Biochimica et Biophysica Acta, 723 (1983) 327–331
Elsevier

BBA Report

BBA 40022

CHARGE DISPLACEMENTS IN PURPLE MEMBRANES ADSORBED TO A HEPTANE/WATER INTERFACE

EVIDENCE FOR A PRIMARY CHARGE SEPARATION IN BACTERIORHODOPSIN

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(Received January 12th, 1983)

Key words: Bacteriorhodopsin; Charge separation; Photovoltage; Purple membrane

Laser flash-induced photovoltages of purple membranes adsorbed to a heptane/water interface were measured with a capacitative electrode. Two signals of opposite polarity were detected in the time range between nanoseconds and seconds: (i) An early charge separation (less than 2 ns), which was steady over the submicrosecond range, and whose rise time was instrumentally limited. The displacement was estimated to be less than 5 Å. (ii) Oppositely directed larger displacements occurred between 10 μs and 100 ms. The results are discussed with respect to those obtained from other experimental systems.

Purple membranes are planar membrane fragments from halophilic bacteria which consist predominantly of bacteriorhodopsin [1,2]. They utilize the energy of visible light to translocate protons across the membrane [3]. Bacteriorhopsin (bR) undergoes a series of spectroscopically characterized intermediates: bR → K → L → M → O [4]. The first intermediate K is formed within picoseconds and lives for some microseconds [5–8]. The primary event is thought to be a *trans-cis* isomerization [9–13]. It has been proposed that a fast charge separation (breakage of a salt bridge) is connected with this geometric change of the chromophore [14,15].

As was shown in various model systems, purple membranes display electrical activity throughout the flash-induced photocycle [16–28]. Because the time resolution in these studies did not exceed 200 ns and because possible signal shaping processes might have interfered, the electrogenic events associated with the bR-K and K-L transitions remained obscure. It is the aim of this paper to deliver experimental evidence for the postulate of a fast charge separation associated with the primary photochemistry of bacteriorhodopsin by means of a direct electrical method.

Recently, a monolayer technique has been described for measuring light-induced interface potential changes at the heptane/water interface in the time domain between nanoseconds and seconds with a capacitative electrode [29,31]. Using this technique, laser flash-induced photovoltages from purple membranes adsorbed to an interfacial layer are reported here. They were recorded with high time resolution and with high pulse fidelity.

Experimental details concerning the heptane/water interface, the capacitative electrode, and the electronic components are described elsewhere [29,32]. The photovoltage was defined as positive when the heptane phase became positive with respect to the subphase. Homogeneous unpolarized illumination was achieved by passing laser flashes through fiber optics. Purple membranes isolated from *Halobacterium halobium* were a gift from

Drs. D. Kuschmitz and B. Hess. The samples were light adapted shortly before the experiments.

The experiments were carried out by first spreading a positively charged detergent, stearylamine, at the heptane/water interface and then letting purple membranes adsorb from the subphase. This yielded stable and reproducible photovoltages after approx. 10 min, proving that purple membranes were asymmetrically adsorbed. Fig. 1 shows photovoltage signals under this condition. In the submicrosecond range the photovoltage resembled a step function (Fig. 1a). Its rise time was 35 ns and corresponded to the rise time of the electrometer amplifier used.

The time range in which the polarity change occurred is shown in Fig. 1b. The zero passage point fell within 5–20 μs for different experiments at pH values 6.0 and 8.0 and at different ionic strengths. Within the same range lie the zero passage points found in comparable studies [23–25,27]. Thereafter, a positive photovoltage developed to a flat maximum between 20 and 100 ms (Fig. 1c). This kinetic phase could be approximated by two exponential functions with time constants of 115 μs and 4.5 ms. Their amplitude ratio was 0.4. Finally, the photovoltage decayed to the baseline within seconds (Fig. 1d).

The photovoltage from bacteriorhodopsin layers formed under different experimental conditions showed the following common features: (1) The photovoltage changed polarity in the microsecond range, going from a small negative phase to a larger positive phase. (ii) Below microseconds the negative photovoltage was like a step function. (iii) Maximal positive photovoltages were reached at about 100 ms after the flash.

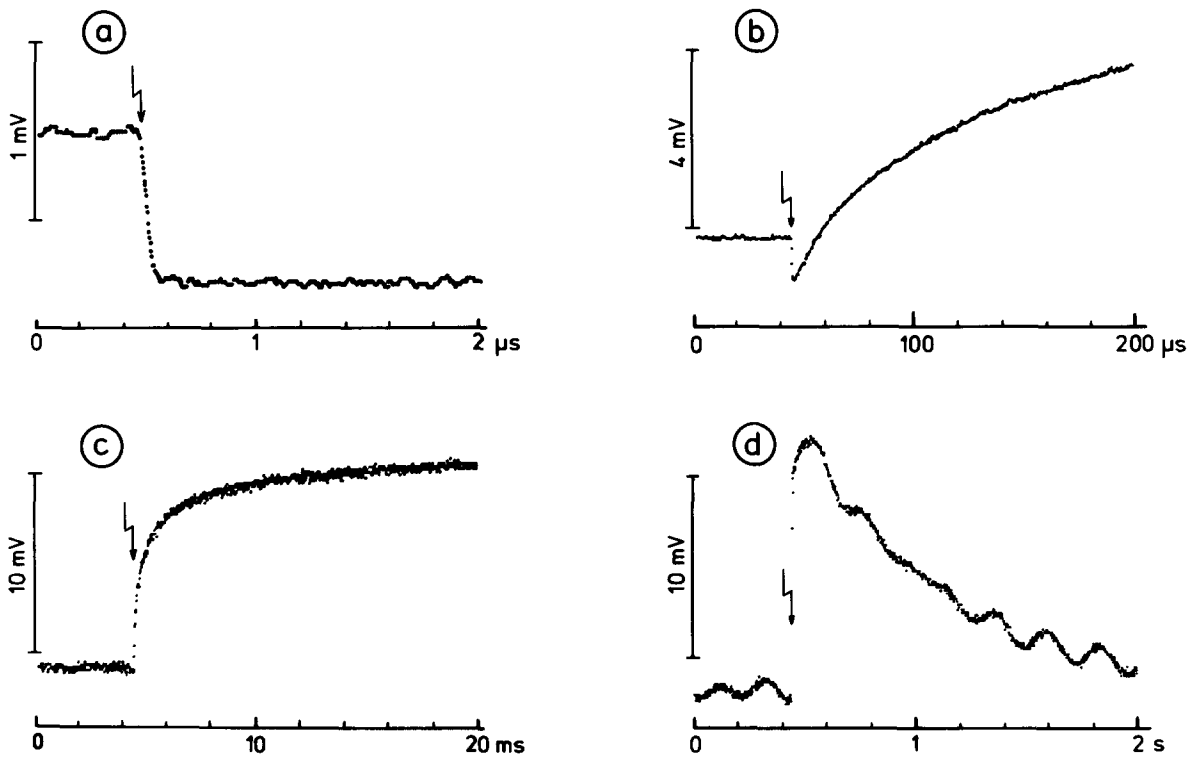

Fig. 1. Photovoltages from purple membranes adsorbed from the subphase to a monolayer of stearylamine at a heptane/water interface. The subphase contained 1 M KCl, 50 mM phosphate buffer, pH 7.0 and 2.3 μM bacteriorhodopsin. The excitation source was a frequency-doubled Nd-Yag laser (wavelength 532 nm) of 10 ns duration and an energy of 3 mJ/cm^2. (a) Photovoltage in the microsecond range. Recording bandwidth: 20 MHz to 10 kHz. (b) Photovoltage in the 100 μs range. Recording bandwidth: 20 MHz to 10 Hz. (c) Photovoltage in the 10 ms range. Recording bandwidth: 1 MHz to 1 Hz. (d) Photovoltage in the seconds range. Recording bandwidth: 100 kHz to d.c.

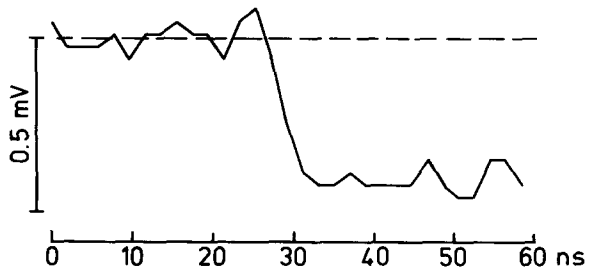

Fig. 2. Photovoltage from a layer of purple membranes under the experimental conditions of Fig. 1. The excitation source was a Q-switched and frequency-doubled Nd-Yag laser with a pulse duration of about 4 ns and an energy of about 1 mJ/cm^2. The recording bandwidth was 100–1 MHz.

The rise time of the early negative phase was studied in separate experiments using a shorter laser flash (4 ns) and a high impedance amplifier of 180 MHz. Fig. 2 shows the rising phase of the photovoltage with the highest time resolution that could be achieved with this setup. Control experiments showed that the rise time was determined by both the duration of the laser flash and the recording bandwidth. This allows an estimation for the upper limit of the charge separation which is about 2 ns [30].

The photovoltage signals were found to be independent of the direction of the incident flash and proportional to the flash energy below 5 mJ/cm^2.

The flash-induced changes of the interface potential caused by purple membranes adsorbed to a heptane/water interface were measured under open circuit conditions. The charge displacements occurred within a planar capacitor (matched to the preamplifier) and, therefore, the relative amplitude of the photovoltage should be proportional to the vectorial component of the charge displacement perpendicular to the plane, if the dielectric medium along the path of the charge displacement is assumed to be homogeneous. With these assumptions, amplitudes of different kinetic phases of the photovoltage can directly be interpreted as relative displacement lengths. Note that similar assumptions are generally necessary for a quantitative interpretation of photocurrents and photovoltages in studies aiming at estimation of displacement distances [25,26].

To summarize the results, the data of Figs. 1 and 2 were replotted on a logarithmic time scale in Fig. 3. The right-hand side of the figure illustrates a tentative scheme of how a single positive charge must be displaced in a bacteriorhodopsin molecule under the assumptions mentioned. The positive polarity of the slow photovoltage shows that the purple membranes adsorbed to the interface with their extracellular sites, in agreement with other studies [16–24,26–29]. The relative contribution of different exponential functions, which are necessary to describe the time course of the photovoltage, yields the relative displacement lengths, which

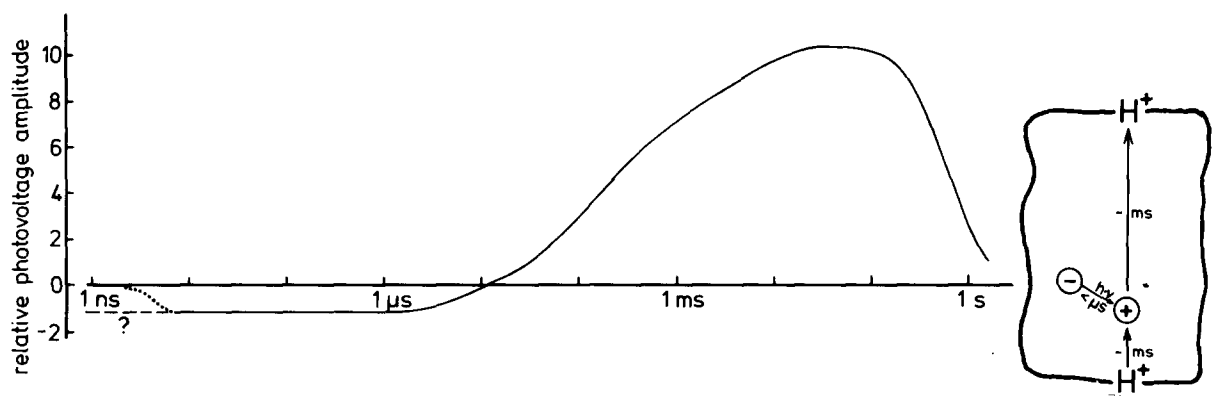

Fig. 3. Plot of the data shown in Figs. 1 and 2 on a logarithmic time scale and a common ordinate. The scheme on the right-hand side illustrates by way of suggestion the displacement of a positive charge (proton) within a bacteriorhodopsin molecule in order to account for the measured photovoltage. It implies that only one charge is displaced, that the displacement is perpendicular to the plane and occurs in a homogeneous dielectric, and that no dipole moment changes of the protein are involved.

correspond to the displacement coefficients introduced in the literature [26,32].

The displacement distance of the early charge separation found in this study is approx. 10-times smaller than that of the overall effect. It amounts to approx. 5 Å if a membrane thickness of 50 Å is assumed. The value is in agreement with the expectation from theoretical considerations [14,15]. Similar values were obtained by Keszthelyi and Ormos [25] who used suspensions of purple membranes oriented by an electric field, and by Drachev et al. [23] who used purple membranes adsorbed to a thin planar collodium film. However, it is at variance to the results of Fahr et al. [26], who discuss displacement about 10-times larger for the fast opposite phase with purple membranes adsorbed to a planar lipid bilayer membrane. The authors interpret this large displacement coefficient as a major conformational change involved in the K-L transition. However, the fast rise time of the first negative process of 2 ns and its decay within some microseconds reported here is only consistent with the interpretation that the negative phase is related to the formation and decay of the K intermediate [24,28].

Under the experimental conditions of Fig. 1, the relative displacements involved in the positive phase were 0.4 (115 μs phase/4.5 ms phase). This ratio lies closer to that given by Keszthelyi and Ormos [25] and Drachev et al. [23,28], but it is significantly smaller than that reported by Fahr et al. [26].

The discrepancy between the displacement coefficients among various authors might be ascribed to the different experimental systems used and to the different measuring methods applied. Fahr et al. [26], for instance, used the voltage-clamp method to record the photoelectric events in a system with high capacitance (lipid bilayer membrane) whereas the current-clamp method was used in this study and in Refs. 16–23 and 28. One problem is that the voltage-clamp method fails to clamp the voltage at high frequencies (above about 1 MHz), which causes the current signals to convert into voltage signals above a transition frequency [33–35]. As a consequence current signals cannot easily be distinguished from voltage signals, although this is possible, if taken into account [33,35]. Recently, Varo [27] compared flash-induced voltage and current signals from the same probe of dried oriented purple membranes and found differences in some time constants. This discrepancy is at present not understood.

Compared to other investigations on the photoelectric activity of oriented purple membranes, the interfacial layer method employed in this study covers a wider time range. It allowed the setting of an upper limit for the rise time of less than 2 ns, for the fast negative component of the photovoltage (Fig. 2). The fast rise time of this phase indicates that the absorption of a light quantum by the chromophore is associated with a primary charge separation in which light energy is stored until its later use by the protein to pump protons, as recently suggested on theoretical grounds [15,36]. Since in the present experiments the kinetics of this charge separation were not time resolved, it could be faster by orders of magnitude as is the formation of K.

Intuitively, one might expect that all charge displacements occurring in bacteriorhodopsin were unidirectional. However, the fast charge separation occurs in a direction which is opposite to that of the proton transloction. At present there is no satisfactory explanation for this fact. In any case, it does not contradict membrane energetics: Considering the small displacement and the high free energy stored (sufficient to pump a proton against an electrical gradient of more than 200 mV [37]), the reverse membrane potential connected with the early charge separation is insignificant.

In the submicrosecond range there was no further relaxation of the photovoltage observed (Fig. 1a). This is in agreement with the life-time of the bathochromic K intermediate. The decay of K and the subsequent formation of L seem to be connected with charge displaments that cause the change of the polarity around 10 μs (Fig. 1b). However, the process seems to be slowed down in purple membranes adsorbed to the interface.

No special efforts have been undertaken to analyze accurately the positive rising phase and the decaying phase for exponential time constants by fit programs, since it was found that too many experimental parameters were involved (cf. Ref. 38) and since it was not the aim of this study. The analysis for two exponential time constants of the rising phase shown in Fig. 1 yielded 115 μs and 4.5

ms. These values would be comparable to the L-M (approx. 30 μs) and M-N (approx. 1 ms) transitions, respectively, if the transitions are assumed to be slower in adsorbed purple membranes. Similar observations were made by others [28].

I would like to thank Dr. U.B. Kaupp for helpful discussions and critical reading of the manuscript. Professor W. Junge generously provided the laboratory facilities. This work was financially supported by the Deutsche Forschungsgemeinschaft.

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