ELECTRIC SIGNALS ASSOCIATED WITH THE PHOTOCYCLE OF BACTERIORHODOPSIN

L. KESZTHELYI and P. ORMOS
Institute of Biophysics, Biological Research Center Szeged H-6701, Hungary

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1. Introduction

Flash-induced biphasic fast electric signals from bacteriorhodopsin (BR) in the purple membrane (PM) of *Halobacterium halobium* were first observed in [1–3]. The observations were made on model systems: PM in thick planar lipid membranes [1], and a not well-characterized interfacial layer of PM built into foam apposed to a thin teflon septum [2,3].

Here we report the measurement of the electric signal on untreated PM suspension (their orientation was achieved by means of an electric field [4]), the existence of at least two long-living components, and the simultaneous measurement of the transiently liberated protons. Additional measurements of the time constants of the changes in light absorption rendered possible the unambiguous designation of the components of the electric signals to the intermediates of the BR photocycle. Similar measurements in a heavy water suspension demonstrated that the complex electric signal reflects the translocation of proton or deuteron in BR. We suggest that the complex electric signal be designated as a protein electric response signal (PERS).

2. Materials and methods

The PM fragments used in the measurements were obtained by a standard procedure from *Halobacterium halobium* strain NRL R_1M_1 [5]. They were suspended in tridistilled water (H_2O) or in D_2O (99.9% in deuterium). Concentrated solutions were poured into a cell of 1 mm thickness ($A \simeq 1.8$; height of suspension column 4–5 mm), resistance $2.5 \times 10^5\ \Omega$.

For orientation and measurement of the electric signal, Pt electrodes at a distance of 8 mm were introduced into the solution. As known from [4] comparatively low electric fields are sufficient to yield a substantial orientation of PM. The scheme of the measuring system is shown in fig.1. The switching unit (home-made) produced:

(i) The biphasic voltage for orientation with the time sequence: T positive, $2T$ zero, T negative ($T = 3$ s);

(ii) A trigger pulse at any time in the first two intervals (T, $2T$) for the laser (Opton dye laser, $\lambda = 580$ nm, pulse length 1 μs, energy 10 mJ) and for the transient recorder (product of the Central Research Institute for Physics, Hungary; smallest conversion time 0.1 μs, conversion range 256 quanta/channel, 1024 channels).

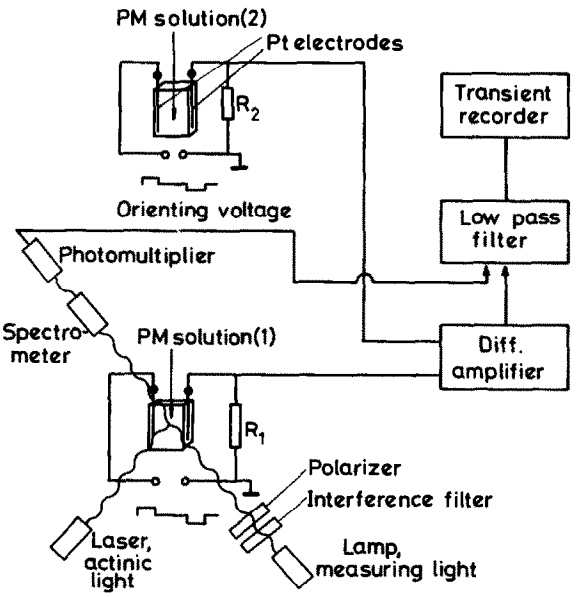

Fig.1. Scheme of the measuring system.

Two identical PM systems were used for electric measurement, because the orienting voltage on the resistance R_1 had to be compensated. The differential amplifier (Keithley 604) received pulses from both PM systems, and at the output only the signal produced by the laser pulse appeared. It could be filtered (Krohn-Hight, 3202 low-pass) before recording. A measuring light system (tungsten lamp of 300 W, interference filters, polarizer, Carl Zeiss (Jena) monochromator and EMI 9862Q/070 photomultiplier) was used to control the orientation [4] and to measure the changes in absorption of PM after the laser pulse. In this case the signal of the photomultiplier was switched to the input of the low-pass filter.

The time-resolution of the whole system was 2 μs (setting the low-pass filter to 0.5 Mc), which was changed when longer time constants were measured.

The data were taken at 20°C.

3. Results and discussion

The dependence of the electric signal on the membrane orientation was measured by flashing the laser at different time t after starting the orientation. In fig.2, (a,b) show the signal at t = 2.5 s, and (c,d) that at t = 3.3 s, measured with different ranges of time and amplitude. In fig.3 the amplitudes of 3 components (see fig.2(a,b)) are represented as functions of the degree of orientation, which was determined separately with polarized light of λ = 570 nm. The results show that components I and II depend only on the orientation, while the amplitude of the long-living component (III) differs markedly in the field-on and field-off cases. The difference must be due to a transient change of the conductivity of the solution.

After establishment of the orientation- and field-dependences of the electric signal, parallel measure-

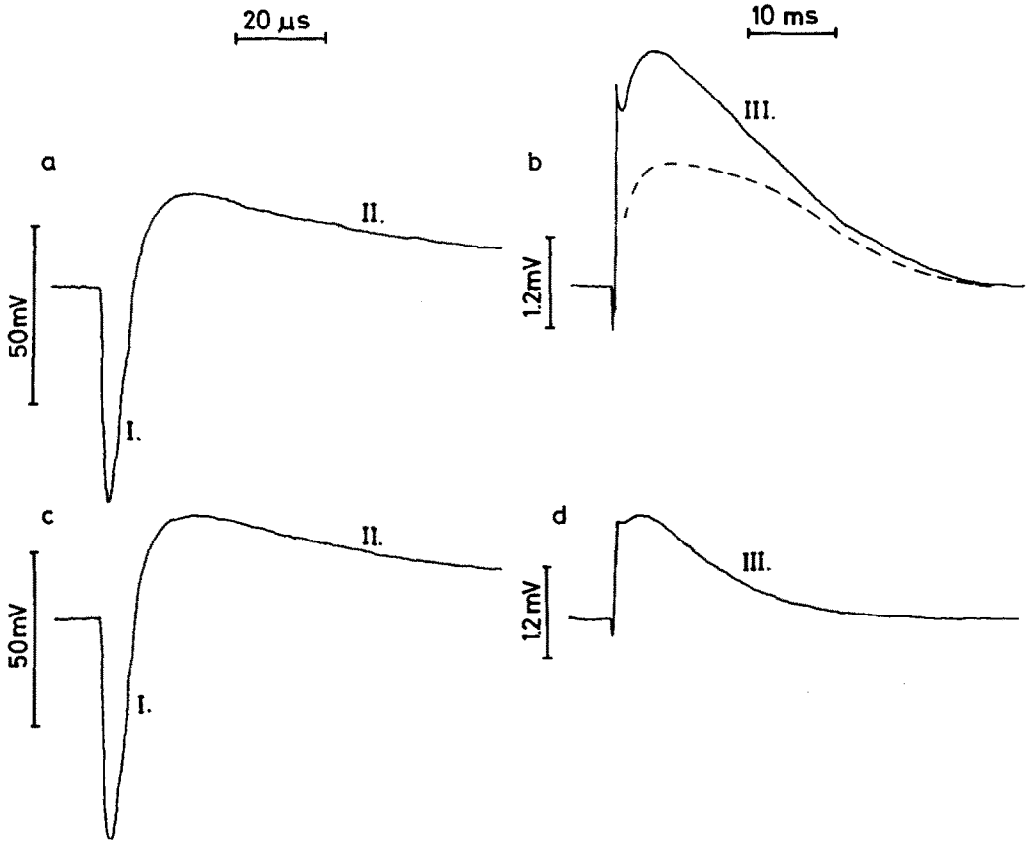

Fig.2. Time-dependence of the electric signals. (a,b) Signals measured at t = 2.5 s (field-on case); (c,d) at t = 3.3 s (field-off case). I–III designate the components of the signal. The dashed line in (b) is the difference between signals (b) and (d) (normalized to component I); i.e., the component originating from conductivity change. PM suspended in H_2O, A = 1.8, T = 20°C, orienting voltage 8.5 V. Distance between electrodes D = 0.8 cm.

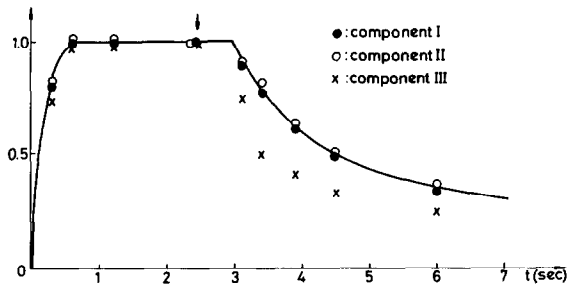

Fig.3. Correlation of the amplitude of the components of the electric signal with the orientation. The degree of orientation [4] of the PM, as measured by polarized light of λ = 570 nm, is given by the solid curve. The data are normalized at the point marked by the arrow. For I–III, see fig.2.

ments of the electric signal and of the light absorption signal at different wavelengths were performed with the aim of correlating the components of the electric signal to the intermediates in the photocycle of BR [6,7]. In a trial experiment it was determined that the light absorption signals do not depend on the orientation. Therefore, the data were taken without orientation. The results are collected in fig.4(a–c). We begin the assignment with a qualitative description of the signals.

Component I of the electric signal is an exponentially falling negative signal, with time constant $\tau_1 \simeq 4\ \mu s$. The same time constant can be observed in both absorption signals (A_{522}) and (A_{408}). A_{522} shows the immediate appearance of the K-intermediate and its decay to the L-intermediate with time constant τ_1. The appearance of A_{408} is delayed by τ_1.

Component II is a positive signal; it falls exponentially, with time constant $\tau_2 \simeq 80\ \mu s$. This is the time constant of the L→M transition, observed in A_{522} as the disappearance of the absorption due to the L_{550} intermediate, and in A_{408} as the onset of the M_{412} form. Figure 4a contains a decomposition of the fast signal.

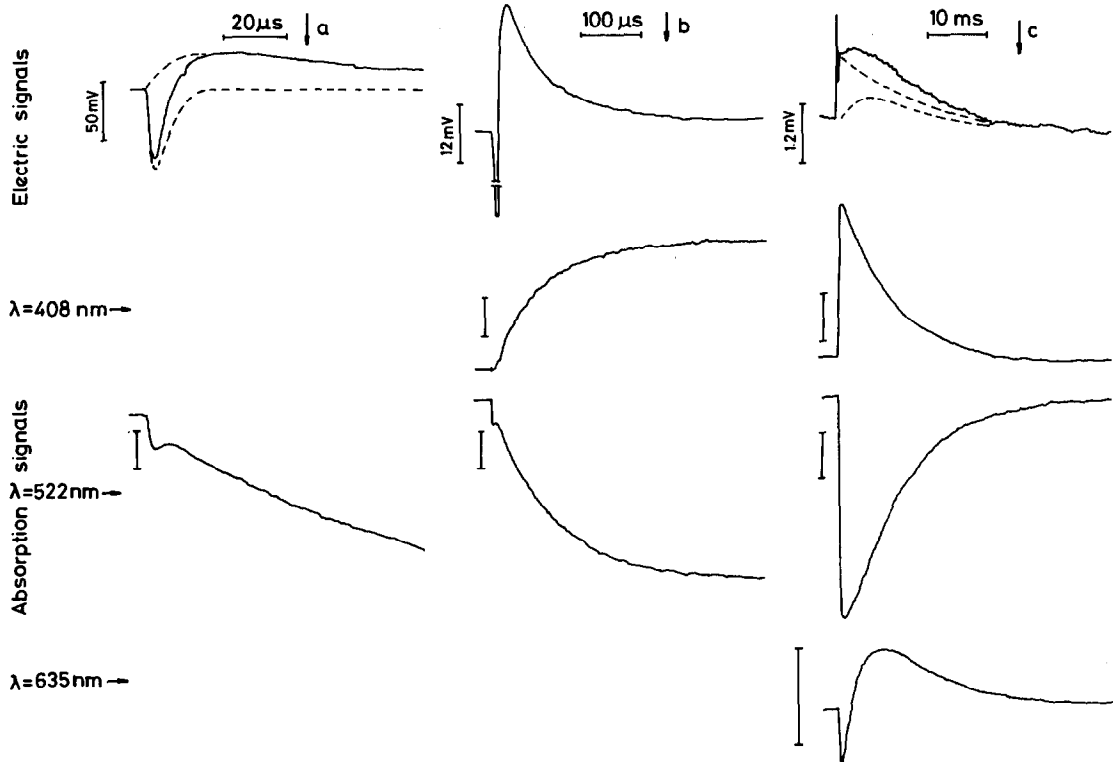

Fig.4. Comparison of electric and light absorption signals at different wavelengths (λ = 408, 522 and 635 nm). (a–c) Signals measured with different time resolution. The electric signals were measured at t = 3.3 s. Dashed lines in column (a) and (c) denote the decomposition of the electric signals. PM in H_2O suspension, A = 1.8, T = 20°C, orienting voltage 8.5 V. The size of bars in absorption signals means $\Delta I/I = 0.1$. Positive signals indicate $\Delta I < 0$.

The long-living component III is a complex signal. The difference between the signals in the field-on and field-off cases is shown in fig.2b. The remaining part is the superposition of an exponentially falling positive signal ($\tau_3 \simeq 8$ ms) and a parent–daughter signal (fig.4c). The decay time of M_{412} in A_{408} is $\tau_3 \simeq 8$ ms. The parent–daughter signal coincides with the time course of the O form, as seen in the A_{635} curve.

The time constants ($\tau_1 - \tau_4$ for the O form) were calculated and are listed in table 1. In the calculations of the time constants, the usual parent–daughter–granddaughter–... equations of radioactive decay (the Buteman equations [8]) were used after modification to take into account that the components in the series may have different amplitudes and signs.

The coincidence of the time constants of the electric components and of the transitions in the BR photocycle indicated that electric analogs of the K→L, L→M, M→O and O→Br transitions were measured in this experiment. The conductivity signal corresponds to the transiently liberated protons.

The photocycle of the BR starts with an extremely fast ($\tau_0 = 11$ ps) transition of BR→K [9]; the electric analog of this, and that of the questionable M→N transition, can not be seen in the electric signal.

The electric and absorption signals were also measured and analysed with the PM in D_2O. Data are also given in table 1. The related time constants are in agreement and a factor of 1.5–5 greater than in the case of H_2O solution. These results corroborate the above assignments. They demonstrate further that the charged particles protons or deuterons do not move freely, but are connected to subgroups of the proteins, because in the event of free movement the isotope effect should have been a factor of 1.4.

Table 1
Time constant data of electric and light absorption signals of successive transitions in the photochemical cycle

Signal type \ Transition	K→L τ_1 (μs)	L→M τ_2 (μs)	M→O τ_3 (ms)	O→BR τ_4 (ms)
Electric	4.4	81	8.0	2.5
	(6.2)	(452)	(22.0)	(4.0)
A_{408}		81	7.9	
		(429)	(20.0)	
A_{522}	3.7	91	8.5	
	(5.1)	(445)	(20.0)	
A_{635}			9.2	2.2
			(21.0)	(4.1)

Data correspond to PM suspended in H_2O, in brackets in D_2O. Assignment is based on the works in [7] and (for the O→BR transition) [10], confirmed by the present study. The time constants of the conductivity signal (fig.2b) caused by the transiently liberated protons in H_2O or deuterons in D_2O experiment were not evaluated in detail

The K→L, L→M, ... transitions are thermally-induced quantum jumps from one state to the next. The electric signals which depend only on the orientation (fig.2,3) have their origin in the displacement current caused by the charge movement between the electrodes. According to the Ramo-Shockley theorem [11], the displacement current is:

$$i = \frac{Q}{D} \cdot v \tag{1}$$

where Q is the charge, v is the velocity of the charge movement, and D is the distance between the electrodes. As v does not have meaning in a quantum transition, we can calculate the induced charge Q_{ind} only. Integration of eq. (1) with respect to time yields:

$$Q_{ind} = \frac{Q}{D} \cdot \int_0^\infty v \mathrm{d}t = \frac{Qd}{D} \tag{2}$$

where d is the displacement of the charge. Q_{ind} charges the capacity C of the circuit, which discharges with time constant RC (R is the measuring resistance, fig.1). If the density of the quantum jumps is taken into account, the time-dependence of the i-th electric signal (in volts) is:

$$V_i(t) = \frac{N R Qd_i}{D} k_i f/k_1, ..., k_i, t/ \tag{3}$$

where N is the number of flash-excited BR photocycles, $f/k_1 \ldots k_i, t/$ is the Buteman function for the i-th component [8], and $k_i = 1/\tau_i$. Equation (3) includes that $V_i(t)$ linearly depends on N. This has been separately checked by measuring the $V_i(t)$ functions by changing the intensity of the exciting light flashes.

Equation (3) renders it possible to calculate d_i, the displacement of the charge in the steps of the photocycle (in the direction perpendicular to the plane of the PM). From the measured data of $V_i(t)$, k_i, N, R and D, values of d_i were calculated (table 2). Σd_i = 10.5 nm, twice the thickness of the membrane [6]. This result very probably expresses the fact that two

Table 2
Displacement of protons in different steps of the BR photocycle

Transition	d_i (nm)	$d_i/2$ (nm)
K→L	−0.3	−0.15
L→M	+1.0	+0.5
M→O	+6.2	+3.1
O→BR	+3.0	+1.5

The error of Σd_i is ≃ 30% because of the difficulty to determine N, the error in ratios of d_i–s is ≃ 5%. In determining N it was taken into account that 25% of the BR take part in the photocycle as evaluated from A_{522} and A_{408} (fig.4) using the molar extinction coefficient data in [7]. $N = 7 \times 10^{14}$, $R = 10^5\ \Omega$

protons are translocated in every photocycle [12]. Accepting this result values of $d_i/2$ express the average displacements of the charges in the steps of the photocycle.

In the K→L transition the protons jump backward ($d_1 = -0.15$ nm), while in the others they move forward. Since the protons are released in the M→O transition and taken up in the O→BR transition [6], it may be stated that the protons move forward during the L→M→O transitions, the sum of their displacements being 3.6 nm; when they are taken up from the other side during the O→BR transition, they move 1.5 nm. Hence, the data prove that the position of the proton donor is near to the inner surface of the membrane, in agreement with [13].

4. Conclusions

This study demonstrates that electric signals are correlated with the steps of the proton-pumping function of the BR protein. It is suggested that the associated electric signal be called a protein electric response signal (PERS). We believe that a PERS should appear in other cases of charges channeling through proteins, and generally in the case of protein functions when charged groups are translocated.

Acknowledgements

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