  Bull. Chem. Soc. Jpn., 57, 3253—3257 (1984)

Enhanced Circular Dichroism and Phase Separation of Azobenzene-Containing Chiral Bilayer[1)]

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(Received May 30, 1984)

An azobenzene-containing chiral amphiphile showed a drastic enhancement of circular dichroism (CD) due to formation of the rigid bilayer assembly. The drastic CD change was caused by phase transition of the matrix bilayer in the case of mixed bilayers with dihexadecyldimethylammonium bromide, but was caused by phase transition of the chiral component in the case of the single component bilayer. Both CD and absorption spectra of the azobenzene-containing chiral bilayer were used for detecting phase separation of mixed membranes. It was pointed out that the CD technique is widely applicable to phase separation studies.

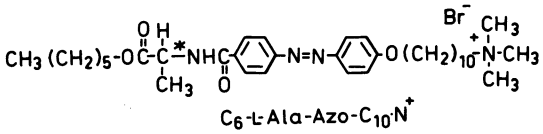
We have shown that synthetic amphiphiles undergo spontaneous formation of bilayers in water.[2,3)] Double-chain ammonium salts are typical examples. In these cases, introduction of the amino acid residue often gives rise to well-developed bilayer assemblies.[4—6)] The amino acid-derived chiral bilayer was found to display very peculiar circular dichroism when the aromatic chromophore was present.[7)] The same is true with bilayers of single-chain amphiphiles, as briefly described.[8)]

Phase separation is one of the most fundamental characteristics of bilayers. The bilayer of the biomembrane is composed of various lipid constituents and their distribution plays critical roles in the control of the physiological function. Detection of the phase separation in the biomembrane has been conducted by calorimetry,[9,10)] the spin probe technique,[11,12)] freeze-fracture electron microscopy,[13)] and recently, by neutron small-angle scattering.[14,15)] In the synthetic system, the spectral shift of the azobenzene chromophore was shown to be especially useful for its detection,[16)] and this technique has been applied to examinations of the interaction of the surface receptor[17)] and of the cluster formation of the reacting species in the catalytic hydrolysis.[18)] The excimer formation of the benzene chromophore was also useful for this purpose.[19)]

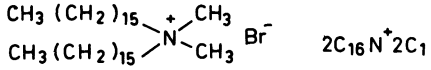
In the present paper, we wish to demonstrate that circular dichroism is a convenient technique for studying phase separation. The large CD enhancement of the chiral bilayer arises from the interaction of spatially fixed chromophores in the gel state and is not observed for fluid (non-interacting) chiral components.[7,8)] Therefore, the CD enhancement can be associated with the cluster formation (in the rigid bilayer matrix). The probe amphiphile used in this study is C_6-L-Ala-Azo-$C_{10}N^+$, and the matrix membrane is $2C_{16}N^+2C_1$. Since this bilayer-forming amphiphile is chiral and contains the azobenzene chromophore, both of CD and absorption spectra are used for detecting phase separation. A comparison of the two spectral methods is discussed.

Experimental

Materials. *p*-(*p*-Hydroxyphenylazo)benzoic acid was prepared by the procedure of Cohen and McGilbery,[20)] mp 246→264 °C (dec) (The arrow indicate the liquid-crystalline range), lit,[20)] mp 268 °C.



C_6-L-Ala-Azo-$C_{10}N^+$



$2C_{16}N^+2C_1$

The benzoic acid was allowed to react with 1,10-dibromodecane to give *p*-[*p*-(10-bromodecyloxy)phenylazo]-benzoic acid in 54% yield after recrystallization from acetic acid/ethyl acetate, mp 195→225 °C. The Williamson product (1 g, 2.2 mmol) and 0.5 g of Na_2CO_3 were added to 10 cm^3 of $SOCl_2$. After the mixture was refluxed for 2 h, excess $SOCl_2$ was removed, and the red-brown residue was condensed with 0.7 g (2.2 mmol) of hexyl-L-alaninate *p*-toluenesulfonate in dry tetrahydrofuran(THF) to give 0.4 g (30% yield) of hexyl *N*-[*p*-[*p*-(10-bromodecyloxy)phenylazo]-benzoyl] alaninate, mp 100—103 °C. Quaternization of this product with trimethylamine in THF produced C_6-L-Ala-Azo-$C_{10}N^+$ in 46% yield, mp room temp→117 °C. The final product was identified by IR and NMR spectroscopies and by elemental analyses. Found: C, 61.2; H, 8.30; N, 8.00%. Calcd for $C_{35}H_{55}N_4O_4Br \cdot H_2O$: C, 61.39; H, 8.24; N, 8.18%.

Preparation of $2C_{16}N^+2C_1$ was described elsewhere.[21,22)]

Measurement. Electron microscopy was performed with a Hitachi H-500 instrument as described before.[2,3)] Differential scanning calorimetry(DSC) was carried out for 20-mmol·dm^{-3} samples with a Daini-Seikosha SSC/560 instrument. The temperature was raised from 0 °C at a rate of 2 °C/min. The details have been described.[22)]

For spectral measurements of single-component bilayers, 5—10 mg of C_6-L-Ala-Azo-$C_{10}N^+$ were dissolved in $CHCl_3$, thin films of the amphiphile obtained by solvent removal were added with distilled water, and the mixture was sonicated for 30 s (Bransonic Cell Disruptor 185) to obtain clear dispersions (5×10^{-4} M, 1 M=1 mol dm^{-3}) and used for spectral measurements after aging for 30 min in ice water.

Aqueous samples of mixed bilayers were similarly prepared from C_6-L-Ala-Azo-$C_{10}N^+$ (5.0×10^{-4} M) and $2C_{16}N^+2C_1$ (5.0×10^{-3} M). The solutions were aged for 10 min at 60 °C and spectral measurements were made after slowly cooling to given temperatures and aging for 20 min. The spectral measurement was subsequently done in the heating process in similar manners.

The absorption and circular dichroism spectra were obtained on a Hitachi 220A spectrophotometer and a JASCO J-40AS spectropolarimeter, respectively. They were equipped with cell holders. The path length of the cell was 1 mm.

Results and Discussion

Electron Microscopy and DSC Study. We have shown that many azobenzene-containing, single-chain amphiphiles form bilayer aggregates.[23] Figure 1 is an electron micrograph of aqueous bilayer of C_6-L-Ala-Azo-$C_{10}N^+$. It is clear that the layered aggregate is formed. The DSC measurement indicates that the aqueous bilayer possess the crystal-to-liquid crystal phase transition at 57 °C (transition range, 40—65 °C) with the enthalpy change of 19.2 kJ/mol.

Absorption and CD Spectra. The absorption spectrum of C_6-L-Ala-Azo-$C_{10}N^+$ possesses maxima at 358 and 234 nm in ethanol and at 359 and 238 nm in the aqueous hexadecyltrimethylammonium bromide (CTAB, 10 mM) micelle, and does not vary with temperature. In contrast, small temperature dependence was found for the aqueous bilayer: λ_{max} are located at 351 and 252 nm at low temperatures and absorption intensities decrease upon raising temperature (Fig. 2a). These reversible spectral changes are apparently related to the phase transition of the bilayer.

The CD spectrum of the amphiphile has the following characteristics: $[\theta]_{355}=+2{,}000$ (maximum) in ethanol, and $[\theta]_{360}=+3{,}000$ (maximum) in CTAB micelle. It is enormously intensified in water as shown in Fig. 2b. At low temperatures, $[\theta]$ is 5.0×10^5 deg·cm^2 dmol^{-1} at 365 nm (maximum) and is -2.9×10^5 at 332 nm (minimum). The spectral intensity decreases drastically at high temperatures (50—70 °C) with sign reversal: $[\theta]_{366}=-1.0\times10^4$ and $[\theta]_{330}=+8.0\times10^3$. The CD enhancement due to temperature reaches fifty fold in magnitude.

The absorption near 350 nm is associated with the transition moment along the long axis of the azobenzene moiety.[24] Therefore, the shape of the low-temperature CD spectrum in this region is indicative of strong exciton coupling among the electric transition moments.[25] The 250-nm absorption corresponds to the transition moment along the short axis of azobenzene. The CD enhancement in this region is smaller by an order of magnitude compared with that in the 350-nm region: $[\theta]_{267}=-50{,}000$ and $[\theta]_{250}=-18{,}000$. However, these values are still greater than those in ethanol (no bilayer formed) or those at high temperatures. The exciton coupling is also apparent.

Figure 3 is obtained by plotting $[\theta]_{365}$ against temperature. The $[\theta]_{365}$ value remains constant at 10—35 °C and drastically decreases at 45—50 °C. As mentioned in the previous section (DSC data), the phase transition of the C_6-L-Ala-Azo-$C_{10}N^+$ bilayer starts at 45 °C and the DSC peak is located at 57 °C. Therefore, the CD enhancement is lost almost completely at the temperature region where the chain melting starts. Virtually the same change is observed in the heating cycle, and hysteresis is essentially absent. It must be noted, however, that the CD in-

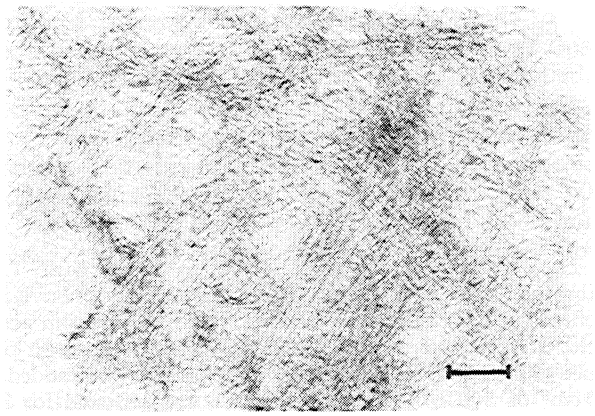


Fig. 1. Electron micrograph of C_6-L-Ala-Azo-$C_{10}N^+$ stained by uranyl acetate, 10 mM; initial magnification, × 40,000, scale bar indicates, 1000 Å.

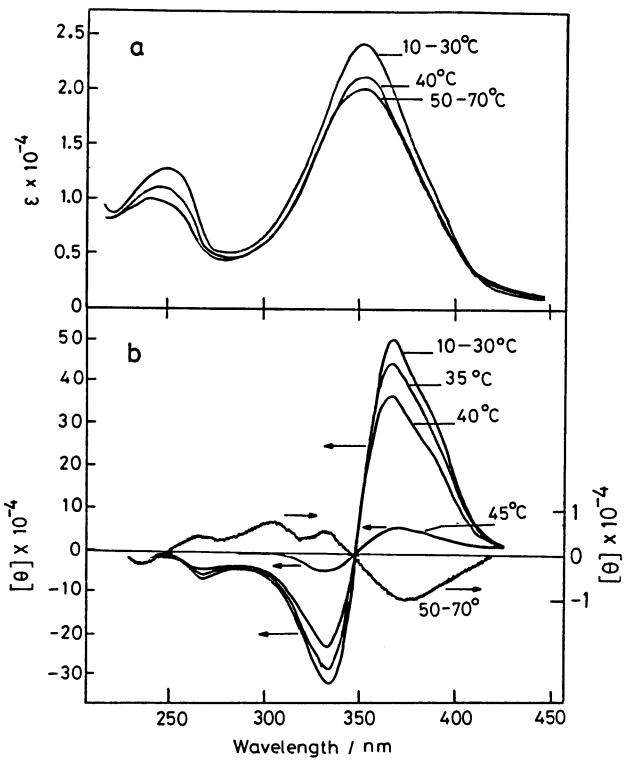


Fig. 2. Temperature dependence of absorption (a) and CD (b) spectra. C_6-L-Ala-Azo-$C_{10}N^+$, 5×10^{-4} M, heating cycle.

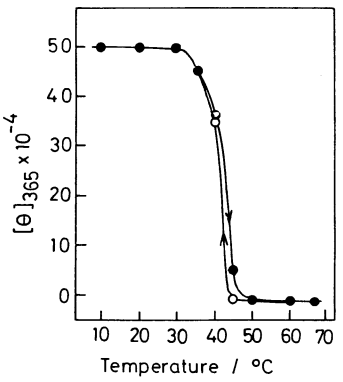
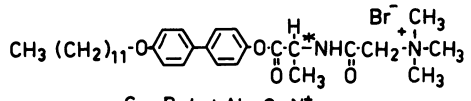


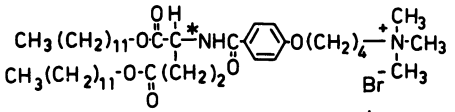
Fig. 3. Temperature dependence of CD intensity. ○: cooling cycle, ●: heating cycle C_6-L-Ala-Azo-$C_{10}N^+$, 5×10^{-4} M.

tensity of the liquid-crystalline bilayer is larger than that of the isolated molecule in ethanol or in CTAB micelle.

These results (exciton coupling and temperature dependence) are essentially the same as those described briefly for bilayers of a biphenyl-containing single-chain amphiphile (C_{12}-Bph-L-Ala-C_2N^+)[8] and a benzene-containing double-chain amphiphile ($2C_{12}$-L-Glu-ph-C_4N^+).[7]



C_{12}-Bph-L-Ala-C_2N^+



$2C_{12}$-L-Glu-ph-C_4N^+

Spectral Changes of Mixed Bilayer. Table 1 summarizes the $[\theta]_{365}$ values of mixed bilayers of C_6-L-Ala-Azo-$C_{10}N^+$ and $2C_{16}N^+2C_1$ at 10 °C. The $[\theta]_{365}$ value decreases with decreasing molar ratios of the chiral component. The CD enhancement is observed, when the molar ratio is not smaller than 1/10.

The enhancement is totally lost with the molar ratios of less than 1/20. As discussed above, the CD enhancement is produced by the exciton coupling of the fixed azobenzene chromophores. Therefore, the CD enhancement in the two-component bilayer indicates the presence of the cluster of the chiral amphiphile. The data of Table 1 suggest that the cluster is only partially formed at the molar ratio of 1/10, and this molar ratio was used in the subsequent experiments.

Table 1. Influence of the composition of a mixed bilayer on CD intensity[a]

$[C_6$-L-Ala-Azo-$C_{10}N^+]/[2C_{16}N^+2C_1]$	$[\theta]_{365}\times10^{-4}$
chiral component only	50
1/2	40
1/5	21
1/10	14
1/20	*ca.* 0.1
1/30	*ca.* 0.1

a) $[C_6$-L-Ala-Azo-$C_{10}N^+]=5\times10^{-4}$ M (constant), 10 °C

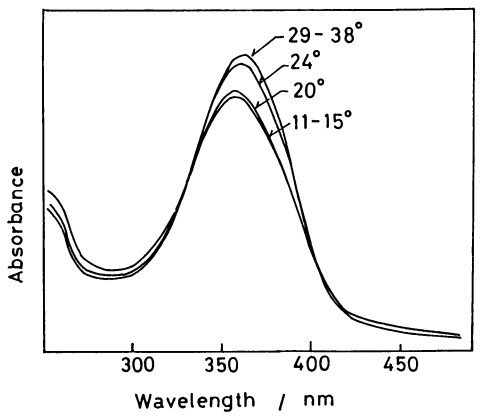


Fig. 4. Temperature dependence of absorption spectra of mixed bilayers. C_6-L-Ala-Azo-$C_{10}N^+$, 5×10^{-4} M, $2C_{16}N^+2C_1$, 5×10^{-3} M, cooling process.

A mixed (1:10) bilayer of C_6-L-Ala-Azo-$C_{10}N^+$ and $2C_{16}N^+2C_1$ was subjected to aging at 60 °C, and absorption and CD spectra were measured in the cooling and, subsequently, heating processes. The absorption spectra in the cooling process are displayed in Fig. 4. Although the overall spectral change is not remarkable, its temperature dependence is more clearly seen in Fig. 5 by plotting the maximum absorbance (A_{max}) and the location of λ_{max} against temperature. Both of A_{max} and λ_{max} change critically at 20—25 °C. Virtually the same temperature dependence was observed in the heating process. The CD spectral shape of the mixed bilayer is the same as those illustrated in Fig. 2b, however, its temperature dependence is different. In the cooling process, the enhanced CD is not observed from 60 °C to 26 °C, and the enhancement starts at *ca.* 22 °C. The temperature dependence of $[\theta]_{365}$ of the mixed bilayer in this process is illustrated in Fig. 6, together with that in the heating process (from 10 °C). The $[\theta]_{365}$ value drastically changes at *ca.* 25 °C in both processes, and this critical temperature agrees with those in absorption spectra (Fig. 5). The hysteresis is small in this system as well.

The 1:10 mixed bilayer gives an DSC peak at 28 °C. This T_c value is almost identical with that of the single-component bilayer of $2C_{16}N^+2C_1$.[22] The DSC peak corresponding to the bilayer of C_6-L-Ala-Azo-$C_{10}N^+$ (57 °C) was not detected. Apparently the two components exist as separate clusters in the rigid bilayer matrix of $2C_{16}N^+2C_1$, but the chiral component cannot form separate domains in the fluid $2C_{16}N^+2C_1$ matrix.

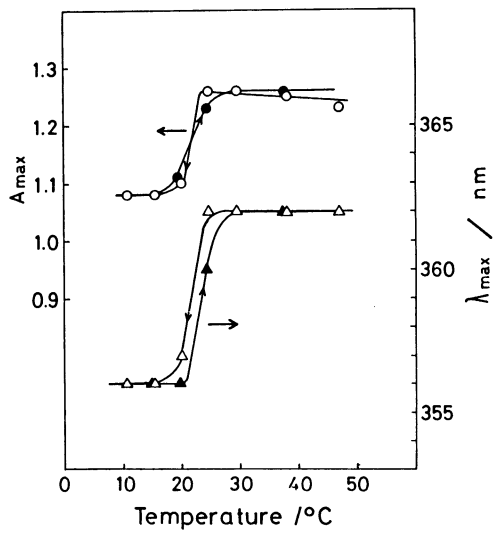


Fig. 5. Plots of A_{max} and λ_{max} against temperatures. C_6-L-Ala-Azo-$C_{10}N^+$, 5×10^{-4} M, $2C_{16}N^+2C_1$, 5×10^{-3} M, ○: cooling process, ●: heating process.

Phase Separation and CD Enhancement. The DSC behavior of the mixed bilayer is consistent with the spectral data. The CD enhancement is not observed at 30—40 °C where DSC data suggest dispersion of C_6-L-Ala-Azo-$C_{10}N^+$ molecules in the fluid $2C_{16}N^+2C_1$ bilayer. The bilayer cluster of the chiral amphiphile would have produced enhanced CD at these temperatures (see Fig. 2b). The phase separation occurs at 22—26 °C where the $2C_{16}N^+2C_1$ bilayer undergoes the liquid crystal-to-crystal phase transition. The domain of the C_6-L-Ala-Azo-$C_{10}N^+$ bilayer is in the crystalline state at these temperatures, and therefore, enhanced CD results. It is noteworthy that the drastic CD change is caused by *phase transition of the matrix bilayer* in the case of the mixed bilayer, but that it is caused by *phase transition of the chiral component* in the single-component bilayer system. It is possible, therefore that mixed bilayers are selected in such a way that large CD changes occur at desired temperatures. These situations are illustrated schematically in Fig. 7.

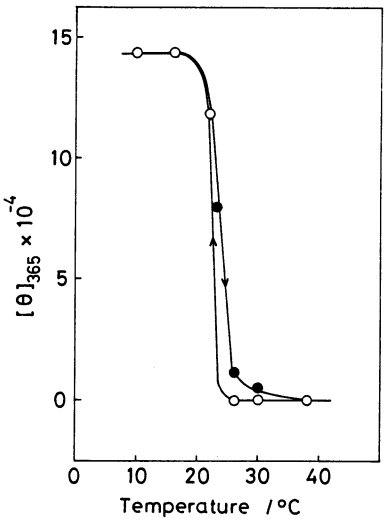


Fig. 6. Temperature dependence of $[\theta]_{365}$ of the mixed bilayer.
C_6-L-Ala-Azo-$C_{10}N^+$, 5×10^{-4} M, $2C_{16}N^+2C_1$, 5×10^{-3} M, ○: cooling process, ●: heating process.

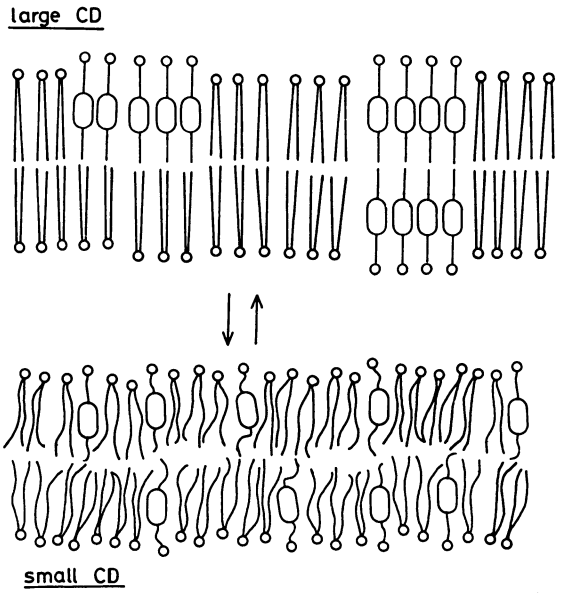
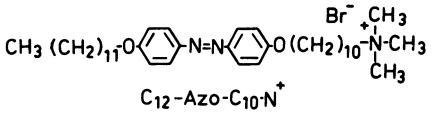


Fig. 7. Schematic illustration of the phase separation.

It was reported from these laboratories that azobenzene-containing bilayers give rise to varied spectral characteristics depending on membrane physical states and chemical structures of the component.[23] For instance, C_{12}-Azo-$C_{10}N^+$ possesses λ_{max} at 330 nm as bilayer clusters and λ_{max} at 355 nm as an isolated chromophore. This spectral shift has been used to detect phase separation. The azobenzene amphiphile used in this study unfortunately displays only a very small difference in absorption spectra, which is not sensitive enough to probe phase separation. Instead, much enhanced CD spectra of the rigid chiral bilayer are a powerful tool for phase separation studies.



Conclusion

An azobenzene-containing, chiral amphiphile exhibited remarkable CD enhancements due to formation of rigid bilayers. This phenomenon was used to study the phase separation behavior in mixed bilayers. Phase separation of synthetic bilayers has been examined by the DSC technique,[18,19] absorption spectroscopy,[16] and excimer formation.[19] The CD technique becomes the fourth methods. The present CD results are consistent with those of the absorption spectrum of the azobenzene chromophore in that phase separation is induced by the liquid crystal-to-crystal phase transition of the matrix bilayer. The spectral shift of chromophores is usually not large enough to made detection of phase separation possible. In contrast, the CD enhancement is observed for many chiral bilayers which possess aromatic chromophores. This indicates that the CD technique is widely applicable to phase separation studies.

We are grateful to Professor K. Yamafuji for the use of a spectropolarimeter.

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1) Contribution No. 733 from Department of Organic Synthesis.
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