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Enhanced Circular Dichroism of Self-Assembled Peptidic Amphiphiles

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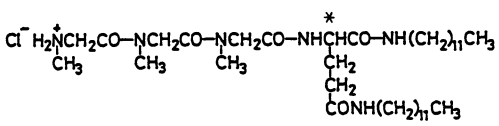
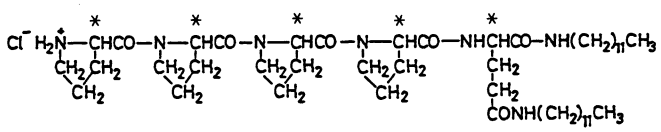
Peptidic amphiphiles self-assembled to show the marked enhancement of circular dichroism in the aggregates. This fact was interpreted as the existence of the inter- and intramolecular strong exciton coupling of the amide and imide chromophores.

Proteins and polypeptides have a large number of amide(NH-CO) and imide [N(alkyl)-CO] groups as a chiroptical chromophore and optical properties of such substances are highly dependent on the specific arrangement of these chromophores in three-dimensional space.[1] The periodic structures found in proteins or polypeptides display multiple types of circular dichroism (CD) spectra according to the combination of α-helical, β-, and random structures. On the other hand, chiral superstructures have been found to be formed by chiral bilayer-forming amphiphiles.[2-7] Recently, we have synthesized some chiral peptidic amphiphiles and investigated their self-assembling properties in aqueous dispersion.[8] As a result, those amphiphiles self-assemble to form multiple aggregated structures based on bilayers. The peptidic amphiphiles would be favorable to correlate the arrangement of amide and imide groups in an aggregate with the morphology of the chiral aggregate. In this communication, we report the enhanced CD of self-assembled peptidic amphiphiles due to the inter- and intramolecular strong exciton coupling of the amide and imide chromophores. In addition, the cooperative rearrangement of the peptide groups is discussed in connection with a change in the morphology of the aggregate.

Peptidic amphiphiles 1 and 2 were synthesized as reported elsewhere.[8] The amphiphile 1 involves no asymmetric center besides the α-carbon of the L-glutamate residue. In contrast, the amphiphile 2 consists of all L-type amino acid residues. Dark-field optical microscope has shown that morphologies of the aggregates formed by 1 and 2 are fine ribbon-like structures (20-50 μm in length and <1 μm in diameter) and spherical vesicles (<1 μm in diameter), respectively.[8] Transparent solutions of those amphiphiles were prepared by sonication (9×10^{-5} — 2×10^{-4} mol·dm^{-3}) and incubated for 1 h — 1 month at 15-20 °C. Judging from



HCl·H-Sar$_3$-Glu(NHC12)-NHC12 **1**



HCl·H-Pro$_4$-Glu(NHC12)-NHC12 **2**

the critical aggregate concentration (CAC) value of each amphiphile determined by the surface-tension measurement (CAC's of 1 and 2 = 3.8 x 10^{-5} mol·dm^{-3} and 3.1 x 10^{-5} mol·dm^{-3}, respectively), at these concentrations the amphiphiles self-assemble to form aggregates. CD spectra were taken on a JASCO J-40A spectropolarimeter equipped with a thermostated cell.

Figure 1 shows the temperature dependence of the CD spectra of 1 in aqueous solution after 4-days incubation at 15-20 °C. The spectra were very sensitive to the measured temperature. The CD intensity shows the enhancement as the temperature decreases. At 22 °C, a large positive Cotton band ([θ]=101000) appeared at 205 nm. With increasing temperature, the ellipticity at maximum decreased gradually. At temperatures above 48 °C, no remarkable Cotton bands were observed. As shown in Fig. 2, the temperature dependence of the ellipticity at 205 nm clearly indicates the transition point around 47 °C. This value is in good agreement with the phase transition temperature (Tm) determined from differential scanning calorimetry (DSC).[8] Oligopeptides generally show weak CD bands in the 200-250 nm region.[1] Actually, at concentrations below the CAC of 1, no chiral activity was observed in the CD spectra. Therefore, the above enhanced CD at lower temperatures implies that strong exciton coupling occurrs among the imide and amide chromophores in the aggregate. In other words, those chromophores are cooperatively arranged in a fixed geometry by self-assembling of the molecules. The fluidity (gel state) of the bilayer-membrane at temperatures below Tm is favorable to construct such a spatial situation. In this way, CD intensity was highly dependent on the membrane fluidity. This fact also suggests that enhanced CD is a result of regulation by the chiral bilayer assembly, rather than by the asymmetric α-carbon of the glutamate residue. Similar CD enhancement was observed for chiral amphiphiles and discussed from the standpoint of the orientation of single chromophore for each amphiphile.[9-10] Generally, periodic arrangement of peptide groups can be found in proteins or poly(amino acids) having more than 100 amino-acid residues. However, our results suggest that such a regular arrangement of the amide and/or imide groups can be attained by self-assembling of the peptidic amphiphiles consisting of only several amino acid residues.

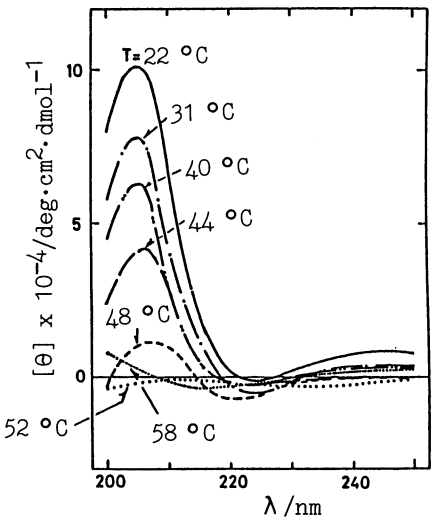


Fig. 1. CD spectra of 1 in water. Aging condition: 15-20 °C, 4 days. [1] = 9.2 x 10^{-5} mol·dm^{-3}

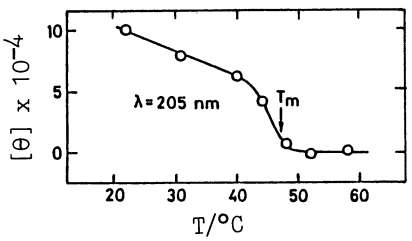


Fig. 2. Temperature dependence of $[\theta]_{205}$.

On the other hand, dark-field optical microscope has demonstrated that the bilayer assembly of 1 undergoes a large morphological change from a long ribbon to a small dust-like aggregate(<1 μm in diameter) at temperatures above Tm.[8] Similar results have already been reported for double-chain ammonium amphiphiles.[10] This fact indicates that the spatial

arrangement of the amide and imide groups in the dust-like assembly has no regularity and the arrangement in the ribbon a fixed regular geometry.

Furthermore, it is worthy of note that gradual CD enhancement was observed in the course of the incubation at 15-20 °C. The peak ellipticity ($[\theta]_{205}$=101000) of the sample incubated for 4 days was twice as large as that incubated for 1 day ($[\theta]_{207}$=56000). Judging from the above discussion, inter- and intramolecular cooperative rearrangement of the amide and imide groups (random → regular) seems to cause a morphological change (small assembly → ribbon) on incubation.

The amphiphile 2 has also given another example of the incubation-time dependence of the CD spectra. Two aqueous solutions of 2 were prepared by incubating at 15-20 °C for 1 h (sample C) and 1 month (sample D). No difference in the apparent transparency was observed for the two samples. As shown in Fig. 3, the sample C displays a CD spectrum giving a negative Cotton band at 205 nm ([θ]= -78500 at 20 °C) and the sample D shifts the minimum to 218 nm ([θ]= -61500 at 24 °C). CD spectra of poly L-proline II and poly D-proline I give a typical negative Cotton band at 206 nm and 218 nm, respectively, suggesting the existence of a helical conformation.[11] Accordingly, the initial spatial arrangement of the four proline-imide chromophores in the assembly might be analogous to that of poly L-proline II. Namely, it seems to be related to a left-handed helix with trans-peptide bond. As the incubation time increases, the imide groups in the assembly may gradually alter the spatial orientation in such a way as to take a left-handed helix with cis-peptide bond. Of course, since there is no direct evidence for the isomerization from trans to cis configuration of the imide groups, nobody can exclude other possibilities for the change in the arrangement and/or configuration of the imide chromophores. In this case, however, no outstanding morphological change was observed by dark-field optical microscopy in the course of the incubation for 1 month.

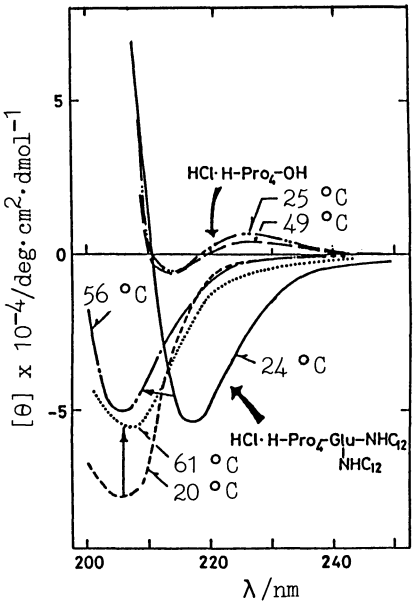


Fig. 3. CD spectra of 2 in water. Sample C at 20 °C (- - - -) and at 61 °C (·····). Sample D at 24 °C (———) and at 56 °C (—·—). HCl·H-Pro$_4$-OH at 25 °C (— - —) and at 49 °C (— — —). Aging condition: sample C (15-20 °C, 1 h) and sample D (15-20 °C, 1 month). [2] = 2.1 x 10^{-4} mol·dm^{-3}

Figure 3 also indicates that the aqueous solution (1.35 x 10^{-3} mol·dm^{-3}) of free HCl·H-Pro$_4$-OH gave two peaks with relatively small ellipticities ($[\theta]_{213}$= -6000 and $[\theta]_{225}$=7000) in the CD spectrum. The CD intensity was not dependent on the peptide concentration. But, introduction of the glutamate residue carrying two long hydrocarbon chains into this peptide by the covalent bond could enhance the optical activity, showing the large negative Cotton band. This result also supports that inter- and intramolecular cooperative interaction of the amide and imide chromophores in the assembly leads to the enhanced CD. In addition, the temperature dependence of the CD spectra of HCl·H-Pro$_4$-OH ($[\theta]_{225}$=7000 at 25 °C, $[\theta]_{225}$=4000 at 55 °C) is much smaller than that of the assembly($[\theta]_{217}$= -54000 at

24 °C, $[\theta]_{205}$= -48000 at 56 °C). The arrangement mode of the chromophores in the assembly plays an important role in such an amplification of the temperature dependence.

The sample C or the sample D was heated to a temperature above Tm (51-55 °C). Two corresponding CD spectra show negative band at 207 nm, having similar feature (Fig. 3). Therefore, the aggregated structure of the two samples at temperatures above Tm is considered to be based on the same arrangement of the chromophores. It should be pointed out here that optical activity remains at temperatures above Tm. This fact is in contrast with the case in the amphiphile 1 having one asymmetric center. Existence of more asymmetric centers in the assembly may contribute to retain the optical activity in a liquid-crystal state of the assembly.

In conclusion, a large enhanced CD found in proteins or poly(amino acids) could be attained by self-assembling of the peptidic amphiphiles. This fact was interpreted as the existence of the inter- and intramolecular regular arrangement of the amide and imide chromophores in the assembly. The situation is schematically illustrated in Fig. 4.

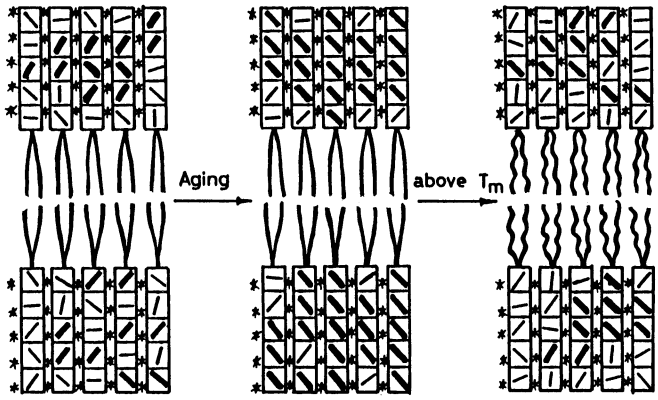


Fig. 4. Schematic illustration of the inter- and intramolecular cooperative rearrangement of the amide and imide chromophores in the assembly of 2. One square corresponds to one amino acid residue and a bold bar represents the chromophore in which a strong exciton coupling occurrs with each other.

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