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Electrode-mediated Rectification of The Differential Photocurrent Response of Dry Bacteriorhodopsin Film

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Abstract Dry bacteriorhodopsin (BR) film was fabricated into sandwich photocell and has differential photocurrent response to rectangular light pulse. And rectification was observed in the dry photocell of indium-tin oxide/BR film/Parafilm/stainless steel, but not of the steel/BR film/Parafilm/ ITO. This is an evidence of electrode-mediated rectification. Measurement of equilibrium potential suggests that the working electrode/BR film interface have different property from that of its counter electrode/Parafilm/BR film interface. The interfacial effect of the electrodes may dominate over that of the orientation of BR. Polarities of acid or base induced transient current confirmed the presence of rectification behavior of the electrode. The results will help to understand the mechanism of differential response of BR film.

Key words differential photocurrent, polarity, bacteriorhodopsin film, electrode-mediated rectification, interfacial effect
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1 Introduction

Bacteriorhodopsin (BR) is the only protein component existing in the purple membrane (PM) of *Halobactrium halobium*. It functions as a light-driven proton pump [1-3], and has been a popular advanced material for the construction of molecular sensors[4-5] or devices based on its photoelectric response[6-7].

Recently, much attention has been made to the differential photocurrent response of BR in the film to a rectangular light pulse[8-10]. The differential response and rectification have been observed in the liquid junction photocell with indium tin oxide (ITO) or tin oxide electrode / BR film / aqueous electrolyte gel / Pt electrode [8, 11]. When a rectangular light pulse is presented to the BR-based photocell, a transient photocurrent appears as a fast-rising spike (D1 component) which subsides exponentially towards the (zero) baseline. When the light pulse is turned off, another transient spike (D2 component) of photocurrent with the reversed polarity appears. That is, the onset and cessation of illumination generate a transient photocurrent of opposite polarity but continuous steady illumination generates no detectable signals. The transient photosignal appears only when there is a change of illumination level, and the polarity of spike reflects the sense of level changes[11]. The same polarities were observed for different orientation of BR. It is rectification. D1 and D2 components have been demonstrated as B3 and B3' components, respectively, in the flash measurement [12]. The brief pulse-induced photocurrent components include several components. B1 is observed on nanosecond time scale and believed to be associated with the retinal photomerization, accompanying the formation of K intermediate and the fast charge separation in the photoexcitation of BR molecule. B2 is observed on the

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microsecond time scale and mainly due to the proton translocation across the membrane. A good correlation between the lifetime of B2 and that of the L-M transition has been found in the pH range from 2.4 to 11.0 when measured under high ionic strength (such as > 40 mmol/L KCl) in a polyacryamine gel[13]. Follow B2 is B3 and B3'. Many efforts have been made to understand its mechanism[12]. One of key problems is how to understand the polarity of the differential photocurrent response. On the other hand, little information about the differential response of dry BR-based photocell is available[14-15].

Here, we present the differential photocurrent response of the dry BR-based photocell and an evidence of electrode-mediated rectification. The interfacial effects of the electrodes dominate over the effect of orientation of bacteriorhodopsin molecules.

2 Materials and methods

Purple membrane was isolated and purified from *Halobacterium halobium* strain R_1M_1 according to the standard procedure[16]. Purple membrane was washed at least three times with deionized and quartz-distilled water (its conductivity was less than 10^{-7} S/cm) and finally suspended in it. The orientation of the BR film on a steel or ITO conductive glass electrode was obtained by the electrophoretic sedimentation method[12]. Several drops of the suspension with pH 3.5 or 7 were placed between two electrodes about 1 mm apart, and the film was formed and dried under a 3 V electric field. The ITO or steel electrode was as anode and another steel sheet as cathode. Two types of oriented films were studied with either the predominant extracellular or cytoplasmic surface facing the working electrode directly, giving orientation patterns of ITO or steel/nBRc film and ITO or steel/cBRn film, respectively. The orientation of BR was confirmed by observation of the polarity of flash-induced B2 photocurrent component, or by comparison with X-type Langmire-Blodegget multilayer film[14]. The relative humidity was obtained by equilibrating the films at least 48 h in the close chamber in an atmosphere that was in equilibrium with saturated salt solution, for example, NaBr for 57% of relative humidity. The expected humidity-equilibrated film was sealed by Parafilm (American National Can, Grrenwich, CT, 06836) and fabricated into photocell with the type of the working electrode/BR film/Parafilm/counter electrode, in which counter electrode was led to ground(Figure 1). Photoelectric signal was picked up by MDY-2 Biomembrane Electric Parameter Meter (made in China)[14]. White light from 30W of halogen lamp and filtered was used as a source.

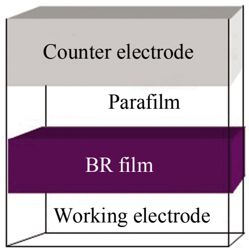

Fig. 1 Diagram of dry bacteriorhodopsin-based photocell

3 Results and discussion

3.1 Differential photocurrent signal of ITO/BR film/Parafilm/Steel

Shown as in Figure 2, the photocurrent signal is typical of differential, as in the wet BR film. The peak value is linearly dependent on the change in the illumination level (Figure 3). Both types of oriented film share the same polarity of photocurrent. A negative spike is observed if onset of illumination, and a positive one if cessation of illumination. In other words, a negative spike corresponds to decreasing of illumination level and a positive one to increasing of light intensity. No photocurrent is observed in the absence of BR film. Therefore, the photoelectric signal results from BR. BR functions as a proton pump. The light-induced release by BR of proton onto the periplasmic side of the purple membrane and the subsequent uptake of proton from the cytoplasmic side

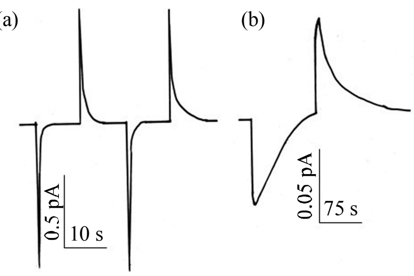

Fig. 2 Photocurrent of dry photocell resulted from ITO/cBRn film/Parafilm/steel

(a) 0.58 nmol/mm² BR molecules, the input resistance (R) was set at $10^7\Omega$. (b) 0.086 nmol/mm² of BR molecules, R was $10^{10}\Omega$. The relative humidities of both BR films were at 57%.

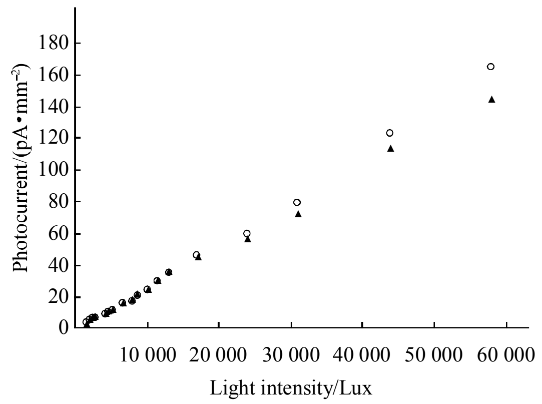

Fig. 3　Photocurrent is linearly dependent on the change in the light intensity

Bacteriorhodopsin molecules of 0.58 nmol/mm² was in the dry photocell with ITO/cBRn film/Parafilm/steel, in which the film was at 43% relative humidity. White light from 30W of halogen lamp and filtered was used as a source. ○ : LIGHT ON; ▲ : LIGHT OFF.

is apparently directional. Opposite orientation resulted in the same polarity of differential photocurrent. This is rectification. In the other words, this rectification occurred in not only the wet BR film, but also the dry BR film. However, in all photocells studied, ITO was as working electrode. How is about other material as working electrode?

3.2　No Rectification was observed in the Steel/BR film/Parafilm/ITO sandwich

The opposite polarity of differential photocurrent spike was observed in the photocell with opposite BR film, that is, the polarity of the differential current of photocell with structure of steel/cBRn film/Parafilm/ITO was opposite to that of steel/nBRc film/Parafilm/ITO, as shown in Figure 4. In the other words, no

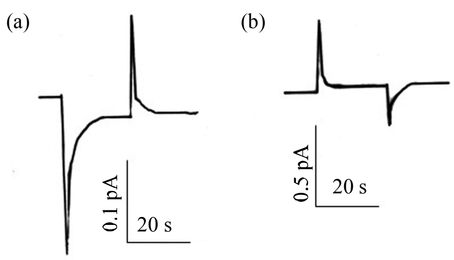

Fig. 4　Photocurrent of dry photocell resulted from steel/cBRn film/Parafilm/ITO

(a) 0.1 nmol/mm² BR molecules. (b) 0.086 nmol/mm² of BR molecules. The film was at relative humidity of 57%, and the input resistance of measuring device was set at $10^9\Omega$.

rectification presented if steel was as a working electrode with its ITO counter electrode. These facts suggest that the rectification of differential response of BR film is electrode-mediated.

3.3　Equilibrium potential

Equilibrium potential results from the interaction between the two electrode interfaces. A positive one was observed in ITO/Parafilm/steel. However, if BR film is inserted into the either electrode and Parafilm interface, respectively, the respect electrode is anode and another counter electrode is cathode, no matter which orientation of BR molecules. This means that the effect of interface itself may be dominant over that of the orientation of BR molecules, although their details are imperfectly clear.

3.4　Rectification behavior of electrode

BR functions as a proton pump. The light-induced release by BR of protons onto the periplasmic side of the purple membrane and the subsequent uptake of protons from the cytoplasic side should result in the change in the proton concentration in the both electrode interfaces. Could the change of proton concentration result in transient current signal? The experimental results have given a positive answer.

Put ITO and steel plates parallel in a square cell with its dimensions of 1 cm × 1 cm × 4 cm, filled with distilled water or 30 mmol/L HCl aqueous solution. Each electrode is tightly next to a wall of cell. When 10 μl 1 mol/L of HCl or NaOH aqueous solution was injected towards and onto the either interface of electrode, a transient current could be observed. The polarities showed that under special conditions, some electrodes could have rectification behavior, and is special to the proton (Table 1).

Table 1　The polarity of acid or base induced transient current of ITO/water/steel*

	Acid	Base
ITO/water interface	Positive (anodic)**	Negative (cathodic)
Steel/water interface	Negative (anodic)	Positive (cathodic)
ITO/30 mmol/L HCl solution interface	Positive (anodic)	Negative (cathodic)
Steel/30 mmol/L HCl solution interface	Positive (cathodic)	Positive (cathodic)

* Steel electrode was grounded. ** The words in parentheses indicate the polarity if the respect electrode was as working electrode and another as its counter electrode.

4 Conclusion

Here evidence of electrode-mediated rectification of differential photocurrent response of BR film to a rectangular light pulse is given. Dry BR film in sandwich photocell with the working/BR film/ Parafilm/counter electrode has differential photocurrent to rectangular light pulse, as in the wet BR film. But in not all cases rectification occurred. The results will redound to understand mechanism of the differential photocurrent response of bacteriorhodopsin film. The mechanism of the differential photocurrent response and the modified model will be studied in future.

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电极介导的菌紫质干膜微分光电流响应的整流性质

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摘要　在长方形光脉冲光照下，菌紫质(bacteriorhodopsin，BR)干膜组装成夹层光电池具有微分光电流响应．在氧化铟锡(ITO)导电玻璃 /BR 膜 / 封口膜 / 不锈钢形成的干膜电池下可观察到整流特性，而在不锈钢 /BR 膜 / 封口膜 /ITO 导电玻璃形成的干膜电池下则观察不到整流特性，这说明是电极介导的整流．平衡电压测定表明：工作电极 /BR 膜表面与对电极 /BR 膜表面有不同的性质，电极的界面效应控制了 BR 的取向．酸与碱产生的瞬间电流极性也证实了电极整流行为的存在．这些结果将有助于了解 BR 膜的微分光电响应．

关键词　微分光电流，极性，菌紫质膜，电极介导整流，界面效应

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