Photoresponsive Permeation Characteristics of a Ternary Composite Membrane of Polymer/Artificial Lipid/Azobenzene Derivative

Atsushi KUMANO, Osamu NIWA, Tisato KAJIYAMA,[†]
Motowo TAKAYANAGI, Toyoki KUNITAKE,*
and Koji KANO**

*Department of Applied Chemistry and *Organic Synthesis,
Faculty of Engineering, Kyushu University,
6-10-1, Hakozaki, Higashi-ku,
Fukuoka 812, Japan*

***Faculty of Engineering, Doshisha University,
Kyoto 602, Japan*

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ABSTRACT: A ternary composite membrane composed of polymer/artificial lipid/azobenzene derivative was prepared by casting from a tetrahydrofuran solution of poly(vinyl chloride) (PVC), dioctadecyldimethylammonium bromide (DOAB) and *p*- ω -hydroxypropyloxy-*p*'-dodecyloxy azobenzene (AZO). AZO dispersed almost molecularly within the multilayer liposome of artificial lipid (DOAB), and even in the ternary composite membrane, AZO exhibited *trans*-*cis* photoisomerization in a similar manner to that in solution. The water or ion permeations through the composite membrane apparently increased upon the irradiation of ultraviolet (UV) light when artificial lipids were in a liquid crystalline state. The water permeability coefficient, *P* depended on the *trans*-fraction of AZO. This suggests that the conformational change due to the *trans*-*cis* photoisomerization of AZO makes compact packing of artificial lipid molecules around AZO loosed, being followed by formation of permeation channel.

KEY WORDS Ternary Composite Membrane / Poly(vinyl chloride) / Artificial Lipid / Dioctadecyldimethylammonium Bromide / Azobenzene Derivative / Photoisomerization / Thermal Isomerization / Photoresponsive Permeation / Permeation /

Lipids in biological systems are amphiphiles composed of both hydrophobic group of two alkyl chains and hydrophilic group of phospholyl group. Biological membranes are in a liquid crystalline state on which their permeation properties depend.^{1,2} One of authors has succeeded in synthesis of totally artificial lipids as a biomembrane model.³⁻⁵ The artificial lipid/water system showed the similar phase diagram to that of biological lipids.⁶ The composite membrane composed of polymer and artificial lipid exhibited a distinct jump of

the water and ion permeability coefficients in the vicinity of crystal-liquid crystal phase transition temperature.⁷ These results apparently indicate that the diffusion process of permeable substances through the membrane is closely related to the thermal molecular motion of lipid molecules.

Photoresponsive systems are well known in nature.⁸ Some of them have their primary biochemical response to light converted into a physiological process by means of a complex pathway. For example, 11-*cis*-retinal, the chromophore of rhodopsin, undergoes a *cis* to *trans* isomerization upon irradiation of

[†] To whom correspondence should be addressed.

light.^{9,10} It has been reported that the light activation of rhodopsin/phospholipid liposomes leads to an increase of membrane permeation.^{11–13} Also, photoresponsive amphiphiles such as azobenzene derivatives were synthesized as a biological lipid model.¹⁴ They have been studied from the viewpoint of permeation control for phospholipid/azobenzene derivative liposomes.¹⁵ These *trans*-azobenzene derivatives have a characteristic of transformation to *cis*-isomers upon an irradiation of wavelength of the 360 nm light, and the latter to the former upon an irradiation of light above wavelength of 420 nm or heating. In the phospholipid liposomes incorporating azobenzene derivatives, the conformational change from a *trans*- to a *cis*-isomers induces the formation of a wide permeation pathway due to the local disorder of chain packing. Therefore, the membrane permeability increased by the irradiation of ultraviolet (UV) light of 360 nm.¹⁵ Also, the facilitated photoresponsive transport of metal cation has been studied for the polymer/liquid crystal/azobenzene-bridged crown ether ternary composite membrane. This system is fairly good example of the carrier mechanism for metal cation transport.¹⁶

Though unilamellar or multilamellar liposomes in solution are a useful biomimetic system for permeation study in a laboratory, they are not advantageous for engineering application, because both mechanical stabilities of liposomes and a thin film with two-dimensional large area are required. In order to prepare the wide and thin artificial membrane having characteristics of biological membrane, the blend systems of polymer and artificial lipids have been studied on the basis of permeation properties. The purpose of this paper is to investigate the water or ion permeation characteristics through a ternary composite membrane of polymer/artificial lipid/azobenzene derivative, which are controlled by the *trans*-*cis* photoisomerization of azobenzene derivative.

EXPERIMENTAL

Materials

The chemical structures of polymer, artificial lipid and azobenzene derivative used are shown in Figure 1. Poly(vinyl chloride) (PVC) was chosen as the matrix substance for the ternary composite membrane because it does not exhibit any apparent thermal transition in the temperature range studied here. PVC was Vinyca 37L (Mitsubishi Monstant Kasei Co., Ltd.) of which number average molecular weight was 66000. Dioctadecyldimethylammonium bromide (DOAB) of artificial lipid was synthesized by stepwise alkylation of dimethylamine. *p*- ω -Hydroxypropyloxy-*p*'-dodecyloxyazobenzene (AZO), the photoreponsive substance, was synthesized by diazo coupling of *p*-(dodecyloxy)aniline and phenol and also, by subsequent reaction with 3-bromopropanol.

A composite membrane of PVC/DOAB/AZO was prepared by casting a 8 wt% tetrahydrofuran (THF) solution of PVC, DOAB, and AZO on the glass plate at 313 K for one day. Then, it was dried *in vacuo* for one week at room temperature. The thickness of the composite membrane was 15–30 μ m. The weight percentages of DOAB and AZO were 13.1 wt% and 1.9 wt%, respectively, and their molar ratio was DOAB/AZO = 5/1.

Characterization of Ternary Composite Membrane

Differential scanning calorimetry (DSC) and X-ray studies were performed in order to investigate the crystal-liquid crystal phase transition behavior of DOAB and a dispersing state of AZO molecules in the composite membrane. Also, transmission electron microscopic observation (TEM) of PVC/DOAB (85/15 in wt%) thin film was performed in order to investigate the aggregation state of DOAB in the composite membrane. A 0.1 wt% THF solution of PVC and DOAB was dropped on a carbon grid and dried for TEM observation.

Figure 1. Chemical structures of components for the composite membrane.

Subsequently, its surface was treated by ion-etching for 10s in order to perform effective staining. Then, the composite membrane was negatively stained with a 0.05 wt% aqueous solution of uranyl acetate.

The composite membrane was irradiated with the light of wavelength of 360 nm for several minutes in order to induce a *trans-cis* isomerization of AZO within the membrane. The light source was a Toshiba 100W super high-pressure Hg lamp. Spectral change of *trans*-AZO in the composite membrane upon UV irradiation was obtained using JASCO 505 spectrophotometer.

Permeation Study through Ternary Composite Membrane

The water and K^+ permeabilities were measured by a normal osmosis method using a KCl aqueous solution of 1 mol dm^{-3} and distilled water separated by the composite membrane. Figure 2 shows a permeation cell used in this work. The quartz glass plate was set on the one end of the cell in order to irradiate the UV light perpendicular to the membrane surface. An effective membrane area was 9.0 cm^2 . The quantity of permeated water through the composite membrane was evaluated from the variation of water level

Figure 2. Permeation cell for photoresponsive study.

height in the capillary. The flux of K^+ was evaluated by measuring the time dependence of K^+ concentration in the downstream side with an atomic absorption spectrophotometer. The UV light was irradiated perpendicular to the membrane surface and the distance between the light source and the membrane surface was 140 mm.

RESULTS AND DISCUSSION

Characterization of Ternary Composite Membrane

Figure 3 shows the X-ray diffraction photograph of the PVC/DOAB/AZO composite membrane. The incident X-ray beam was perpendicular to the membrane surface. The diffraction pattern apparently indicates the existence of DOAB crystallite on the basis of the magnitude of diffraction spacings. The long spacing of 2.98 nm for the PVA/DOAB/AZO composite membrane corresponds to that of 2.8 nm for DOAB/AZO (5/1) mixture. Though these magnitudes of the long spacing were different from those of DOAB (3.6 nm) and AZO (5.6 nm) themselves, it seems apparent that there exists a layer structure of DOAB/AZO in the PVC matrix since a ratio of the reciprocals of the long spacings for the 1st and 2nd order scatterings is 1:2.

Figure 4 shows the transmission electron micrographs for the blend thin film of PVC/DOAB, which was negatively stained with uranyl acetate. In the magnified area (right photograph), the layer structure was ap-

Figure 3. X-Ray diffraction photograph and its schematic representation of PVC/DOAB/AZO composite membrane.

PVC/DOAB=85/15

Figure 4. Transmission electron micrograph of PVC/DOAB composite membrane (left) and enlarged one of selected area in the left photograph encircled by broken lines (right). The composite membrane is negatively stained with uranyl acetate.

parently observed. The spacing between dark striations was 4.0–4.6 nm and was consistent with the mean thickness of bilayer of DOAB.⁶ This result indicates that DOAB multilayer structure exists even in the polymer matrix.

Figure 5 shows the DSC curves of DOAB, AZO, DOAB/AZO (5/1 in molar ratio) and

the composite membrane. DOAB (dry) exhibited the endothermic peak corresponding to the crystal (K)-liquid crystal (Meso) phase transition, $T_{K \rightarrow M}$ at 357 K. The melting temperature of AZO was 399 K. The phase transition temperature of DOAB in the DOAB/AZO mixture (dry) shifted to lower tempera-

ture compared with that of DOAB itself (from the case (1) to the case (3)) and furthermore, the melting behavior of AZO disappeared (from the case (2) to the case (3)). These DSC results indicate that AZO molecules disperse almost in a molecular level within the multilayer of DOAB. $T_{K \rightarrow M}$ of DOAB in the PVC/DOAB/AZO composite membrane was 343 K in case of a dry condition and was depressed to 317 K in case of a wet condition. This result suggests that the polar head group of artificial lipids was bound to water, even though in the composite membrane. $T_{K \rightarrow M}$ of DOAB (dry) in the ternary composite membrane became

Figure 5. DSC curves of DOAB, AZO, DOAB/AZO mixture and composite membrane. K, Meso and Iso designate crystal phase, mesophase and isotropic phase, respectively.

slightly lower than that for the DOAB/AZO system (from the case (3) to the case (4)). This slight depression of $T_{K \rightarrow M}$ may suggest that DOAB in the composite membrane aggregates in a relatively unstable state such as the reverse bimolecular aggregation, in which the hydrophobic portion of hydrocarbon chains faces to the outer direction in the outermost layer of DOAB multilayer and contacts with the polymer matrix. This type of aggregation may be easily formed due to considerable affinity between the hydrophobic portion of artificial lipid and polymer.

Photo- and Thermal Isomerizations of AZO in Composite Membrane

Photo- and thermal isomerizations of AZO in the composite membrane was studied on the basis of the UV spectroscopic measurement. The composite membrane was interposed between the quartz glass plates and set in the spectrophotometer. Figure 6 shows the spectral change of the ternary composite membrane upon irradiation of the 360 nm UV light at 283 K. At this temperature, DOAB molecules were in a crystalline state (below $T_{K \rightarrow M}$). The absorption maximum of *trans*-AZO was observed at 360 nm. The peak height decreased with an increase in irradiation time of the 360 nm UV light. On the other hand, the

Figure 6. Spectral change of the ternary composite membrane as a function of irradiation time of 360 nm light at 283 K.

Figure 7. Decrease in *trans*-fraction of the ternary composite membrane as a function of irradiation time of the 360 nm UV light at 328 K.

absorption intensity around 300 nm and 470 nm increased due to the concomitant formation of *cis*-AZO. This means that the *trans*-*cis* photoisomerization of AZO molecule takes place even in the ternary composite membrane similarly to the case in solution. Since the *cis*-isomer is more bulky than the *trans*-isomer,¹⁷ the regular alignment or compact packing of DOAB molecules may be disordered by the *trans*-*cis* isomerization of AZO derivatives which are molecularly dispersed in the bimolecular layer of DOAB. Also, in the case of a liquid crystalline state of DOAB above $T_{K \rightarrow M}$, the *trans*-*cis* photoisomerization behavior was observed in the similar manner to that below $T_{K \rightarrow M}$ mentioned above.

Figure 7 shows the *trans*-AZO fraction as a function of irradiation time of the 360 nm UV light at 328 K (above $T_{K \rightarrow M}$). Figure 7 was obtained by using the ternary composite membrane in the wet condition, which was a similar condition to permeation experiment. The *trans*-fraction was evaluated from the change of the absorption peak height at 360 nm. The base line was drawn between 300 and 430 nm on the spectrum of curve 1 in Figure 6, taking polymer scattering and overlapping of *cis*-AZO absorption into consideration. The peak height from the base line at 360 nm before irradiation of the UV light was assumed to be equal to a unity. Figure 7 tells us that the isomerization reaches gradually to the equilib-

Figure 8. Time dependence of thermal isomerization from *cis*- to *trans*-AZO in the dark as a function of measuring temperature.

rium state at about 50–60% of the *trans*-fraction due to the *cis*→*trans* reverse transformation.

In order to investigate the photoresponsive control of the water or ion permeation for the ternary composite membrane, the temperature dependence of the thermal isomerization rate must be considered, because the thermal isomerization as well as the photoisomerization is caused at a higher temperature during irradiation of the UV light. The thermal isomerization reaction of AZO in the composite membrane was evaluated by measuring the time dependence of the absorption maximum at 360 nm in the dark. The composite membrane was irradiated by the 360 nm light up to attaining the equilibrium on the *trans*→*cis* photoisomerization and then, was left in the dark at several temperatures as shown in Figure 8. Figure 8 shows the temperature dependence of thermal isomerization of AZO from the *cis* to *trans* form. The percentage of thermal isomerization was determined by the magnitude of reincrease in the absorption peak at 360 nm in the dark. As shown by the curve 1 in Figure 8, the thermal isomerization could be neglected below 283 K since it was so slow in comparison with the photoisomerization. The rate of ther-

mal isomerization was considerably fast at around 328 K and this was an inevitable reverse reaction upon the photoisomerization above 328 K. A plot of the logarithm of the *cis*-fraction against the thermal isomerization time exhibited a fairly good first-order reaction. This reaction rate constant, k was $3.08 \times 10^{-4} \text{ s}^{-1}$ at 328 K. Since a certain fraction of *cis*-AZO transforms to *trans*-AZO due to the thermal isomerization after light off upon permeation experiment, the true fraction of *trans*-AZO was evaluated by using the value of k .

Water or K^+ Permeation through Photoresponsive Ternary Composite Membrane

Figures 9 and 10 show the photoresponsive effect on the time dependences of the permeation quantity of water, q at 328 K and K^+ concentration in the downstream side at 321 K, respectively, for the PVC/DOAB/AZO ternary composite membrane. As mentioned before, the DOAB/AZO mixture was in a liquid crystalline state in the composite membrane at these temperatures. The UV irradiation period was 30 min for water permeation experiment and 60 min for K^+ one, respectively, as indicated by light on and light off. Permeation of water and K^+ were apparently accelerated upon irradiation of the 360 nm light when the DOAB/AZO mixture was in a liquid crystalline state. Since *cis*-AZO is more bulky than *trans*-AZO, it is reasonable to consider that the DOAB multilayer becomes more disordered state with an increase in the *cis*-AZO fraction due to the *trans*→*cis* photoisomerization. Therefore, the apparent increase in permeability to water or K^+ may be explained by an increase in the number and/or the fraction of effective channel or vacancy for permeation.

On the other hand, in the case below $T_{\text{K} \rightarrow \text{M}}$, the permeation of water or K^+ was not detectably affected by irradiation of the 360 nm light, though the *trans*→*cis* isomerization certainly occurred. Generally speaking, water

Figure 9. (a): Change in quantity of water permeation, q at 328 K affected by light on or off. (b): Photoisomerization effect on relative water permeation, Δq at 328 K.

Figure 10. Change in quantity of K^+ permeation at 321 K affected by light on.

molecules or K^+ can not diffuse through the DOAB multilayer in a crystalline state. Though the molecular arrangement in a crystalline state may be locally disordered by the *trans*→*cis* photoisomerization, possibility of the continuous vacancy passing through the DOAB multilayer seems to be very low.

Therefore, the permeabilities to water and K^+ did not apparently change by the irradiation of the 360 nm UV light below $T_{K \rightarrow M}$. These results of permeation experiment indicate that the permeation properties are strongly dependent upon the phase transition behaviors of artificial lipids and also, the permeabilities to water and K^+ are remarkably facilitated upon the irradiation of the UV light. Accordingly, it is reasonable to consider that the DOAB/AZO forms the continuous domain within the composite membrane and also, water or ion passes mainly through such continuous domain in a liquid crystalline state.

Indeed, the various types of the aggregation states of DOAB multilayer may coexist in the composite membrane. It is considered as extreme examples that the bilayer surface of DOAB aggregates parallel or perpendicular to the composite membrane surface resulting in formation of the multilayer structure. Though the aggregation and dispersing states of DOAB multilayer have not been investigated in detail, it is undoubted from the temperature dependence of the photoresponsive permeation that DOAB multilayer effectively contributes to water or K^+ permeation in the ternary composite membrane.

The value of the permeation rate, dq/dt gradually decreased with time after turning off the 360 nm UV light as shown in Figure 9(a). After turning off the light, the fraction of *trans*-AZO increased again with time, because *cis*-AZO transformed thermally to *trans*-AZO at 328 K. This behavior is resulted in a decrease in channel or vacancy in the DOAB multilayer, in other words, an increase in the ordered region of DOAB molecular chain packing. Consequently, the rate of water permeation decreased gradually with time after light off and it approached to that before irradiation of the UV light.

As discussed above, the permeability to water or K^+ through the ternary composite membrane was affected by the packing state of DOAB molecules, in other words, the fraction

Figure 11. Change in diffusive permeability coefficient, P to water with *trans*-fraction of AZO.

of *trans*-AZO. Figure 11 shows a plot of the diffusive permeability coefficient, P to water against the *trans*-AZO fraction. The *trans*-fraction was evaluated by using the data in Figure 7 and the value of k . Upon photoisomerization, the *trans*-fraction reached to 0.59 at 30 min after irradiation at 360 nm (Figure 7). This experimental value of 0.59 corresponded to the simultaneous processes from photoisomerization and thermal isomerization during irradiation of the UV light at 328 K. The degree of the reverse reaction from *cis*-AZO to *trans*-AZO induced by thermal isomerization after turning off the UV light at 328 K was calculated by the rate constant, k . Also, the variation of P was evaluated by the slope of the permeation curve in Figure 9(a). As shown in Figure 11, the magnitude of P decreased with an increase in the *trans*-AZO fraction and, in other words, water diffusion through the DOAB multilayer became easily with an increase in the *cis*-AZO fraction. Therefore, Figure 11 indicates that the *trans*→*cis* isomerization of AZO may induce disordering of compact packing of DOAB molecules around AZO, resulting in an increase in number of channel or vacancy for water permeation in the multilayer of DOAB.

The permeation mechanism of the photoresponsive ternary composite membrane above $T_{K \rightarrow M}$ could be schematically explained as shown in Figure 12 on the basis of the photoresponsive characteristic permeation of water and K^+ . When AZO molecules have the *trans*-conformation, DOAB bilayer can retain the

Figure 12. Schematic representation of formation of channel in composite membrane due to local disordering of DOAB molecular aggregation.

compact packing. Though permeable substances can pass through the fluid region of DOAB bilayer above $T_{K \rightarrow M}$, its permeability is not prominent because of considerable compact packing of DOAB molecules. On the other hand, when AZO molecules transform to *cis*-conformation by irradiation of the UV light, the transmembrane channels may be formed in the DOAB bilayer due to bulkiness of the *cis*-AZO as shown in Figure 12. Therefore, it is apparent that permeable substances pass through the composite membrane with the higher fraction of *cis*-AZO more easily than that with *trans*-AZO conformation. Consequently, the permeability to water or K^+ for the ternary composite membrane above $T_{K \rightarrow M}$ increased significantly by irradiation of the UV light, according to the postulated mechanism shown in Figure 12.

CONCLUSION

From DSC, X-ray and morphological studies, it was concluded that the DOAB/AZO mixture was capable of formation of multilayer structure in the polymer/DOAB/AZO ternary composite membrane, and AZO molecule dispersed almost monomerically in the DOAB multilayer. The aggregation state of DOAB molecules was controlled by the conformational change of AZO molecules.

AZO molecules in the composite membrane exhibited photo- or thermal isomerizations upon irradiation of the 360 nm UV light or heating, respectively, in the similar manner to that in solution.

The permeability of the composite membrane to water or K^+ increased by irradiation of the 360 nm UV light in the case of the liquid crystalline state of DOAB (above $T_{K \rightarrow M}$). The detectable increase of water or K^+ permeation through the ternary composite membrane has not been observed by irradiation of the UV light below $T_{K \rightarrow M}$. It was concluded from these results that the transmembrane channels were effectively formed by the *trans*→*cis* photoisomerization of AZO upon irradiation of the 360 nm UV light when DOAB was in a liquid crystalline state, and water or K^+ permeated along these channels through the DOAB multilayers. Therefore, permeable substances became more easily to transfer across the DOAB bilayer membranes accompanied by *cis*-conformation of AZO.

REFERENCES

1. M. C. Block, L. L. M. van Deenen, and J. DeGier, *Biochim. Biophys. Acta*, **443**, 1 (1976).
2. K. Inoue, *Biochim. Biophys. Acta*, **339**, 390 (1974).
3. T. Kunitake, *J. Macromol. Sci. Chem.*, **A13**, 587 (1979).
4. Y. Okahata and T. Kunitake, *J. Am. Chem. Soc.*, **101**, 5231 (1979).
5. T. Kunitake and Y. Okahata, *J. Am. Chem. Soc.*, **102**, 549 (1980).
6. T. Kajiyama, A. Kumano, M. Takayanagi, Y. Okahata, and T. Kunitake, *Contemporary Topics in Polym. Sci.*, **4**, 829 (1984).
7. T. Kajiyama, A. Kumano, M. Takayanagi, Y. Okahata, and T. Kunitake, *Chem. Lett.*, 645 (1977). A. Kumano, T. Kajiyama, M. Takayanagi, and T. Kunitake, *Rep. Prog. Polym. Phys. Jpn.*, **25**, 793 (1982).
8. B. F. Erlanger, *Ann. Rev. Biochem.*, **5**, 267 (1976).
9. G. Wald, *Nature (London)*, **219**, 800 (1968).
10. T. G. Ebrey and B. Honig, *Q. Rev. Biophys.*, **8**, 129 (1975).
11. A. Darszon, M. Montal, and J. Zarco, *Biochem. Biophys. Res. Commun.*, **76**, 820 (1977).
12. D. F. O'Brien, N. Zumbulyadis, F. M. Michaels, and R. A. Ott, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 5222

- (1977).
13. D. F. O'Brien, *Photochem. Photobiol.*, **29**, 679 (1979).
 14. T. Kunitake, Y. Okahata, M. Shimomura, S. Yasunami, and T. Takarabe, *J. Am. Chem. Soc.*, **103**, 5401 (1981).
 15. K. Kano, T. Tanaka, T. Ogawa, M. Shimomura, and T. Kunitake, *Photochem. Photobiol.*, **34**, 323 (1981).
 16. A. Kumano, O. Niwa, T. Kajiyama, M. Takayanagi, K. Kano, and S. Shinkai, *Chem. Lett.*, 1327 (1983).
 17. G. C. Hampson and J. M. Robertson, *J. Chem. Soc.*, 409 (1941).