

## Analyses and proofs of multiexponential process of bacteriorhodopsin photoelectric response

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A bacteriorhodopsin (bR)-based photodetector is prepared with the structure of stainless steel slide/bR film/aqueous electrolyte gel/copper electrode. The photoelectric response signal to a 10 ns laser pulse is measured. A theory on the bR photoelectric kinetics is developed based on the concept of charge displacement current and the bR photocycle rate equations. Comparison between the theoretical and experimental results proves the multiexponential process of the bR photoelectric response to a short laser pulse. The decay time constants and amplitudes of each exponential component are obtained by data fitting. © 2001 American Institute of Physics.  
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Bacteriorhodopsin (bR) found in the purple membrane (PM) of *Halobacterium salinarum* is a retinal protein. It functions as a light-driven proton pump in the living organism. 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 is 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 compared to other proteins, for instance, it can retain its native structure even at 140 °C and keep active for several years.<sup>1</sup> Therefore bR has attracted attention for numerous technical applications, especially in the fields of optical information processing<sup>2-4</sup> and visual simulation.<sup>5-7</sup> Much research has shown that illumination of an oriented bR sample results in a photovoltage (or photocurrent). The characteristic of the photovoltage is its 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 ( $\mu\text{s}$ – $\text{ms}$ ), which are concerned with the transfer of protons in the bR molecule and are regarded as being closely related to the photocycle. Although the photoelectric properties of bR have been studied for numerous years, most studies are emphasized on the experimental aspects such as the measurement of the ultrafast response,<sup>8</sup> the dependence of response signal on the environment,<sup>9</sup> the improvement of differential photocurrent,<sup>10</sup> the development of some practical optoelectronic devices,<sup>5-7</sup> and so on. There lacks a systematical theory describing the whole photoelectric kinetics of the bR, especially with a quantitative theory. In this article, we try to analyze the photoelectric response of bR to a short laser pulse based on the concept of charge displacement current

and the rate equations of bR photocycle. The derived kinetic equation indicates it is a multiexponential process, which is finely proved by our experimental results.

Purple membrane is isolated from the  $R_1M_1$  strain according to the conventional method.<sup>11</sup> 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 ( $\sim 2$  V) is applied between the electrodes. Under 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 532 nm wavelength, 10 ns duration, and 10 Hz repetitive rate. The photoelectric response signal is coupled into a 300 MHz digital oscilloscope. Through an IEEE488 GP-IB interface card, a PC microcomputer running on our self-developed software controls the oscilloscope to acquire data, store wave forms, and process the experimental results. The experiment is performed at room temperature.

A bR molecule, once excited by visible light, takes a very rapid charge separation of the protonated Schiff base from its counter ion. This ultrafast step converts photoenergy into 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 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 a resistance  $R$  and a capacitance  $C$  in 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  $\tau$  and intensity  $I_0$ . Given  $n_i$  ( $i=0\sim6$ ) is the population of each state and define the excitation rate constant  $k_0 = \eta\sigma I/(h\nu)$ , where  $h\nu$  is the excitation photon energy and  $\eta$ ,  $\sigma$ , respectively, stand for the quantum transition efficiency and the absorption cross section of the bR ground state. We have the following rate equations of the bR photocycle:

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

$$\dot{n}_{i+1} = k_i n_i - k_{i+1} n_{i+1} \quad (i=0\sim5, k_6=0). \quad (3)$$

Holding Eqs. (1), (2), and (3), we get the transient photovoltage wave form in the measuring circuit,

$$\begin{aligned} V(t) &= \sum_{i=0}^5 V_i(t) \otimes n_{i+1} \\ &= \frac{Q}{DC} \left[ d_0 k_0 e^{-t/RC} \otimes n_0(t) \right. \\ &\quad \left. + \sum_{i=1}^5 (d_i - d_{i-1}) k_i e^{-t/RC} \otimes n_i(t) \right], \quad (4) \end{aligned}$$

where  $\otimes$  stands for the convolution operation. 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  $N_1 \approx N(1 - e^{-k_0 \tau})$  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),

$$V_f(t) = \frac{N_1 Q R d_0 k_0}{\theta D (1 - k_0 R C)} (e^{-k_0 t} - e^{-t/RC}). \quad (6)$$

Here we have supposed any decays back into the bR ground state are negligible.

For the slower stages of the photovoltage, because of the approximate conditions:  $k_i \gg k_{i+1}$  ( $i=1\sim4$ ) and  $1/RC \gg k_i$  ( $i=1\sim5$ ), we have

$$n_i(t) \approx N_i e^{-k_i t} \quad (i=1\sim5), \quad (7)$$

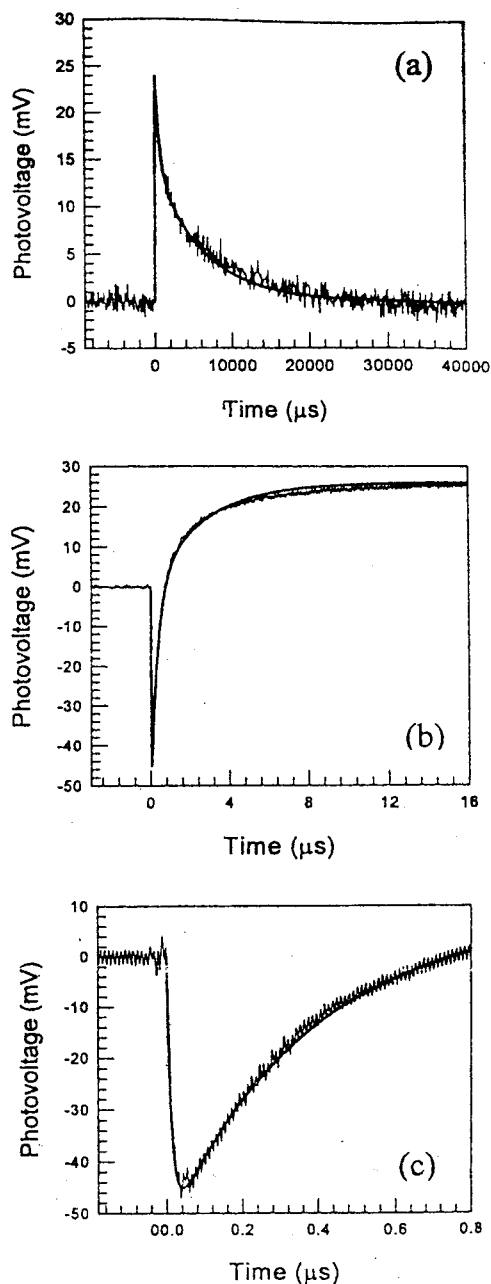


FIG. 1. Photoelectric response signals of the bR film photodetector (stainless steel slide/bR film/aqueous electrolyte gel/Cu electrode) measured on three different time scales. Each curve consists of 500 dots. Excitation source: 532 nm, 10 ns laser pulse, averaging number: 64. The thick solid lines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

and then

$$V_s(t) = \frac{N_1 Q R}{D} \sum_{i=1}^5 (d_i - d_{i-1}) k_i e^{-k_i t}. \quad (8)$$

Thus the total bR photoelectric kinetic equation is a seven-exponential function with 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

TABLE I. Decay time constants and amplitudes of the exponential components, fitted from the experimental data of Fig. 1 ( $\tau_i = 1/k_i$ ).

$i$	0	1	2	3	4	5
$\tau_i$ ( $\mu$ s)	$1.2 \times 10^{-2}$	$9.09 \times 10^{-2}$	2.68	35.7	559	$6.17 \times 10^3$
$a_i$ (mV)	53	0.762	-27.24	0.508	11.49	14.48

negative and the slow peak is positive, which reveals the characteristics of the multiexponential process. To quantitatively prove the observation, we use the Marquardt algorithm nonlinear least-squares<sup>5</sup> fit to fit the above experimental curves according to the theoretical Eq. (9). It is impossible to fit the seven-exponential equation in one curve fitting, because there are too many parameters to be determined; even the fitting results for different time base curves are very different. Here we adopt three-step correlated regional fitting to determine the parameters of Eq. (9). This is based on the fact that in certain time ranges some relatively faster components can be ignored and some relatively 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 time base 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. In the step fitting the fast time base 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. By this way the decay time constants and amplitudes of the components are fitted out and listed in Table I. The fitting curves are in good agreement with the experimental curves.

From the values of the 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 a 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 it occurs within 5 ps. As for the intermediate  $N$ , it is a dubious component. Here we could not make a judgment.

In summary, we have analyzed and proved that the photoelectric response of the bR film photodetector to a short laser pulse is a multiexponential 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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