

Multi-exponentially Photoelectric Response of Bacteriorhodopsin *

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A thin oriented bacteriorhodopsin (bR) film is deposited on a stainless steel slide by use of the electrophoretic sedimentation method. A junction is made with electrolyte gels having a counterelectrode to construct a bR-based photoelectric detector. The photoelectric response signal to a 10 ns laser pulse is measured. A theory on the photoelectric kinetics of bR is developed based on the concept of the charge displacement current and the bR photocycle rate equations. Comparison between the theoretical and experimental results proves that the bR photoelectric response to a short laser pulse is a multi-exponential process. The decay time constants and amplitudes of each exponential component are obtained by data fitting.

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Bacteriorhodopsin (bR) found in the purple membrane of the Halobacterium salinarium (previously called the Halobacterium halobium) is a retinal protein and functions as a light-driven proton pump. Upon absorption of photons, it initiates a photocycle with the primary photochemistry of 13-trans to 13-cis isomerization of the all-trans retinal within hundreds of femtosecond, and then followed by a series of spectroscopically distinct thermal reactions spanning the picosecond to millisecond time domain. The bR is highly durable and stable in ambient conditions as compared to other proteins. For example, it can retain its native structure even at 140°C and keep active for several years.¹ So bR has attracted attention for numerous technical applications, especially in the fields of optical information processing²⁻⁴ and visual simulation.⁵⁻⁷ Many researches have shown that illumination of an oriented bR sample results in a photovoltage (or photocurrent). The characteristic of the photovoltage is the bipolarity, i.e., the initial phase of the photovoltage is negative and ultrafast (ps), which reflects the very early photoinduced charge separation of the retinyl chromophore, and the followed components are positive and slower ($\mu\text{s}\sim\text{ms}$), which are concerned with the transfer of protons in the bR molecule and regarded as being closely related to the photocycle. Although the photoelectric properties of bR have been studied for years, most studies are restricted to the experimental aspects, such as the measurement of the ultrafast response,⁸ the dependence of response signal on the environment⁹ and the measuring impedance,¹⁰ the mechanism of differential photocurrent,¹¹ the development of some practical optoelectronic devices^{5,6} and so on. What is missing is a

systematical theory describing the whole photoelectric kinetics of bR, especially with a quantitative theory.

In this letter, we analyse the photoelectric response of bR to a short laser pulse based on the concept of the charge displacement current and the rate equations of the bR photocycle. The derived kinetic equation indicates it is a multi-exponential process, which is finely proved by our experimental results.

Purple membrane (PM) is isolated from the R₁M₁ strain according to the conventional method.¹² A drop of PM aqueous suspension is placed on a stainless steel slide and covered with a second electrode 1 mm away. Then a potential (~ 2 V) is applied between the electrodes. Under the electric field, the PM fragments partly orient, migrate and finally deposit onto the anode and retain their orientation when the electrodes are separated. The bR film photodetector is constructed as a sandwich-type electrochemical cell comprising junctions of stainless steel slide/bR film/aqueous electrolyte gel (1 mm thick)/counterelectrode (copper), where the Cu electrode is ring-shaped so as to direct the incident light onto the bR film. The viscous gel is composed of 10% carboxymethyl chitin and 1 M potassium chloride (pH6). The bR film photodetector is illuminated by a Q-switched double frequency Nd:YAG laser with a 532 nm wavelength, 10 ns duration and 10 Hz repetition rate. The photoelectric response signal is directly coupled into a 300 MHz digital oscilloscope. Through an IEEE488 GP-IB interface card, a PC microcomputer running with our specially developed software controls the oscilloscope to acquire data, store waveforms and process the experimental results. The experiment is performed at room temperature.

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A bR molecule, once excited by the visible light, takes a very rapid charge separation of the protonated Schiff base from its counter ion. This ultrafast step converts photoenergy into an electric potential, which drives protons of the amino acid sequence of the bR to transport running the proton pump. Because a large number of bR molecules are orderly arranged, this rapid charge separation and the protons' movement in the bR molecules can induce a measurable displacement current in the external circuit. The bR film photodetector can be simply equivalent to a light-driven displacement current source with resistance R and capacitance C in the circuit. Assuming there is a charge Q in a bR molecule moving between two electrodes over a distance D , then the charge induced voltage in the measuring circuit is

$$V_i(t) = \frac{Qd_i}{DC} e^{-t/RC}. \quad (1)$$

where d_i is the displacement of the charge transiting from position i to position $i + 1$. In a laser flash experiment, many bR molecules are excited, therefore, many protons move. Suppose the bR photocycle has five intermediates and takes five-step thermal relaxations. Assign the five intermediates to 1, 2, 3, 4, 5 and assign the stimulated bR ground state to 0 and the thermal restored bR state to 6. The laser pulse shape is simplified by a rectangular function having duration time τ and intensity I_0 . Assuming that n_i ($i = 0 - 6$) is the population of each state and the excitation rate constant $k_0 = (\eta\sigma/h\nu)I_0$, where $h\nu$ is the excitation photon energy, η and σ represent the quantum transition efficiency and the absorption cross section of the bR ground state, respectively. We have the following rate equations of the bR photocycle:

$$\dot{n}_0 = \begin{cases} -k_0 n_0, & 0 < t < \tau, \\ 0, & t \geq \tau, \end{cases} \quad (2)$$

$$\dot{n}_{i+1} = k_i n_i - k_{i+1} n_{i+1}, \quad (i = 0 - 5, k_6 = 0). \quad (3)$$

From Eqs. (1)–(3), we obtain the transient photovoltage waveform in the measuring circuit:

$$V(t) = \sum_{i=0}^5 V_i(t) \otimes \dot{n}_{i+1} = \frac{Q}{DC} [d_0 k_0 e^{-t/RC} \otimes n_0(t) + \sum_{i=1}^5 (d_i - d_{i-1}) k_i e^{-t/RC} \otimes n_i(t)]. \quad (4)$$

The first convolution item describes the excitation process and the remaining items express the thermal relaxations.

During excitation ($0 < t < \tau$), Eq. (2) gives

$$n_0(t) = N e^{-k_0 t}, \quad (5)$$

where N is the population of the bR ground state. Considering that the total population of level 1 is $N_t \approx$

$N(1 - e^{-k_0 \tau})$ after excitation and letting $\theta = 1 - e^{-k_0 \tau}$ which represents the excitation efficiency, we get the fast stage of the photovoltage, i.e., the first item in Eq. (4):

$$V_f(t) = \frac{N_t Q R d_0 k_0}{\theta D (1 - k_0 R C)} (e^{-k_0 t} - e^{-t/RC}). \quad (6)$$

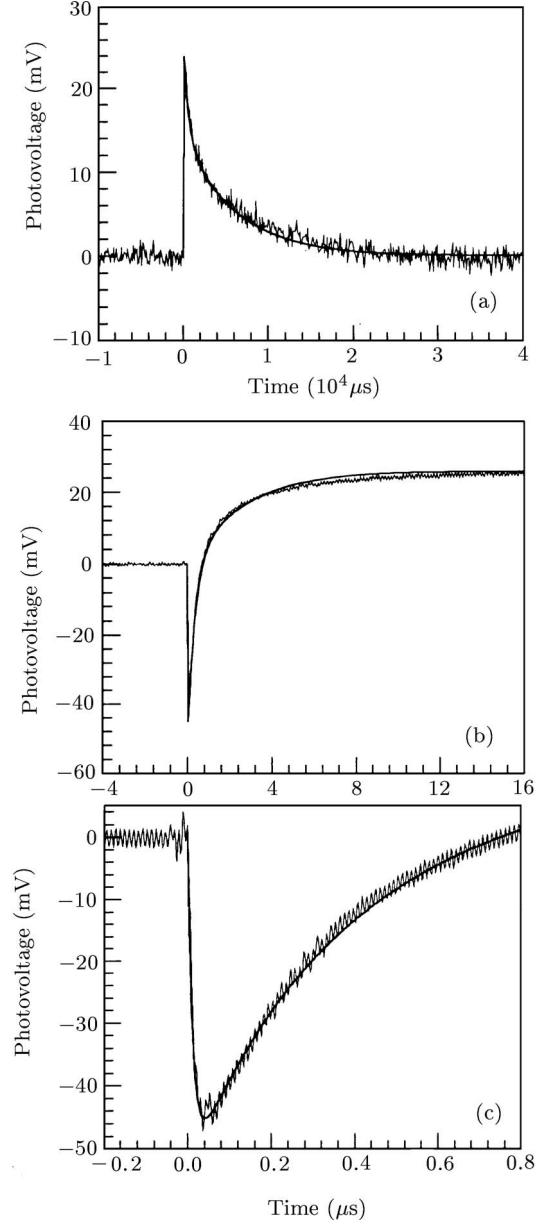


Fig. 1. Photoelectric response signals of the bR film photodetector (stainless steel slide/bR film/aqueous electrolyte gel/Cu electrode) measured on three different timescales. Each curve consists of 500 dots. Excitation source: 532 nm, 10 ns laser pulse, averaging number: 64. The solid lines are fitting curves.

For the slower stages of the photovoltage, because of the approximate conditions: $k_i \gg k_{i+1}$ ($i = 1 - 4$) and $1/RC \gg k_i$ ($i = 1 - 5$), we have

$$n_i(t) \approx N_i e^{-k_i t} \quad (i = 1 - 5), \quad (7)$$

and then

$$V_s(t) = \frac{N_t QR}{D} \sum_{i=1}^5 (d_i - d_{i-1}) k_i e^{-k_i t}. \quad (8)$$

Thus the total bR photoelectric kinetic equation is a seven-exponential function in the following form:

$$V(t) = a_0(e^{-k_0 t} - e^{-t/RC}) + \sum_{i=1}^5 a_i e^{-k_i t}. \quad (9)$$

The photoelectric response signal of the bR film photodetector to 10 ns laser pulse is shown in Fig. 1. The fast peak is negative and the slow peak is positive, which reveals the characteristics of the multi-exponential process. To quantitatively prove the observation, we use the Marquardt algorithm of non-linear least squares fit to fit the above experimental curves according to the theoretical Eq. (9). It is impossible to try the seven-exponential equation in one curve fitting, because there are too many parameters to be determined, even the fitting results for different timebase curves are very different. Here we adopt the three-step correlated regional fitting to determine the parameters of Eq. (9). This is based on the fact that in certain time ranges some relative faster components can be ignored and some relative slower components can be treated as constants; therefore the fitting equation can be simplified in this time range. We first proceed a two-exponential fitting with the slowest timebase curve [Fig. 1(a)] to determine the rate constants k_4 , k_5 and the amplitudes a_4 , a_5 . Then to determine the parameters of components 2 and 3 from Fig. 1(b), the above fitted items of components 4 and 5 should be added into this two-exponential fitting equation. For the fast timebase curve [Fig. 1(c)], a three-exponential fitting is applied, whose fitting equation also includes the already obtained items of the components 2, 3, 4, 5, but now the items of the components 4 and 5 can be substituted just by their amplitudes a_4 and a_5 . To keep $V(t) = 0$ at $t = 0$, the constraint of $a_1 + a_2 + a_3 + a_4 + a_5 = 0$ must be satisfied. In this way, the decay time constants and amplitudes of the

components are obtained and listed in Table 1. The fitting curves are in close agreement with the experimental curves.

Table 1. Decay time constants and amplitudes of the exponential components, fitted from the photoelectric response signal of the bR film photodetector ($\tau_i = 1/k_i$).

i	0	1	2	3	4	5
τ_i (μ s)	1.2×10^{-2}	9.09×10^{-2}	2.68	35.7	559	6.17×10^3
a_i (mV)	53	0.762	-27.24	0.508	11.49	14.48

From the values of time constants, we can assign the bR photocycle to a model: bR \rightarrow K \rightarrow KL \rightarrow L \rightarrow M \rightarrow O \rightarrow bR. The primary step bR \rightarrow K is photoinduced ultrafast process reflecting the charge separation of the chromophore. In our experiment this step is seen as the very fast negatively falling edge of Fig. 1(c). Because the measuring circuit has a limited bandwidth, this ultrafast signal is not truly resolved. Reference 8 has proved that it occurs within 5 ps. As for the intermediate N, it is a dubious component, from which we could not make a judgment.

In conclusion, we have analysed the photoelectric response of the bR film photodetector to a short laser pulse and have proved that it is a multi-exponential process. The theoretical equation of the bR photoelectric kinetics is confirmed by the experimental curve fitting with the three-step correlated regional fitting method. This theory is also applicable to other photoelectric materials.

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