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Orientation and immobilization of bacteriorhodopsin in polyacrylamide gel membranes

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Summary

Electric field application in the gel entrapment process by acrylamide-bisacrylamide system caused orientation of bacteriorhodopsin (in the form of purple membrane isolated from Halobacterium halobium). The observed photoelectric current upon illumination of the gel membrane was enhanced by this oriented immobilization and it was stable for considerable periods. Addition of ionic monomer resulted in further increase in the observed photocurrent and slight modifications in the photo-response behavior.

Biological membranes have considerable structural asymmetry or anisotropy such as seen in lipid compositions and protein distribution/orientation, which constitute a major basis of the high functions of the biological membranes(1). For modeling these 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 responsive to a given electric field. Electric field was given during the preparation of the synthetic membrane, normal to the membrane plane, to influence the distribution of the ionic substance to be fixed. When the protein has an electric dipole, not only the distribution but its orientation is influenced by applying an external electric field.

Bacteriorhodopsin (BRp) was found in purple membranes (PM) within the cell membrane of Halobacterium(4) and is known for its unique property of proton pumping upon illumination, which has attracted keen attentions as an information- or energy-transducing material(5). In these attempts, the orientation of BRp in the reconstituted matrix was, however, major problem to obtain effective transduction and most of the previous studies have noted interfacial orientations. 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 synthetic material systems(7). These attempts in some cases required a special ion-exchanging property for the membrane material and/or complicated polymerization techniques and other cases provided less practical materials.

Our electro-static field method can be applied to a wide variety of membrane materials with very simple procedure. In the previous report we presented an example of orientation of PM in a resin membrane derived from a photocrosslinkable prepolymer(8). In this paper we further apply the same principle for a combination of PM and polyacrylamide gel membrane, which is a well known substance for enzyme entrapment.

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Experimentals

Purple membrane was isolated principally according to the procedure reported(4). Membranes were prepared with an equipment illustrated in the previous report(8). A buffer solution (Hepes, pH 7) containing purple membranes (7.4mM final, as determined by optical absorbance (at 560nm)=63000), acrylamide (1.9M), bisacrylamide(0.1M), 2-mercaptoethanol (10mM) and riboflavin(0.1mM) was placed in a cell between two sheets of indium oxide evaporated polyester film (IOTO film 100L-BK-12, Toray Co.,Japan) with the conductive surface facing to the solution. In some cases 0.1M ionic comonomer (methacrylic acid (MAA, from Wako Pure Chemical, Japan), 2-dimethylaminoethyl methacrylate (DMA, from Tokyo Kasei, Japan), 2-acrylamido-2-methylpropanesulfonic acid (TBAS, from Nitto Chem.Ind., Japan) or trimethylaminoethylmethacrylate (QDM, from Nitto Chem.Ind., Japan)) was added. An electric field was applied between the film below the solution and the point-type counter electrode placed over the top film, the latter being taken as the ground level, with 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 mercury lamp (Ushio UM102, 100W) for 90min to complete the polymerization. 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 500μm). As a control, a membrane without application of electric field was prepared by the similar procedure. The obtained membrane was washed in cold water overnight and stored in the cold.

The photocurrent was measured with an electrometer (Takeda Riken, Japan; TR8651) connected to the two IOTO films, while illuminating by two halogen-lamps (100W) from the both sides of the membrane (distance 50 cm, in ambient temperature).

Results and Discussion

Figure 1 shows the typical photocurrent behavior of the BRp-membrane obtained with the application of +500V, 0V and -500V, respectively. The photocurrent was drastically enhanced by application of the electric field during the membrane preparation in the present manner and the direction was inverted by the inversion of the polarity. The maximum current was achieved within several seconds after the initiation of the illumination and it kept practically constant for about one minute or decreased slowly.

Similar results were obtained for the membranes prepared with different electric potentials but the current measured under the same condition was

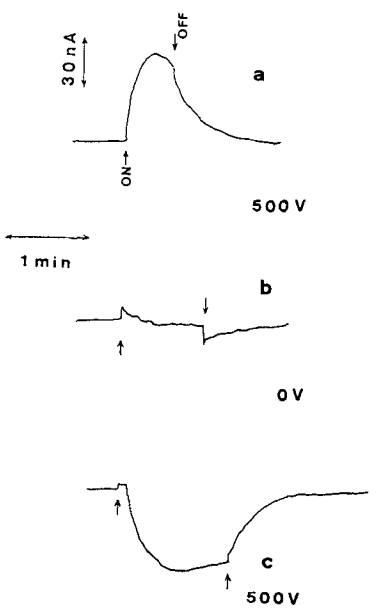

Fig.1. Photocurrent observe for an immobilized BRp(PM) membrane prepared with application of +500V(a), 0V(b) and -500V(c).

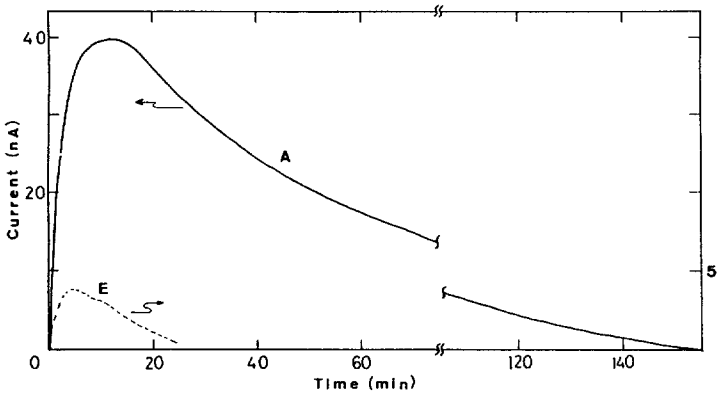

Fig.2. Change in the photocurrent behavior of BRp membrane during prolonged illumination.
—— (A), present result; --- (E), result of prepolymer membrane(8)

dependent on the voltage applied to the preparation; under the present conditions the application of 600V yielded the highest output, which is considerably higher value than that observed in the case of the prepolymer membrane(8). When the illumination was prolonged, the observed current decreased gradually and become practically zero after 150-180 minutes (Fig.2), which is far longer than the case of prepolymer membrane(curve E in Fig.2)(8).

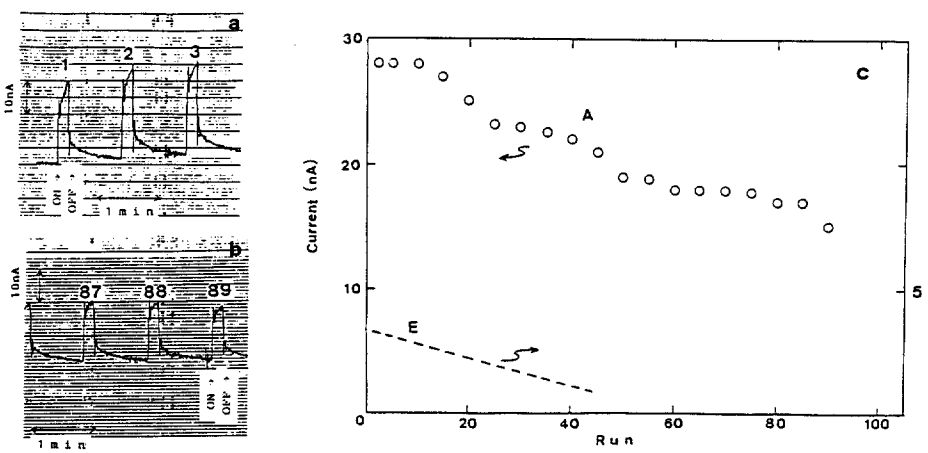

Fig.3. Change in the photocurrent behavior of BRp membrane during repeated (continual) illuminations with intervals (a,b; number shows run-number) and the withering profile of the present polyacrylamide system (A) compared with the result of prepolymer system (E) (c).

It remained relatively high for longer time, however, when the illumination was continually repeated (e.g. 1 min interval in the dark between 10s illuminations)(Fig.3). The photocurrent value itself is also higher in the present case than the case of prepolymer, which is shown in Fig.3 by a dotted curve(E). 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. After a rest the concentration gradient of proton will be evened by the thermal diffusion of the proton in the gel membrane.

Addition of ionic comonomer in the polymerization mixture resulted in a slight modification of the photo-response profile of the obtained PM-membrane and about 50% increase of the attained maximum photocurrent. Figure 4 shows the examples for the addition of anionic comonomer, MAA (a) and TBAS (b), and cationic comonomer, DMA (c) and QDM (d). A part of the reason of the increase in current would be related with the higher conductivity of these ionic gel membranes than the control acrylamide gel membrane.

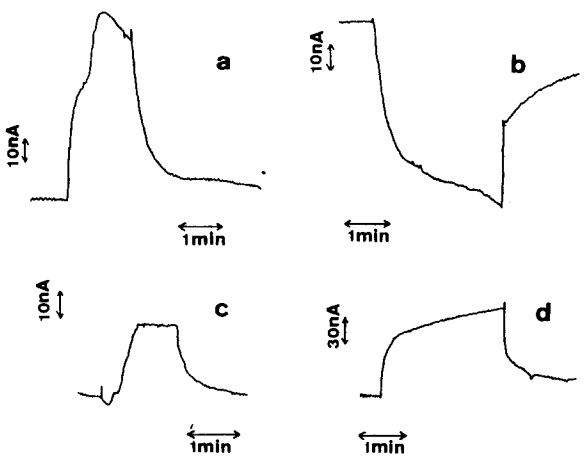

Fig.4 Influence ionic comonomer addition. (a) AAM with +500V applied during the membrane preparation, (b) TBAS with -500V, (c) DMA with +500V, (d) QDM with +500V.

The attempts to orientate PM or BRp in gel membrane were grouped into i) surface orientation method and ii) field orientation method by Caplan and Fisher (7-c). Our principle here applied appears to be classified into the second group but when we consider the indirectly (or non-electrophoretically) applied electric field on a medium with high dielectric constant we can understand that the electric potential drops steeply near the charged face of the conductive side of IOTO plate and therefore the orientation of PM will be realized only at the neighboring region of the interface, which is inherent for any other "surface" orientation method, into which, thus, our method is also to be grouped. Existence of ionic comonomer might, at least to some extent, influences or enhances the interfacial orientation.

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