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PHOTO-INDUCED POLYPEPTIDE MEMBRANE POTENTIAL CHANGES BY cis-trans ISOMERISM IN SIDE-CHAIN AZOBENZENE GROUPS

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Photo-induced membrane potential changes were observed for solid membrane of poly(L-glutamic acid)(PGA) with azobenzene groups in the polymer side chains (azo-modified PGA). The increase in membrane potential indicates an increase in negative fixed charge concentration of the membrane, i.e., an increase in the degree of dissociation of the L-glutamic acid CO_2H side chains in the membrane *via* the trans to cis isomerization of the azo groups on UV irradiation.

While it is now generally accepted that the membrane potential change of natural membranes plays an essential part of the transmissional function between cells and their environment, the mechanism by which perturbations in the outside of the cell can be transmitted to its interior remains unexplained. On the basis of biological functions several model systems$^{1)}$ have appeared to elucidate the relation between a photocontrolled transmissional function (membrane potential) of the membrane and a photoresponsive group in the membrane. This report shows the photo-induced membrane potential changes of the azo-modified PGA membrane which is caused by the co-operative effect owing to two functional groups, i.e., the photoreaction of the azobenzene side chains and the dissociation of the L-glutamic acid CO_2H side chains in the membrane. We have reported the photocontrol of ion permeabilities of the azo-modofied PGA membrane.$^{2)}$

PGA polymers incorporated with azobenzene groups were synthesized by the condensation reaction of PGA with *p*-phenylazoaniline in dimethylformamide at 70 °C, as reported by Ciardelli.$^{3)}$ The content of the azo groups in the polymer was regulated by altering the reaction time. The maximum mol% of the azo groups in the polymer obtained was 12%. The trans to cis photoisomerization in azo-modified PGA membrane in aqueous solution was induced by UV irradiation (100 W high-pressure mercury lamp) at 25 °C. On the basis of the changes in absorbance at 350 nm, it was estimated that 40% of the trans form were converted into the cis isomers. The UV irradiation did not affect the main-chain α-helix structure of the azo-modified PGA membrane, as confirmed by CD spectra. Membranes of ca. 10 μm thickness were prepared for membrane potential measurements by casting a 2% chloroform solution of the azo-modified PGA on flat glass plates and allowing the solvent to evaporate. The dependence of the membrane potential, $\Delta(\Delta\psi)$, of the azo-modified PGA membrane containing 3 mol% azo groups on UV irradiation was observed with a KCl concentration ratio across the membrane of $c_1/c_2 = 4$ at 25 °C. The results are shown in Table 1. The membrane potential of the azo-modified PGA membrane during irradiation is 13% higher than the membrane potential in the dark at pH 6.2. However, the UV irradiation did not affect the membrane potential of the azo-modified PGA membrane at pH 9.0, indicating that the degree of dissociation of L-glutamic acid CO_2H side chains is high at pH 9.0 even in the dark. Table 1 also shows the effect of UV irradiation on the membrane potential of poly(γ-methyl L-glutamate)(PMG) membrane, which has no dissociable side chains, with entrapped 3 mol% of *p*-phenylazoaniline[4)] (azo-entrapped PMG). $\Delta(\Delta\psi)$ value of the azo-entrapped PMG membrane is independent of the UV irradiation. This result indicates that the trans to cis isomerization of the azo groups in the membrane does not directly affect the membrane potential. It appears, therefore, that the increase in membrane potential of the azo-modified PGA membrane is owing to an increase in the degree of dissociation of L-glutamic acid CO_2H side chains. Changes in water content[5)] of the azo-modified PGA membrane containing 12% azo groups were also observed on UV irradiation, which can be associated with the decrease in hydro-

Table 1. Effect of UV irradiation on the membrane potential, $\Delta(\Delta\psi)$, of azo-modified PGA membrane containing 3 mol% azo groups at pH 6.2 and pH 9.0, and of azo-entrapped PMG membrane containing 3 mol% azo groups at pH 6.2. ($\Delta(\Delta\psi)$: mV, concentration of KCl: c_1=0.001, c_2=0.00025 mol/l, 25 °C)

	$\Delta(\Delta\psi)$ in the dark	$\Delta(\Delta\psi)$ UV irradiation
Azo-modified PGA (pH 6.2)	29.3	33.0
Azo-modified PGA (pH 9.0)	34.0	34.0
Azo-entrapped PMG (pH 6.2)	12.8	12.8

phobicity of the side chain azobenzene groups (with increase in the content of cis isomer), i.e., the water content of the membrane increases from 0.165 wt% in the dark to 0.200 wt% after UV irradiation. This result implies that acid side-chain dissociation of the azo-modified PGA at pH 6.2 may be accelerated by the enhancement of water uptake by the membrane due to UV irradiation. Thus, the photo-induced increase in the membrane potential of the azo-modified PGA membrane can be explained in terms of a co-operative effect between the photo-reaction of the azobenzene side chains and the dissociation of the L-glutamic acid side chains in the membrane *via* the enhancement of water uptake by the membrane on UV irradiation. Figures 1 (a) and (b) show the increase and recovery cycles of membrane potential and the changes in absorbance at 350 nm of the azo-modified PGA membrane containing 12% azo groups, which were achieved at 60 °C by switching the high-pressure mercury lamp on and off, respectively. The photo-induced changes of the membrane

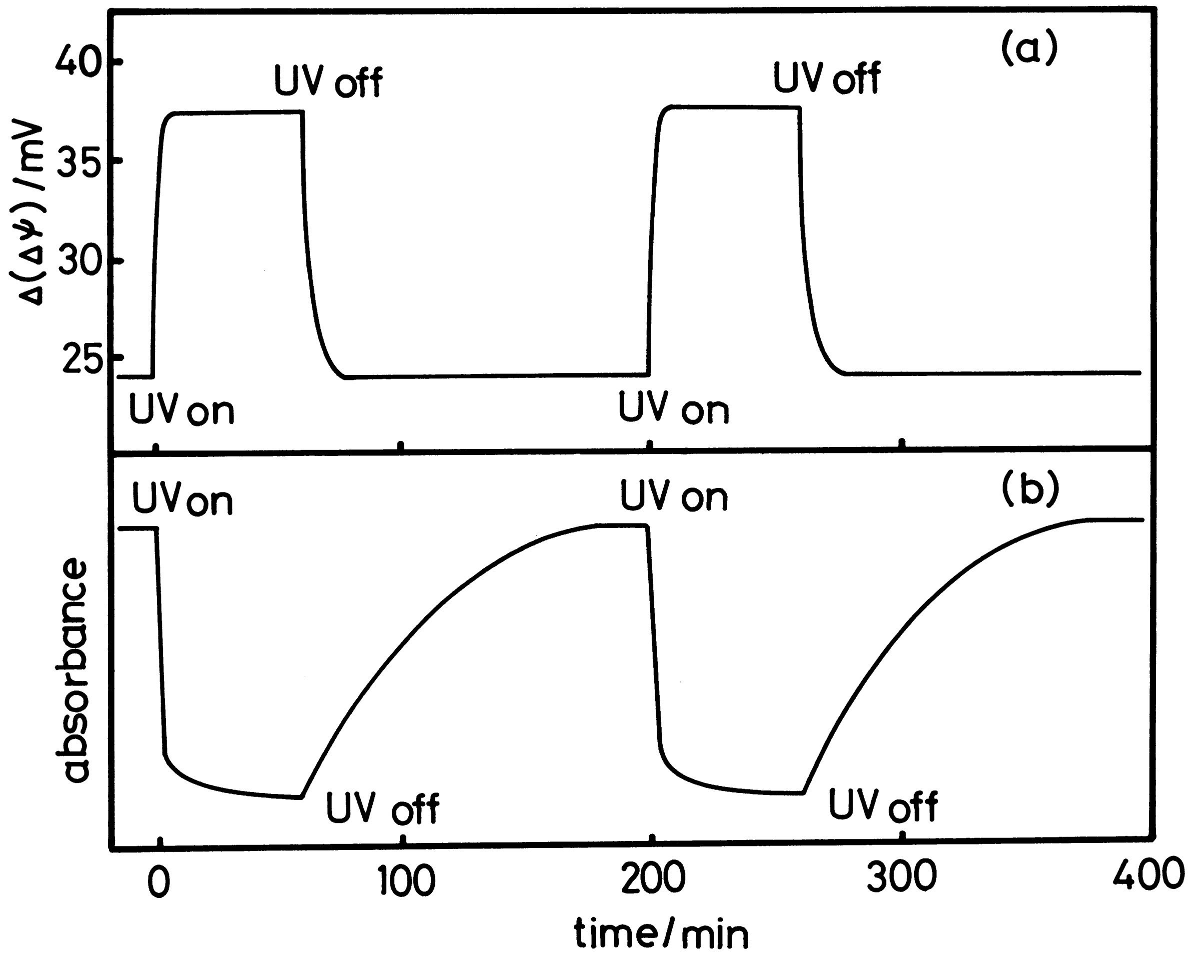

Fig. 1 (a). Photo-induced membrane potential changes of the azo-modified PGA membrane containing 12% azo groups at pH 6.2 (concentration of KCl : c_1 = 0.001, c_2 = 0.00025 mol/l, 60 °C.)
(b). Changes in absorbance at 350 nm of the membrane at 60 °C.

potential occur concomitantly with the absorption changes at 350 nm, however, the photo-induced increase and decrease of membrane potential occurs at a rate faster than that at which the absorption intensity at 350 nm changes. The membrane potential of our membrane system is owing to the Donnan potential (surface potential) differences at the membrane/solution interfaces, assuming that the diffusion potential is negligibly small for KCl-membrane systems. Therefore, the photo-induced membrane potential changes in Fig. 1 (a) may be owing to the surface potential changes *via* the rapid trans to cis isomerization of the azo groups at the surface of the membrane. On the other hand, the rate of the changes in absorbance at 350 nm of the azo-modified PGA membrane may reflect not only the faster photoisomerization of the azo groups at the membrane surface but also the slower photoreaction of the azo groups of the inner parts of the membrane. This is the reason why the rate of the membrane potential change of the azo-modified PGA membrane is faster than that of the change in absorbance at 350 nm of the membrane. The photo-induced ion permeability changes through the azo-modified PGA membrane, which are mainly determined by the properties of the inner parts of the membrane, were entirely consistent with the absorption change at 350 nm of the membrane.$^{2)}$

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4) On the basis of the changes in absorbance at 370 nm of the azo-entrapped PMG membrane containing 3% azo groups, it was estimated that 25% of the trans forms were converted into the cis isomers at 25 °C.

5) For experimental method of the measurement of the degree of hydration of the membrane see: T. Kinoshita, S. Hayashi, A. Takizawa, and Y. Tsujita, J. Macromol. Sci., Phys., B17, 39 (1980).

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