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Oriented immobilization of bacteriorhodopsin in synthetic polymer membranes by use of electric static field

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Summary

Application of an electric field during the process of resin formations induced oriented immobilization of bacteriorhodopsin (BRp) in the form of purple membrane, isolated from Halobacterium halobium, within a membrane prepared from a photo-crosslinkable resin prepolymer through entrapment. The observed photoelectric current upon illumination of the membrane was highly enhanced by this oriented immobilization.

Structural asymmetry of biological membranes, as seen in lipid compositions and protein distribution/orientation, constitutes one major basis of the high functionality of these membranes such as selective permeation and anisotropic transport(1). To construct models for some of these points, we have prepared asymmetric charged membranes by using a static electric field(2) and applied this procedure to the preparation of enzyme-immobilized membranes with an asymmetric distribution of the protein(3).

This method is based on the ionic nature of co-monomer or enzyme proteins to be responsive to a given electric field. During the preparation of the synthetic membrane an electric field was applied perpendicular to the membrane plane in order to influence the distribution of the ionic substance to be fixed. When the protein has an electric dipole, its orientation is influenced by applying an external electric field and by the present procedure we can alter this orientation.

Bacteriorhodopsin (BRp), located in purple membranes within the cell membrane of Halobacterium(4), has a unique property of proton pumping upon illumination, and has attracted considerable attention as an information- or energy-transducing material(5).

Orientation of BRp in the reconstituted matrix is, however, one major difficulty in obtaining effective transducing materials and most of the previous studies have noted accidental orientation or some interfacial forces. Application of a relatively low external electric field(6) has been shown to induce orientation of purple membranes in dispersed solutions and this electric property has been explored to improve the overall efficiency of the light-conversion in a synthetic material system(7). These attempts, however, required a special ion-exchanging property for the membrane materials or complicated polymerization techniques.

Our electro-static field method can be applied to a wide variety of membrane materials with very simple procedure. In the present example, photo-crosslinkable resin prepolymers(8) derived from polyethyleneglycol was used as the membrane matrix.

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Experimentals

Figure 1 shows the membrane preparation equipment. A buffer solution (Hepes 0.1M pH 7) containing purple membranes (7.4mM final, as determined by optical absorbance and ε(at 560nm)=63000) and a prepolymer (called ENT-2000 with the Mr≒2000 of the prepolymer main chain (I): 100mg/400µl final) was placed in a cell and sandwiched between indium oxide evaporated polyester film (IOTO film 100L-BK-12, kindly donated by Toray Co.,Japan) with the conductive surface facing the solution. An electric static field was applied between the film below the solution and the point-type counter electrode placed over the top of the film by a high voltage power supply (PS2510, Advantec, Japan). After incubation for 30 to 60 min in the dark with the electric field applied, near UV light was emitted by a UV-lamp (National, FUL 14BA-37-K) for 5 to 7 min to complete solidification of the prepolymer solution. This lamp has the energy maximum at 370 nm and a preliminary study proved that an illumination of a purple membrane solution by this lamp for a prolonged period under similar conditions caused no detectable change in the spectrum behavior of purple membrane. 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 250µm). As a control, a membrane without application of electric field was prepared by a similar procedure. The obtained membrane was washed in cold water overnight and stored in the cold.

The photocurrent was measured using an electrometer (Takeda Riken; TR8651) connected to the two IOTO-films sandwiching the membrane while illuminated by two halogen-lamps (100W) from both sides of the membrane (distance 50 cm, in ambient temperature), as illustrated in Fig. 2.

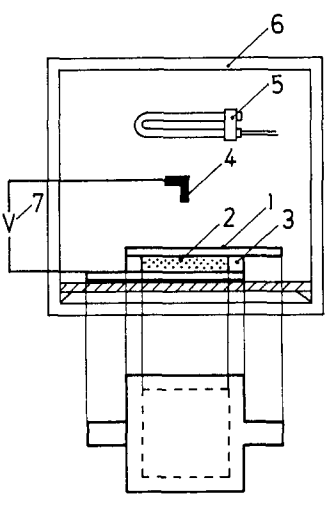
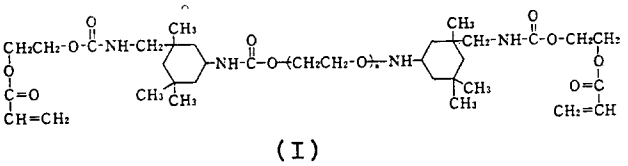


Fig.1. Membrane preparation cell. 1,IOTO film; 2, prepolymer solution containing BRp; 3,silicone frame; 4,counter electrode; 5, lamp; 6, incubate box; 7, constant voltage source.



(I)

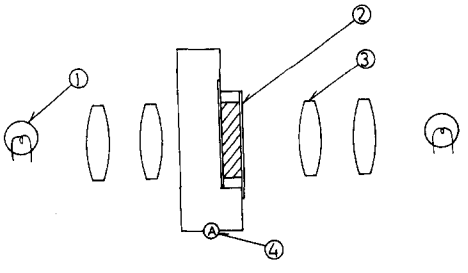


Fig.2 Apparatus for the photocurrent measurement. 1,halogen lamp; 2,membrane covered with IOTO films; 3,lens; 4,electrometer.

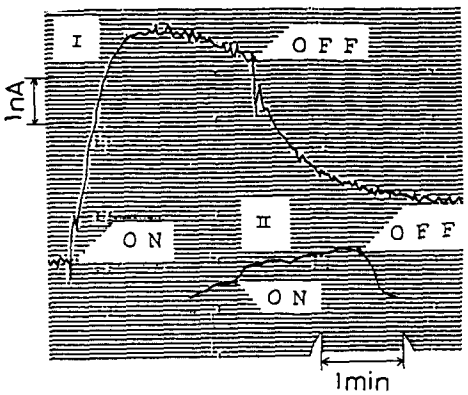


Fig.3. Photocurrent observed for an immobilized BRp membrane prepared with (I) or without (II) application of 500V.

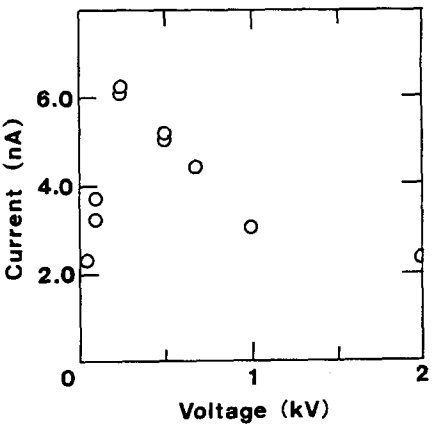


Fig.4. The effect of voltage applied during the membrane preparation on the maximum observed photocurrent.

Results and Discussion

Figure 3 shows the typical photocurrent behavior of the BRp-membrane obtained with the application of 500V compared with the result of the control. The photocurrent was drastically enhanced by application of the electric field during the membrane preparation in the present manner. The maximum current was achieved within several seconds after the initiation of the illumination and it kept practically constant for about one minute.

Similar results were obtained for the membranes prepared under different electric potentials but the current measured under the same condition was dependent on the voltage applied to the preparation, as shown in Fig.4. Under the present conditions, 200-300V yielded the highest output and this result can be understood in relation to the subtle balance of the permanent and induced dipole moments of BRp. The direction of the electric current is consistent to the reported directions of the dipole moment(6-e) and the proton stream produced by this protein(4).

When the illumination was prolonged, the observed current decreased gradually and become practically zero after 40 to 50 minutes (Fig.5). It remained relatively high for a longer time, however, when the illumination was continually repeated (e.g. 1 min interval in the dark between 10s illuminations)(Fig.6). After a rest in the dark for more than a few minutes the membrane exhibited the initial amount of the photocurrent and this is explained by an accumulation of the hydronium ion on one side of the membrane and the resultant free energy difference canceling the photo-driven proton-pump motive force. This membrane will be porous enough for the hydronium ion and it attains equilibrium after a short period in the dark.

Since this membrane contains a considerable amount of medium solution, the membrane resistance is relatively low (ca. 10K) while the photocurrent is relatively high. Therefore, the measurement of membrane potential was difficult and the amperometric detection is preferable as a photo-electric device.

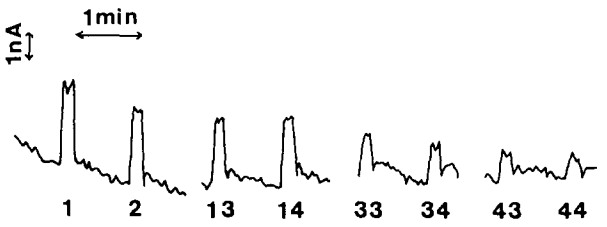


Fig.5. Change in the photocurrent behavior of BRp membrane during prolonged illumination.

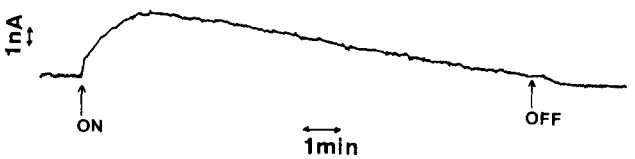


Fig.6. Change in the photocurrent behavior of BRp membrane during repeated (continual) illuminations with intervals.(Numbers indicate run numbers.)

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