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Orientation of bacteriorhodopsin in non-aqueous polymer membrane

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Summary

Electric field orientation of bacteriorhodopsin (BRp) in the form of purple membrane (PM) in synthetic polymer membranes made from poly(hydroxymethyl methacrylate) or photo-crosslinkable propyleneoxide-based prepolymer was achieved by combining once-desalted and liophilized PM sample and ethylene glycol as a dilutant, which was detected by an increase in photoelectric current or membrane potential, respectively.

Bacteriorhodopsin (BRp) locates in the cell membrane of *Halobacterium* (1) as a complex with lipid (purple membrane, PM) has a unique property of proton pumping upon illumination, which has attracted keen attention as an information- or energy-transducing material (2). Controlling the orientation of BRp in the reconstituted matrix is one major difficulty in obtaining effective transducing materials. Application of a relatively low external electric field was shown to induce orientation of PM in dispersed solution (3) and this property was used in several attempts to improve the efficiency of the light-conversion in reconstituted systems (4-9).

In order to construct models of biological membranes, especially with respect to their structural and spatial asymmetry, we have prepared synthetic membranes by using a static electric field (10-14), which is based on the ionic or dipolar nature of proteins to be responsive to a given electric field given during the preparation of the synthetic membrane perpendicular to the membrane plane non-electrophoretically influencing the distribution or orientation of the protein, such as BRp.

In the previous studies (13,14) we used aqueous systems because of the lability of the protein in non-aqueous medium. When we consider the indirectly applied electric field on a medium of high dielectric constant such as water, however, we know that the electric potential drops steeply near the conductive plate and the orientation of BRp is realized only at the neighboring region of the interface. One way to overcome this disadvantage is to use a medium of lower dielectric constant, but most of organic solvents are known to denature PM. Here we report that a combination of once-desalted and liophilized PM sample and a dilutant ethylene glycol was successfully applied.

Experimental

Polymer membrane containing BRp was prepared with an apparatus as previously described (13). Ethylene glycol solution of PM containing

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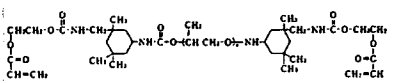
propyleneoxide-based photo-crosslinkable prepolymer (called ENTP (I), (15)) or hydroxymethyl methacrylate (HEMA, II) and benzoin ethyl ether as a sensitizer was placed in a flat cell covered by two sheets of indium oxide evaporated polyester film (IOTO-film, 100L-BK-12, kindly donated by Toray Co.,Japan) with the conductive surface facing to the solution. This composition, especially solvent, was selected after several preliminary tests of stability and activity. An electric static field was applied between the film below the solution and the point-type counter electrode placed over the top film by a high voltage power supply (PS2510, Advantec, Japan). The voltage value mentioned below is of that given to the former with reference to the latter electrode. After incubation for 30 to 60 min in the dark with the electric field applied, near UV light was emitted to complete solidification of the membrane. Under similar conditions it was checked that no detectable change in the absorbance spectrum of purple membrane was caused.

The size of the obtained polymer membrane was usually 2cmx2cm and the thickness was controlled by a silicone-rubber frame placed between two sheets of IOTO film (usually 500um). As a control, a membrane without application of electric field was prepared by a similar procedure. The obtained membrane was then washed in cold water overnight and stored in the cold. Thus it should be noted that though the membrane was prepared in non-aqueous circumstances its photo-activity was measured in aqueous ones.

The photocurrent was measured using an electrometer (TR8651; Takeda Riken, Japan) connected to the two IOTO-films sandwiching the membrane while illuminated by two halogen-lamps (100W) from both sides of the membrane (distance 50cm, in ambient temperature). Membrane potential was measured with a cell composed of two equal volume compartments containing 0.1M KCl attached with salt-bridges and Ag/AgCl electrodes. Ionic (KCl) permeability was measured with a measuring cell with conductance electrodes.



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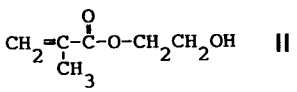

II

Results and Discussion

Figure 1 shows the typical photocurrent behavior of the HEMA-made BRp-membrane obtained with an application of -500V, compared with the result of the control. The photocurrent was enhanced by application of the electric field during the membrane preparation in the present manner. The maximum current was achieved soon after the initiation of the illumination.

The direction of the electric current is consistent with the reported directions of the dipole moment (3) and the proton stream produced by BRp (1), which means that at least a part of the immobilized BRp is oriented along the electric field and the vectorial sum of the proton stream caused by light energy is a certain value.

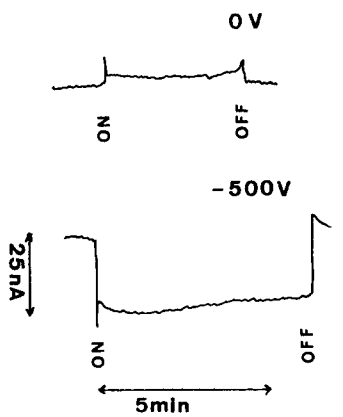

Fig.1. Photo-current observed for BRp immobilized in HEMA membrane prepared with or without application of -500V.

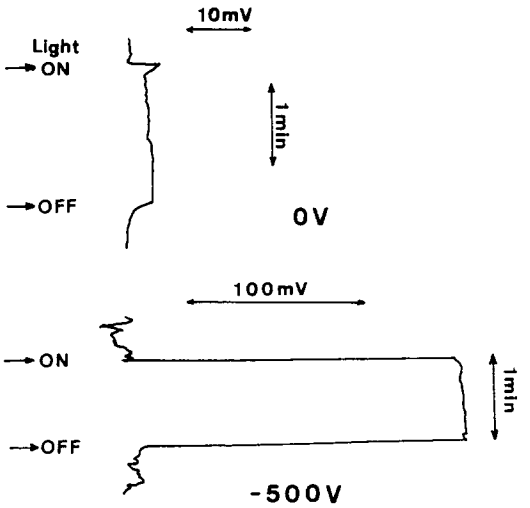

Fig.2. Change in the photo-induced membrane potentials of ENTP-entrapped BRp by application of electric potential during membrane preparation process.

Figure 2 shows the typical photo-induced membrane potential of the ENTP-made BRp-membrane obtained with an application of -500V or 0V. The membrane potential was highly increased by application of the electric field during the immobilization process in the present manner. Though anisotropic photo-activity of BRp was not detected as membrane potential in the cases of BRp membranes made of hydrophilic materials such as ethyleneoxide-based prepolymer (ENT) or acrylamide (13,14), the present ENTP membrane showed clear difference in photo-induced membrane potential and to the contrary it was hard to measure the photo-induced current in this system. These are explained in terms of low ionic permeability of this membrane system as compared in Table 1.

Table 1. Comparison of Ionic Permeability of the Membranes used for Immobilization of BRp*

Property \ Membrane type	ENT	ENT/ENTP** (50/50)	ENTP	HEMA
Permeability (cm/s)	4.8×10^{-4}	3.8×10^{-4}	1.5×10^{-7}	2.5×10^{-6}
Diffusion Coeff. (cm^2/s)	2.4×10^{-5}	1.9×10^{-6}	7.4×10^{-9}	1.3×10^{-7}

* Properties of the membrane containing no BRp, measured at 25°C with 0.1M KCl. Membrane area 0.78cm^2.
** weight content

This property also limits the observed photo-current value of HEMA membrane. Though the observed current of HEMA membrane (Fig.1) was comparable to that of e.g. aqueous polyacrylamide membrane system (13), it doesn't always mean that the degrees of orientation of BRp in two systems are comparable, since the current value is dependent on the membrane resistance. Evaluation of the degree of orientation by means of diffraction measurement of the membrane is under way.

Thus by selecting the solvent and entrapping materials, BRp (in the form of purple membrane) was shown to be immobilized in an oriented manner in non-aqueous matrix by applying external electric field. This will be extended to versatile utilization of the interesting functions of this protein.

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