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PHOTOINDUCED POTENTIOMETRIC RESPONSE OF POLY(VINYL CHLORIDE)/SPIROBENZOPYRAN/CROWN ETHER COMPOSITE MEMBRANES MODIFIED WITH UREASE

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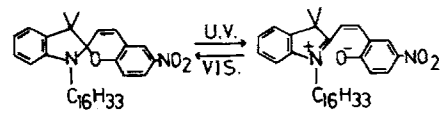
Photoresponse of the poly(vinyl chloride) membranes, which contain spirobenzopyran and crown ether, covered with a urease layer was studied in the presence and absence of urea. In the absence of urea, UV light irradiation induced more than 160 mV of membrane potential change, whereas the photoresponse decreased with an increase in the concentration of urea in the solution. The effects of such operating variables as crown loading and pH and ionic strength in the aqueous phase on the potentiometric response were also elucidated in the presence of urea. The results were explicated using the fact that the local concentration on NH_4^+ and H^+ ions changed as a result of the urease-catalyzed decomposition reaction of urea.

The photochromic behavior of spiropyran derivatives has been extensively investigated in solutions,[1,2] monolayers,[3] and polymer membranes.[4] We have engaged in developing spiropyran compounds as a photosensitive component to regulate such chemical and physical properties of artificial membranes as ionic permeability and membrane potential. It has been reported that the membrane potential changes when the polymer membranes containing spiropyran derivatives are photoirradiated.[5–18] In the course of our study on the photoinduced potentiometric response of the poly(vinyl chloride) (PVC) membranes doped with 1′-hexadecyl-3′,3′-dimethyl-6-nitrospiro-[2H-1-benzopyran-2,2′-indoline] (**1**), the following were found; (1) **1** exhibits normal photochromism in the plasticized PVC membrane; (2) UV light irradiation of the membrane induces membrane potential change of more than 100 mV with a response time of 1–2 min; (3) the photoinduced potential changes arise from the change



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in charge density on the membrane surface (i.e., surface potential change), which stems from the protonated form of photogenerated open-**1** (a positively charged species); (4) the content of open form of **1** is higher on the UV-irradiated surface than that on the nonirradiated surface (i.e., formation of asymmetric membrane), and (5) in the case that the membrane is doped with ionophore, the magnitude of photoresponse depends considerably on the ionic concentration in the aqueous phase.

Recently we have found that the photoresponse of the PVC/**1**/ nonactin membrane depends on the enzymatic reaction at the membrane surface.[16,18] The present paper reports the enzyme reaction-modulated photoresponse of the PVC/**1**/ crown ether membranes.

EXPERIMENTAL

Materials

Poly(vinyl chloride) (PVC) (polymerization degree is 1,000; from Wako Co., Ltd.,) was used without further purification. Di-2-ethylhexyl phthalate (DEHP), tetrahydrofuran (THF), urea, glutaraldehyde (GA), and crown ether (dibenzo-18-crown-6) were of extra pure reagent grade. Urease and bovine serum albumin (BSA) were purchased from Sigma Co. The synthetic procedure and anlyatical data of **1** were reported elsewhere.[14]

Preparation of membrane

A PVC/**1**/crown ether membrane was prepared by pouring the mixture of PVC (250 mg), DEHP (0·5 ml), **1** (30 mg), an appropriate amount of crown ether, and THF (20 ml) onto a flat Petri dish (8·5 cm diameter) and allowing the solution to evaporate. The thickness of the membrane thus prepared was *ca.* 0·15 mm. After the membrane had been glued to the top of a glass tube (see Figure 1), the surface of the PVC membrane was coated with an enzyme layer by pouring a mixture of an equal amount of 10% urease solution, 10% BSA solution, and 8% GA. Thus a thin layer (10 μm or less) of the immobilized enzyme formed on the PVC membrane. The enzyme layer was covered with a nylon mesh for improving surface adhesion.

Membrane potential measurement.

A U-shaped glass cell (Figure 1) was used for all measurement at 25°C. The effective membrane area of the cell was 0·78 cm^2. The C_2 side solution was stirred gently. The electrode in the C_1 solution was earthed. The composition of the electrochemical cell for the membrane

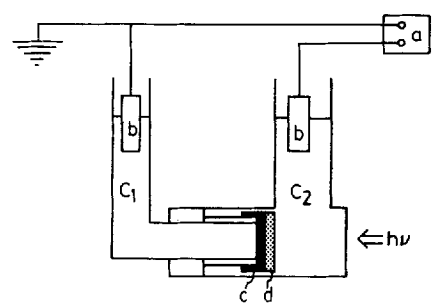

Figure 1. Schematic illustration of the cell for membrane potential measurement. a;potentiometer, b;Ag/AgCl electrode, c;PVC/**1**/crown ether membrane, d;urease layer

potential measurement was as follows; Ag/AgCl | 0·1M $(CH_3)_4NCl$ | electrolyte solution (C_1) | PVC/**1**/crown ether membrane | enzyme layer | urea solution (C_2) | 0·1M$(CH_3)_4NCl$ | Ag/AgCl. The pH of the solutions was regulated with a modified Britton-Robinson buffer (LiOH was used in place of NaOH). Before use the membrane was conditioned for *ca.* 15 h by soaking it in the buffer under dark conditions. The light source was a 500 W xenon lamp, and cut-off filters Toshiba UVD-35 and O-55 were used for isolating UV (320 nm<λ<400 nm) and visible(λ>550 nm) light, respectively. Noise level was *ca.* ±1 mV in the present experimental conditions.

RESULTS AND DISCUSSION

It is well established that the photochromic behavior of spiropyran derivatives is highly sensitive to the environmental conditions such as polarity of solvent and viscosity of medium, etc.[19–21] Figure 2 shows absorption spectra of the PVC/**1**/crown ether membrane before and after UV light irradiation. The membrane exhibited no absorption maximum in the range of 400 nm <λ before irradiation. Upon exposing the membrane to UV light, the membrane turned purple within 1–2 min and an absorption maximum appeared at 564 nm, confirming the formation of open-**1**.[1–4] The original transparent membrane was recovered within 30 sec under visible light irradiation. The coloration/decoloration was reversible. These results show that the photochromic behaviour of the PVC/**1**/crown ether membrane resembles that of the PVC/**1** membrane,[14] suggesting no undesirable effect of crown ether on the photochromism of **1**.

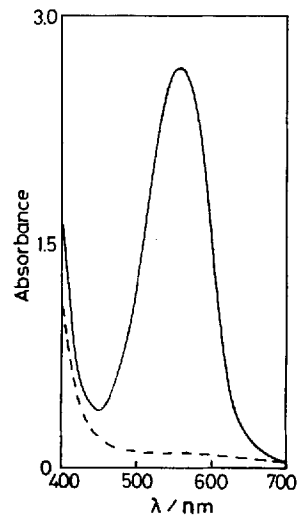

Figure 2. Absorption spectra of PVC/**1**/crown ether membrane before (---) and after (—) UV light irradiation

Potentiometric properties of the PVC/**1**/crown ether membrane without an enzyme layer were studied under photoirradiation. Figure 3 shows a typical potential change of the membrane induced by UV and visible light irradiation. UV light irradiation induced *ca.* −160 mV of negative shift in membrane potential [$\Delta(\Delta\phi)$ = 160 mV], and the potential was reversibly recovered to the original value by visible light irradiation. The photoresponse of the

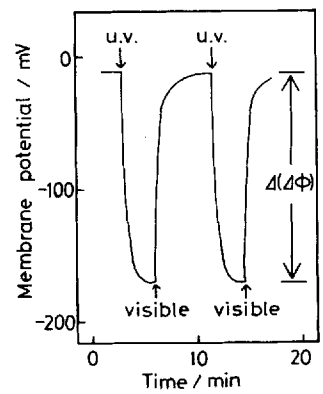

Figure 3. A typical photoresponse of PVC/**1**/crown ether membrane in 5 mM buffer at pH 7·0. Crown ether content; 0·77 w/w%

membrane can be explained by the same mechanisms as for the PVC/**1** membranes without crown ether; A surface potential change at the membrane/solution interface, associated with the photoinduced electrically positive charges arising from the protonated form of open-**1**.[14]

Figures 4 and 5 show the effects of pH and NH_4Cl concentration on the magnitude of photoinduced membrane potential. Photoresponse of the PVC/**1**/ crown ether membrane considerably depended on the pH of the aqueous phase (Figure 4). The $\Delta(\Delta\phi)$ exhibited high values in neutral and slightly acidic regions and was suppressed in alkaline pH. The pH dependence of the $\Delta(\Delta\phi)$ value seems to reflect the protonation equilibrium at the membrane surface between the open-**1** and its protonated form. The protonated form of **1** contributes to the change in surface potential, while the open-**1** itself does not because of its electrical neutrality.

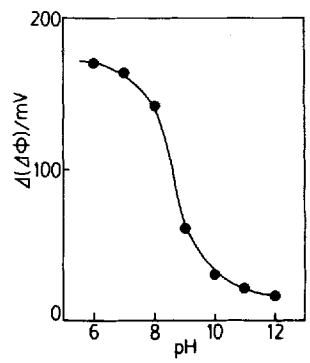

Figure 4. Effects of pH on the magnitude of photoinduced membrane potential change in 5 mM buffer. Crown ether content; 0·77 w/w%

The concentration of NH_4^+ ion is another factor which affects the magnitude of $\Delta(\Delta\phi)$ value. Figure 5 clearly reveals that the higher concentration of NH_4^+ ion inhibits a high response in $\Delta(\Delta\phi)$. This behaviour can be explained by the same idea that was applied for analyzing the results for the photoresponse of the PVC/**1**/valinomycin and the PVC/**1**/nonactin membranes in the presence of K^+ and NH_4^+ ions, respectively.[15,17] The idea is that the

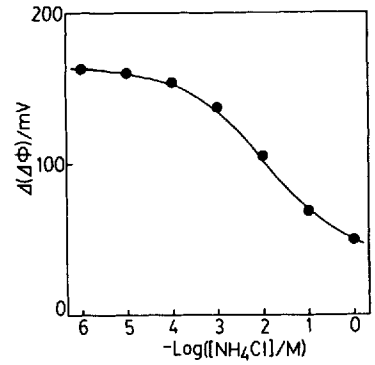

Figure 5. Effects of NH_4Cl concentration on the photoinduced membrane potential change in 5 mM buffer at pH 7·0. Crown ether content; 0·77 w/w%

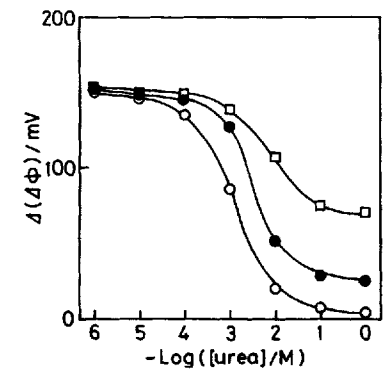

Figure 6. Effects of crown ether content on the $\Delta(\Delta\phi)$ value of the urease-modified membranes in the presence of 10^{-6}–1 M urea. Crown ether content; 0·06 (—□—), 0·13 (—●—), and 0·77 w/w% (—○—). 1 mM buffer (pH 7·0) was used

specific adsorption of NH_4^+ ion on the membrane surface inhibited in part the generation of photoinduced potential change, since the crown ether used is a strong binder for NH_4^+ ion.[22] It should be noted here that the $\Delta(\Delta\phi)$ value can be regulated by pH and NH_4^+ ion concentration in the solution.

The above results stimulated us to construct a membrane system in which the photoresponse in membrane potential can be regulated by enzymatic reaction at the membrane surface. We coupled the PVC/**1**/crown ether membrane with urease, which decomposes urea to ammonia and carbon dioxide with the consumption of H^+ ion.

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^-$$

The photoinduced potentiometric response of the urease-modified PVC/**1**/crown ether membrane was similar to that of urease-free membranes shown in Figure 3, except that the membrane potential changed upon addition of urea in the C_2 side solution and that the $\Delta(\Delta\phi)$ value was dependent on the urea concentration in the solution. Figure 6 shows the $\Delta(\Delta\phi)$ values of the urease-modified membranes with various crown ether contents. Below 10^{-4} M

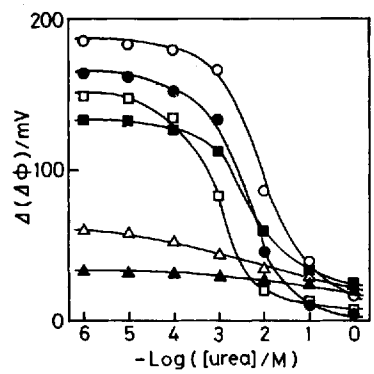

Figure 7. The pH dependence of the photoresponse of the urease-modified PVC/1/crown ether membrane in the presence of urea. The pH of the solution; 5·0 (—○—), 6·0 (—●—), 7·0 (—□—), 8·0 (■) 9·0 (—△—), and 10 (—▲—). 1 mM buffer was used. Crown ether content; 0·77 w/w%

urea, the $\Delta(\Delta\phi)$ values read 135–145 mV for all membranes, while the photoresponse was suppressed by increasing the concentration of urea. The effect of enzymatic reaction on the $\Delta(\Delta\phi)$ value was more significant in the membrane with higher crown ether loading, showing the essential role of crown ether. It is evident that the changes in the local concentrations of H^+ and NH_4^+ ions are due to the origin of the enzymatic reaction-dependent photoresponse.

The catalytic activity of enzymes is usually sensitive to ambient pH. Additionally, in the present system, the $\Delta(\Delta\phi)$ value of the PVC membrane depended on the pH of the solution as shown in Figure 4. For these reasons, we checked the effects of pH on the photoresponse of the urease-modified PVC/1/crown ether membrane over the range of pH 5.0–10 (Figure 7). In pH 5·0-8·0, the $\Delta(\Delta\phi)$ values normally depended on the concentration of urea. However, the dependence of $\Delta(\Delta\phi)$ value on the urea concentration disappeared in the media of pH 9·0 and 10. The reasons for this may be that the immobilized urease loses its catalytic activity in pH 9.0 and 10 and that the magnitude of $\Delta(\Delta\phi)$ of the PVC/1/crown ether membrane itself reduces in alkaline pH region.

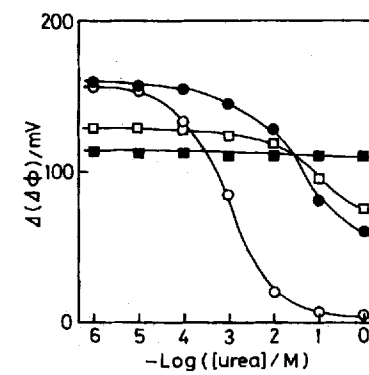

Figure 8. Effects of buffer capacity on the photoresponse of the urease-modified PVC/1/crown ether membrane in the presence of urea. The concentration of buffer; 1 (—○—), 5 (—●—), 40 (—□—), and 100 mM (—■—). The pH was constant at 7·0. Crown ether content ; 0·77 w/w%

Figure 8 shows how the $\Delta(\Delta\phi)$ depends upon the buffer capacity in the presence of 10^{-6}–1 M urea. When the buffer capacity was higher, the $\Delta(\Delta\phi)$ value depended upon the concentration of urea to much less extent. In 100 mM buffer, the photoresponse was practically independent of the urea concentration. These results clearly suggest the significant role of the local pH changes at the membrane surface in determining the magnitude of photoresponse.

CONCLUSIONS

The photoinduced potentiometric response of the PVC/**1**/ crown ether membrane modified with ureas was considerable, dependent on the concentration of urea in the solution. The results originated from the local concentration changes of H^+ and NH_4^+ ions produced by the decomposition reaction of urea catalyzed by urease.

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REFERENCES

1. H. Gross, H. Durr and W. Rettig, *J. Photochem*, **26**, 165–178 (1984).
2. M. Gehrtz, C. Brauchle and J. Voitlaunder, *J. Am. Chem. Soc.* **104**, 2094-2101 (1982).
3. D. A. Holden, H. Ringsdorf, V. Deblauwe and G. Smets, *J. Phys. Chem.* **88**, 716–720 (1984).
4. M. Kryszewski and B. Nadolski, *Pure Appl. Chem.* **49**, 511–522 (1977).
5. S. Kato, M. Aizawa and S. Suzuki, *J. Membr. Sci.* **1**, 289–300 (1976.
6. S. Kato, M. Aizawa and S. Suzuki, *J. Membr. Sci.* **2**, 39–47 (1977).
7. S. Kato, M. Aizawa and S. Suzuki, *Kobunshi Ronbunshu*, **34**, 793–799 (1977).
8. I. R. Bellobono, S. Giovanardi, B. Marcandalli, S. Calgari and D. Nassari, *Polym. Photochem.* **4**, 59-67 (1984).
9. I. R. Bellobono, B. Marcandalli, E. Selli and S. Cargalli, *Photogr. Sci. Eng.* **28**, 162–165 (1984).
10. M. Irie, A. Menju and K. Hayashi, *Nippon Kagaku Kaishi*, 227–232 (1984).
11. J. Anzai, A. Ueno and T. Osa, *J. Chem. Soc., Chem. Commun.* 688-689 (1984).
12. J. Anzai, H. Sasaki, A. Ueno and T. Osa, *Chem. Lett.* 1445–1448 (1985).
13. J. Anzai, H. Sasaki, A. Ueno and T. Osa, *Makromol. Chem., Rapid Commun.* **7**, 133–137 (1986).
14. J. Anzai, Y. Hasebe, A. Ueno and T. Osa, *Kobunshi Ronbunshu*, **43**, 683–689 (1986).
15. J. Anzai, Y. Hasebe, A. Ueno and T. Osa, *Bull. Chem. Soc. Jpn.* **60**, 1515–1516 (1987).
16. J. Anzai, Y. Hasebe, A. Ueno and T. Osa, *Maku (Membrane)*, **12**, 165–167 (1987).
17. J. Anzai, Y. Hasebe, A. Ueno and T. Osa, *Bull. Chem. Soc. Jpn.* **60**, 3169–3173 (1987).
18. J. Anzai, Y. Hasebe, A. Ueno and T. Osa, *J. Chem. Soc., Perkin Trans. II.* submitted.
19. N. W. Tyer. Jr., and R. S. Becker, *J. Am. Chem. Soc.* **92**, 1289–1294 (1970).
20. V. A. Kuzmin, Y. N. Malkin and E. R. Zakhs, *Photogr. Sci. Eng.* **23**, 20–26 (1979).
21. C. Lenoble and R. S. Becker, *J. Phys. Chem.* **90**, 62–65 (1986).
22. C. J. Pedersen, *J. Am. Chem. Soc.* **89**, 7017–7036 (1967).
23. C. Walsh, *Enzymatic Reaction Mechanisms*, W. H. Freeman and Co. New York (1979), pp. 84–86.