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I. Primary electrogenic processes in bacteriorhodopsin probed by photoelectric measurements with capacitative metal electrodes

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Purple membranes in suspension were electrically oriented between metal electrodes forming a planar capacitor and the flash-induced charge displacements were measured as a photovoltage. This geometry brought about unprecedented high time resolution, even at low ionic strengths. An electrical transmission window of this capacitative cuvette for open circuit photovoltage was characterized. The photovoltage from purple membranes displayed two phases of opposite polarity. The early negative phase resembled a step function in the submicrosecond range. When excited with picosecond laser flashes, this phase showed an instrumentally limited rise-time of about 380 ps, proving a primary charge separation in bacteriorhodopsin which is faster than or equal to 100 ps. The separated charges are stable for about 1 μs. Within the experimental resolution and within the time range between 380 ps and 1 μs, there was no relaxation which could be attributed to a protein relaxation or to a K′ intermediate. The photovoltage decayed with different time constants of the order of 1 μs, depending on the conductance of the medium. Of the same order were the cell time constant and the time constant of the decay of the spectral K intermediate. This hampered the evaluation of the correlation between the photovoltage and the K-L transition. Another positive phase had a smaller amplitude and displayed a time constant of 30 μs which did not depend on the conductivity of the medium. This phase was approx. 40% faster than the decay of the spectroscopically measured L-intermediate. The latter result indicates that charge displacements occur in the time range of the L-M transition, and are not exactly correlated to spectroscopic transients in the visible range.

Introduction

Isolated purple membranes from halophilic bacteria consist of a two-dimensional lattice of the chromoprotein, bacteriorhodopsin. Upon exposure to light, protons are pumped from one membrane side to the other (for a review, see Refs. 1 and 2). Due to their asymmetric surface charge, the membrane sheets can be oriented by an electric field [3–5]. When the membranes are oriented this way and excited by a flash, the electrogenic pumping process leads to an electrical polarization of the suspension medium. This polarization has been measured as flash-induced photoelectric signals in different experimental systems [4–12].

The analysis of the amplitude and the kinetics of photovoltage or photocurrent signals is important for unraveling the charge displacements accompanying the proton pumping mechanism and the correlation to the spectral transitions. Rayfield [13], for instance, called attention to the possibility that conformational changes of the protein associated with charge displacements may escape spectroscopical detection. For different reasons, differences between electrical and spectroscopical data have never been unambiguously demonstrated. Rigorous experiments require simultaneous spectroscopic and photoelectric measure-

ments under the same experimental conditions and a demonstration that the electrical signals are not distorted or shaped by relaxations due to the measuring system and the instrumentation.

In the past, two classes of experimental systems have been used to study photoelectric effects from purple membranes: (i) adsorption to thin planar membranes or to interfaces (capacitative systems) [13–22] and (ii) electrical orientation in suspensions (dielectric polarized systems) [4–11]. In view of the desirable identical conditions for optical and electrical measurements as well as for undistorted signal recording, both classes exhibit specific problems. The little material adsorbed to thin membranes implies small optical density and thus limits time-resolved flash spectroscopy. In addition, different populations of adsorbed purple membranes (for example, first and secondary adsorbed layers) may give rise to multiphasic electrical and/or optical signals.

These shortcomings of the membranous systems are absent in the macroscopic dielectrically polarized system, since they are homogeneous and allow the simultaneous recording of electrical and spectroscopic data [4–10]. However, the photoelectric signals from these systems seem to be quite susceptible to frequency limitations of the measuring system and the instrumentation [11].

One indication for the presence of signal distortions is the observation of a photovoltage in the form of short transients in cases where the molecular reaction would be expected to produce a step function like photovoltage [11,12,23,24]. This expectation holds for the photosynthetic charge separation [26–27], but most likely also for the primary charge separation in bacteriorhodopsin [12,22,29–31]. For both cases, step functions like open-circuit photovoltages were obtained by the capacitative system of a heptane/water interface and a capacitative electrode [22,28,32]. In contrast, photovoltages as short transients were observed from the respective dielectrically polarized systems [11, 12,23–25].

Another indication for the presence of signal distortions is the dependence of the kinetics of the photovoltage on instrumental parameters (like the impedance of the preamplifier) and the conductivity of the medium. Examples have been reported for purple membranes [11] and for chloroplasts [24,25]. On the other hand, the independence of the kinetics on these parameters may serve as a criterion for undistorted signals. This argument will be applied in the present study.

The first part of this communication describes how a macroscopic dielectrically polarized system can be converted into a capacitative-like system which is capable to transmit a step-function photovoltage if the molecular reaction is a fast-stabilized charge separation. This was achieved by two planar metal electrodes which were so closely adjusted that they formed a planar capacitor whose capacitance was larger than the parasitic capacitance. The material under investigation then represents the dielectric of the capacitor. By applying a charge injection technique, the measuring cuvette was electrically characterized and a defined frequency window for open circuit voltage was assessed.

The second part of this communication aims at investigating the early charge displacements in bacteriorhodopsin with respect to (i) a primary picosecond charge separation associated with the *trans-cis* isomerization of the chromophore of bacteriorhodopsin, (ii) possible dielectric relaxations of the protein induced by this charge separation, (iii) charge displacements connected with the K′ intermediate, as proposed by Ormos et al. [8], (iv) the correlation between a microsecond transient of the photovoltage and the decay of the spectral K intermediate and (v) the correlation between a 30-μs transient of the photovoltage and the decay of the spectral L intermediate.

Special attention was paid to distortions of the kinetics of the photovoltage by transition frequencies due to the experimental system and the instrumentation. The variation of the medium conductance served to distinguish between electrical relaxations originating from molecular charge displacements and those originating from the measuring system.

Materials and Methods

The two electrodes forming the capacitative cuvette were platinum plates (20 × 8 mm). They were vertically mounted in a plastic holder containing small spacer to maintain a 0.8 mm separation of the plates (Fig. 1). The electrodes, the

holder and the electric driving circuit for the orienting voltage were placed in a metal box (Faraday cage).

Two couples of small mercury batteries (1.5 V) delivered the orienting voltage to the Pt-electrodes as shown in Fig. 1. Either of the batteries was switched by one of the two Reed relays. The relays were triggered by photo-Darlington transistors. These in turn were triggered by an infrared light-emitting diode (LED) positioned near the outer side of the metal housing. This construction was chosen in order to avoid any leading-in cable which could conduct electrical noise into the Faraday cage. The LED was triggered by a programmable delay pulse generator which determined the duration of the orienting voltage and the delay for the flash trigger.

For the time domain $t > 35$ ns, 10 MHz electrometer preamplifiers with 3 pF input capacitance were used (M and S Elektronik, type EMV 80). Any other operational amplifier of more than 10 MΩ input resistance and less than 10 pF input capacitance is equally suitable. For the time domain 1 ns $< t <$ 100 ns, a low-noise impedance converter (custom-built with GaAs field-effect transistors) was used with an input capacitance of 3 pF, an input resistance of 18 kΩ, a gain of 1 and a limiting frequency of about 5 GHz. Maximal time resolution and further amplification (needed for impedance converter) was achieved by 3.15 GHz amplifiers of 50 Ω impedance (B & H Electronics, type DC-3002LN).

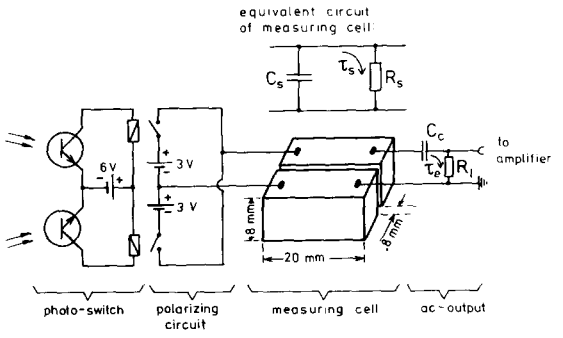

Fig. 1. Scheme of the capacitative cuvette, the electronic circuitry for the electrical preorientation and the equivalent circuit of the cuvette.

The signals were recorded either by a 100 MHz transient recorder (Biomation, type 6500) or by a 1 GHz oscilloscope with channel plate intensifier (Tektronix, type 7104). A trace on the screen of the oscilloscope from a single shot was picked up and digitized by a nocticon camera (Thomson SCF, model TSN 1150) with a resolution of 256 × 256 points. For storage and averaging, the signals were transferred to a computer (Digital International Ltd., PDP 11/34).

The water used was 3-times distilled. Glycerol contained 13% water. Ethylene glycol was 99.4% pure. Other solvents and chemicals had commercial purity. The resistance of the media was measured with a Wayne Kerr RCL-bridge, working at 1 kHz. Purple membranes from *Halobacterium halobium* were a gift of Drs. D. Kuschmitz and B. Hess. The membranes were light-adapted before use. All experiments were carried out at room temperature 21 ± 2°C.

Experimental details concerning the orientation of purple membranes are described elsewhere [4,5]. Purple membranes in highly viscous media were oriented with 1.5 V for 200 ms. After a delay of 200 ms, a single flash was given. A deposition of purple membranes on the electrodes was prevented by switching the orienting voltage with alternating polarity for subsequent measurements. Purple membranes in low viscous media (distilled water) were orientated for only 10 ms. No more than one pulse of orienting voltage of a given polarity was applied within a minute in order to avoid a deposition of purple membranes on the electrodes.

The excitation source was a frequency-coubled ($\lambda = 532$ nm) Nd-Yag laser system purchased from J.K. Lasers. The laser was operated either Q-switched (10-ns pulses) or mode locked (30-ps pulses). The flashes passed through a light-pipe before they reached the cell. Excitation energies were on the order of 1 mJ/cm^2.

Flash spectroscopic measurements were carried out with 10-ns pulses and with the identical suspensions as used for the electrical measurements. The measuring light source was a tungsten lamp. Absorption changes indicating the K-L and L-M transition were measured at 630 nm. They were detected with a Si-photodiode (FMD 100) at an electrical bandwidth setting of 100 MHz.

Results and Discussion

Electrical characterization of the capacitative cuvette

To construct a macroscopic measuring cuvette with a broad frequency window for open-circuit measuring conditions, the cell capacitance should be considerably larger than the minimal stray capacitance attainable (typically 10–15 pF). The cuvette was realized by separating two Pt-electrodes (area of 1.6 cm^2) by 0.8 mm. With water as dielectric, a cell capacitance of 142 pF is calculated.

To achieve open-circuit measuring conditions during a flash experiment, it was necessary to disconnect the supply batteries from the electrodes for that time (otherwise the plates were short-circuited). Although the separation could principally be achieved by electronic elements (i.e., diodes or switching transistors), a galvanic separation by means of relais was preferred, since the latter prevented high-frequency ringing. In the GHz range it was additionally necessary to block the supply leads by a few windings around a ferrit core in the close vicinity of the electrode plates (not shown in Fig. 1).

In order to protect the preamplifiers from the supply batteries for the orienting voltage, the cell was a.c.-coupled to the preamplifier (Fig. 1). The time constant, τ_e, of this component was set to $\tau_e = 10$ ms by a coupling capacitor, $C_c = 5$ nF and a load resistance, $R_1 = 2$ MΩ. This *RC*-element determined the lowest limiting frequency for signal transmission (Fig. 2a). It is sufficiently low not to interfere with other lower limiting frequencies discussed in the following.

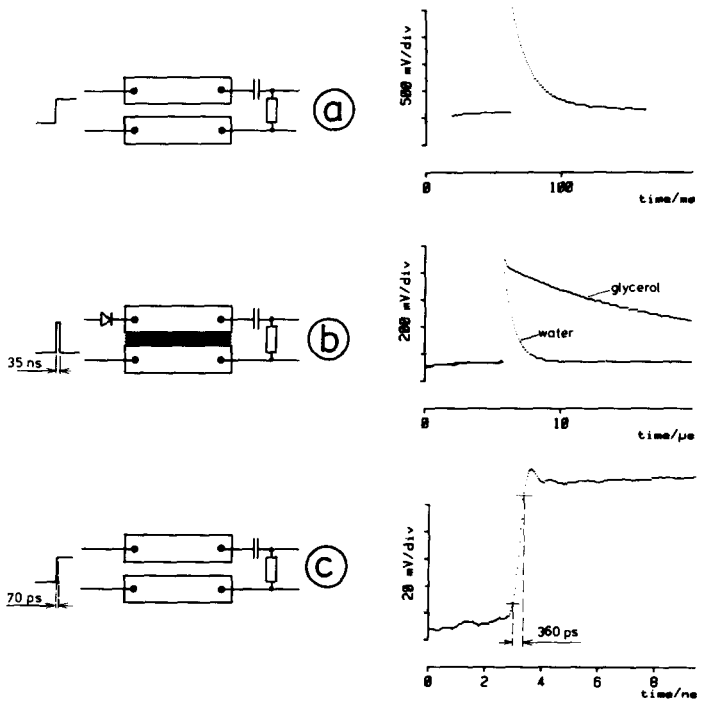

Fig. 2. Electrical transmission characteristics of the capacitative cuvette as tested with step and delta function pulses as indicated on the left side. The traces illustrate the time domains where relevant transitions occur. (a) *RC*-decay caused by the a.c.-coupling of the output. (b) Two examples showing the dependence of the *RC*-decay caused by the discharge of the cell capacitance via the resistance of the medium in the electrode gap on the conductivity of the medium (compare Table I). The cell was charged by a pulse of a duration of 35 ns via a silicon diode (charge injection) and the discharge was monitored with an electrometer amplifier. The blocking resistance of the diode was 10 MΩ. (c) Maximal rise-time of the present apparatus. Pulses of 70 ps rise-time were sent through the empty measuring cell and the *RC*-component. The transmitted pulse was picked up by an ultrafast impedance converter, amplified 10-times by a 3 GHz amplifier, and recorded on a 1 GHz oscilloscope.

The cell capacitances calculated from the dimensions of the cell and the dielectric constants are for glycerol ($\varepsilon = 42.5$) $C_s = 75$ pF and for ethylene glycol ($\varepsilon = 38$) $C_s = 67$ pF. Since the stray capacitance lies parallel to the cell capacitance, both form a capacitative voltage divider which diminishes the actually measured voltage [33]. In order to keep this effect small, the cell capacitance should be as high as possible. Assuming a stray capacitance of $C_e = 10$ pF, the measured voltage is diminished by 6.6% in the case of water and by 13% in the case of ethylene glycol.

One lower limiting frequency of the capacitative cuvette is given by the discharge of the cell capacitance, C_s, through the external load resistance, R_1. In view of a broad transmission window, this limiting frequency should be as low as possible. At a cell capacitance of $C_s = 100$ pF and a load resistance $R_1 = 2$ MΩ this time constant is 200 μs.

In practice, the useful frequency window of open-circuit voltage is further restricted by still another relaxation. It is caused by the discharge of the capacitor formed by the electrodes, C_s, through the resistance of the medium, R_s, between the metal plates. Its time constant $\tau_s = R_s C_s$ was experimentally determined by a charge injection method [34] for two representative solvents, water and glycerol (Fig. 2b). A short, 35 ns, pulse was sent via a diode onto the electrode plates as indicated on the left side of Fig. 2b. The resulting decay was single exponential. Its time constant did neither depend on the test pulse amplitude (1–200 mV), nor on whether or not pulses of orienting voltage were given prior to the charge injection pulse. Numerical values for several media are listed in Table I.

The time constant of the above decay was approximately equal to the one independently calculated from the RC-time, formed by the cell capacitance, C_s, and the ohmic resistance of the medium, R_s, according to $\tau_s = R_s C_s$ (Table I). The cell capacitance was calculated from the space between the electrodes and the dielectric constant of the medium. The resistance was measured with a resistance bridge. Although there seem to be small systematic deviations between both methods, the numbers in the last two columns agree sufficiently well. The deviation may be ascribed to a higher impedance of the Pt-electrodes at 1 kHz (operating frequency of the bridge) as compared to about 1 MHz (corresponding frequency of the charge injection experiment).

The relaxations determining the lower limiting frequency of the measuring cell are summarized on a logarithmic time scale in Fig. 3. The figure includes some experimentally determined lower limiting frequencies for other solvents (1 mM KCl in water, distilled water, 2 M sucrose in water, ethylene glycol (99.5%) and glycerol (83%)).

The highest time resolution of the present set-up was given by the limiting frequency of the oscilloscope: $\tau_u = 360 \pm 15$ ps (Fig. 2c). It was tested by connecting one end of the electrode plates to a step function generator with a rise-time of 70 ps and recording the transmitted pulse by a 1 GHz oscilloscope. A much higher time resolution can be

TABLE I

COMPARISON OF THE CELL TIME CONSTANT τ_s AS DETERMINED BY TWO DIFFERENT METHODS

In one method the decay time constant was calculated as a product of the measured resistance and the cell capacitance, calculated from the cell geometry and the dielectric constant. In the other method τ_s was measured by charge injection as described in the text.

Medium	Dielectric constant ε	Calculated capacitance C_s (pF)	Measured resistance R_s (kΩ)	Calculated decay τ_s (μs)	Measured decay τ_s (charge injection) (μs)
H_2O_{dist}	80	142	5.0 ±0.2	0.7 ±0.4	0.7 ±0.04
Glycerol (87%)	50	89	90 ±2	8.0 ±0.2	11 ±0.5
Ethylene glycol	42	74	90 ±4	6.7 ±0.4	11.5 ±0.5
2 M sucrose	(77)	(136)	34 ±3	(4.6 ±0.4)	5.0 ±0.3
2 M sorbitol	(77)	(136)	5.5 ±0.3	(0.75±0.3)	0.94±0.05
2 M sorbitol + 1 mM KCl	(77)	(136)	0.62±0.02	(0.08±0.002)	0.12±0.05

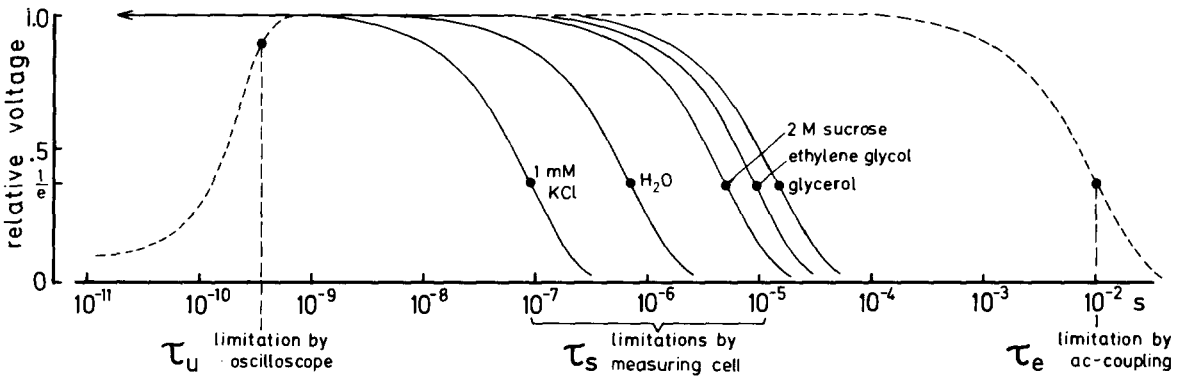

Fig. 3. Schematic representation of the electrical transmission characteristics of the capacitative cuvette under the assumption of an infinitely fast step polarization process of the dielectric. The curves were derived from traces like in Fig. 2. The lower limiting frequency of the time window that can be used for an open-circuit voltage measurement is indicated for 1 mM KCl, distilled water, 2 M sucrose, ethylene glycol and glycerol (87%). The time constants τ_s and τ_e are defined in Fig. 1. The upper limiting frequency is labeled with τ_u and given in the present set-up by the limiting frequency of the oscilloscope $\tau_u = 350$ ps.

expected with faster recording electronics, since, assuming an indefinitely fast change of the dielectric polarization of the medium between the plates, the field lines reach the electrode plates with the velocity of electromagnetic radiation. Due to the small cell geometry, this consideration predicts a theoretical maximal time resolution of a few picoseconds, irrespective of the medium conductivity.

In summary, under open circuit measuring conditions and at low ionic strengths there exists an open time window of at least 2–3 orders of magnitude which can be used for measurements of undistorted open circuit photovoltage, whose amplitude is a measure for the distance between the separated charges and whose kinetics display the kinetics of the charge displacements. At high frequencies the window is restricted by the upper limiting frequency of the detecting device (see Fig. 3). At low frequencies it is restricted by an *RC*-relaxation of the cell resulting from the cell capacitance and the resistance of the medium.

The preceding discussion referred to the measurement of the open circuit voltage (current clamp). The alternative measurement of photocurrent was not considered, since in the high frequency range $f > 1$ MHz the conditions required for a voltage clamp at the charge-separation sites at the membranes under investigation cannot be attained due to a finite access resistance even at highest ionic strengths.

Photovoltages from oriented purple membranes

A primary charge separation

Fig. 4a shows the photovoltage of oriented purple membranes in 2 M sorbitol evoked by a train of picosecond flashes. The single flashes were spaced by 7 ns. Within this time, the photovoltage decayed almost to the baseline with a time constant of 5–6 ns. As discussed in the previous subsection this decay can be attributed to the discharge of the cell capacitance ($C_s = 130$ pF) through the input resistance of the preamplifier ($R = 50$ Ω). Accordingly, the photovoltage decay is not related to a molecular charge displacement. This conclusion is further substantiated by the experiment shown in Fig. 4b where the photovoltage did not decay when the 50 Ω preamplifier was replaced by an impedance converter (voltage follower) of an input resistance of 18 kΩ. Doubling the stray capacitance did not markedly alter the signal.

At high impedance, the photovoltage signals evoked by the train of picosecond pulses, accumulated (data not shown). The signals looked like step functions in a stair. However, there was strong ringing around 1 ns, which was caused by the unmatched impedance between measuring cell and impedance converter (compare Fig. 3 in Ref. 35).

Fig. 4c and d shows the rising phase of the photovoltage at the highest possible time resolu-

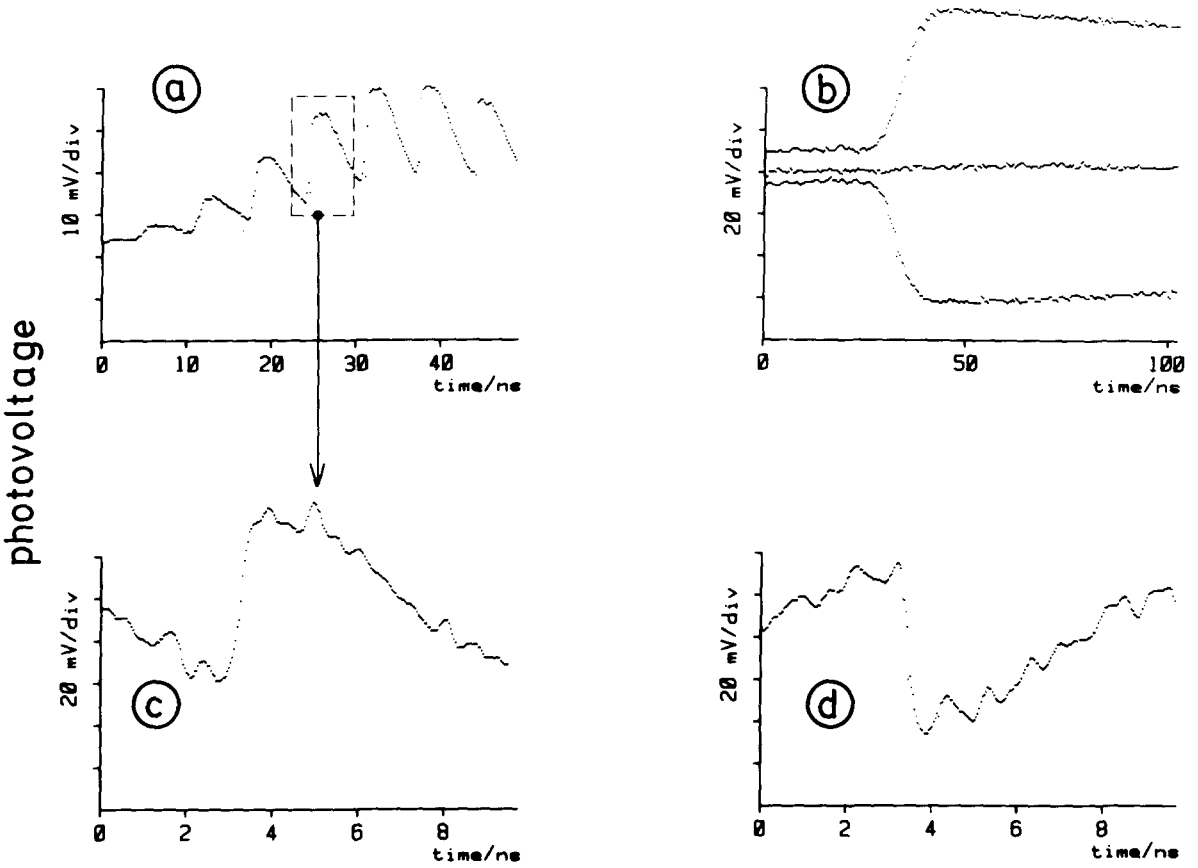

Fig. 4. Fast photovoltages in different time domains from oriented purple membranes in 2 M sorbitol. (a) Excitation by a train of picosecond pulses ($\lambda = 532$ nm). The preamplifiers (3.15 GHz) had 50 Ω input resistance which caused an *RC*-decay after each picosecond pulse. (b) Excitation by 10 ns flashes at $\lambda = 532$ nm. The photovoltage was measured by an impedance converter with an input resistance of 18 kΩ. The upper, middle and lower trace were taken with positive orienting voltage, no orienting voltage and negative orienting voltage, respectively. (c) and (d) Rise-times of the photovoltage evoked by one picosecond flash of the train at positive- and negative-orienting voltage. One point corresponds to 39 ps.

tion of the present set-up. The depicted traces reflect one picosecond pulse out of the full train. One digital point corresponds to 39 ps. The analysis of 20 different traces yielded a 10–90% rise-time which fell within 9 and 10 points. Taking into account the rise-time of the oscilloscope (350 ps) and that of the preamplifier (111 ps, both calculated from the producers specification), the measured rise-time indicates a maximal reaction time of the molecular process [35] of:

$$\tau_{10-90\%} = \sqrt{(390\text{ ps})^2 - (384\text{ ps})^2} = 70\text{ ps}$$

The polarity of the photovoltage depended solely on the polarity of the orienting voltage: when the orienting voltage was reversed the resulting photovoltage was the mirror image (Fig. 4c and d), and with no orienting voltage applied prior to the flash there was no detectable flash response. This holds also in the 100-ns range as shwon in Fig. 4b. The upper and lower traces correspond to positive and negative orienting voltages, respectively, whereas the middle trace results from a flash of the same energy but 4 min after the last experiment. The rise-time of these signals was determined by the duration of the laser flash (10 ns). The slight decay, with an estimated time constant of 1 μs, is composed of the lower *RC*-limita-

tion (130 pF × 19 kΩ = 2.3 μs) and charge displacements due to the K-L transition (see below). The maximal amplitude of the photovoltage in this time range was, within the experimental accuracy of ±20%, equal to that in the picosecond range when normalized to the same flash energy.

The results presented so far can be explained by a rapid light-induced charge separation occurring in bacteriorhodopsin which is faster than or equal to 100 ps. This result is in agreement with that of Groma et al. [12] who succeeded in measuring this charge separation with a time resolution of 60 ps using also a capacitative measuring system, namely dried oriented purple membranes sandwiched in a metal capacitor.

It is obvious that the early charge separation verified by the photoelectric data corresponds to the cleavage of a salt bridge associated with the geometrical change of the *trans-cis* isomerization of the retinal at its binding site in the apoprotein, as proposed on theoretical grounds [29–31]. The charge separation may serve as an energy reservoir for the absorbed light quantum. It is filled within picoseconds by the *trans-cis* isomerization and made empty in the millisecond time range by the energy needed for the proton pumping mechanism.

In agreement with other photoelectric studies, the polarity of the early phase was opposite to the slower phases. This substantiates recent resonance Raman studies which show that the Schiff base proton is initially displaced in a direction opposite to that of proton pumping [36].

The primary charge separation in bacteriorhodopsin is basically different to that in photosynthesis: the cleavage of a salt bridge is of electrostatic nature whereas the photosynthetic charge separation is due to a redox reaction, between an electron donor and an electron acceptor separated by the membrane thickness.

If the limiting frequencies of the present set-up are taken into account, the photovoltage in the submicrosecond range is like a pure step function. This is in agreement with other photovoltage measurements, where purple membranes were adsorbed to a heptane/water interface [22]. Thus, the primary charge sparation in bacteriorhodopsin is also a step-function-like process as suspected in Refs. 11 and 22. This indicates that the rapidly separated charges are stabilized in the protein up to at least 100 ns and that there are no further electrical relaxations in that time range. This result does not support significant protein relaxation in this time range. It also shows that charge displacements due to the K-K′ transition which were found by Ormos et al. [8], must be smaller than the signal-to-noise ratio of the traces in Fig. 4. Accordingly, they should not exceed 10% of the primary charge separation.

Electrical relaxations around 1 μs and the correlation to the spectral K-L transition

This section is concerned with relaxations of the photovoltage between 100 ns and 10 μs and their correlation to the spectroscopically defined K and L intermediates. The data shown result from only the positive polarity of the orienting voltage, since mirror images were obtained for the negative polarity.

Around 1 μs the photovoltage decayed close to the baseline for all suspension media tested (Fig. 5a, b and c), ending at slightly negative levels for times $t > 10$ μs (best seen in Fig. 5a). To investigate whether this decay time parallels the decay of the K intermediate or a relaxation of the measuring system, purple membranes were suspended in media of different conductivity. In parallel experiments, using the identical suspensions, the *RC*-decay of the measuring cell itself was measured by charge injection as described before (Fig. 5d, e and f) and the kinetics of the decay of the K intermediate were measured by the absorption change at 630 nm (Fig. 5g, h and i).

When the purple membranes were suspended in 2 M sorbitol, the exponential time constant of the decay of the photovoltage was 1.5 μs (Fig. 5a). When the membranes were suspended in distilled water, the decay was twice as fast, 0.79 μs (Fig. 5c), and when they were suspended in sorbitol containing 1 mM KCl the decay was shortened to 0.56 μs (Fig. 5b). In contrast, the spectroscopically measured K-L transition showed the same time constant of 1.5 μs for all three suspension media (Fig. 5g, h and i).

The accelerated decay of the photovoltage signals in suspension media of increasing conductivity went parallel to a decrease of the lower limiting frequency of the measuring cell (τ_s in Figs. 1 and

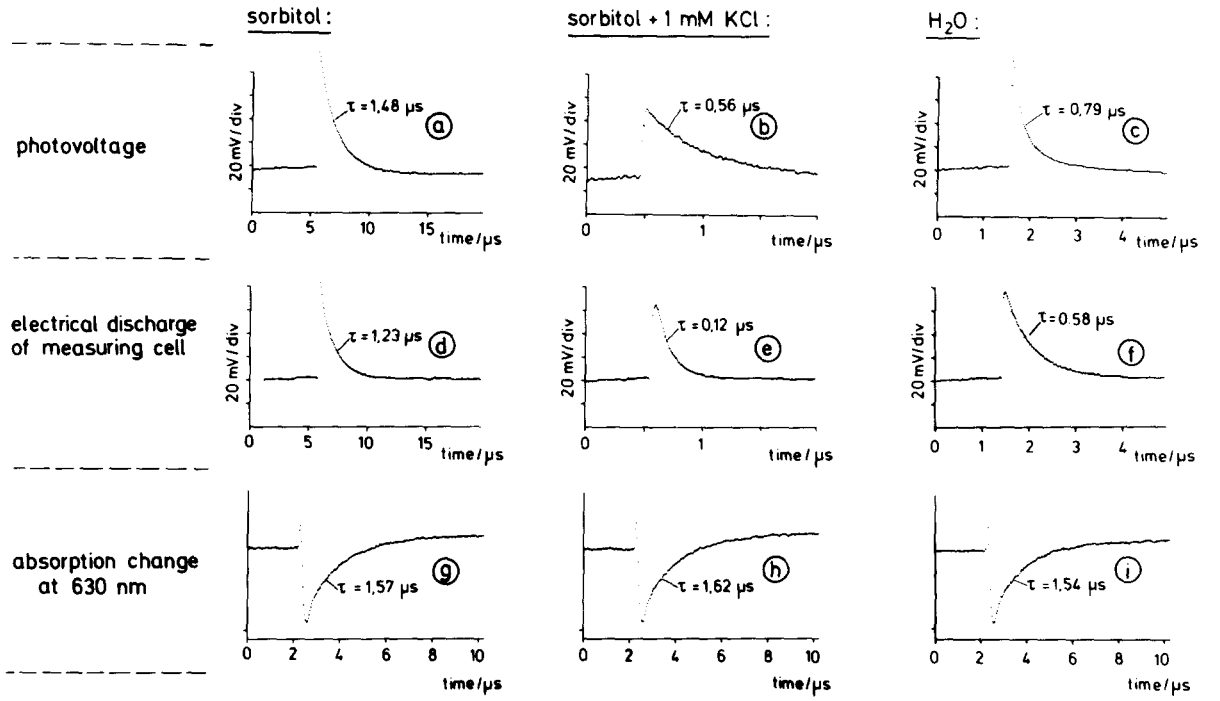

Fig. 5. Comparison between the photovoltage, the discharge kinetics of the capacitative cuvette and the decay of the K-intermediate for purple membranes in different suspension media (sorbitol concentration, 2 M). A single flash at 532 nm (1 mJ/cm^2) was given 200 ms after switching off the orienting voltage. All electrical signals were recorded by an electrometer amplifier. The discharge kinetics of the cell was measured by charge injection as shown in Fig. 2. The formation and decay of the K intermediate was measured at 630 nm by flash spectroscopy at an electrical bandwidth of 100 MHz.

3). The dependence of the photovoltage on the conductivity suggests that in this particular time range the photovoltage is shaped by *RC*-relaxations, since the spectroscopic transition was proved not to depend on the conductivity. It may be considered unlikely that the charge displacements in bacteriorhodopsin are accelerated by higher ionic strengths, whereas the spectral transition is not. Therefore, the data do not allow for a clear-cut statement on the correlation between the electrical signals and the spectral K-L transition.

The problem of a transition frequency

In the foregoing, the decay of the photovoltage around 1 µs was attributed the lower limiting frequency of the measuring cuvette. It is conceivable, however, that on a microscopic level the photovoltage across the purple membranes could principally also decay by ion fluxes around the membrane sheets [23,25,37]. The latter relaxation would also limit 'open circuit' measuring conditions. Nonetheless, if slower charge displacements occur, there can still photoelectric signals be measured after these decays (e.g., Fig. 6). They may be regarded as a voltage drop at the resistance of the medium by balance currents. Therefore, photoelectric signals observed after these relaxations ought be interpreted as photocurrents. According to this picture the signals are necessarily smaller than the true voltage across the membranes.

A more detailed discussion will be published elsewhere. We note here that there exists a transition frequency, f_t, at which open-circuit conditions convert into short-circuit-like conditions, even if the electrodes are connected to high-impedance amplifiers. The transition frequency will depend on the source capacitance of the measuring system, as well as on the conductivity of the suspension medium (estimated range: 100 kHz $< f_t <$ 3 MHz). If the photoelectric signals are much faster or

much slower than f_t, then the kinetics may not depend on the conductivity. Therefore, it is recommended to use the dependence or independence of the kinetics on the ionic strength as a general criterion for distorted or undistorted photoelectric signals.

Electrical relaxations around 50 μs and the correlation to the decay of the L intermediate

The photovoltage in the time range 10 μs < t < 200 μs had a sign opposite to the early phase and a much smaller amplitude (Fig. 6a). As discussed above, the small amplitude is due to the fact that the response in this time range reflects a voltage drop at the resistance of the suspension induced by the displacement currents of the purple membranes. Because of the different meaning of the photovoltage below and above the transition frequency, f_t, the amplitude of the slower phase is not directly comparable with the amplitude of the early phase.

As seen in Fig. 6a the amplitude and decay kinetics of the photovoltage were almost the same in suspension media of similar resistance, sorbitol and distilled water (Table I). However, when the resistance was diminished by addition of 1 mM KCl, the photovoltage decreased below the noise level at the given amplification (Fig. 6a). This small amplitude can be explained by a smaller voltage drop in the medium of higher conductivity. Doubling the stray capacitance altered the amplitude and the kinetics only insignificantly.

In a recent study, the author reported photovoltage signals from purple membranes adsorbed to a heptane/water interface under unadulterated open-circuit conditions [22], where the amplitude of the slow phases was larger than the amplitude of the fast phase. Assuming that the amplitude is proportional to the distance of the charge displacement, it was concluded that the early charge separation contributed only 10% to the total electrogenic step. The present result, although it shows

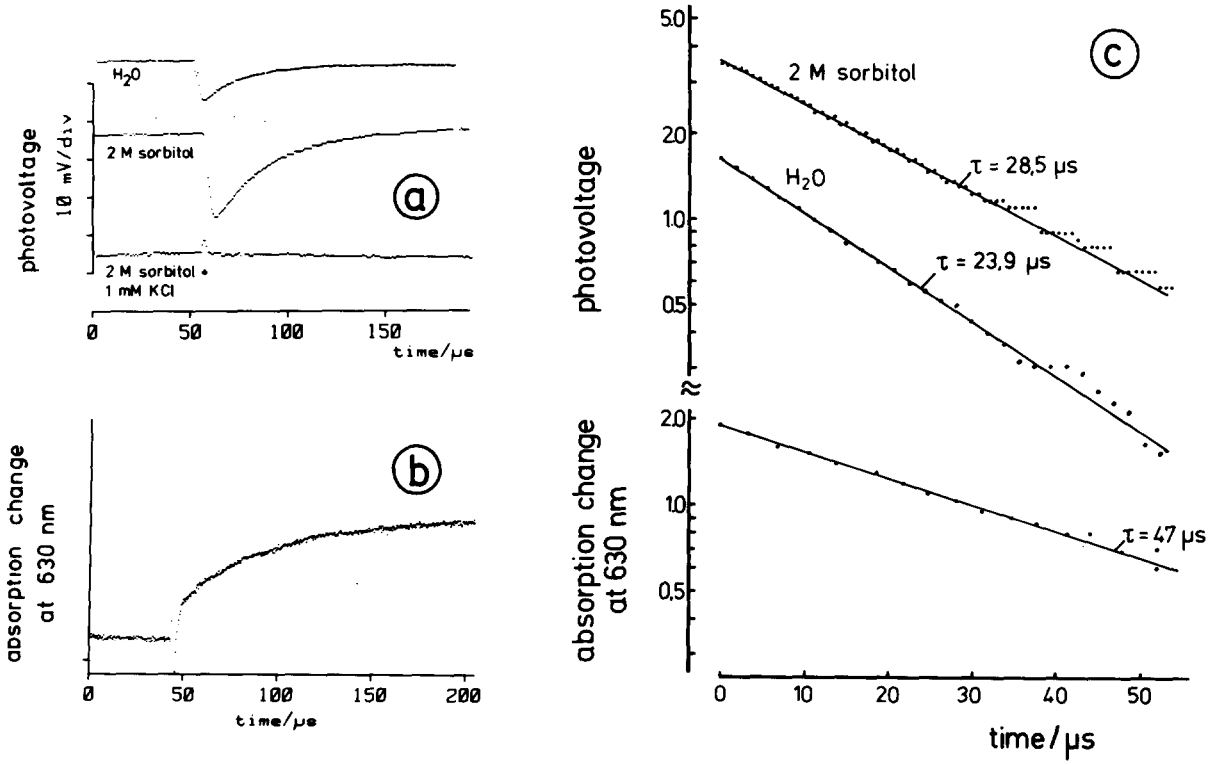

Fig. 6. (a) Flash-induced photovoltage around 100 μs from purple membranes in different suspension media as indicated. (b) Absorption change at 630 nm indicating the decay of the L-intermediate. All three media displayed indistinguishable kinetics. (c) Semilogarithmic plot of the left-hand traces.

the inverse amplitude ratio, is consistent with this interpretation, since the open-circuit conditions are met in the submicrosecond range but not in the range above 10 μs. To analyze the displacement coefficients from the present data would require a detailed equivalent circuit for the suspended purple membranes in the dielectric of the capacitor which is not yet established.

Semilogarithmic plots of the decay phases are shown in Fig. 6c. The time constants for distilled water and sorbitol were 27.5 and 28.5 μs, respectively. Increasing the amplification and the flash energy allowed the observation of the photovoltage also in 2 M sorbitol with 1 mM KCl, which displayed a time constant of 33 μs (data not shown). The time constants agree well with that found for the photocurrent of purple membranes adsorbed to planar bilayers, when a single exponential analysis was applied [21]. They do not agree with that found for the photovoltage of purple membranes adsorbed to a heptane/water interface [22]. In the latter system the photocycle was probably slowed down due to the presence of organic solvent. Neither of the two studies contained spectroscopical control measurements.

The time constants of the photovoltage were about 1.5-fold faster than the time constant of 47 ± 2 μs measured for the decay of the L-intermediate in the same suspension media (Fig. 6c). The faster kinetics of the photovoltage could possibly correspond to an intermediate, X (kinetically between L and M), which was observed by Raman spectroscopy [38]. However, the discrepancy between electrical and spectroscopical detection was not found with purple membranes in a macroscopic dielectrically polarized system [5,10].

Since in the present study the difference between the electrical and spectroscopical detection exceeded the experimental error, and since the decay kinetics of the photovoltage was much the same in media of different conductivity, this result is indicative of charge displacements due to a conformational change of the protein, which occur at some distance from the pocket of the retinal binding site, such that the spectral properties of the chromophore remain unaffected.

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