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Photofluidization of Phospholipid Membrane Induced by Isomerization of Azobenzene Amphihiles at Varying Depth in the Membrane

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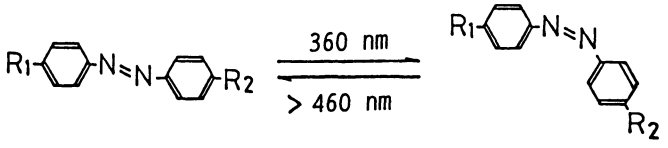
Induced circular dichroism (ICD) in the mixed bilayer of azobenzene amphiphiles with dipalmitoyl-L-α-phosphatidylcholine was closely related to membrane fluidity controlled by trans-cis photoisomerization of the chromophore. Degree of the photochemical perturbation on membrane properties was the strongest when the azobenzene moiety was located in the middle of the long alkyl chain.

The mode of interaction of foreign molecules such as metal ions, surfactants, aneasthetic drugs and proteins with phospholipid bilayers is a current topic[1] in view of controlling membrane properties relevant to biological and physiological applications. Jain proposed that the mode of interaction could be classified into four categories according to the location of the structural perturbers; near the polar group(Type 1), near the glycerol backbone(Type 2), in the C_1-C_8 region(type 3) and in the region between C_9 to the terminal methyl group of acyl chain(Type 4).[2] For the purpose of understanding the dynamic action of a perturber buried in different depth, perturbation should be generated instantaneously <u>in situ</u>, which is only possible by means of a photochemical process.

In this study, we are going to describe the changes in membrane fluidity brought about by a sudden structural change(i.e. photoisomerization) of azobenzene containing amphiphiles incorporated in varying depth in the membrane of dipalmitoyl-L-α-phosphatidylcholine bilayer. The structural change in the membrane was reflected sharply in the degree of induced circular dichroism(ICD) appearing at the absorption wavelength of azobenzene chromophore.



trans form cis form

R_1 : $-O(CH_2)_m O\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}O(CH_2)_2\overset{+}{N}(CH_3)_3$

R_2 : $-O(CH_2)_{n-1}CH_3$

	1	2	3
m	3	6	10
n	12	9	5

Apart from the interest in dynamic modulation of membranes of biological relevancy, photochemically triggered phase change is another important aspect since the concept will lead to a new approach to amplified image recording as has been demonstrated in micelles and liquid crystals.[3,4]

The amphiphiles containing azobenzene moieties, 1-3, were prepared based on the procedure reported by Okahata et al.[5] Dipalmitoyl-L-α-phosphatidylcholine (l-DPPC) was obtained from Sigma Chemical Co. The lipid was used without further purification. The mixed liposomal solutions were prepared by the ethanol-injection method[6] in order to obtain completely transparent solution. The concentrations of azo-amphiphile and l-DPPC were 5 x 10^{-5} M and 5 x 10^{-4} M, respectively. ICD spectra were recorded on a JASCO J-500 spectropolarimeter at 25 °C. Calorimetric scans were performed on a SEIKO I&E SSC-5000 calorimeter operating at a heating rate of 1 °C/min or 5 °C/min. The heating curves were shown in Fig. 2. Lipid suspensions were prepared by vortex agitation of dry lipids with 50 mM tris-HCl buffer (pH 7.4) above the phase transition temperature of l-DPPC. A total lipid concentration of 1 wt% was used in the calorimetric experiments. Trans-cis photoisomerization was carried out after preparation of lipid suspensions with a monochromatized light from a 500 W xenon lamp.

Three kinds of the amphiphiles having the same head group as DPPC and an azobenzene moiety (trans form) in different sites of a hydrocarbon tail were incorporated into the bilayer membrane of l-DPPC. When the azobenzene moiety was located near the head group (1) or at the center of the hydrocarbon tail (2), the azobenzene amphiphiles were successfully incorporated into the bilayer membrane: no individual micelle formation nor stacking of the chromophores in the bilayer was observed at 25 °C by absorption spectra.[7] On the other hand, an amphiphile containing the azobenzene moiety located near the end of tail (3) was found not to form a stable mixed bilayer. Immediately after preparation, 3/DPPC dispersion was completely transparent, but on standing for a short period it became turbid, indicative of the instability of the 3/DPPC system. The amphiphiles 1 and 2 exhibited a positive ICD with λ_{max} = 360 nm in l-DPPC bilayer, while no ICD was observed when they were incorporated into dl-DPPC bilayer membrane. The intensity of ICD ($[\theta]_{max}$) was found to depend strongly on temperature in both 1/DPPC

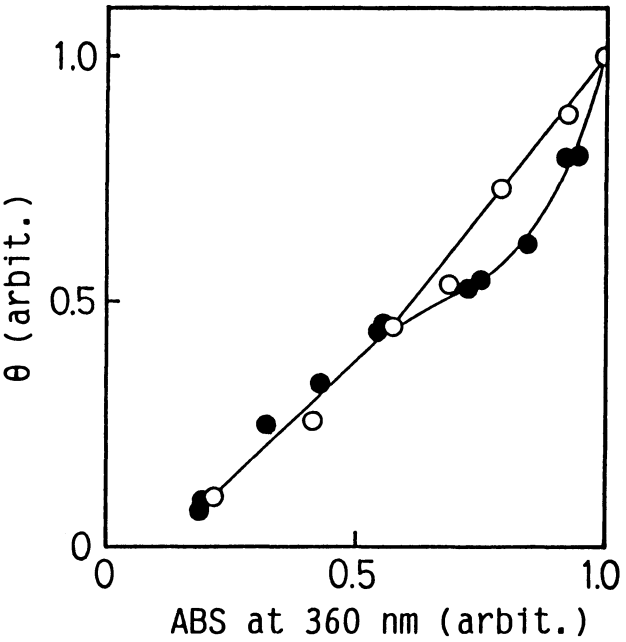


Fig. 1. Changes in ICD on photoirradiation of l-DPPC bilayer membranes containing azo-amphiphiles at 25 °C. Trans content is expressed by the absorbance at 360 nm, a characteristic absorption band of trans isomer.
[Azo] = 5x10^{-5} M, [l-DPPC] = 5x10^{-4} M;
1/l-DPPC, ○ ; 2/l-DPPC, ●

and 2/DPPC mixtures. At low temperatures (15-20 °C), $[\theta]_{max}$ values were 2 x 10^4 deg·cm^2/dmol in both cases. $[\theta]_{max}$ decreased drastically with increasing temperature, and no ICD was detected above 35 °C, which is close to the gel-to-liquid crystalline phase transition temperature (T_m) obtained from DSC measurement. These observations strongly suggest that the ICD observed at the $\pi - \pi^*$ transition region of trans azobenzene chromophores arises from the closely-packed 1-DPPC molecules whose mobility in the membrane is highly restricted.[8,9]

Trans → cis photoisomerization of the azobenzene moiety of 1/1-DPPC and 2/1-DPPC bilayers reduced the intensity of ICD as shown in Fig. 1. After prolonged irradiation, absorbance at λ_{max} became as low as 5% of the initial value. Decrease in absorbance at 360 nm for cis isomer can not fully account for the observed decrease in θ. Structural perturbation arising from the cis isomer (bent form) upon the closely-packed matrix molecules is another factor to reduce the ICD. Trans → cis isomerization of 2 caused quite different effects on the loss of ICD from that of 1. Change in θ was well related to the amount

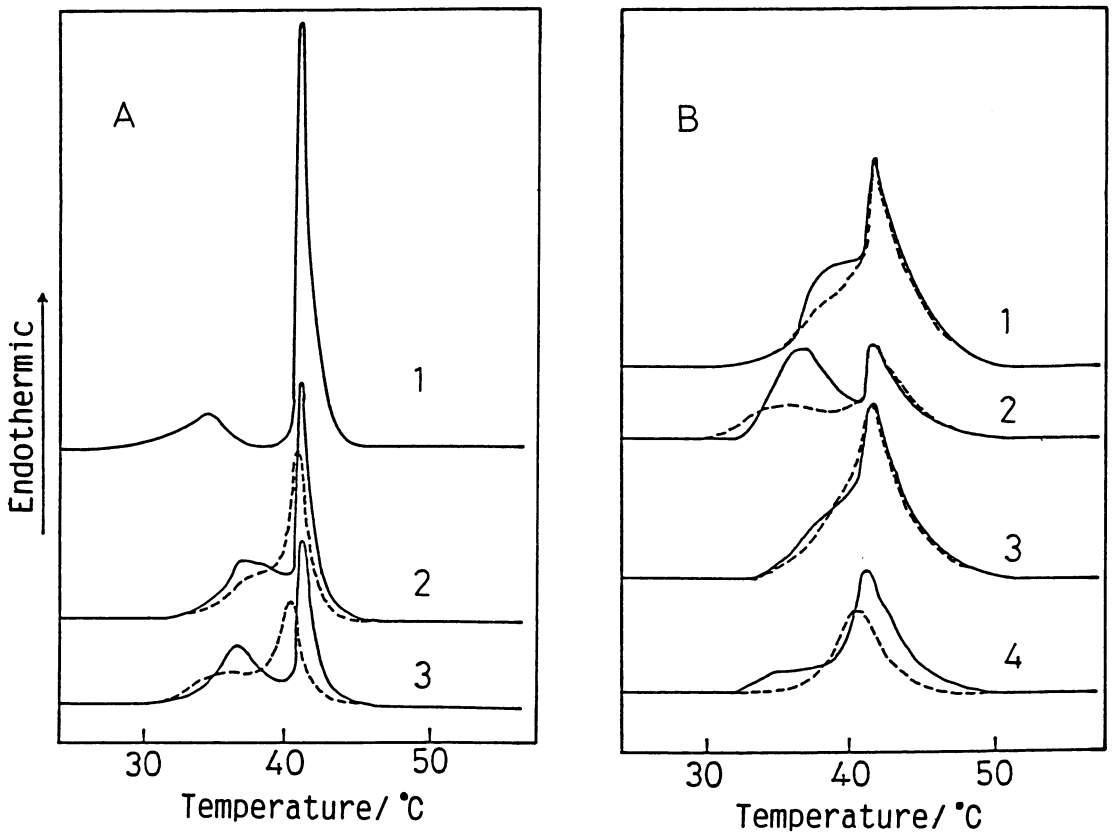


Fig. 2. Differential scanning calorimetry thermograms of 1-DPPC bilayer membranes containing azo-amphiphiles.
A; Effects of trans isomer on the 1-DPPC membrane measured at a heating rate of 1 °C/min.
1, pure DPPC; 2, [Azo]/[DPPC] = 1/10; 3, [Azo]/[DPPC] = 2/10
———— , 1/1-DPPC; — — — — — , 2/1-DPPC
B; Effects of trans-cis isomerization on the 1-DPPC membrane measured at a heating rate of 5 °C/min. 1, [1]/[DPPC] = 1/10
2, [1]/[DPPC] = 1/1; 3, [2]/[DPPC] = 1/10; 4, [2]/[DPPC] = 1/1
———— , trans isomer; — — — — — , cis isomer

of trans → cis isomerization in the case of 1/l-DPPC, whereas a larger decrease in θ was observed at the initial stage of the photoreaction in the case of 2/l-DPPC. This non-linear response of θ against the amount of photoreaction in 2/l-DPPC demonstrates site-dependent perturbation on membrane structure. This is well explicable on the basis of calorimetric measurements (Fig. 2). Reverse photoisomerization (cis → trans) restored the ICD in both systems, indicating that the change in ICD was completely reversible.

As shown in Fig. 2A, the mixed membrane of the trans isomer of 1 and l-DPPC gave two separate endothermic peaks on heating. The sharp peak at 40.8 °C corresponds to that of pure DPPC (40.9 °C), suggesting phase separation; one domain is composed of a random mixture of 1 and DPPC and the other is a pure DPPC domain. With the procession of trans → cis isomerization, the peak height at lower temperature side alone was reduced while the another was unaffected (Fig. 2B, curves 1 and 2). This is the manifestation that the structural change in the azobenzene moiety does not extend to the DPPC domain. On the other hand, the trans-2/DPPC membrane exhibited a broad peak with a small shoulder which shifted to lower temperature compared with that of pure DPPC, implying that the two components are molecularly mixed and the packing of DPPC is disordered in the presence of 2. Moreover, Fig. 2B (curves 3 and 4, dashed lines) revealed that the trans → cis photoisomerization brought about further shift and broadening.

These effects will account for the non-linear photoresponse shown in Fig. 1. In 1/l-DPPC, each domain containing 1 is isolated so that molecular environment responsible for ICD is not affected by trans → cis isomerization occuring in other domains. Consequently, the intensity of ICD is proportional to the chromophore concentration. In 2/l-DPPC, however, ICD of remaining trans isomers is non-lineraly related to the degree of trans → cis isomerization, since disordering of the molecular arrangement of DPPC results abruptly by a small portion of photoisomerization to cis-azobenzene. This suggests a possibility of amplifying photoresponse.

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