Generation of Photoinduced Steady Current by Purple Membrane Langmuir-Blodgett Films at Electrode-Electrolyte Interface

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(Received October 21, 2002; CL-020894)

The Langmuir-Blodgett films of purple membrane (PM) immobilized on a gold electrode produced steady cathodic photocurrents under cathodic polarization of the electrode in aqueous electrolyte. The photocurrent is attributable to proton-reducing current that responds to proton release from light-excited bacteriorhodopsin. Effect of DC electric field at the water-air interface on the orientation of PM monolayer film was investigated.

The photosensitive membrane protein of *Halobacterium salinarum*, bacteriorhodopsin (BR), conducts unidirectional proton pumping reaction of high quantum efficiency (about 0.7).[1,2] Initiated by photoisomerization of the retinal Schiff base, vectorial relay of proton transfer occurs within the BR molecule, being driven by pK_a shifts in deprotonation and protonation equilibria of key amino acid residues associated each other in hydrogen-bonding networks.[3] Proton is finally released from the extracellular surface of BR and serves itself to form a pH gradient for ATP synthesis. Immobilization of BR as a device element has been subjects of intense studies for applications to energy conversion[4], and optical memories[5], and sensors.[6] In order to realize molecular orientation of BR for eliciting its photochemical functions, various methods have been attempted. BR is more negatively charged on its cytoplasmic surface at neutral pH. This nature has been used for electrostatic adsorption and orientation of BR in a polymer gel[7] and on a charged surface.[8] BR molecules are originally perfectly oriented in purple membrane (PM), a natural cell wall containing two-dimensional BR crystal. A relatively thick layer of oriented PMs can be obtained by electrophoretical deposition technique[9] and has been widely used for optoelectric measurements.[10] We have also studied photoelectric behavior of electrodeposited films of PM in an electrochemical cell.[11] A ultrathin film of BR of well-defined surface density and thickness, however, is difficult to be obtained by the electrophoretical methods. One solution has been shown by the antibody-assisted organization of BR monolayer film on a solid surface by sophisticated multiple processes.[12] We report here an attempt to prepare oriented Langmuir-Blodgett films of BR for eliciting the proton pumping function of BR and its electrochemical application to proton reducing (water splitting) reaction.

PM fragments were isolated and purified from S9 strain of *Halobacterium salinarum* by usual manners.[13] A spreading solution for monolayer formation was prepared by suspending PM fragments in a mixture of water, hexane, and dimethylformamide (vol ratio 5:5:1) and agitated to form an opaque emulsion.[13] Langmuir film was prepared on a circular trough filled with a distilled water. To apply a DC electric field perpendicular to the water surface (i.e., supernatant film), a couple of flat electrodes were placed in parallel in the aqueous phase and air phase, respectively, separating the electrodes by 5 mm and an electric field of 5–6 kV/cm was applied to the electrode dipped in the aqueous subphase. The above emulsion was applied to the aqueous surface between the both electrodes to form a monolayer film of PM. The PM film was transferred to the surface of gold electrode (vacuum-sublimed gold layer on a glass substrate) at a constant surface pressure of 20 mN/m by horizontally attaching the electrode plane to the monolayer. The PM-deposited electrode was removed from the water surface after removal of the surrounding monolayer. The electrode was thereby coated by a single monolayer that had been exposed to a high DC field. The above coating process was repeated to deposit a multilayer of PM. A reference multilayer film without the DC field treatment was stacked on the electrode in field-free condition by means of alternate Y-type deposition.

The electrode was set in an electrochemical cell with a platinum counterelectrode, an Ag/AgCl reference electrode, and an aqueous electrolyte containing 0.1 mol/L KCl and phosphate buffer of pH 7.0. Light source was a 500 W xenon arc lamp in combination with an infrared cut-off filter and a Toshiba short cut filter of L42. Photocurrent was measured with use of a Toho Technical Research potensiostat 2020 and a Gould 420 digital storage oscilloscope. Photocurrent action spectra was measured by a system (Bunkoh-Keiki Co.) comprising a lock-in amplifier, monochromator, xenon arc lamp, and power meter that allows correction of photocurrents for constant incident photon number.

Visible light irradiation to the Langmuir-Blodgett films with and without DC field treatment equally caused cathodic photocurrents, which comprised rapid transient components and steady-state component. Figure 1 shows a typical response profile obtained in cathodically polarized condition at −0.4 V vs Ag/AgCl. Transient cathodic and anodic currents on the onset of light switching on and off, respectively, are the characteristic differential-type response of BR.[5] As previously studied, this transient current is originated at an oxide layer present on the surface of Au electrode.[15] It is a nonfaradaic, capacitic current, which rapidly responds to a pH change brought by proton release and uptake reactions of BR. Following this capacitic current, a steady-state cathodic current remained, which is a faradaic current indicating an electrochemical reduction of certain species in the electrolyte. Figure 2 shows action spectrum of the steady-state photocurrent. The spectrum matched well the optical absorption of the chromophore of BR, i.e., retinal Schiff base, except that, despite week incident light used, there occurred no steady-current response in shorter-wavelength region (<450 nm).

Although the transient currents were measurable in all the range of applied electrode potentials, the amplitude of photocurrent showed a distinct potential dependence as shown

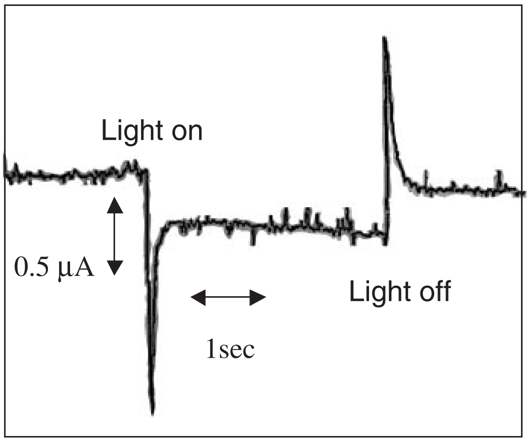

Figure 1. Time course of photocurrent response at a PM multiplayer (10 layers) deposited on a gold electrode. PM monolayer was prepared by exposure to DC field. Photocurrent is cathodic and measured at −0.4 V vs Ag/AgCl.

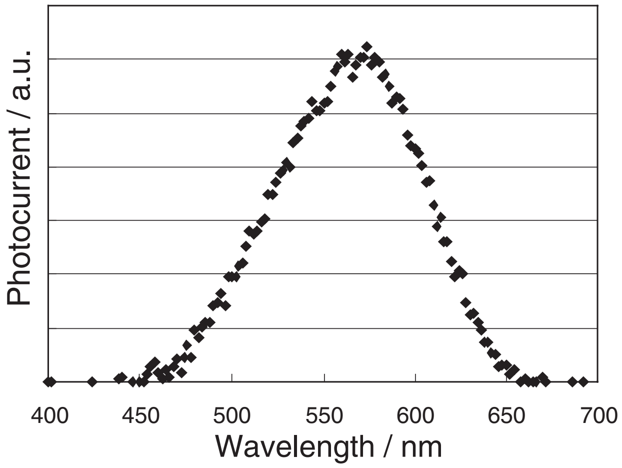

Figure 2. Action spectrum for steady cathodic photocurrent of 10 PM layers on gold electrode. Electrode potential, −0.6 V vs Ag/AgCl.

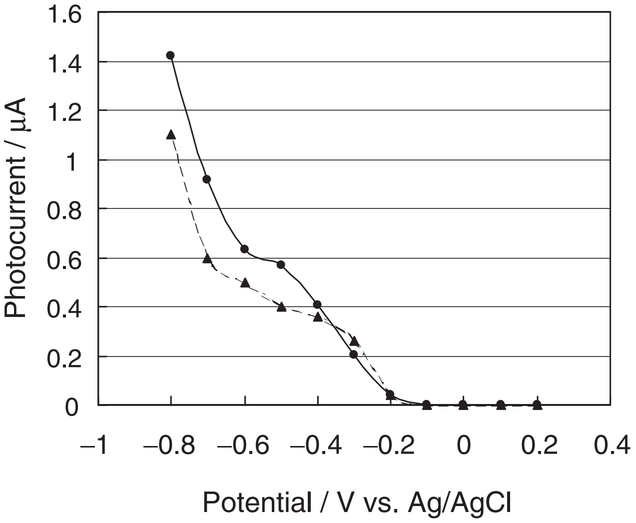

Figure 3. Electrode potential dependences of steady photocurrent for PM multilayers (10 layers) of DC-field applied preparation (solid line) and no field application (dashed line).

in Figure 3. It occurred at potentials more negative than −0.3 V vs Ag/AgCl and was steadily increased by cathodic polarization of electrode. No photocurrent occurred at a BR-free Au electrode. At the potentials where photocurrents occurred, a background cathodic current (dark current) always existed. This current is regarded as a proton-reducing current based on a rationale that $E_{H^+/H_2} \fallingdotseq -0.25$ V vs Ag/AgCl and a concomitant overpotential for hydrogen evolution. Accordingly, steady-state photocurrent is interpreted as an increase in proton-reducing current, which is caused by an increased proton concentration at the electrode surface on the proton-releasing reaction of BR.

Theoretically, with a PM film of random orientation, proton release at the extracellular (proton-releasing) surface of BR in contact with the electrode surface occurs with probability of 50%. This is assumed to be enhanced when PM sheets were oriented so that the extracellular surface of BR is oriented towards the electrode. This effect was examined with a DC field-applied Langmuir-Blodgett film of PM. DC electric field was applied by setting a positive electrode in the aqueous phase. The more negatively charged side of PM, i.e. cytoplasmic surface of BR, must thereby direct towards the aqueous phase. This process gives a PM LB film immobilized on the electrode with its extracellular (proton-releasing) side of BR directed towards the electrode surface. In Figure 3, the result of field-applied LB film was compared with nontreated LB film where amplitudes of currents were corrected for the amount of BR immobilized on the electrode surface. The field-treated LB film gave higher amplitude of photocurrent than the nontreated (random) LB film. The observed difference in photocurrent amplitude is considered to reflect the orientation state of PM sheets. The amplitude of the field-oriented film is however much lower than the theoretically predicted level (twice increase in photocurrent), apparently due to an imperfection in the degree of field-applied orientation. Atomic force microscopic observation revealed that some of purple membrane sheets in a LB film are folded, indicating that the film formation process should be improved to realize efficient orientation of the membrane sheet.

For quantitative assessment of molecular orientation, it is necessary to establish a method to discriminate the sidedness of BR molecule by direct detection, e.g., morphological observation[7] or protein surface labeling technique.[12] A method by the latter technique, by preparing the fluorescent-labeled anti-BR antibody, to determine the molecular orientation is under investigation.

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