CHEMISTRY LETTERS, pp. 421-424, 1980.

PHOTORESPONSIVE MEMBRANES. REGULATION OF MEMBRANE PROPERTIES BY PHOTOREVERSIBLE cis-trans ISOMERIZATION OF AZOBENZENES

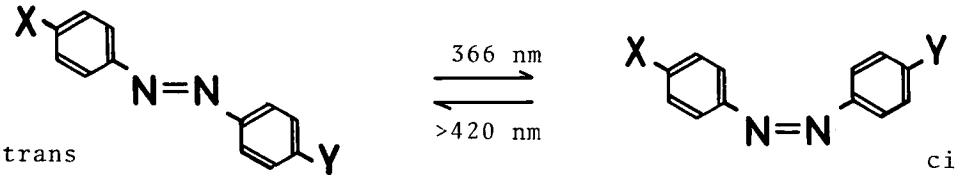
Koji KANO, Yasuhiko TANAKA, Teiichiro OGAWA, Masatsugu SHIMOMURA*, Yoshio OKAHATA*, and Toyoki KUNITAKE*

*Department of Molecular Science and Technology, Graduate School of Engineering Sciences, Kyushu University and *Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812*

Photoresponsive membranes have been constructed by incorporating the amphiphatic alkylammonium salts containing azobenzene chromophores into dipalmitoylphosphatidylcholine liposomes.

Several light-regulated processes have been known in the biological system. Visual excitation is initiated by light absorption of a visual pigment, rhodopsin, in the receptor cell membrane. Electronic excitation of rhodopsin leads to isomerization of 11-cis-retinal to the all-trans-form. This is followed by conformational changes of the protein (opsin), the permeation of Ca^{2+} across the membrane, and the conductance change in the retinal photoreceptor cell membrane.[1] The model systems have been widely studied by using artificial lipid membranes, such as bilayer lipid membranes (BLM)[2] and liposomes,[3] to deduce the mechanism of the vision. Phytochrome is also a photoresponsive bile pigment to control the life of plants. It has been assumed that the photoinduced configurational change of the bilitriene structure provides the conformational change of phytochrome protein, which influences the permeability of the phytochrome loading membrane.[4]

These photobiological aspects lead us to study the construction of the completely artificial photoresponsive membrane systems. To utilize light as an on-off switch, the photoreceptor has to have the following features: 1) the photoinduced configurational change is reversible and reproducible, 2) the configurational change provides the large geometrical change, and 3)the quantum yields of the photoreactions are high. In this study, azobenzene was chosen as a photoresponsive chromophore which fits our aim. trans-Azobenzene derivatives are very close to planar in



trans cis

structure. In contrast, one of the benzene rings of the cis-isomer occupies a plane that places it at an angle of about 56° from the plane of the azo nitrogens and the other ring.[5]

The amphiphatic azobenzenes used in the present study were listed and abbreviated as the following:[6]

C_2Azo: X = $Br^-(CH_3)_3\overset{+}{N}-(CH_2)_2-O-$, Y = $CH_3-(CH_2)_{11}-O-$
C_4Azo: X = $Br^-(CH_3)_3\overset{+}{N}-(CH_2)_4-O-$, Y = $CH_3-(CH_2)_{11}-O-$

Sonication (bath-type, Branson B-12, 80-W) of a mixture of dipalmitoyl-D,L-α-phosphatidylcholine (DPPC) and an azobenzene derivative in water produced an almost clear solution. During sonication, the temperature of the sample solution was kept at 50-60°C and nitrogen gas was bubbled through the sample solution. The negative-stain electron micrographs showed the formation of unilamellar vesicles with diameter of 250-300 Å at the mole ratio ([azo]/[DPPC]) up to 1/5. The vesicle structure disappeared when the mole ratio was greater.

Upon irradiation of the 366-nm light (an Ushio 500-W super high-pressure Hg lamp with a Toshiba UV-D35 filter), the absorption of trans-azobenzene chromophore (λ_{max} = 358 nm) in DPPC membrane disappeared together with the appearance of the absorption of the cis-isomer (λ_{max} = 442 nm). At the photostationary state, more than 80% of trans-azo derivatives was converted to the cis-isomers. This configurational change was photoreversible. The cis-isomers quantitatively regenerated the trans-isomers upon irradiation with >420-nm light.

The difference in the membrane feature between the liposomes loaded trans- and cis-azobenzene derivatives was studied by measuring the rates of osmotic shrinkage and/or of release of bromothymol blue (BTB) from membranes.

Osmotic Shrinkage. The unilamellar vesicles were prepared from 7.3 mg of DPPC and appropriate amounts of C_2Azo and/or C_4Azo in 2.0 ml of water. The liposome solution was diluted by 10-fold with water. Osmotic shocks were provided by rapidly injecting 50 μl of 1.0 M NaCl into 2.0 ml of the liposome solutions placed in a thermostated quartz cell (1 cm^2). The final concentration of the hyperosmolar NaCl solution was 2.44 x 10^{-2} M. Rates of the turbidity change were followed at 220 nm by a JASCO UVIDEC 505 spectrophotometer. Figure 1 shows the typical time-dependent

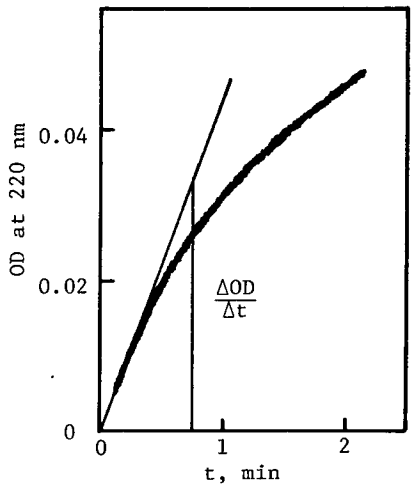


Fig. 1. Time-dependent absorption change of trans-C_4Azo loading DPPC liposomes at 25°C: [trans-C_4Azo]/[lipid] = 1/10. The optical density at t = 0 was chosen arbitrarily.

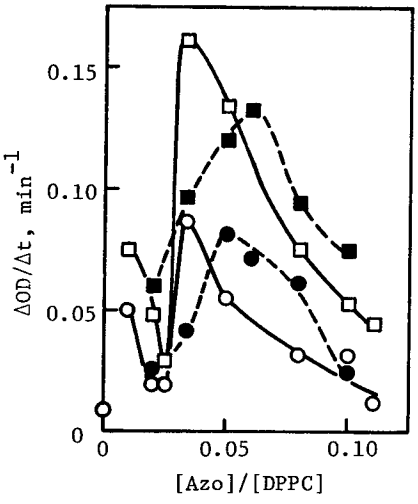


Fig. 2. Effects of the azobenzene derivatives on the shrinkage rates of the DPPC liposomes at 25°C: —○—, trans-C_4Azo; —□—, cis-C_4Azo; --●--, trans-C_2Azo; --■--, cis-C_2Azo. The cis-isomers were prepared by irradiating the liposome solutions at 366-nm light for 5 min.

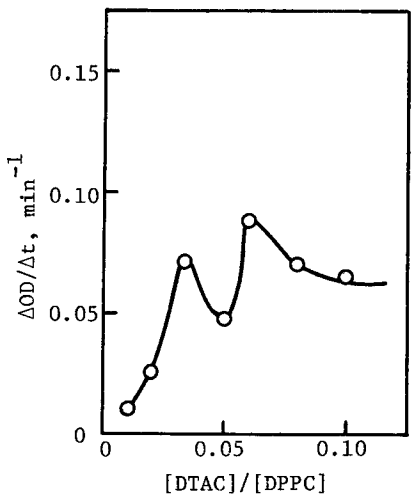


Fig. 3. Effects of DTAC on the shrinkage rates of DPPC liposomes at 25°C.

differencial absorbance change of the C_4Azo loading DPPC liposome solution after an osmotic shock. The rate of the osmotic shrinkage ($\Delta OD/\Delta t$) was defined as shown in Fig. 1. The effects of entrapped trans- and cis-azo derivatives on the shrinkage were complex as shown in Fig. 2. At the all azo concentration range, however, it was clearly indicated that the shrinkage rate of the cis-isomer loading liposomes was much faster than those of the trans-isomer loading liposomes. In the case of C_4Azo, the two optimum conditions were observed in the osmotic shrinkage rates. As Fig. 3 shows, the alkylammonium salt without the azobenzene chromophore (dodecyltrimethylammonium chloride, DTAC) entrapped in the membrane also accelerated the osmotic shrinkage of DPPC liposomes and provided two maximum shrinkage rates when the amounts of entrapped DTAC were varied. Therefore, the complex shape in the C_4Azo concentration profile for the osmotic shrinkage may be due to the influence of the positively charged head groups of C_4Azo, not the azobenzene chromophore itself.

The enhanced shrinkage by cis-azobenzene derivatives may be interpreted in term of channel formation for water permeation across the membrane. Since the trans-azobenzene derivatives are very close to planar and linear in structure, it is likely that the structure of the lipid bilayers is not perturbed by the coexisting azobenzene derivatives. On the other hand, the geometry of the cis-isomers requires the separation between the molecules of amphiphatic alkylated azobenzenes and phospholipid to provide the channel. In this step, the long alkyl chains bound with azobenzene play an important role. There is no difference in shrinkage rates between trans- and cis-azobenzene loading liposomes. The long alkyl chains seem to function like protein in the photoresponsive biological phenomena (vide supra). The deceleration in the osmotic shrinkage at higher concentrations of the azo derivatives may be ascribed to the permeation of inorganic salt (NaCl) across the membrane due to the formation of larger channels.

Bromothymole Blue Release from Membrane.

The channel formation in the case of the liposomes containing the cis-azobenzene derivatives was supported by the following results. A mixture of 7.3 mg of DPPC and appropriate amounts of C_4Azo and/or C_2Azo and BTB in 2.0 ml of aqueous KH_2PO_4 (0.01 M) solution (pH 5.0) containing 0.1 M NaCl was sonicated and the resulting liposome solution was passed through a Sephadex G-50 column to remove free BTB. In all cases, the entrapment of BTB into the DPPC unilamellar liposomes was almost 100%. The liposome solution was diluted by 6-fold with the same buffer. The same volumes of the liposome and NaOH (pH 12.0) solutions were mixed and introduced to a thermostated quartz cell (1 cm^2) by using a Union Giken mixing apparatus. The pH-values of the mixed solutions were ca. 9.5. At lower pH, BTB is so hydrophobic that it is kept in the lipid bilayer. BTB is in the dianion form at higher pH and becomes soluble in water. The rate

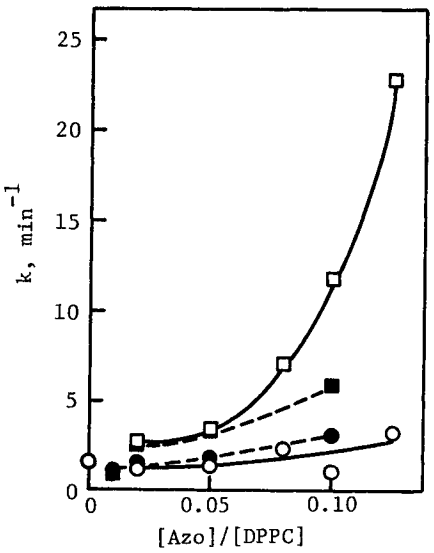
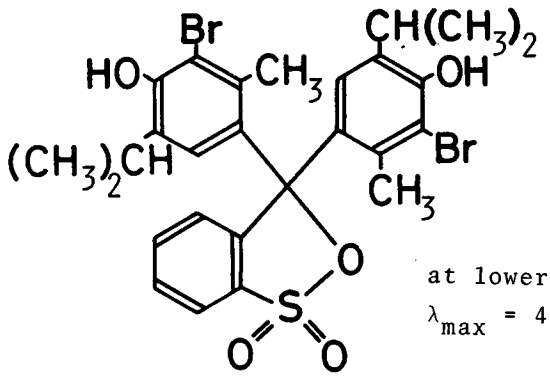
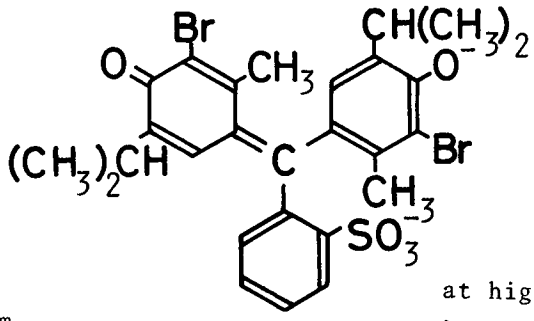


Fig. 4. Effects of azobenzene derivatives on the rates of the BTB release from DPPC liposomes at 25°C: —○—, trans-C_4Azo; —□—, cis-C_4Azo; --●--, trans-C_2Azo; --■--, cis-C_2Azo. The cis-isomers were prepared by irradiating the liposome solutions at 366-nm light for 5 min.



at lower pH
λ_{max} = 418 nm



at higher pH
λ_{max} = 617 nm

of the BTB release from the liposomal membranes was followed by measuring the optical density changes of the BTB dianion at 617 nm. The first-order rate constants for the BTB release (k) were determined by using the following equation:

$$k = \frac{1}{t}\ln\left(\frac{OD_\infty - OD_0}{OD_\infty - OD_t}\right) \qquad (1)$$

where OD is the optical density at 617 nm and the subscripts 0, t, and ∞ represent the times after the external pH of the liposome solution was changed. Figure 4 shows the effects of the trans- and cis-azobenzene derivatives on the BTB release. For both of C_2Azo and C_4Azo, the rate of BTB release from the cis-isomer loading liposomes was larger than those from the trans-isomer loading liposomes. Especially, the BTB release was markedly accelerated in the case of the cis-C_4Azo loading liposomes at the mole ratios of C_4Azo to lipid above 0.05. This C_4Azo concentration is similar to that where the rate of shrinkage starts to decrease (see Fig. 2). This finding suggests that the large channels in the liposomal membranes are formed at the higher concentrations of cis-C_4Azo. Inorganic salt (NaCl) and/or BTB leak to the external aqueous phase by permeating through the channels provided by the cis-C_4Azo.

This is the first report on the photoresponsive lipid bilayer membrane ever constructed by completely artificial materials. The detailed study is in progress.

References and Note

1) W. A. Hagins, Ann. Rev. Biophys. Bioenerg., 1, 131 (1972).
2) H. Ti Tien, Bioelect. Bioenerg., 5, 318 (1978).
3) D. F. O'Brien, Photochem. Photobiol., 29, 679 (1979).
4) S. B. Hendricks and H. A. Bortwick, Proc. Natl. Acad. Sci. USA., 58, 2125 (1967).
5) J. Bieth, S. M. Vratsanos, N. H. Wassermann, A. G. Cooper, and B. F. Erlanger, Biochemistry, 12, 3023 (1973).
6) The preparation of C_4Azo and C_2Azo will be reported elsewhere.

(Received February 8, 1980)