) was evaporated in a flask, and the resulting peptide thin film was hydrated with 3 mL of pure water with a bath-type sonicator followed by a probe-type sonicator, until the milky white dispersion became slightly turbid. The dispersion was eluted twice through polycarbonate filter with 0.4-μm pores to obtain a transparent dispersion. The dispersion was used immediately for the subsequent experiments.

Circular dichroism (CD) spectra of the HA16B dispersion were recorded at room temperature on a JASCO J-600 CD spectropolarimeter using optical cells of 0.5-cm path length. Dynamic light scattering (DLS) was measured at room temperature on Malvern 7032 multicorrelator with an optical cell of 1-cm diameter, and the data were analyzed by the system 4700C. The diffusion coefficient, D, was extracted from the measured autocorrelation function by a cumulants method.[18] The polydispersity of the size distribution is expressed to C/B^2 when the logarithm of the correlation function, $g(\tau)$, fit to a power series of the correlation time.

$$\ln\{g(\tau)\} = A + B\tau + C\tau^2 + ...$$

The average hydrodynamic radius of postulated rigid sphere was calculated from D using the Stokes−Einstein equation.

$$R = k_B T/6\pi\eta D$$

where η is the viscosity of water and T is the absolute temperature.

Micrographs of transmission electron microscopy (TEM) were taken using JEM-120 EX JEOL. Sample preparation for TEM was carried out by the following two methods. (1) Negative stain: 10 μL of the peptide dispersion (6×10^{-5} M) was put on a collodion-coated copper grid and sacked out with a piece of filter paper after 1 min. A 10 μL portion of aqueous ammonium molybdate solution (0.5 wt %) was placed on the grid after it was dried up and sacked out with a piece of filter paper, followed by immediate examination. (2) Cold-stage method (cryo-TEM): 10 μL of the aqueous dispersion of peptide at a concentration of 7×10^{-5} M was put on a holey carbon-coated grid and quickly sacked out with a piece of filter paper. The grid was dipped into liquid ethane quickly and examined immediately.

Results and Discussion

The CD spectra showed a double-minimum pattern, characteristic of α-helical conformation,[17] independent of concentration (Figure 2). It is hard to consider that the hydrophobic helical peptide is molecularly dispersed in water. Therefore, most of the α-helical peptide molecules should form a self-assembly in water. The formation of self-assembly was confirmed by DLS[18] measurement (Figure 3) showing the presence of particles with a very narrow distribution of sizes. The average diameter determined by the cumulants method was 75 nm with a polydispersity of 0.11, indicating also a narrow size distribution. Furthermore, two kinds of TEMs showed that the particles are spherical and quite uniform in size (Figure 4). The TEM[19] taken with a dried dispersion negatively stained with ammonium molybdate showed the diameter of the particles to be ca. 40 nm (Figure 4a). The cryo-TEM[21,22] taken with a frozen dispersion without staining showed the particle diameter to be ca. 30 nm (Figure 4b). The different values of particle size should be due to flattening of the particles during drying procedure in the former TEM. Though these values are significantly smaller than the average diameter determined by DLS, it might be overestimated due to a repulsive Coulombic interaction between charged particles.[23] Especially in pure water, the long Debye screening length[24] can cause such large overestimation. It is, therefore, concluded that the peptide molecules are assembled to form spheres of 30−40 nm diameter in water. In the present case, a micellar structure of the assembly should be discarded because of far larger diameter of the particle than 2.5 nm of the molecular length. The sharp size distribution suggests the formation of an assembly with a regular structure.

According to the theoretical prediction by Israelachvili[12] on the molecular shape and the self-assembling manner

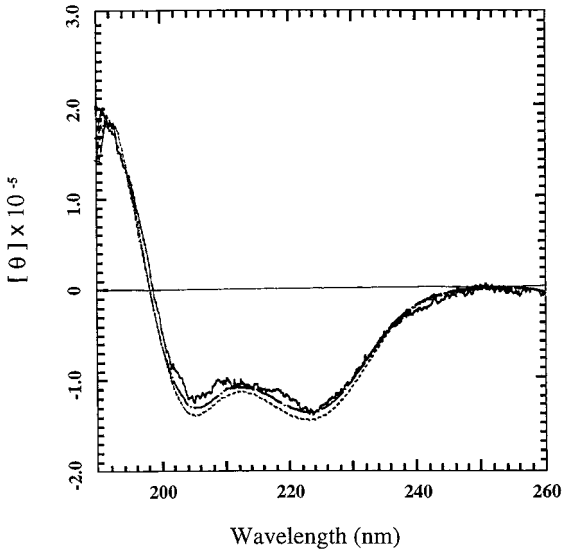

Figure 2. CD spectra of HA16B dispersion in water at the peptide concentrations of 1.9×10^{-6} M (—), 1.9×10^{-5} M (— - —) and 1.9×10^{-4} M (- - -). The peptide concentration was determined by the ninhydrin assay.

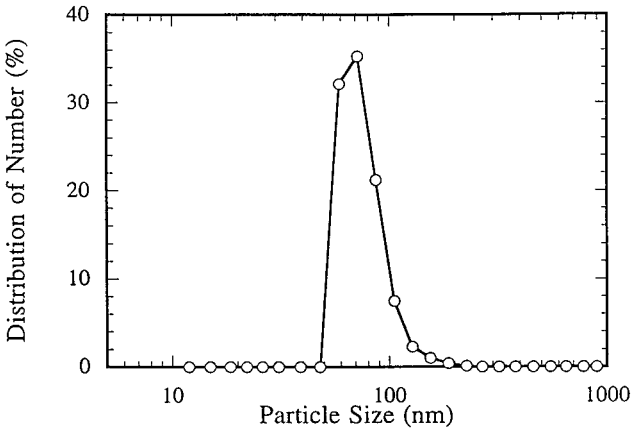

Figure 3. Particle size distribution of HA16B assembly determined by DLS at a peptide concentration of 2.0×10^{-5} M in water at room temperature. The average diameter of the particle determined by the cumulants method was 75 nm and polydispersity of 0.11.

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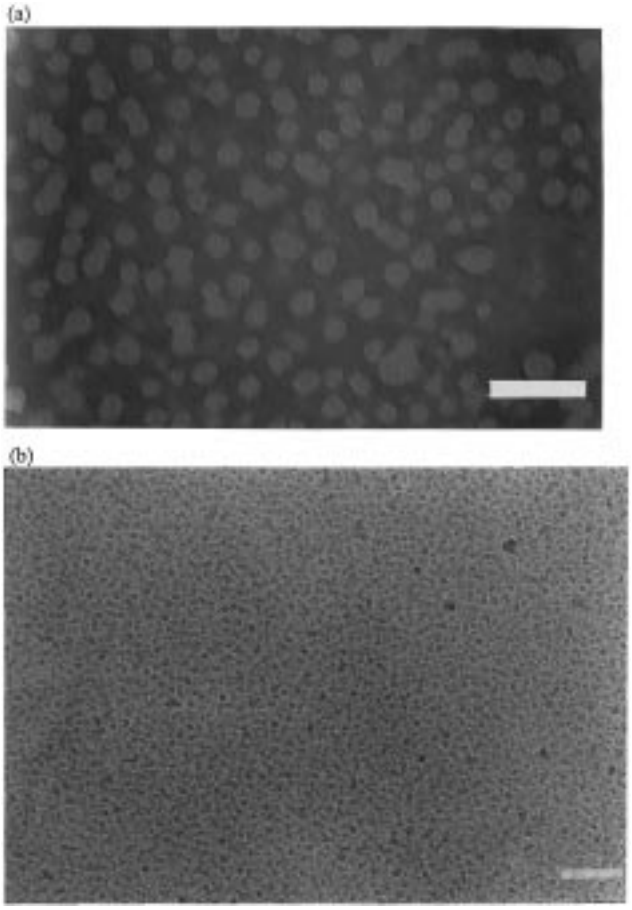

Figure 4. (a) TEM image of HA16B assembly negatively stained by ammonium molybdate. The white bar represents 200 nm length. (b) Cryo-TEM image of HA16B assembly. The white bar represents 400 nm length.

of amphiphilic molecules, the molecules forming a vesicular assembly are necessary to have a cylindrical shape with a large hydrophilic headgroup (truncated cone with the critical packing parameter, v/a_0l_c, of $^1/_2-1$). The ammonium group on the N-terminal of the peptide molecule seems to be too small to be the necessary hydrophilic headgroup for a large cross-section area of the helix, HA16B. However, at the N-terminal of the helix there are three amide hydrogens which are free from intramolecular hydrogen bond. Therefore, these amide groups should be hydrated in the aqueous medium and act as a part of hydrophilic headgroups. Since the cotton effect in CD spectra (Figure 2) was significantly smaller than that of HA16M solution in ethanol, the helical structure could become loose partially at the N-terminal. These should result in comparable cross-section size of the hydrophilic part with that of the hydrophobic helix. The theory about the critical packing parameter is adaptable for the molecular assembly with high mobility in the hydrophobic region. Some Langmuir monolayers of peptides having the helical part the same as HA16B are reported to undergo the phase transition between a liquid phase and a solid phase.[10,11] At least during the assembly formation, the peptide is considered to maintain high mobility like a liquid state.

The balance of hydrophilicity and hydrophobicity seems to be very delicate. Although the assembly was stable for more than 3 weeks in degassed pure water stored in a refrigerator, addition of small amount of electrolyte solution or water-soluble dyes to the transparent dispersion caused immediate precipitation of the peptide. The precipitation might have been caused by destruction of hydrophilic−hydrophobic balance due to the decreasing of the headgroup cross section, a_0, as a result of increasing ionic strength in the dispersion.[13] The high sensitivity to the environmental fluctuation might affect the assembly size during the freezing process. This can explain the several large particles that were observed in the cryo-TEM image (Figure 4b) despite the sharp size distribution from DLS. The TEM image shown in Figure 4a could be obtained only in the samples stained after the grid was completely dried up. The assembly that adhered and deposited on the grid might be able to endure the staining process. The primary amphiphilicity is also an important factor to form the molecular assembly. The fully protected peptides, BA16M and Boc-(Ala-Aib)$_8$-OCH$_2$C$_6$H$_5$, were not dispersed in water with the same procedure. Another peptide having a smaller C-terminal group, TFA·H-(Ala-Aib)$_8$-OCH$_3$, did not form a molecular assembly large enough to be detected by DLS even at a high peptide concentration of 1.0×10^{-4} M. This fact lets us speculate that HA16B assembly has a bilayer structure with the hydrophobic helix tail associating together at the center of the membrane, although the interior water was not confirmed with an inclusion of water-soluble dyes due to the precipitation of the peptide nor with neutron diffraction due to the particle size distribution. The stable helical structure might be another important factor that directs vesicular assembly. Once intermolecular hydrogen bonds are formed randomly among the peptide molecules, they may lead to amorphous aggregates of the peptide instead of the regular assembly. Such aggregates can be avoided due to the stable intramolecular hydrogen bonds in the helical structure. It is expected for another peptide having a stable and more hydrophobic helix part, for example TFA·H-(Leu-Aib)$_8$-OCH$_2$C$_6$H$_5$, to form an assembly sturdy enough to endure the sizing procedures and to enable the structural investigation with neutron diffraction in further study.

These observations suggest that HA16B forms a vesicular structure composed of a thin peptide membrane. We propose that this kind of the peptide assembly should be called *Peptosome* in analogy with liposome.

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