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Spontaneous Separation of Enantiomeric Components in Synthetic Bilayer Membranes

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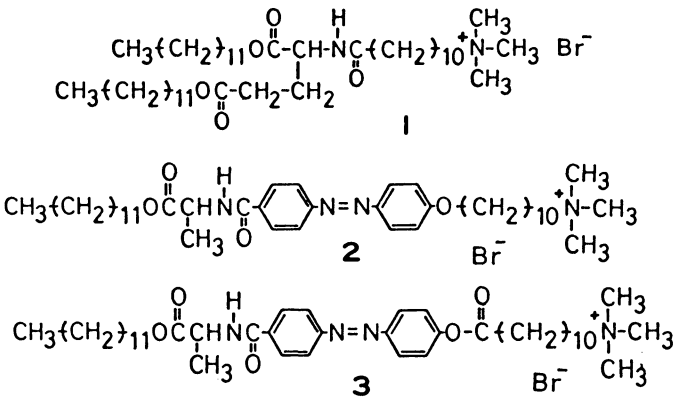
Enantiomeric components in bilayer membranes of chiral ammonium amphiphiles which contain the azobenzene unit separate spontaneously from each other, as evidenced by absorption spectral changes and electron microscopic observation of helical superstructures.

Synthetic bilayer membranes which are composed of chiral amphiphiles possess unique properties. For example, circular dichroism is markedly enhanced in certain chromophore-containing membranes,[1-3] and helical superstructures are formed in some cases by incubation at temperatures below the gel to liquid-crystal phase transition (T_c).[4-7] The chiral bilayer environments induced circular dichroism of bound achiral dyes,[8,9] and were used as matrices of enantioselective reactions.[10] However, fundamental structural aspects of these membranes remain to be elucidated. We describe in this paper detection of component mixing and separation in chiral bilayer membranes.

The chiral amphiphiles used in this work are shown below. Preparation of didodecyl-N-(11-trimethylammonioundecanoyl)-L-glutamate bromide (**1**) was reported elsewhere.[11] Dodecyl-N-[4-[4-(10-trimethylammoniodecyloxy)phenylazo]benzoyl]-L(D, DL)-alaninate bromides (L-**2**, D-**2**, DL-**2**) and dodecyl-N-[4-[4-(11-trimetylammonio-undecanoyloxy)phenylazo]benzoyl]-L(D)-alaninate bromides (L-**3**, D-**3**) were newly synthesized.[12] The three isomers of **2** could be readily dispersed in water. Their absorption maxima are located at 320 nm immediately after dispersion, which, upon 4-h incubation at 40 °C, shift to 350 nm and helix formation ensues (cf. Fig. 3). No spectral changes are observed in the temperature range from 10 to 70 °C after further incubation. Amphiphiles **3** give transparent aqueous dispersions by warming and no helices are observed. Their absorption maxima are located at 303 nm (at 35 °C), and prolonged incubation does not induce any spectral change.



Azobenzene-containing components

in bilayer membranes often exhibit large absorption shifts in the region of 300-400 nm, depending on the orientation and aggregation of the chromophore.[13] This unique spectroscopic property was applied to detection of component mixing and separation in bilayers of **2** and **3**. The mixing of L-**2** and D-**2** and the separation of DL-**2** can not be detected by absorption spectroscopy, since the spectral behavior of these membranes is identical. Thus, we examined miscibility of bilayers of **2** and **3**, to begin with. When these dispersions, separately prepared, are combined at 15 °C without sonication, their absorption spectra are simply the sum of independent spectra of **2** (λ_{max}; 350 nm) and **3** (λ_{max}; 303 nm), irrespective of whether they are D- or L-isomers. The spectra turn to those of single maxima at 345 nm when the mixtures are incubated. The rate of the spectral change in the combination of bilayers of the same chirality was always faster than that of the opposite chiralities at the same incubation temperature. For example, when bilayer of **3** was added to bilayer of **2** possessing the same chirality, the 350-nm peak is intensified at the expense of the 300-nm peak arising from **3**, and the change completed within two hours at 35 °C (Figs. 1A and 1C). On the other hand, mixtures of bilayers of opposite chiralities show much less changes under the same conditions (Figs. 1B and 1D). The peculiarity of the spectral change caused by the chirality difference was not observed in mixed bilayers of **1** and **3**. The disappearance of the 300-nm peak means mixing of two components of **2** and **3**. Therefore, bilayers of **2** and **3** are more miscible, when they possess the same chirality.

We next investigated separation of binary bilayers into enantiomeric components. The binary bilayers of **2** and **3** were prepared by warming an aqueous dispersion of equimolar amounts of **2** and **3** at 60 °C and incubated for 1 h. Figure 2 displays spectral changes due to cooling of the incubated dispersions. Temperature

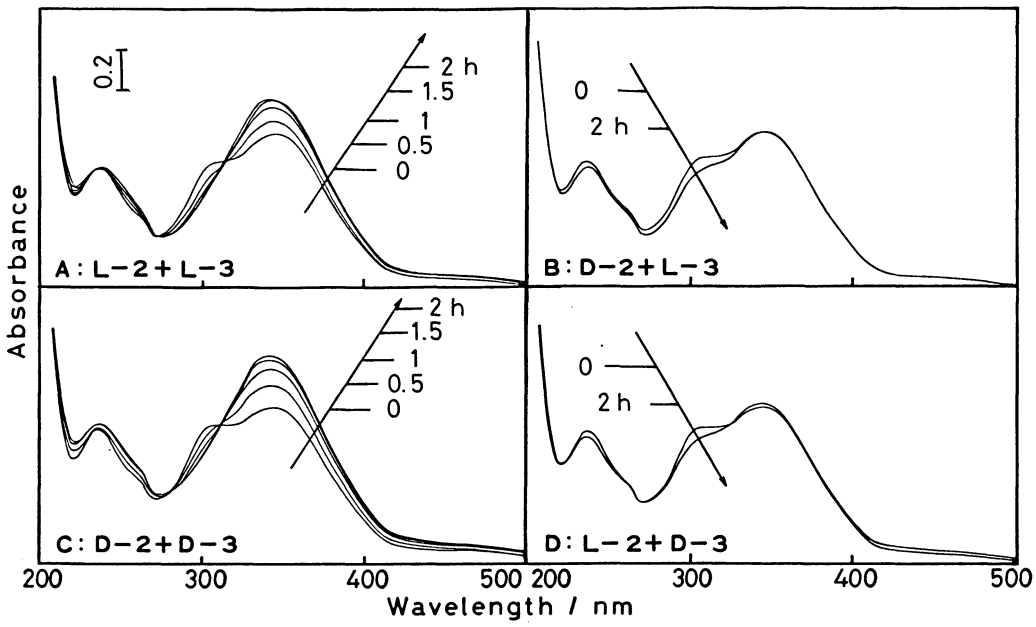


Fig. 1. Spectral changes of mixed bilayer membranes due to incubation at 35 °C. [**2**] = [**3**] = 2.5 x 10^{-5} M. Bilayer **2** was incubated for 4 h at 40 °C to form helices (λ_{max}; 350 nm)and then cooled to 35 °C. Spectral measurement was started upon addition of aqueous dispersions of **3** (λ_{max}; 303 nm).

was lowered at the 10 °C interval and the spectral measurement was made after keeping the sample at the respective temperature for 1 h. The spectral pattern of a binary bilayer of L-**2** and L-**3** is slightly changed by lowering temperature, as shown in Fig. 2A. In contrast, a binary bilayer of D-**2** and L-**3** gives larger spectral changes (Fig. 2B). The increase in the spectral intensity at 300 nm is indicative of the formation of domains of component **2**. A virtually identical spectral change was observed for the chirally reversed combination of L-**2** and D-**3**. It is clear that component separation is promoted between components of opposite chirality.

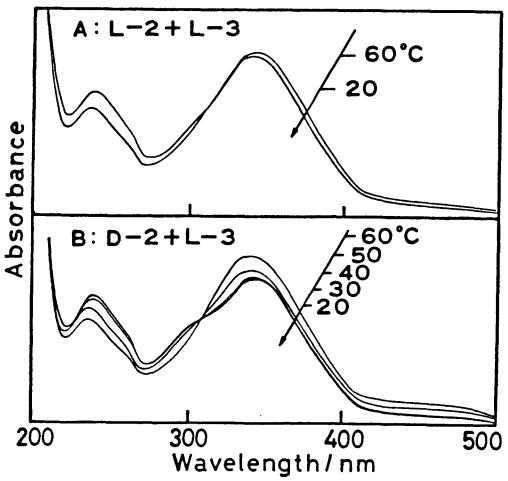


Fig. 2 Temperature dependence of absorption spectra of binary membranes of **2** and **3**. [**2**] = [**3**] = 2.5 x 10^{-5} M.

Separation of racemic bilayer into enantiomeric components was indicated by electron microscopic observation. Figure 3 contains electron micrographs of aqueous dispersions of **2**. Amphiphiles L-**2** and D-**2**, separately, form straight fibrils with a constant diameter of 100 Å (Figs. 3A and 3C). These fibrils are transformed into helices by incubation. A helical sense can be

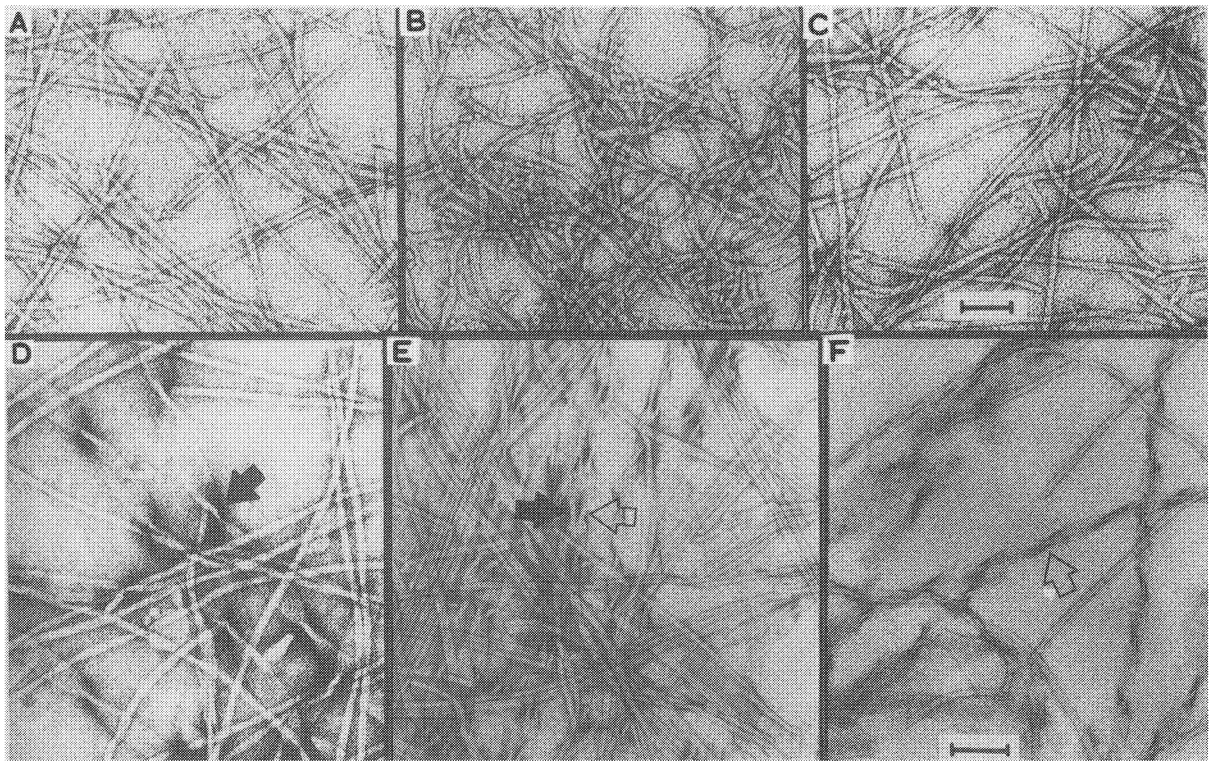


Fig. 3. Electron micrographs of aqueous dispersion of **2** (1 mM) stained by uranyl acetate. Scale bar. (⟷) 1000 Å. Original magnifications: x30000 (A, B, C), x50000 (D, E, F). Arrows point to helices: ⇨(right handed), ➡(left handed). Aging conditions: A, L-**2**, 30 °C, 30 min; B, DL-**2**, 40 °C, 10 min; C, D-**2** 40 °C, 10 min; D, L-**2**, 30 °C, 24 h; E, DL-**2**, 40 °C, 4 h; F, D-**2**, 40 °C, 4 h.

determined from the direction of the shadow of the deposited staining agent as will be discussed elsewhere.[14] The helical senses for L-**2** and D-**2** are left-handed and right-handed, respectively (Figs. 3D and 3F). On the other hand, fibrils initially formed from DL-**2** are irregular in shape and in diameter (100-120 A), which suggest that there are some distortion in this bilayer aggregate composed of chirally different molecules (Fig. 3B). Upon further incubation, both types of the helices become observable as noted by arrows (Fig. 3E), indicating that the racemate is separated into enantiomeric aggregates. Simultaneous observation of opposite helices was also made for an aqueous 5:1-mixture of L-**1** and D-**1** by the use of an optical microscope equipped with dark-field condenser,[15] and for a racemic diacetylenic phospholipid by scanning electron microscopy.[16]

In conclusion, we demonstrated that spontaneous separation of the enantiomeric components occurred in binary bilayers.

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12) Amphiphiles **2** was synthesized in the same procedure as described in reference 3. L-**2**: Found: C, 63.33; H, 8.89; N, 7.08%. D-**2**: Found: C, 62.97; H, 8.91; N, 7.02%. DL-**2**: Found: C, 63.39; H, 8.97; N, 7.14%. Calcd for $C_{41}H_{67}N_4O_4Br \cdot H_2O$: C, 63.30; H, 8.94; N, 7.20%.
Syntheses of **3** were conducted as follows: 4-[4-(11-bromoundecanoyloxy)phenylazo]benzoic acid (**4**) was prepared from 4-(4-hydroxyphenylazo)benzoic acid and 11-bromoundecanoyl chloride in the presence of triethylamine. Dodecyl-L(D)-alaninate p-toluenesulfonate was condensed with **4** by use of diethyl cyanophosphonate in the presence of triethylamine, and the product was quaternized. L-**3**: Found: C, 63.05; H, 8.30; N, 6.94%. D-**3**: C, 63.02; H, 8.30; N, 6.99%. Calcd for $C_{42}H_{67}N_4O_5Br \cdot 0.5H_2O$: C, 63.30; H, 8.60; N 7.03%.
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