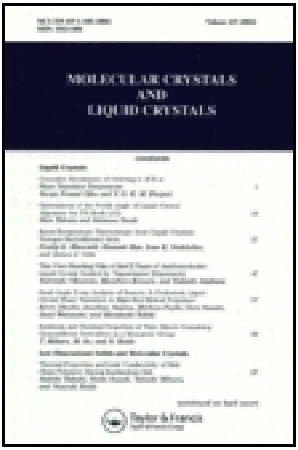

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Polarity Change of the Transient Photoelectric Signals by Orientation and pH of Purple Membrane Films

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Oriented purple membranes produce the transient photoelectric signal when irradiated by a pulsed light. This photoelectric signal contains three main components(each called B1, B2, and B3) with different lifetimes ranging from picosecond to millisecond. B1 and B2 are the internal vectorial components since the reflection of the inner charge change of the membrane. Therefore, the direction of B1 and B2 depends on the orientation of purple membrane in the film. However, B3 is not the vectorial components because the direction of B3 does not depend on the orientation of purple membrane in the film but depends on the pH of the film modified electrode in electrolyte solution.

Keywords; polarity change; oriented purple membrane; photoelectric signal

INTRODUCTION

Bacteriorhodopsin(bR) is the light-sensitive protein present in the purple membrane(PM) of *Halobacterium salinarium* which is found to have a proton pump function[1]. Light absorption induces a photocycle of bR which includes a series of intermediates;

$$bR_{568} \underset{500fs}{\overset{h\nu}{\rightarrow}} J_{625} \underset{5ps}{\rightarrow} K_{610} \underset{2\mu s}{\rightarrow} L_{550} \underset{60\mu s}{\overset{-H^+}{\rightarrow}} M_{412} \overset{+H^+}{\rightarrow} N_{520} \underset{\sim ms}{\rightarrow} O_{640} \rightarrow bR_{568}$$

As a result of this photocycle, protons are translocated from the cytoplasmic(CP) side to the extracellular(EC) side of the cell membrane, which generates an electric potential and a pH gradient. Oriented purple membrane produces an electrical signal when irradiated by a light pulse. In the present work the transient photocurrent signals have been observed from different orientation and pH.

MATERIAL AND METHODS

PM was isolated and purified from *Halobacterium salinarium* strain ET 1001. The PM film on indium thin oxide(ITO) glass electrode was obtained by electrophoretic sedimentation(ES) method.

The photoelectric measurement was carried out by using pulsed laser as excitation source. The pulsed laser is from the second harmonic(532 nm) of a Nd:YAG laser(Quanta-Ray; DCR-3, Spectra Physics Co.). The photocurrent was measured with a Keithley Model-428 current amplifier(Keithley Instruments Inc.).

RESULTS AND DISCUSSION

Figure 1 shows the transient photocurrent signals for oriented PM films excited by a pulsed laser. It can be seen that there are three components in the photoelectric signal. The B1 component is believed to be associated with the retinal photoisomerization, accompanying the formation of the bR $\rightarrow$ K and the fast charge separation in the photoexcitation of PM. The B2 component is mainly due to the proton movement across the membrane, and a good correlation between the lifetime of B2 and that of the M-formation[2].

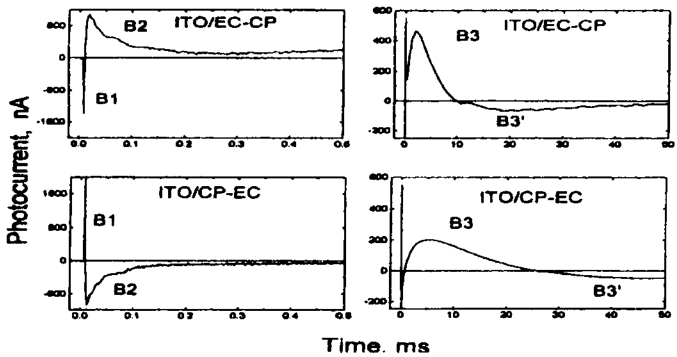

FIGURE 1. Membrane orientation effect on the photocurrent components.

The rise time of the B3 component coincides with that of the proton appearance from EC surface to solution as determined by Dencher et al. using a pH dye in solution[3]. This suggests that B3 results from the proton accumulation near the PM film modified ITO side in solution. When the EC side faces the ITO surface(ITO/EC-CP), B2 and B3 are positive but B1 is negative. However, when the orientation of PM is reversed(in this case the CP surface faces the ITO; ITO/CP-EC), the direction of B1 and B2 changed while B3 is still positive. Thus it can be seen that B1 and B2 are related to the vectorial sum of the internal charge with the PM.

Figure 2 shows the transient photocurrents at two pHs under pulsed laser excitation. The comparison between the B1 and B2 signals at low pH and at high pH demonstrates that the current directions for B1 and B2 are independent of pH. The pH affects markedly the polarity of B3. It can be seen that B3 has reversed direction at high pH versus low pH and so does B3'. Further, it was identified that B3 is responsible for the observed differential photocurrent upon CW light excitation[4]. The pH dependence of B3 component can be explained by proton release into the solution at $pH > 6.0$, whereas proton uptake from the solution occurs at $pH < 5.0$ with a delayed proton release[5].

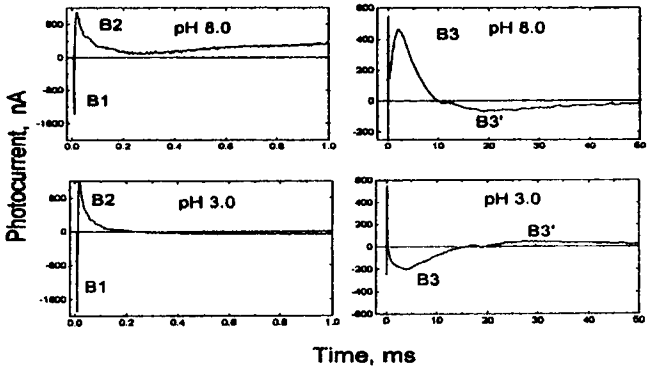

FIGURE 2. pH effect on the photocurrent components.

CONCLUSION

It has been shown that it is possible to orient the direction of the dipoles of PM molecules by the ES film deposition. The direction of vectorial photocurrent components(B1 and B2) proves the degree of orientation of PM in the film. The origin of nonvectorial component, B3, is from the average local pH change near the PM modified ITO surface.

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