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# Mechanisms of Pulse Response and Differential Response of Bacteriorhodopsin and Their Relations¶

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## ABSTRACT

**Bacteriorhodopsin (BR) films are oriented and deposited on indium tin oxide conductive glass by using electrophoretic sedimentation and Langmuir–Blodgett methods to construct sandwich-type photocells, respectively. The pulse response photoelectric signal of the BR photocell under pulsed laser and the differential response photoelectric signal under irradiation of interval light are measured. The origins of these two types of photoelectric responses and their correlations are analyzed. The pulse response signal initiates from the ultrafast charge separation of the retinal and the proton translocation followed by the deprotonation and reprotonation of the Schiff base and its surrounding amino acids. This is a quick response and is the preceding reaction of the differential response. The differential response signal is caused by the charging and discharging of the continuous proton current of the BR light-driven proton pump at light-on and light-off, which is a slow process. The differential response is related to not only the construction of the BR photocell but also the coupling mode of measurement. To observe the differential response signal, the BR photocell must have large enough $B_3$ and $B_3'$ components in its pulse response as well as an alternative coupling mode to measure it.**

## INTRODUCTION

Bacteriorhodopsin (BR) is one of the rhodopsin protein families (rhodopsin, halorhodopsin, etc.). It consists of a chromophore-retinal and a seven-times folded α-helix amino acid chain in its molecule composition. Because of its special conformation, it has the function of a light-driven proton pump. When irradiated by light, a photoelectric signal can be generated in BR. This was discovered in former studies on the characteristic of a light-driven proton pump in BR. When BR is embedded in a membrane and irradiated with continuous light, protons flux can be initiated from one side of the membrane to the other. Because of the insulation of lipid bilayer, the proton potential would not disappear through concentration diffusion in a short time. Thus electric potential is formed between the two sides of the membrane. This direct current is very important to the photosynthesis of adenosine triphosphate in *Halobacterium salinarium* (1). A short pulse irradiation on BR results in a transient photoelectric signal (2,3), whereas interval light stimulation cause a differential photocurrent according to the changes of the light intensity (4,5). These phenomena were referred in many articles. So far it is clear that the BR photoelectric response to pulse light is caused by the charge separation of the chromophore and the intramolecular protons translocation, which result in a displacement current that is regarded to be closely related to the photocycle (3). The interpretation to the differential photocurrent remains controversial, though there were many experiments and even applied researches on this aspect (6,7). Hong (4) proposed a BR membrane equivalent circuit model to interpret the BR differential response, but it was too complex to determine many circuit parameters in this model. Wang (5) studied the characteristics of different oriented BR films responding to the pulse laser and the interval light. He thought that the differential response was related to the pulse response and was caused by the slower components in pulse response. But he did not elucidate the origin and the determining factors of these slower components. In this article, the mechanism of BR photoelectric responses was experimentally studied and analyzed. As a result, we propose a new model that gave a new interpretation to the pulse photoelectric and the differential photoelectric responses as well as their relations.

## MATERIALS AND METHODS

Purple membrane (PM) was isolated from the $R_1M_1$ strain according to the conventional method. PM fragments were directionally deposited on a transparent conductive indium tin oxide (ITO) glass by using electrophoretic sedimentation and Langmuir–Blodgett (LB) methods. The procedure of depositing PM was described in detail in references (8) and (9). The type of these oriented BR membranes is ITO/CP-EC, *i.e.* the cytoplasmic (CP) membrane is attached to the ITO surface. The BR film photocell was constructed as a sandwich-type electrochemical cell comprising junctions of ITO–BR film–aqueous electrolyte–counterelectrode. The structure is shown in Fig. 1. In electrophoretic sedimentation, the counterelectrode was ITO, the electrolyte was composed of 0.6% gelose and 1 mol/L potassium chloride (pH7). In BR-LB film, the counterelectrode was Au, the electrolyte was 1 mol/L potassium chloride (pH 7). All the structure was sealed with glue and mounted within a copper box. The effective light sensitive area was 5 mm in diameter. Similar photocells without BR films were also constructed to make a comparison measurement. The photoelectric signal was directly measured by a digital

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*Abbreviations:* AC, alternative couple; BR, bacteriorhodopsin; CP, cytoplasmic; DC, directive couple; EC, extracellular; ITO, indium tin oxide; LB, Langmuir–Blodgett; PM, purple membrane.

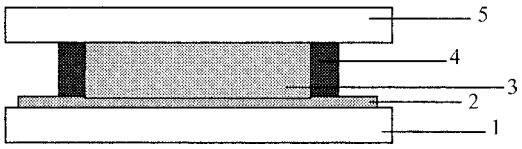


**Figure 1.** The structure of oriented BR film photocell. 1 represents ITO glass with the conductive side to the oriented BR film. 2 represents the oriented BR film deposited on ITO. The type of the oriented BR membrane is ITO/CP-EC, *i.e*. the cytoplasm of BR is attached to the conductive side of ITO glass. BR film is oriented and deposited on ITO by electrophoretic sedimentation and LB methods, respectively. 3 represents electrolyte. In electrophoretic sedimentation BR photocell, the electrolyte is composed of 0.6% gelose and 1 mol/L potassium chloride (pH7). In BR-LB photocell, the electrolyte is 1 mol/L potassium chloride (pH7). 4 represents Teflon spacer with 0.6 mm thickness. 5 represents counterelectrode. In electrophoretic sedimentation BR photocell, the counterelectrode is ITO glass. In BR-LB photocell the counterelectrode is Au. All the structure is sealed with glue and mounted in a copper box. The effective light sensitive area is 5 mm in diameter. The output electric signal is directly coupled into a digital oscilloscope with the ITO substrate connected to negative electrode and the counterelectrode to positive electrode.

oscilloscope (TDS680B, Tektronix Inc., Beaverton, OR) with the ITO substrate connected to negative electrode and the counterelectrode to positive electrode.

Laser pulse was generated by a Q-switched double frequency Nd: YLF laser (GSD-20-527L, Photonics Industries International Inc., Bohemia, NY) with 527 nm wavelength, 100 ns pulse width and 1 kHz repetition rate. The average output power is 530 mW. Interval light was obtained by using a halogen lamp and a mechanical shutter. The average light power was about 200 mW. Electric signal was measured by a digital oscilloscope with 1 MΩ input impedance. A rectangular waveform generator was used to simulate the effects of how differential response happens and how directive coupling (DC) and alternative coupling (AC) modes of oscilloscope affect the measuring waveforms.

## RESULTS AND DISCUSSION

### Response of photocell without BR to light illumination

The photocells without BR were first tested by pulsed laser and interval light. No photoinduced electric signal was observed except the light independent current, the so-called dark current, which was from potential differences between electrodes. This confirmed that the following results of the BR photocells came solely from the BR itself rather than the cell assembly.

### Response of BR to pulsed laser

Pulse laser illumination on the oriented BR photocell results in a bipolar signal (a faster negative signal and a slower positive signal) shown in Fig. 2. The characteristic of the signal is determined by the structure of the BR photocell. The surface of BR is bound with bivalent metal cations, charge amino acids and polar phospholipids to form an asymmetrical distribution of charges on the membrane surface. This results in a dipole moment that points from the CP to the extracellular (EC) side (4). When illuminated, the retinal is initiated with a photochemical reaction of all-*trans* to 13-*cis* isomerization and a polarization of electron clouds in the retinal, which make the cytoplasm to have positive potential with respect to the EC side. Thus a displacement photocurrent from the positive to negative electrodes within the photocell is produced, whereas in external circuit the current is from negative to positive (Fig. 3A). This process forms the negative signal. Consequently, the proton pump is initiated. A proton is transferred from Schiff base to the receptor-Asp85, then to the EC side. At the same time,

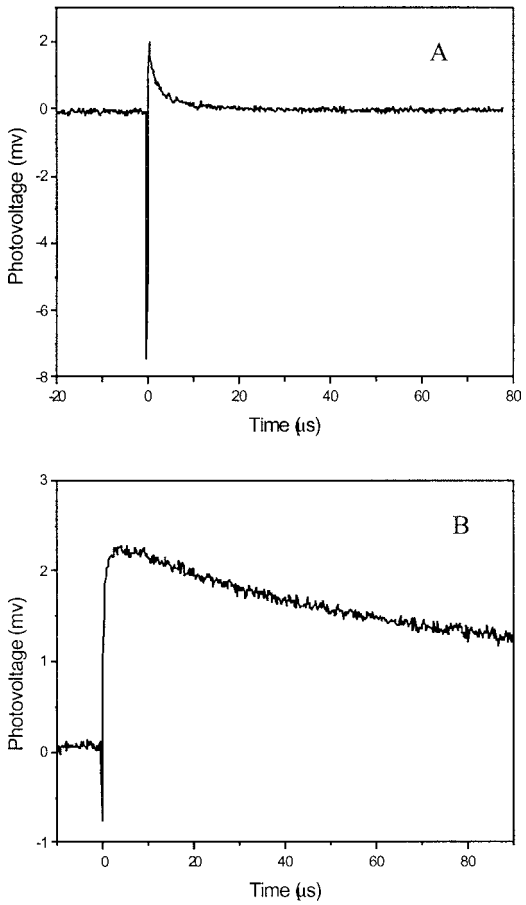


**Figure 2.** The response signals of the oriented BR photocell to pulsed laser. Pulse laser is generated from a Q-switched double frequency Nd: YLF laser (Photonics Industries International Inc.) with 527 nm wavelength, 100 ns pulse width, 1 kHz repetition rate and 530 mw average power. The output signal is measured by a digital oscilloscope (Tektronix Inc.). In the figures, bipolarity signals (a faster negative signal and a slower positive signal) are measured when the oriented BR photocells are illuminated by pulsed laser. A: The electrophoretic sedimentation BR photocell response to the pulse laser. B: The BR-LB photocell response to the pulse laser. The figures show that different structures of BR photocells have different response waveforms. The ratio of the positive signal to the negative signal and the relaxation time of the positive signal are quite different for both the photocells.

the doner-Asp96 transfers a proton to Schiff base and takes in a proton from the cytoplasm. As a result, a proton is pumped from the cytoplasm to the EC side. The direction of photocurrent in this process is from negative to positive electrodes within the photocell, whereas in external circuit it is from positive to negative, which forms the positive signal (Fig. 3B).

### Response of BR to interval light illumination

The response of BR photocell to interval light illumination is complex. It was found that the appearance of differential response was related to the construction of the BR photocell as well as the coupling mode of measurement. In the experiment, the electrophoretic sedimentation BR photocell showed no differential response, whereas the BR-LB photocell had a differential response. However, different waveforms were observed when using different coupling modes of oscilloscope—DC or AC (shown in Fig. 4A,B). Only in AC coupling mode, the differential response was observed.

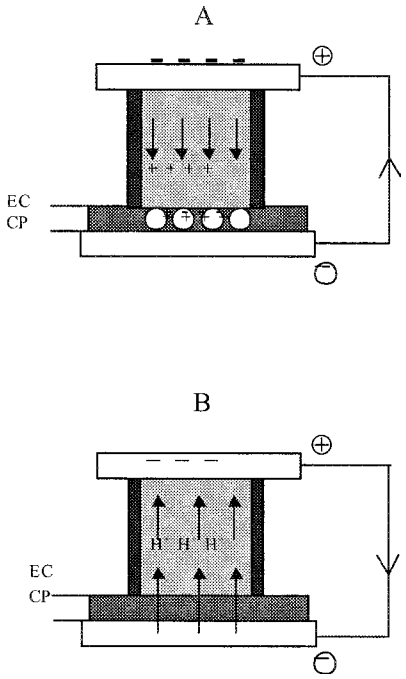


**Figure 3.** The mechanism of bipolarity signal. A: The mechanism of the fast negative component. This signal is produced by the photochemistry of all-*trans* to 13-*cis* isomerization and the polarization of electron cloud in retinal, which results in a quick charge separation and causes the EC side of BR membrane to be negatively charged and the cytoplasm positively charged. Thus positive ions in the electrolyte are attracted to the interface of BR membrane and negative ions leave for the counterelectrode. In the external circuit, the direction of photocurrent is from negative to positive electrodes, resulting in the negative signal. This photocurrent is a displacement current because there is no protons flow. B: The mechanism of the slow positive component. This signal is produced by the proton pump, which pumps protons from the CP to EC side to form a continuous conductive proton flow. The direction of the photocurrent is from positive to negative electrodes in the external circuit, whereas from negative to positive electrodes in internal circuit.

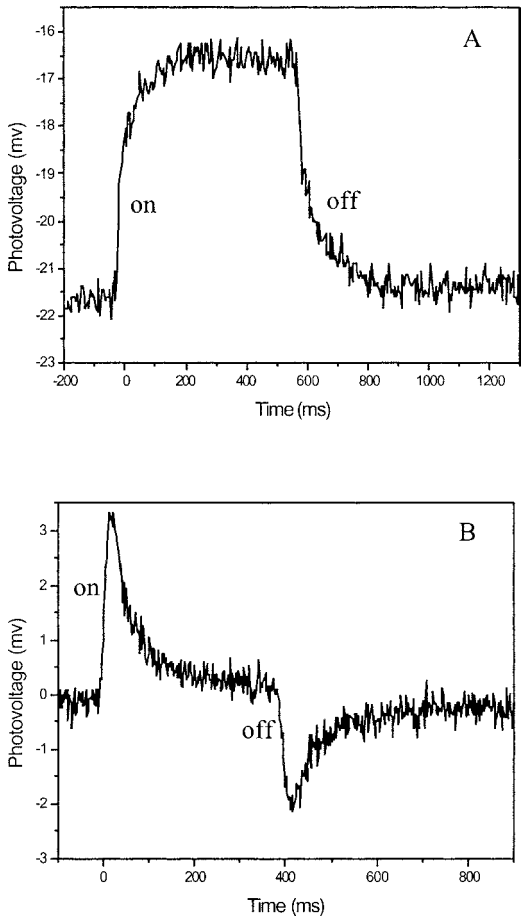


**Figure 4.** Waveforms of the BR-LB photocell response to the interval light, measured by the digital oscilloscope DC and AC modes at 1 MΩ input impedance. A: Waveforms obtained at DC. B: Waveforms obtained at AC. All the measuring conditions are same except for the coupling mode. The waveform in B is the so-called differential response. Obviously the differential response is related to the coupling mode of oscilloscope.

In the measurement, all conditions were same except for the coupling mode of oscilloscope. It was probably that the same original photocurrent was deformed by different measuring modes. To check this hypothesis, we performed a simulation experiment as follows: a rectangular waveform with 1 s cyclic time was generated, then measured by DC and AC coupling mode of oscilloscope at 1 MΩ input impedance, respectively. It could be seen that the rectangular signal was slightly deformed when measured by DC mode, whereas in AC mode it was completely changed into a differential signal. In fact, in the internal circuit of oscilloscope, the AC coupling mode is different from the DC coupling mode just because of serially connected high-pass filtering capacitor in the input circuit. Therefore direct current and slow signal are resisted, only fast components can pass through. However, in DC mode, both low and high frequency components can pass through. According to Fig. 4, we supposed that the original response waveform of the BR photocell to interval light was likely to be a rectangular one, which actually corresponded to the process of opening and closing of the light-driven proton pump. It is certain that for any photocell, if it can generate a rectangular signal to the interval light and serially connected a high-pass filtering capacitor in its output circuit, a differential response signal will be obtained.

## Relations between the pulse response and the differential response

Comparing the pulse response waveforms of different BR photocells, we found that the negative component remained unchanged (approximately equal to the excitation laser pulse in time), whereas the shape of the positive component was quite different. This difference might be the reason whether the differential response occurred or not. In Fig. 2 the positive component of electrophoretic sedimentation BR photocell is low in amplitude and fast in relaxation, whereas the BR-LB photocell gives a reverse result, which causes the former to have no differential response and the latter to have one. This implies that the differential response is closely related to the shape of the positive component. Hong (4) and Wang (5) studied the positive components. They thought that they were composed of a faster component $B_2$ and two slower components $B_3$ and $B_3'$. Quoting their results, a better interpretation is given in this article. $B_1$ is the negative component. $B_2$ results from the fast proton displacement when BR illuminated, which occurs near the surface and inside of the BR membrane and is a positive signal. $B_2$ component is only related to the characteristic of BR itself, like the $B_1$ component. That is, to any

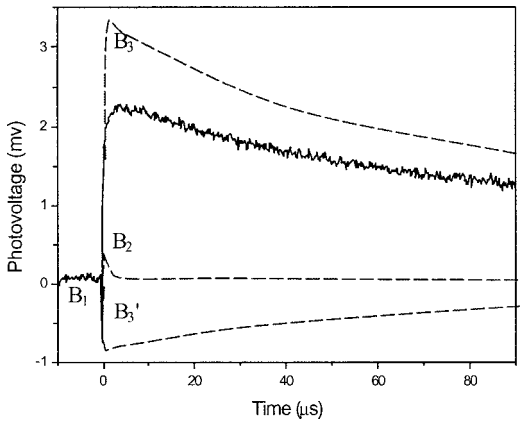


**Figure 5.** Decomposition of the pulse response signal of the BR-LB photocell. The waveform can be divided into four components: $B_1$, $B_2$, $B_3$ and $B_3'$. $B_1$ is a negative component, which is a displacement current generated by the fast charge separation of retinal. $B_2$ results from the fast proton displacement that occurs near the surface and inside of the BR membrane and is a positive signal. $B_1$ and $B_2$ are only attributes to the properties of the BR molecule. $B_3$ is a conductive current generated by the light-driven pump proton, which flows from the negative to positive electrodes within the photocell, charging the photocell. $B_3'$ is a reverse current formed by proton gradient diffusion built up between the membrane's two sides by the light-driven proton pump. $B_3$ and $B_3'$ are slow components. They are responsible for the differential response. There will be no differential response if the $B_3$ and $B_3'$ components are small.

BR photocell, the polarities of $B_1$, $B_2$ and their ratio are certain. The differences are in $B_3$ and $B_3'$ components. The formation of $B_3$ is thought to be the charging process of the photocell. In this process, the light-driven pump proton generates a conductive current, which flows from the negative to positive electrode within the photocell, charging the photocell. The rising edge of the photovoltage and the positive pulse of the differential response are determined by $B_3$, which is also a positive signal. $B_3'$ is thought to be a reverse current formed by proton gradient diffusion, which is built up between the membrane's two sides by the light-driven proton pump. Because of the high resistance of PM, it is a slow negative signal. When light is off, the photocell discharges in two ways; one is from the positive to negative electrode through the external circuit and the other is through the $B_3'$. The falling edge of the photovoltage and the negative pulse of the differential response are related to $B_3'$. According to this analysis, the pulse response of the BR-LB photocell can be decomposed into several components as shown in Fig. 5. For the electrophoretic sedimentation BR photocell, no differential response was detected because the values of $B_3$ and $B_3'$ were very small.

What is related to $B_3$ and $B_3'$? According to this experiment, it is likely that $B_3$ and $B_3'$ are related to the electrolyte and the counterelectrode. In the BR-LB photocell, a solution electrolyte and an Au counterelectrode were used. In the electrophoretic sedimentation BR photocell, a gel electrolyte and an ITO counterelectrode were used. But compared to our previous results (10), if the ITO counterelectrode was substituted by a copper electrode, the differential signal could also be observed. So, the effect of electrolyte can be excluded. $B_3$ and $B_3'$ are mostly related to the counterelectrode.

## CONCLUSION

The photocell based on oriented BR membrane is a rather complex photoelectric system. The response characteristic is related not only to the properties of BR molecular but also to the BR surrounding conditions and the electrode material. The native function of BR molecule is to generate a negative displacement signal from the primary photoinduced charge separation of retinal and consequently a positive displacement current from the light-driven proton pump, in which deprotonation and protonation of Schiff base and its surrounding amino acids cause protons transferring from CP to EC sides. These are fast, photoinduced responses happening inside the BR molecule. Afterward are slow processes, during which the light-driven proton pump generates a continuously conductive proton current to charge the photocell through the electrolyte and the photocell discharges through the external and internal circuits. These two processes are responsible for the BR photocell generating the direct current under continuous light illumination and the differential response to the change of light intensity. It is proved that the measuring methods and the counterelectrode material directly affect the differential signal whether it can be observed or not. The mechanisms of pulse response and differential response of BR are different but also related. The pulse response is the early response and the base of differential response. The appearance of $B_3$ and $B_3'$ components of the pulse response signal determine whether the differential response happens or not. $B_3$ and $B_3'$ are related to BR surrounding conditions such as humidity, pH value, *etc*. They are also directly related to the counterelectrode material.

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