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Photoelectrochemical Behavior of Purple Membrane Langmuir–Blodgett Films at the Electrode–Electrolyte Interface

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Langmuir–Blodgett films of the halobacterial purple membrane were formed on SnO_2 conductive electrodes. Generation and the behavior of bacteriorhodopsin-induced photocurrents at the interface of electrode and aqueous electrolyte were investigated under controlled potentials in an electrochemical cell. Efficient and rectified photocurrents were obtained, demonstrating significant dependence on the electrode potential and pH of the electrolyte.

Bacteriorhodopsin (bR) existing in the halobacterial purple membrane has proved itself to be a rare photosensitive protein endowed with various functions that are useful for construction of future molecular devices. Among recent successful applications of *in vitro* bR are those for optical computers and memories$^{1)}$ which utilize rapid photochromism or nonlinear optics of this retinal protein. Another potential application of bR is for the design of photoelectric devices, taking advantage of its charge transfer function as a proton pump. bR–based photoelectric cells have been studied extensively in both dry$^{2-4)}$ and wet$^{5,6)}$ systems in attempts to fabricate light–sensors modeled on visual reception.$^{3)}$ *In vitro* photophysics of bR and its intermediates has further contributed to help clarify the photochemical mechanisms associated with this protein.$^{4,6,7)}$ Experimentally, however, the photoelectric study of bR has so far been based on limited kinds of cell configurations, either dry sandwich–type voltaic cells with metal–bR–metal junctions$^{2-4,7)}$ or liquid junction cells comprising a bR–loaded membrane separating two aqueous solutions.$^{5)}$ These systems inevitably involve practical problems; electric outputs of dry cells are highly affected by humidity change$^{2)}$ and, in liquid junction cells, indirect contact between electrodes and bR with inserted liquid phases causes difficulty in acquiring a rapid and efficient response. In this communication, we provide a new method of exploiting the light conversion function of *in vitro* bR, based on an electrochemical junction cell which lends itself to efficient photocurrent production.

Purple membrane (PM) was isolated from the S9 strain of *Halobacterium halobium*, cultured in our laboratories, according to the conventional method of Oesterhelt and Stoeckenius.$^{8)}$ A pure water suspension of PM fragments with an optical density of 5–10 (560 nm) was mixed with hexane and DMF at a volume fraction to the suspension of 1.0 and 0.2, respectively, and emulsified by agitation on a Voltex mixer. A resultant small amount of emulsion was carefully applied on the surface of pure water in a Langmuir film balance to form an interface film of PM. The film showed a stable pressure–area isotherm with an average

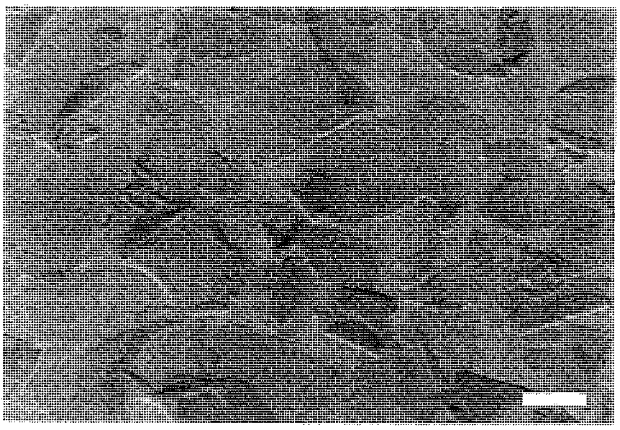

Fig. 1. Electron microscopic observation of a single PM layer transferred on to a carbon-coated micro grid under surface pressure of 20 mN/m. Inserted bar is equivalent to 0.5 μm.

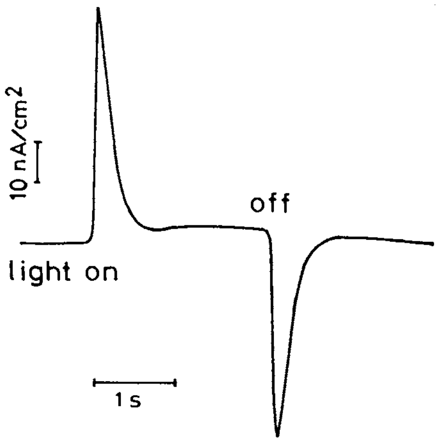

Fig. 2. Photocurrent time response of a PM LB film (2 layers) coated on SnO_2 electrode under pressure of 30 mN/m. Electrode was polarized at -0.35 V vs. SCE and irradiated with green light.

film area per bR molecule of 800–900 Å^2.[9] A single layer of PM was deposited on a carbon-coated micro grid for transmission electron microscopy and a typical result is shown in Fig. 1, where PM fragments have an average size of 0.5–1.0 μm.[10] A Langmuir-Blodgett (LB) film of PM was formed on an SnO_2 transparent electrode (5 x 5 mm in size, thickness of SnO_2 layer of 4500 Å, a specific resistance of 4 x 10^{-4} Ω cm) by depositing PM layers under a controlled surface pressure of 20–30 mN/m by the modified horizontal lifting method.[11] The PM-coated electrode thus prepared was set in an electrochemical cell containing an aqueous electrolyte of 0.1 mol/dm^3 KCl together with a platinum counter electrode and a saturated calomel reference electrode (SCE). The electrode was irradiated with green light from a 150 W xenon arc lamp equipped with IR-cut and band-pass filters and photocurrent was measured under regulation of the electrode potential with a Toho Technical Research potentiostat, Model 2020. The setup of the electrochemical cell employed here is fundamentally based on the method reported previously.[12]

Visible light irradiation to the PM electrode caused transient photocurrent under suitable regulation of the electrode potential. Figure 2 depicts a typical response profile obtained with an LB film consisting of 2 PM layers. Photocurrent always occurred in the cathodic direction at the onset of irradiation and rapidly decayed to a stationary level (almost equal to the background dark current) to give a negative stroke when light was switched off. This characteristic response can be interpreted as a sort of displacement current caused by movements of dipole and/or proton in a bR molecule following the photoisomerization of retinal. That the origin of the photocurrent is bR was confirmed by its action spectrum (see Fig. 3) which agreed fairly well with the characteristic broad absorption of bR over the wavelength region 450–650 nm.

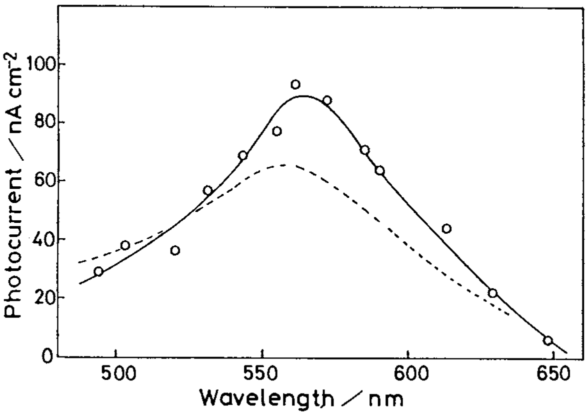

Fig. 3. Photocurrent action spectrum obtained for a stack of 8 PM layers (20 mN/m) on SnO_2 in aqueous KCl electrolyte (0.1 mol dm^{-3}) at pH 7. Electrode potential was -0.4 V vs. SCE. Interference filters were employed for monochromatic irradiation. Dotted line shows optical absorption of bR (dry PM LB film) in arbitrary unit.

Various factors influencing the photoresponse of bR were therefore investigated with respect to the maximal transient of the cathodic photocurrent.

The magnitude of the bR-induced photocurrent significantly depended upon the electrode potential with its profile being affected by the pH of the electrolyte. Figure 4 exhibits photocurrent-potential characteristics obtained for a stack of 6 PM layers in contact with electrolyte solutions of different pHs. At any pH the direction of photoresponse was maintained to be cathodic, indicating that a light-driven transfer of positive charge occurs from the electrolyte to the electrode side. Apparently, the photocurrent tends to be enhanced by increased cathodic polarization of the SnO_2 electrode. However, under highly cathodic conditions (at potentials more negative than -0.4 V vs. SCE), photocurrent is suppressed, accompanied by a noticeable increase in the background dark current due to the electrolysis, H^+/H and O_2/O_2^- , which may be responsible for the interference in the photocurrent. The photocurrent enhancement under cathodic polarization can be rationalized as an electrostatic effect of the electrode which promotes charge displacement in bR molecules favorable for generation of cathodic photocurrent.

The pH of the electrolyte highly affected the photoresponse, as revealed by the potential dependence profiles (Fig. 4). Figure 5 depicts a pH dependence of photocurrent measured at a constant electrode potential of -0.35 V vs. SCE. The result may imply both electrochemical effects and biological phenomena due to bR. Electrochemically, the surface potential of SnO_2 as well as other oxide electrodes is a function of electrolyte pH and is shifted negatively with a raise in pH.[13] An increase in pH is thus assumed to promote electrostatically the cathodic photocurrent and may account for the pH effect around pH region 5-8. In acidic and alkaline conditions, however, biological changes in bR itself must be mainly responsible for the lowering of photocurrent. It is well established that bR undergoes deactivation in its proton pump at both very low and high pHs,[14] in particular producing deionized blue membranes at low pH.[15] Suppression of photocurrent in the acidic ($pH<4$) and alkaline ($pH>9$) regions probably reflects this phenomenon.

The present electrochemical photocell sensitized by the solid-liquid interfacial layer of bR is definitely an efficient light conversion system. Under optimum conditions for electrode potential and pH, quantum efficiency of the photocell was estimated to be on the order of 10^{-2} for cells employing 1 to 10 layers of PM.

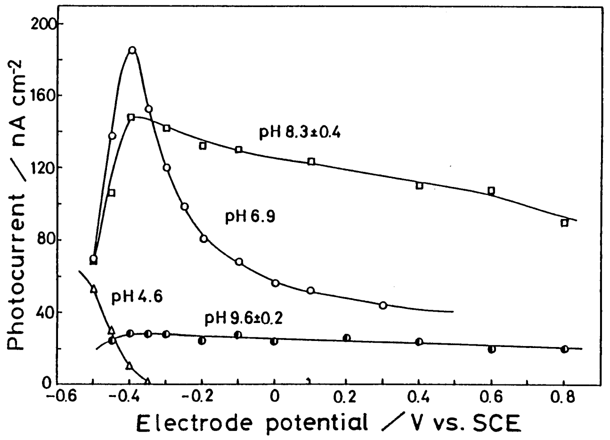

Fig. 4. Dependence of the photocurrent on the electrode potential at different pHs for a stack of 6 PM layers (20 mN/m). Irradiation was made by green light.

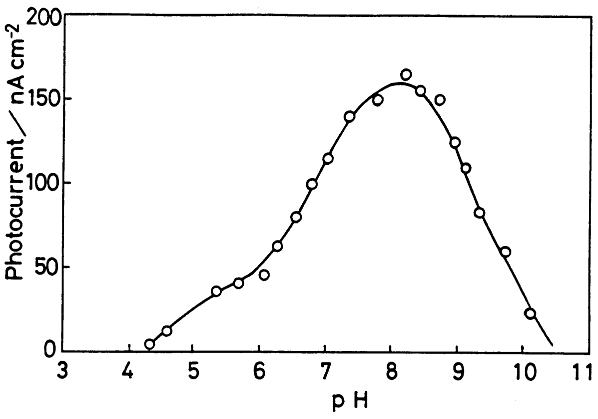

Fig. 5. Effect of pH on the photocurrent at an controlled electrode potential of -0.35 V vs. SCE. PM film used was the same as in Fig. 4. pH was adjusted by HCl/NaOH titration.

As a light conversion system, the essential advantages of this wet type photocell over previous dry photovoltaic cells[2–4,7] are that photoelectric conversion is achieved in the quantum mode and the output is free from the influence of ambient humidity. The former guarantees linearity of electric output against intensity of input light, an important prerequisite to light detectors.

The detailed mechanism for the generation and rectification of photocurrent has not been clarified. We assume that the negatively charged SnO_2 electrode is exclusively detecting a cathodic fraction of charge displacements which would be vectorially random among bR molecules in the present LB films.[16]

Excellent stability of *in vitro* bR against ambient light and atmosphere enables fabrication of a sufficiently durable light sensor. As a visual pigment analogue, bR is expected to exhibit a unique light-sensing function as presented by its differential responsivity to light intensity (Fig. 2). Rapid offset behavior in its response to a given light intensity resembles the light adapting behavior of visual perception.[9] We are presently extending this study in an effort to further develop the potential functions of bR's light sensing capability.

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10) As seen in Fig. 1, a single layer of PM (a monolayer of PM) comprises a 2–dimensional dispersion of PM fragments in which bR molecules are roughly aligned with a monolayer thickness (ca. 50 Å). In a built–up LB film of more than 6 layers, vacancies between PM fragments (see Fig. 1) as well as underlying electrode surface are virtually covered with superposed PM s.
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14) The pK's for the decrease in bR's proton release are 2.8 and 9.1, according to Ref. 6. See also for proton pump dropping at high pH, P. O. S. Ormos and L. Keszthelyi, *Biochim. Biophys. Acta*, **809**, 181 (1985).
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16) Orientation of PM s (bR molecules) in the direction vertical to the electrode plane was not particularly regulated in the present study and is virtually random between PM s.

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