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FORMATION OF HELICAL SUPER STRUCTURE FROM SINGLE-WALLED BILAYERS BY AMPHIPHILES WITH OLIGO-L-GLUTAMIC ACID-HEAD GROUP

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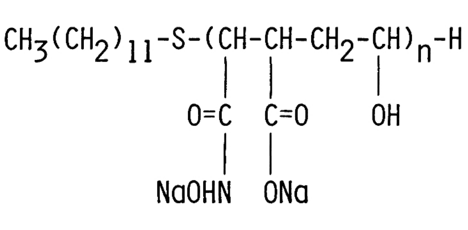
Double-chain alkyl amphiphiles with oligo-L-glutamic acid-head group were prepared. These compounds were dispersed in water at pH 8 to 9 to form bilayer aggregates, in which helical and hollow structures were contained. New CD bands were observed in the solution.

In recent years, many membrane-forming amphiphiles, which give the unique aggregate morphologies[1-5] and physicochemical properties[6-9] and regulate various chemical reactions,[8-11] have been synthesized. Amino acids were often applied as structural units, and the bilayers constituted by these chiral compounds showed enhanced optical activities[6,12,13] and substrate specificities.[8,10,11] We have reported that surfactants with the hydrophilic copolymer-head group(1) also formed the multi-lamellar vesicles in the presence of Cu^{2+}.[14,15] In this communication, we wish to report that the double-chain alkyl amphiphiles with oligo-L-amino acid (glutamic acid)-head group (2, $2C_mGlu_n$) formed highly ordered aggregates, including helical super structures formed from molecular bilayers. A related observation was done independently by Kunitake and coworkers,[16] and their finding is arranged for simultaneous publication in this journal.



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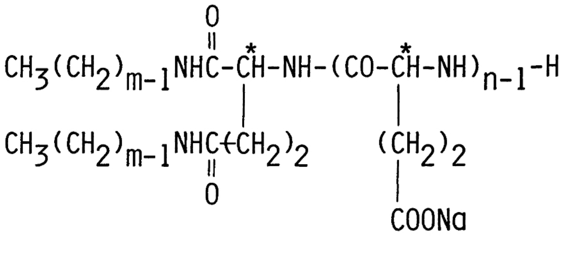

2, $2C_mGlu_n$ ($m = 12$ and 16)

Compounds 2 were synthesized as follows. L-Glutamate dialkylamides[17] were prepared by coupling of long-chain alkylamines ($m = 12$ and 16) and N-carbobenzoxy-L-glutamic acid by diethyl phosphorocyanidate/triethyl amine and by the subsequent debenzyloxycarbonylation by H_2/Pd. The polymerization[18] of N-carboxyanhydride(NCA) of γ-benzyl L-glutamate by the amine obtained in tetrahydrofuran and the following hydrolysis by NaOH[19] produced 2. The degree of polymerization (n) was determined by NMR spectra[20)] and confirmed by elemental analyses.[21)] The value of n agreed with the ratio of NCA to amine.

2 were dispersed in water to give slightly turbid solutions (pH 8-9).[22)] Electron microscopies of these solutions provided significant informations. As shown in Fig. 1, $2C_{12}Glu_{14}$ and $2C_{16}Glu_{14}$ produced lamellar structures (pre-vesicles in $2C_{16}Glu_{14}$), of which widths were 30 - 50 Å and 60 - 100 Å, respectively. These thicknesses are nearly equal to those of their molecular bilayers. In addition, the endothermic behavior was observed in the DSC measurement of these solutions: the peak-top temperature for $2C_{12}Glu_{14}$ and $2C_{16}Glu_{14}$ were 34.5 and 65.5 °C, and 56.5 and 81.5 °C, respectively. Kunitake et al. described that the double-chain compounds with polyethyleneglycol-head group formed molecular bilayers such as vesicles or lamellae (disks) with the thickness of 50 - 100 Å.[2)] In this study a peculiar aggregate form was discovered in $2C_{12}Glu_{14}$ (aggregate A in Fig. 1-a, magnified in Fig. 1-b). We consider that the aggregate A was constituted by twisting of a ribbon-type bilayer because the width of hydrophobic layer was 30 - 40 Å (in Fig. 1-b) and the aggregate possessed the gel-to-liquid crystal phase transition (in DSC measurement). Although such a helical super structure was found in the fibril of poly-γ-benzyl-L-glutamate,[23)] the complex of poly-L-lysine and poly-L-glutamic acid,[24)] and the gel solution of 12-hydroxystearic acid,[25)] this study provided the first example of the super structure constituted by synthetic bilayers.

On the other hand, a paired bilayer was also observed (aggregate B in Fig.1-a, magnified in Fig. 1-c). It is difficult to consider that this aggregate was formed by overlapping of two sheets of single-layer lamellae, because the length of the lamellar aggregate was too close to that of each pair. Therefore it may be

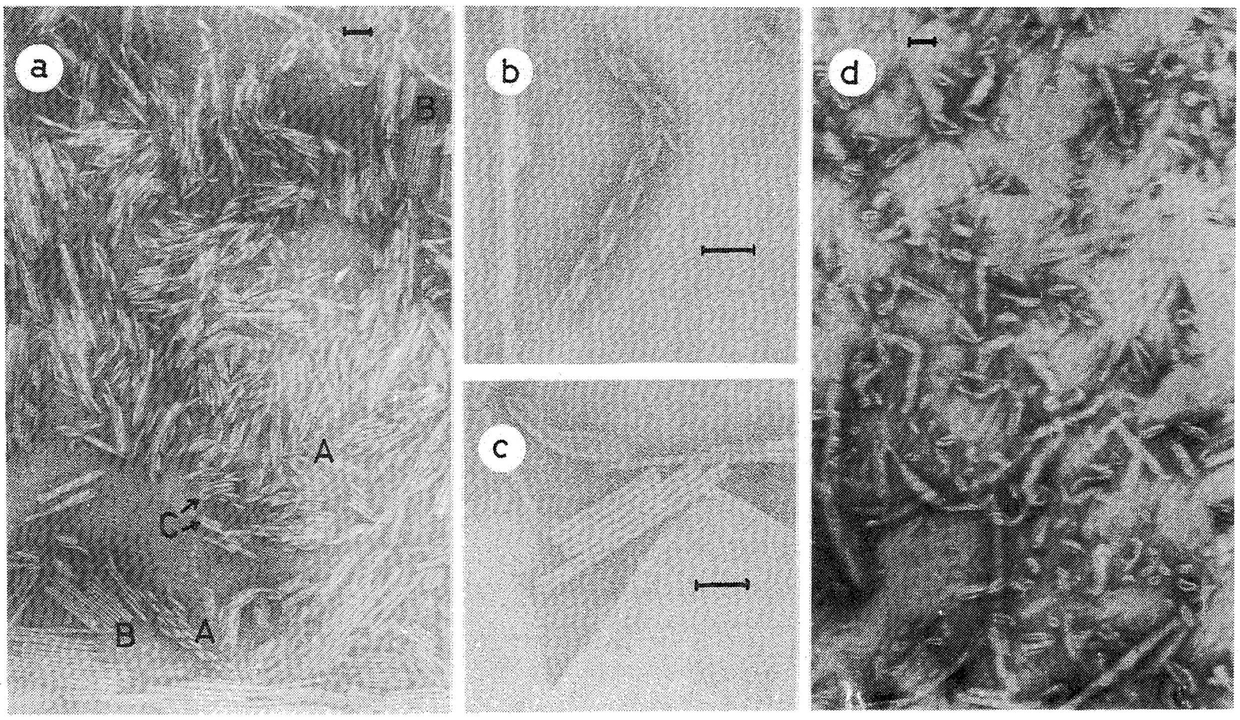

Fig. 1. Electron micrographs of $2C_{12}Glu_{14}$ (a - c) and $2C_{16}Glu_{14}$ (d)
0.1wt%, stained by molybdate. ⟼ 500 Å

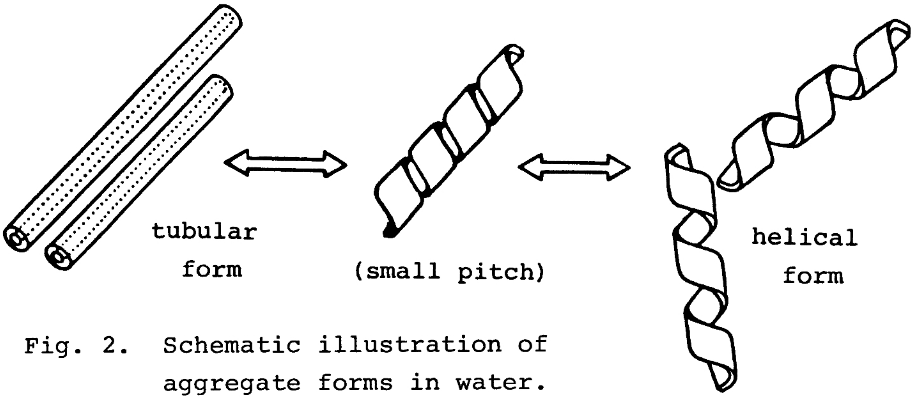

Fig. 2. Schematic illustration of aggregate forms in water.

a tubular (hollow) structure (as shown in Fig. 2) and the black line between the lamellae belongs to the inner aqueous phase. The tubular structure was proposed by Kunitake.$^{3)}$ It seems that this tubular structure is related to the above-mentioned helical structure, because there were other types of aggregates (for example, aggregate C in Fig. 1-a) whose morphologies were in between forms A and B.

It was expected that the super structure had large effects upon CD. As shown in Fig. 3, the CD spectra of $2C_{12}Glu_{14}$ and $2C_{16}Glu_{14}$ were different from that of random coil. However in dilute solutions ($\approx 10^{-6}$ M) or in the presence of an anionic surfactant (SDS), the CD spectra approached that of the random coil. Therefore the unusual CD is attributable to aggregation in water.

The formation of the super structure constituted by molecular bilayers were revealed through this study and Kunitake et al.$^{16)}$ Although the driving force of bilayer to twist is not clear, physicochemical characterization of these helices is in progress.

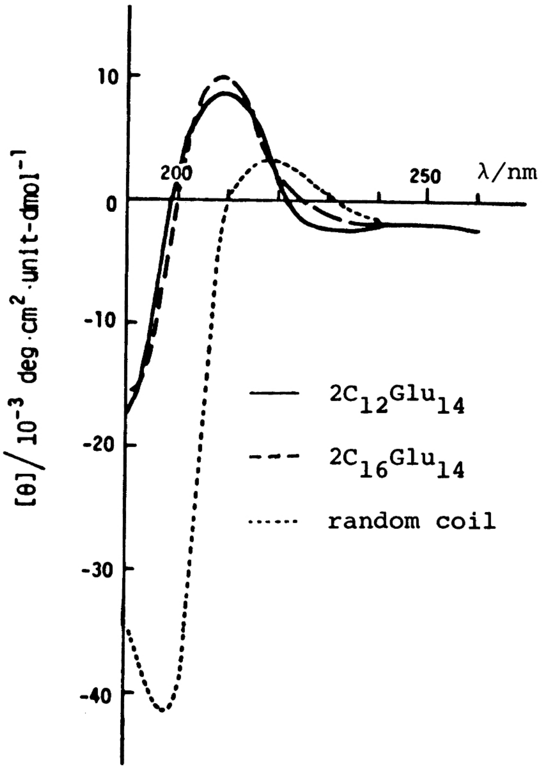

Fig. 3. CD spectra of $2C_mGlu_{14}$ at pH 9.0, 25°C, 5.0×10^{-4}M.

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We extend our application to these authors for communication of their data prior to publication.
17) L-Glutamate dialkylamide (m=12): mp 115 - 118 °C, Found C, 72.18; H, 12.65; N, 8.72%. Calcd for $C_{29}H_{59}N_3O_2$ C, 72.19; H, 12.34; N, 8.71%. (m = 16): mp 105 - 108 °C Found C, 73.13; H, 12.79; N, 6.81%. Calcd for $C_{37}H_{75}N_3O_2$ C, 73.13; H, 12.69; N, 6.91%.
18) H. Uchio, H. Ihara, T. Izuno, T. Ide, K. Taniguchi, and K. Yamada, Yukagaku, in press.
19) Condition of hydrolysis: in methanol solution containing equimolar amounts of NaOH for ester residue, within a few minutes.
20) ^{1}H-NMR data of $2C_mGlu_n$ in CF_3COOH: δ1.36 (a, $-(CH_2)_{m-2}-$), δ2.20 - 2.60, 2.80 (b, $-CH_2CH_2-$, Glu), δ3.50 (c, $-CH_2-N-$), δ4.76 (d, $-CO-\overset{*}{C}H-N-$).
The value of n was determined by peaks a and d.
21) Elemental analyses of $2C_mGlu_n$ (m = 12, n = 6): Found C, 52.19; H, 7.91; N, 8.49%. Calcd for $2C_{12}Glu_{6.0}1.4H_2O$ C, 52.91; H, 7.78; N, 8.82%. (m = 12, n = 11): Found C, 44.70; H, 6.25; N, 8.55%. Calcd for $2C_{12}Glu_{11.4}6.8H_2O$ C, 44.70; H, 6.25; N, 8.62%. (m = 12, n = 14): Found C, 38.41; H, 6.16; N, 7.62%. Calcd for $2C_{12}Glu_{13.9}29.3H_2O$ C, 38.41; H, 6.58; N, 7.62%. (m = 16, n = 14): Found C, 42.55; H, 6.39; N, 7.23%. Calcd for $2C_{16}Glu_{13.6}18.4H_2O$ C, 42.55; H, 6.76; N, 7.67%.
22) On sonification by Branson Sonifier 145 (bath type) at 70 - 80 °C for 15 min or Branson Sonifier 185 (probe type) for 2 min.
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