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The Preparation of Self-Organized Nano-Particles Composed of Peptide-Based Amphiphiles and the Optical Resolution Properties

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Amphiphilic block copolymer of poly (peptide-*co*-ethylene glycol) containing ligands (pyridyl moiety) in the side chains of the polypeptide was prepared. The amphiphile was soluble in water to form self-organized nano-particles. TEM measurement showed that the self-organized nano-particle seems to be a vesicular aggregate. On the other hand, the complex formation of the side-chain pyridyl ligand with Cu^{2+} in vesicular aggregates was confirmed by the UV and CD spectra indicating the ternary complex formation. Furthermore, L-Tryptophan shows higher affinity to vesicular aggregates-Cu^{2+} system than D-Tryptophan.

Key words: Amphiphilic block copolymer, polypeptide, aggregate, complex formation, solubilization, optical resolution

1. INTRODUCTION

In the past studies[1)], it was confirmed that amphiphilic block copolymer consisting of hydrophilic poly (ethylene glycol) (PEG) and hydrophobic polypeptide formed aggregates in aqueous solution in which α-helical polypeptide segment is in the hexagonal packing and its specific solubilization behaviors were observed based on such the α-helix aggregate structure.

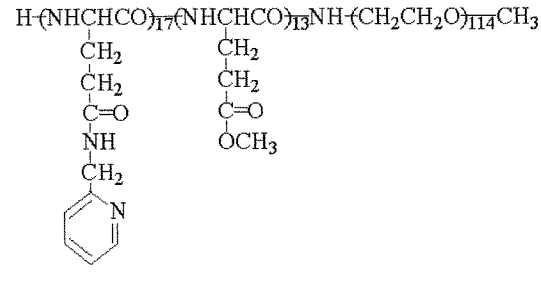
On the other hand, some proteins are known to create the active site by the complex formation with metal ions.[2~4)] We reported that α-helical polypeptide, in which the pyridyl moiety was introduced as a ligand, formed the ternary complex (e.g. pyridyl moiety-copper ion (Cu^{2+})- amino acid) depending on the D- and L-isomers. [5)] This implied an active site formation for optical isomers.

In this study, amphiphilic block copolymer (poly (peptide-*co*-ethylene glycol)) containing ligands (pyridyl moiety), which can form the complex with transition metal ions, in the side chains was prepared. The structure of its self-organized nano-particle and the ternary complex formation behaviors in aqueous solution were studied. Furthermore, the optical resolution properties of this nano-particle were examined.

2. EXPERIMENT

Amphiphilic block copolymer of poly (γ-methyl L-glutamate-*co*-ethylene glycol) containing pyridyl moiety in the side chains was prepared.

Poly (ethylene glycol) containing a terminal amino group (NH_2-PEG; Mw=5050) was used as an initiator. First, NH_2-PEG and *N*-carboxy anhydride of γ-methyl L-glutamate (MLG-NCA) were dissolved in *N,N*-dimethylformamide (DMF), respectively. Then MLG-NCA solution was added to the stirred NH_2-PEG solution slowly and the mixture was stirred for 140h at room temperature. After confirming the disappearance of the peaks of MLG-NCA (1780 and 1850 cm^{-1}) by a FT-IR measurement, the solution was poured into diethyl ether. As a result of ^{1}H-NMR analysis of the block copolymer in trifluoroacetic acid (TFA), the polymerized degree of PMLG segment in the polymer was estimated to be 30 ($PMLG_{30}$-PEG). Furthermore, to introduce pyridyl moiety in the side chains of PMLG, $PMLG_{30}$-PEG was dissolved in 2-amino methyl pyridine, afterward the solution was stirred at 60℃ for 72h. The introduction degree of pyridyl moiety was calculated at about 55% by ^{1}H NMR analysis of the block copolymer in TFA ($P(2PLG_{17}/MLG_{13})$-PEG) (Scheme 1).



Scheme 1 Chemical structure of $P(2PLG_{17}/MLG_{13})$-PEG.

To estimate the complex formation in aqueous solution, circular dichroism and absorption spectrum measurements were applied by use of a circular dichrometer J-820K (JASCO) and a UV/VIS spectrophotometer V-550 (JASCO).

Atomic force microscope (AFM) (Nano-Scope IIIa (Digital Instruments) and Transmission electron micrograph (TEM) (H-800, Hitachi) were used to observe the shape and size of the aggregates. The mica was used as the substrate for the AFM measurement. A few drops of P(2PLG$_{17}$/MLG$_{13}$)-PEG solution and P(2PLG$_{17}$/MLG$_{13}$)-PEG solution containing Cu^{2+} were dripped on cleaved mica surface. After a few minutes, the substrates were freeze-dried.

Semiequilibrium dialysis method[6, 7] was applied to estimate the optical resolution of a pair of amino acid. In this case, D- and L-Tryptophan (D-Trp and L-Trp) were used as guest molecules. The concept of semi-equilibrium dialysis method is shown in Figure 1. P(2PLG$_{17}$/MLG$_{13}$)-PEG solution, which was prepared at

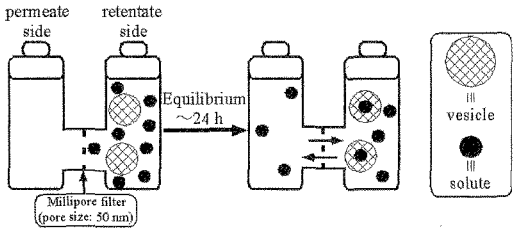


Figure 1 Schematic illustration of the apparatus for semiequilibrium dialysis method.

30 times molarity of the critical aggregation concentration (CAC=1.6 × 10^{-6} M ; the value was calculated by the fluorescence probe method which used pyrene as a fluorescent probe[8, 9]), Cu^{2+} (three times molarity of pyridyl moiety ([Cu^{2+}] /[2PLG] =3)) and D-Trp or L-Trp were added in the right side of the semiequilibrium dialysis cells (retentate side). And in the left side of the semiequilibrium dialysis cells (permeate side), Cu^{2+} as same molarity as the retentate side was added. After 24h when the concentration of guest molecule in both sides comes up to the equilibrium state, molarity of the guest molecule in the permeate side was determined by a fluorescence spectrum measurement and solubilization equilibrium constants (K) were calculated by using follow equation (1),

$$K=X/c \qquad (1)$$

where X is the intra-aggregate mole fraction of the guest molecule and c is the molarity of the guest molecule in the permeate side. By plotting K with X, solubilization isotherms were obtained.

3. RESULTS AND DISCUSSION

The secondary structure of peptide segment in P(2PLG$_{17}$/MLG$_{13}$)-PEG aggregate in aqueous solution was first examined by CD spectroscopy. The spectrum showed a typical right handed α-helix pattern, although a trough appeared at 227 nm with a shoulder at 210 nm, indicating formation of α-helix aggregation. A similar CD pattern was also observed in the case of P(2PLG$_{17}$/MLG$_{13}$)-PEG aggregate containing Cu^{2+}.

Subsequently, we estimated the shape and the size of P(2PLG$_{17}$/MLG$_{13}$)-PEG aggregates by AFM. As a result (Figure 2 (a)), spherical aggregates were observed. And the range of the diameter of the aggregates was from 30 to 90 nm. Figure 2 (b) showed an AFM image of P(2PLG$_{17}$/MLG$_{13}$)-PEG aggregates containing Cu^{2+}. The shape of the observed aggregates was also spherical and the range of the diameter (from 30 to 90 nm) was

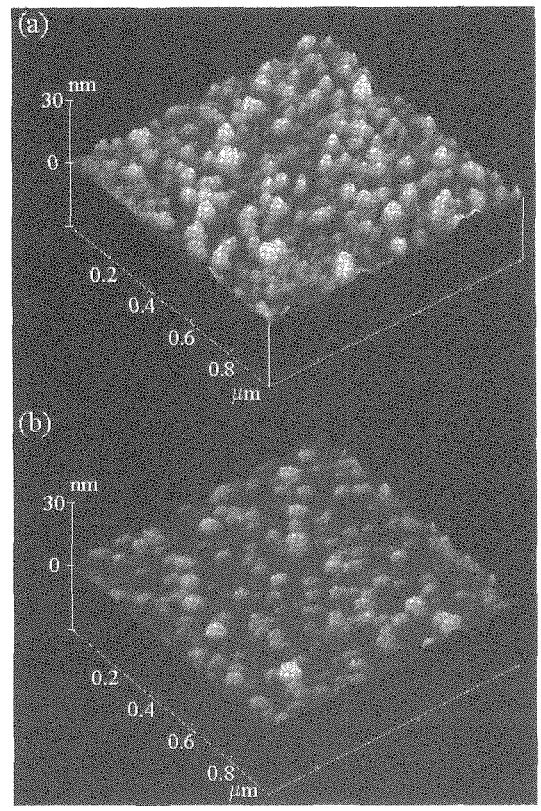


Figure 2 AFM images of (a) P(2PLG$_{17}$/MLG$_{13}$)-PEG aggregates and (b) P(2PLG$_{17}$/MLG$_{13}$)-PEG aggregates containing Cu^{2+} on cleaved mica surface.

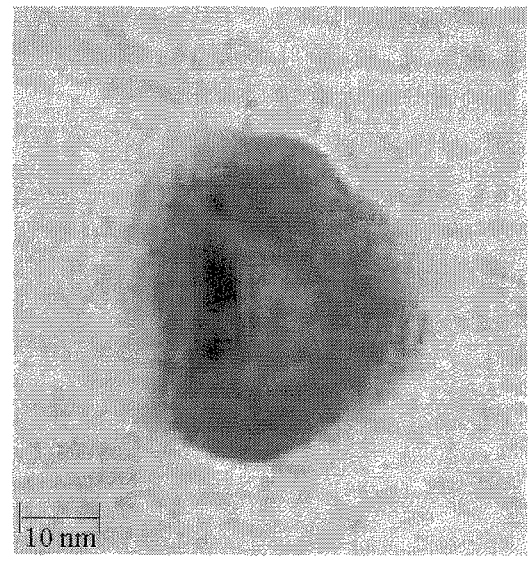


Figure 3 Transmission electron micrograph of P(2PLG$_{17}$/MLG$_{13}$)-PEG containing Cu^{2+} in aqueous solution.

almost equal to that of the aggregates in Figure 2 (a). This indicates that the complex formation of pyridyl moiety with Cu^{2+} in the aggregates has no effect on the shape and size of the aggregates

TEM observation (Figure 3) also showed that the diameter of the aggregates was about 40 nm, which was in the range of the diameter observed by AFM. It should be noted here that the space inside the aggregate was found. It is considered that the water may be in this aggregate interior. Therefore, it was suggested that the $P(2PLG_{17}/MLG_{13})$-PEG aggregate containing Cu^{2+} formed a vesicular aggregate in aqueous solution.

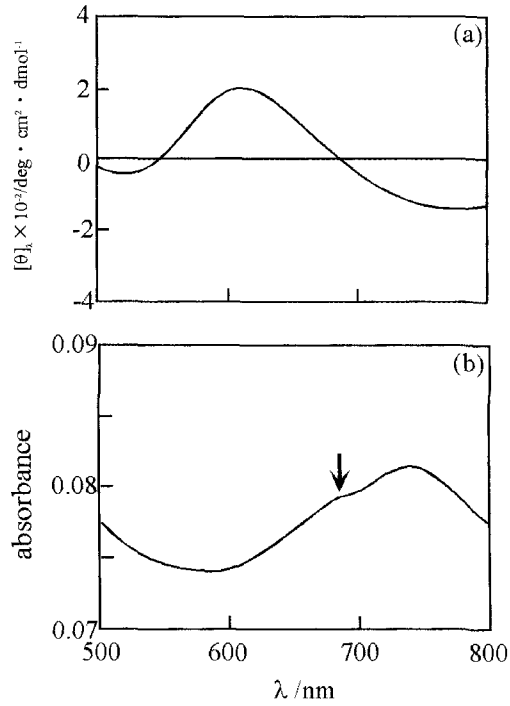


Figure 4 Circular dichroism (a) and absorption spectra (b) of $P(2PLG_{17}/MLG_{13})$-PEG containing Cu^{2+} in aqueous solution at pH 6. $[Cu^{2+}]=2.8\times10^{-3}$ M.

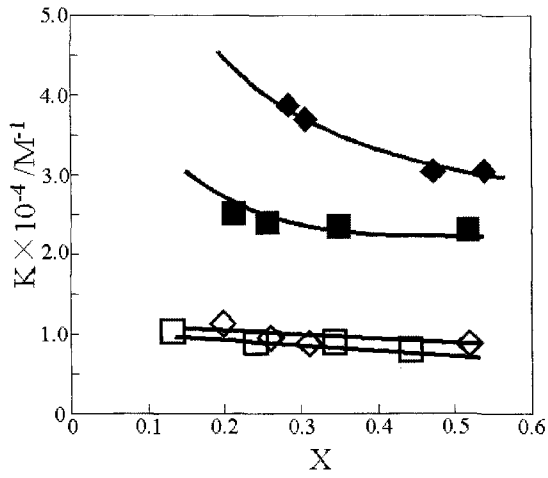


Figure 5 Dependence of the solubilization equilibrium constants for D-Tryptophan (□) and L-Tryptophan (◇) in $P(2PLG_{17}/MLG_{13})$-PEG vesicles and for D-Tryptophan (■) and L-Tryptophan (◆) in $P(2PLG_{17}/MLG_{13})$-PEG vesicles containing Cu^{2+} on intravesicular fraction of D-Tryptophan and L-Tryptophan.

On the other hand, the CD and UV measurements were performed to confirm the complex formation of the side-chain pyridyl ligand with Cu^{2+} in vesicular aggregates in aqueous solution. In the CD spectrum (Figure 4 (a)), an asymmetric couplet was observed at around 680 nm. This agreed with the absorption band (see an arrow in Figure 4 (b)) in the vicinity of 680 nm observed as a shoulder in the spectrum. This absorption band observed as a shoulder is based on the *d-d* transition of pyridyl moiety with Cu^{2+}.[5,10] Accordingly, it was confirmed that pyridyl moiety in the side chains of the polypeptide and Cu^{2+} formed the 1:1 complex in the vesicular aggregate.

From the result of Figure 4, it is considered that the complex formation of pyridyl moiety-Cu^{2+} in vesicular aggregates possesses another site with which a guest molecule can form the ternary complex. So, by utilizing the ternary complex formation of the vesicular aggregate, we tried to perform the optical resolution of a pair of amino acid. Figure 5 shows solubilization isotherms of D-Trp and L-Trp in vesicular aggregates with and without Cu^{2+} estimated by semiequilibrium dialysis method. Both D-Trp and L-Trp showed the higher solubilization equilibrium constant, K value, for the $P(2PLG_{17}/MLG_{13})$-PEG vesicle containing Cu^{2+} compared with that of the vesicle without Cu^{2+}. Because it is considered that in both D-Trp and L-Trp, the ternary complex, pyridyl moiety-Cu^{2+}-Trp, is formed in vesicular aggregates. Furthermore, in the case of $P(2PLG_{17}/MLG_{13})$-PEG vesicles, there are not significant differences for K values between D-Trp and L-Trp. In the case of $P(2PLG_{17}/MLG_{13})$-PEG vesicles containing Cu^{2+}, however, K values of L-Trp were higher than those of D-Trp. This indicates that L-Trp can form the steadier ternary complex in vesicular aggregates because of higher affinity to vesicular aggregates-Cu^{2+} system than D-Trp.

The solubilization behaviors of D-Trp and L-Trp in vesicular aggregates containing Cu^{2+} seem to be obeyed Langmuir type adsorption. To analyze this quantitatively, the following equation[7] (2) was used,

$$K=K_0(1-BX)^2 \qquad (2)$$

where K_0 is the limiting value of K at X=0 and B is half the number of host molecules constituting a site for the adsorption of a guest solute. From solubilization isotherms obtained in Figure 5 and eq. (2), K_0 and B values for D-Trp and L-Trp were calculated, respectively (Table 1). As a result, K_0 value for L-Trp

Table 1 Parameters for D-Tryptophan and L-Tryptophan in $P(2PLG_{17}/MLG_{13})$-PEG vesicles containing Cu^{2+}.

	D-Trp	L-Trp
K_0/M⁻¹	3.06×10^4	5.19×10^4
B	0.45	0.50

was higher than that of D-Trp. Accordingly, this also indicates that L-Trp shows higher affinity to vesicular aggregates-Cu^{2+} system than D-Trp. In addition, it was confirmed that the number of host molecules constituting a site for the adsorption of a guest in vesicular aggregates was about one pyridyl moiety-Cu^{2+} pair for one D- and L-Trp, respectively.

4. CONCLUSION

In this study, we succeeded in preparation of self-organized polypeptide nano-particles which can form the ternary complex in aqueous solution. It was suggested from TEM observation that the self-organized nano-particle was composed of a vesicular aggregate. Furthermore, 1:1 complex formation of the side-chain pyridyl ligand with Cu^{2+} was confirmed, and this complex further interact with D-Trp and L-Trp to produce the ternary complex by Langmuir type adsorption manner. It was suggested that L-Trp showed higher affinity to vesicular aggregates-Cu^{2+} system compared with D-Trp.

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