

Lipid Membrane Analogues. Formation of Highly-oriented Structures and their Phase Separation Behaviour in Benzene

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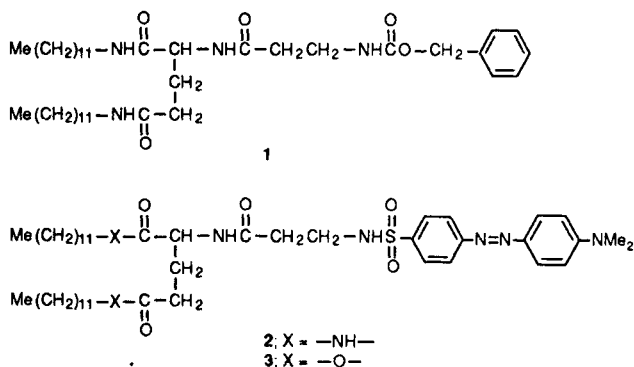
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L-Glutamate derivatives **1** and **2** having two *N*-dodecylamide groups formed highly-oriented aggregates in benzene, which underwent phase transition and phase separation behaviours analogous to those of lipid membranes.

It is well-known that amphiphiles with double-chain alkyl groups form bilayer membrane structures in water.¹ Recently, Ishikawa *et al.* have reported that some compounds composed of double-chain perfluoroalkyl groups and a single-chain alkyl group could form bilayer membrane structures in cyclohexane.² The driving force of the bilayer formation in cyclohexane is attributable to the highly solvophobic property of the perfluoroalkyl groups which provide extremely low solubility parameters³ much lower than those of the cyclohexane. On the other hand, we have reported that double-chain alkyl lipids with three amide groups per molecule could form single-walled helical bilayer aggregates in dilute aqueous solutions and this special morphology was stabilized by intermolecular hydrogen bonds among lipids.⁴ In this communication, we report that lipid analogues **1** and **2** without special solvophobic parts such as perfluoroalkyl groups but with three amide groups a molecule can be self-assembled in organic solvents (especially in benzene), and that the aggregates show phase transition and phase separation phenomena.

Compound **1** was dissolved in dry benzene at 70 °C to provide a clear solution. When the solution was cooled to room temperature, typical gelation was observed. The phase transition temperatures were measured at between 45 and 85 °C (peak-top temperature, $T_c = 72$ °C) by differential scanning calorimetry (DSC).† Compound **2** containing a chromophoric group also showed phase transition in benzene at temperatures between 34 and 75 °C ($T_c = 50$ and 64 °C), although its solubility in benzene was limited. The λ_{max} of **2** in benzene was located at 425 nm at temperatures above the phase transition temperature. When the temperature was lowered from 60 to 20 °C, a remarkable λ_{max} shift from 425 to 387 nm was observed. This λ_{max} shift included a chirality change. The circular dichroism (CD) spectra showed that **2** in benzene provided strong exciton couplings around 387 nm at 20 °C ($[\theta]_{370} = 5.0 \times 10^4$, $[\theta]_{410} = -4.9 \times 10^4$ deg cm² dmol⁻¹), whereas the degree decreased at temperatures around the phase transition temperature and very weak exciton coupling was observed in a chloroform solution. Similar λ_{max} shift and chirality enhancement has been reported in aqueous bilayer membranes from chiral chromophore-containing synthetic lipids.⁵ These phenomena are explained by the formation of highly oriented (bilayer membranes) structures with head-to-head chiral-stackings of chromophoric groups in water.



† The DSC measurement was carried out using a sealed AG cell.

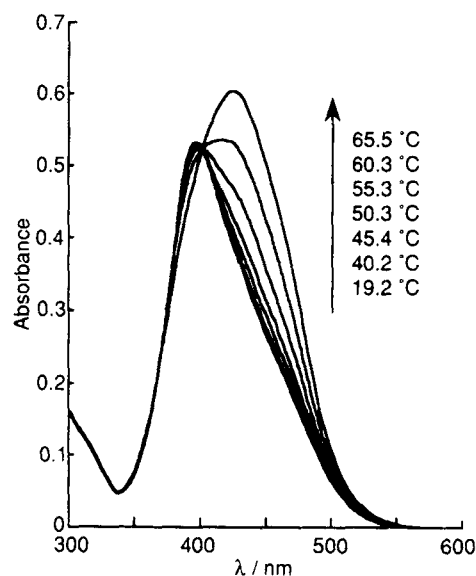


Fig. 1 Temperature dependence of visible spectra of **2** in the presence of **1** in benzene. $[\mathbf{1}] = 2.0$ mmol l⁻¹, $[\mathbf{2}] = 2.0$ mmol l⁻¹. Path length of cell; 0.1 mm.

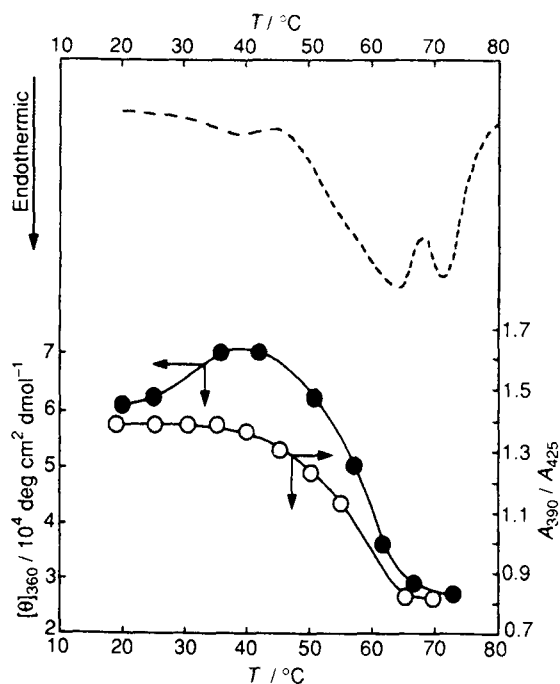


Fig. 2 Temperature dependencies of absorption ratio between 390 and 425 nm and $[\theta]$ value at 360 nm in a benzene solution containing **1** and **2**. Dotted lines represent a DSC thermogram in the mixture. $[\mathbf{1}] = 2.0$ mmol l⁻¹, $[\mathbf{2}] = 2.0$ mmol l⁻¹.

Therefore, it is estimated that compound **2** also forms highly oriented structures in benzene analogous to aqueous bilayer membranes.

A mixture of **1** and **2** (1 : 1) dissolved in dry benzene at 70 °C provided a clear solution with λ_{max} of 425 nm and $[\theta]_{360}$ of 2.9×10^4 deg cm² dmol⁻¹. However, λ_{max} shifts and chirality enhancement were observed with gelation at room temperature. Fig. 1 shows the temperature dependence of the visible spectra in the mixture. The spectra at 20 °C is close to that of the benzene solution of compound **2** alone. In addition, these gels showed increase of CD strength around the absorption of the chromophoric groups. Fig. 2 shows the temperature dependencies of the absorbance (*A*) ratio (A_{390}/A_{425}) and the strength of $[\theta]_{360}$ in the CD spectra. The remarkable temperature dependencies are closely related to the phase transition behaviour of the mixture (the DSC thermogram is presented by dotted lines in Fig. 2). These results indicate that compound **2** separates from compound **1** at temperatures below the phase transition temperature. Such phase separation phenomena in lipid membrane systems are often observed in water.⁶ However, this is the first example in a pure organic system.

The aggregation morphologies of **1** and **2** in benzene have not yet been specified. However, the X-ray patterns in the cast films of their benzene solutions contain sharp reflections at 32.22 and 25.52 Å in **1** and **2**, respectively. These values correspond to the thicknesses of tilted single layers of **1** and **2** with molecular lengths of 33 and 38 Å [estimated from their Corey–Pauling–Koltun (CPK) models]. The IR spectra at room temperature provided a peak at 3300 cm⁻¹. This peak is assigned to that of N–H stretching vibration under hydrogen bonding.⁷ Therefore, the λ_{max} and chirality enhancement occurring with gelation is attributed to the formation of highly oriented structures stabilized by hydrogen-bonding interac-

tion. This assumption is supported by additional experimental data: (i) the ester type compound **3** corresponding to **2** showed neither λ_{max} shift nor chirality enhancement. (ii) When trifluoroacetic acid was added to the benzene solution of **1**, phase transition was not observed at any temperature. It is assumed that trifluoroacetic acid suppresses the hydrogen-bonding interaction.

In conclusion, highly oriented structures in benzene were produced using lipid analogues without special solvophobic groups. This system showed critical phase separation behaviour, one of the most important phenomena in biomembrane functions.

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References

- 1 T. Kunitake and Y. Okahata, *J. Am. Chem. Soc.*, 1977, **99**, 3860; Y. Murakami, A. Nakano and H. Ikeda, *J. Org. Chem.*, 1982, **47**, 2137.
- 2 Y. Ishikawa, H. Kuwahara and T. Kunitake, *J. Am. Chem. Soc.*, 1989, **111**, 8530; Y. Ishikawa, H. Kuwahara and T. Kunitake, *Chem. Lett.*, 1989, 1737.
- 3 C. Hirayama, H. Ihara, S. Nagaoka, K. Kato and K. Kashiara, *Chromatographia*, 1992, **33**, 413.
- 4 H. Ihara, M. Takafuji, C. Hirayama and D. F. O'Brien, *Langmuir*, 1992, **8**, 1548; H. Ihara, M. Yamaguchi, M. Takafuji, H. Hachisako, C. Hirayama and K. Yamada, *Nippon Kagaku Kaishi*, 1990, 1047.
- 5 T. Kunitake, N. Nakashima, M. Shimomura, Y. Okahata, K. Kano and T. Ogawa, *J. Am. Chem. Soc.*, 1980, **102**, 6642.
- 6 N. Nakashima, K. Morimitsu and T. Kunitake, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 3253; M. Shimomura and T. Kunitake, *Chem. Lett.*, 1981, 1001.
- 7 K. Tahara, H. Suzuki and M. Honma, *Hyomen*, 1981, **19**, 688.