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INFLUENCE OF STRAY CAPACITANCE AND SAMPLE RESISTANCE ON THE KINETICS OF FAST PHOTOVOLTAGES FROM ORIENTED PURPLE MEMBRANES

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Flash-induced photovoltages were measured with metal electrodes in two experimental systems of purple membranes oriented by an electric field. One system consisted of a suspension of purple membranes cooled to 80 K. The photovoltage evoked by a xenon flash lamp displayed a single phase with a fast rise and a slow *RC*-decay. The signal shape is consistent with a fast charge separation occurring before the decay of the K-intermediate. The other system consisted of purple membranes embedded and stabilized in polyacrylamide gel. At room temperature, the photovoltage, evoked by a 10 ns laser flash, displayed a negative phase in the submicrosecond range and a slower positive one. The shape of the signals were altered in a complex manner by the stray capacitance and the ionic strength. The rise-time of the negative phase was approx. 14 and approx. 40 ns at ionic strengths of 10 and 1 mM, respectively. The initial peak amplitudes of the photovoltage from both experimental systems depended on the external capacitance in an inverse manner, indicating that both experimental systems were not impedance-matched. The evaluation of kinetic data of molecular reactions from measurements of the photovoltage is discussed.

Introduction

Purple membranes consist of a two-dimensional lattice of bacteriorhodopsin (BR). Bacteriorhodopsin utilizes light to pump protons from one side of the membrane to the other [1]. Upon flash illumination a series of spectroscopic intermediates which are closely related to the proton pumping mechanism occurs. Since the process of proton translocation is electrogenic, many studies have been carried out to monitor the electrical activity of bacteriorhodopsin with metal electrodes and to relate it to spectroscopical transitions [2–21]. In one of these studies, an early charge separation, similar to a step function, was reported. This separation rose faster than 2 ns and remained stable for microseconds, i.e., for approximately the life-time of the K-intermediate [21]. This submicrosecond phase, which is oppositely directed to the slower phases, was first reported as a negative transient by Skulachev et al. [9,10].

Several experimental systems are known to achieve the necessary asymmetry. They can be distinguished according to whether purple membranes are oriented like planar membranes separating two conducting phase [2–12,15–18,21] (Capacitative systems) or oriented in suspensions of macroscopic dimensions [13,14,19,20] (dielectrically polarized systems). The present study is concerned with the electrical properties of only the latter system, in which a measuring cuvette is dielectrically polarized by the charge displacements within individual membrane fragments and

in which the photoelectric activity is measured as open circuit photovoltage with two electrodes separated by more than 8 mm. To deduce the magnitude and kinetics of molecular-charge displacements from photovoltage signals, it is essential to know whether signal-shaping processes are involved and complicate the analysis.

As an example for the problem in question, we mention the photosynthetic charge separation, which is known to occur with picoseconds and to be stable for milliseconds as shown by flash spectroscopical methods [22–25]. Hence, the flash-induced molecular reaction is a voltage step across the photosynthetic membrane. A photovoltage of such form could be measured in a capacitative system where chloroplasts or thylakoid vesicles were spread at a heptane/water interface and the resulting interfacial layer was sandwiched between a planar capacitor [26–28]. However, the shape of the photovoltage changed dramatically when a dielectrically polarized detection system was used which was based on the light gradient in a suspension of chloroplasts or other photosynthetic vesicles [29,30]. In this system, flashes evoked short transients. Their kinetics and polarity depended strongly on experimental parameters, like ionic strength, size of the vesicles, excitation wavelength, flash duration and stray capacitance [29,30]. At present, not all of the observations are understood. One difficulty is the complex achievement of the asymmetry (small light gradients in spherical vesicles).

In contrast to chloroplasts or photosynthetic vesicles, purple membranes have a simple planar geometry, are electrostatically asymmetric and can easily be oriented by electric fields [31–33]. This facilitates to elucidate the relation between molecular function and electrical signals. On the other hand, the analysis is more complicated as compared to the simple step function of photosynthesis, since at room temperature several phases of charge displacements fall within the time-range of interest (10 ns–200 μs) [2–21]. Therefore, we will describe first experiments at 80 K where only one intermediate is formed [19,34] and a step function for the photovoltage could be expected [21]. This experimental system consisted of a suspension of purple membranes which was frozen in liquid nitrogen in the presence of an orienting voltage. After disconnecting the supply voltage, the influence of the external capacitance on the open circuit photovoltage was studied. Other experiments were carried out at room temperature where the intermediates K, L and M are formed, and the photovoltage is expected to be multi-exponential. In this experimental system purple membranes were embedded and stabilized in polyacrylamide gel in the presence of an orienting voltage. The influence of the external capacitance and the conductance of the medium on the open-circuit photovoltage was studied.

Materials and Methods

Preparation of purple membranes

Purple-membrane fragments were prepared from *Halobacterium halobium* strain R_1M_1 by standard procedures [35]. They were purified by washing in tridistilled water. The samples were light-adapted shortly before carrying out the experiments.

Experiments at low temperature

For these experiments purple membranes were suspended in a glycerol/water mixture (40% v/v). The measuring cuvette had dimensions of $1 \times 0.5 \times 0.1$ cm. Two Pt-wire electrodes were 0.8 cm apart. In the presence of an orienting voltage of 7 V, the cuvette was immersed into liquid nitrogen. Under this condition, the resistance of the sample was more than $1 \cdot 10^{10}$ Ω. The electrodes were shunted with a resistor of $4.7 \cdot 10^5$ Ω, and with different capacitors to increase to stray capacitance. The preamplifier was a high-impedance amplifier (Burr-Brown, type 3554) working at a gain of 100. The capacitance of only the sample and the electrodes was about 1 pF. Without additional external capacitances, the whole system had a stray capacitance of 190 pF.

Preparation of oriented purple membranes in gel

Purple membranes were suspended in 7.5% acrylamide. The pH was adjusted to the value of 7.5. At this pH value, the membranes have their maximal dipole moment [33]. In the presence of an orienting voltage of 20 V/cm [30], polymerization was initiated by 0.2% ammonium-persulfate and 0.2% TMED (N,N,N',N',-tetramethylethylen-

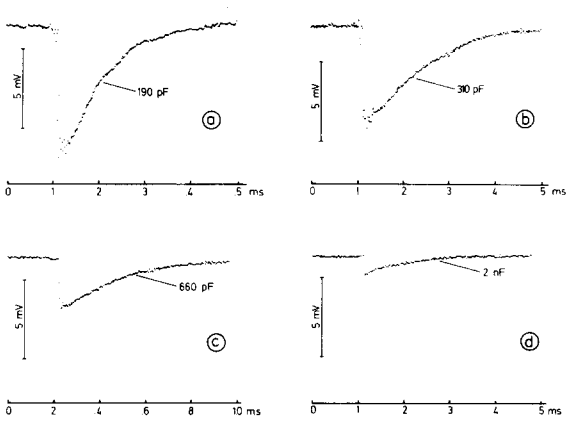

Fig. 1. Dependence of the photovoltage from oriented purple membranes at 80 K on the external capacitance (numbers given in the figure). The membrane fragments were suspended in glycerol/water. The orienting voltage was 7 V. Excitation by a discharge flash. Note the different scaling factors on the time base.

ediamine). Polymerization had finished within some minutes. The gel was stored in distilled water at 4 °C until use. To change the ionic strength in the sample, the gel block was dialyzed for 50 h against the respective medium exchanging it three times. The measuring cuvette for the experiments with gel was the same as the one used in Ref. 30. Its stray capacitance was 12 pF. The external capacitance was increased by soldering further capacitors parallel to the electrodes. The preamplifier was an electrometer amplifier with an input capacitance of 3 pF and a limiting frequency of 10 MHz (EMV 80, MS Electronik).

Flash excitation

The excitation source for the experiments at low temperature (Fig. 1) was a 3 μs flash from a xenon discharge lamp. The light from this lamp was filtered by a green glass filter ($\lambda_{max} = 530$ nm, $\Delta\lambda = 70$ nm). For the experiments at room temperature (Fig. 2), a 10 ns flash from a Q-switched and frequency-doubled Nd-Yag laser was used. The laser beam passed through a light guide with scrambled fibers to achieve homogeneous illumination of the sample.

Results and Discussion

Experiments at low temperature

The influence of the external capacitance on the photovoltage from electrically oriented purple membranes in glycerol/water at 80 K is shown in Fig. 1. According to Ref. 19, at this low temperature there is only one electric transition known, the K′-formation, which could be responsible for the photovoltage [34]. Since the K′-intermediate is stable at this temperature and in the depicted time-range, one would expect the photovoltage to look like a step-function if the signal transmission would be undistorted. As seen in Fig. 1, this was not the case. The peak amplitude decreased and the decay became slower with increasing external capacitance.

A quantitative analysis of the traces shown in Fig. 1 revealed that the decay kinetics were identical to the RC-time formed by the sum of all external capacitances and the external load resistance ($4.7 \cdot 10^5$ Ω). Furthermore, the product of

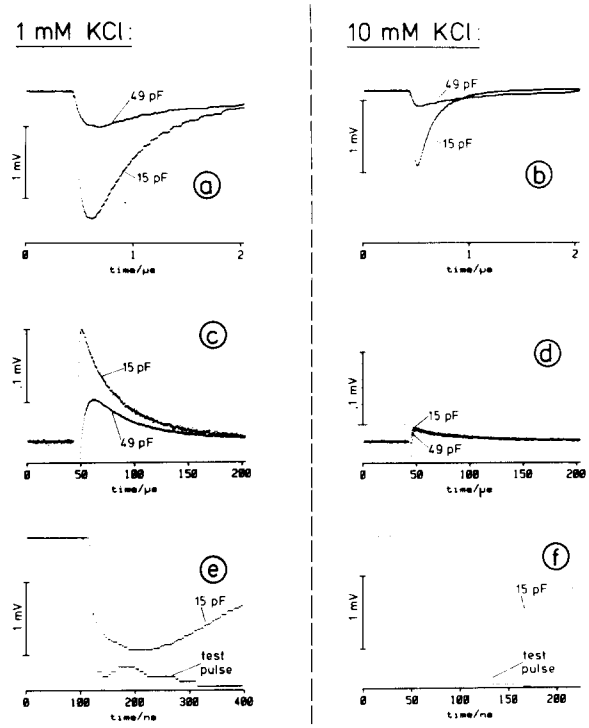

Fig. 2. Dependence of the photovoltage from oriented purple membranes embedded in gel at room temperature on the external capacitance (numbers given in the figure). The gel was either dialyzed in 1 mM (a, c and e) or 10 mM KCl (b, d, and f). The sample resistances were 140 kΩ and 25 kΩ, respectively. The traces of the test pulses represent the rise time of the apparatus. Excitation by 10 ns laser flashes at 530 nm of 1 mJ/cm^2. Note the different scaling factors.

the peak amplitude and the external capacitance was constant in all cases tested. Thus, this experimental system obeys the basic law for capacitors, $Q = CV$: at the time the flash is fired, the purple membranes in the cuvette inject a charge pulse of constant charge onto the capacitances of the electrodes and cables.

Summarizing these experiments at 80 K, we conclude that a fast charge separation (not time-resolved) occurs in bacteriorhodopsin which is associated with the formation of the K′-intermediate [19], and that the data of Fig. 1 are consistent with the proposal that the separated charge is stable within the time-range investigated. The decay kinetics seen in Fig. 1 are due to *RC*-relaxations of the electrical circuit.

Experiments at room temperature

The photovoltage from oriented purple membranes embedded in polyacrylamide gel at room temperature was measured under open-circuit conditions using a 15 MHz electrometer preamplifier (Fig. 2). The influence of the stray capacitance on the signal is shown at two representative ionic conditions and two time-ranges. In agreement with other studies [9–17,19–21], the photovoltage displayed a fast negative phase in the time domain smaller than or approximately equal to 2 μs (Fig. 2a and b) and a positive phase in the time-domain larger than 2 μs (Fig. 2c and d).

The amplitude of the fast negative phase depended strongly on the external capacitance at the two ionic strengths investigated (Fig. 2a and b). The increase of the external capacitance, C_a, caused a slower decay of the negative phase. When the gel was dialyzed in 1 mM KCl and $C_a = 15$ pF, the signal decayed with an exponential time-constant of $\tau = 530$ ns and at $C_a = 49$ pF with one of $\tau = 1300$ ns. The quotients of the decay time-constant and external capacitance are 35 kΩ and 26.5 kΩ, respectively. These numbers are smaller than the measured resistance of the sample $R = 140$ kΩ using a resistance bridge (operating frequency, 1 kHz) and the same electrode couple. If the decay would be assumed to reflect an *RC*-decay of the total capacitance and the sample resistance, one would expect a slower decay than actually observed. The measured faster decay may be caused by charge displacements connected with the K-L transition.

A similar result was found when the gel was dialyzed in 10 mM KCl. Under this condition the sample resistance decreased to $R = 25$ kΩ and the decay became accelerated to $\tau = 205$ ns at $C_a = 15$ pF and to $\tau = 690$ ns at $C_a = 49$ pF (Fig. 2b). Here the quotient between decay time-constant and external capacitance was 13.7 and 14.1 kΩ, respectively. These numbers are smaller than the sample resistance of 25 kΩ measured by a resistance bridge. As argued before, this deviation may be due to charge displacements connected with the K-L transition which accelerates a pure *RC*-decay.

Hence, the data of this experimental system in the time-range smaller than or approximately equal to 2 μs support a view which assumes an early charge separation in purple membranes and the injection of a pulse of constant charge onto the capacitance of the electrodes and the cables, and a subsequent discharge through the resistance of the sample.

Since the data given above show deviations from this statement, it should be considered as a first approximation. On one hand, deviations may occur by the different involvement of the resistance of the sample itself, and the resistance of the water phase connecting the electrodes to the sample surface. Both resistances were not distinguished here. On the other hand, the decay kinetics, especially at low ionic strength, are probably affected by charge displacements associated with the decay of the K- and the formation of the L-intermediate ($\tau \approx 2$ μs).

Although the voltage transient originating from the early charge separation has virtually decayed within some microseconds (Fig. 2a and b), there was a further photovoltage of smaller amplitude and positive sign which developed to a maximum, some microseconds after the flash (Fig. 2c and d). This signal was much smaller at 10 than at 1 mM KCl. The kinetics depended on the external capacitance in the case of 1 mM KCl but not in the case of 10 mM (compare Fig. 2c and d).

The insensitivity of the kinetics of the photovoltage to the external capacitance after about 10 μs indicates that the measuring quantity under this condition was not a 'true' photovoltage. We interpret this signal as a photocurrent. Assuming that at $t = 0$ a purple membrane sheet of the capacitance C_m is charged to the voltage V and that no

charge displacements take place, then V will decay with a time constant $\tau_i = R_s C_m$, where R_s stands for the effective resistance of the electrolyte surrounding the membrane. This fast ($t \ll \tau_i$) voltage shall be considered as 'true' voltage. If, however, charge displacements take place in the time range $t \gg \tau_i$, then the corresponding voltage across the membrane decays faster than the kinetics of the charge displacement and only a residual voltage remains, which represents the voltage drop at R_s of the discharge current. From this interpretation it is expected that this slow ($t \gg \tau_i$) voltage is proportional to the resistance of the medium or that at different salt concentrations the ratio of the voltage equals the ratio of the resistances.

A test of this relation can be obtained from Fig. 2c and d, traces with 15 pF. The quotient of the positive peaks at the two ionic strengths is 7.3, whereas the quotient of the sample resistances yields 5.6. This is a reasonable agreement, since there is still an influence of the stray capacitance. When in the experiment of Fig. 2c the electrometer was replaced by a current amplifier, the measured photocurrent displayed the same kinetics (data not shown). This indicates the ohmic behaviour of the system in this time-range.

Like the decay kinetics, the kinetics of the rising phase at low ionic strength appeared also to be determined by a RC-relaxation. The experiments for studying this effect were carried out with an appropriate impedance converter (voltage follower) having a limiting frequency of 500 MHz, an input resistance of 18 kΩ and an input capacitance of 3 pF. The rise of the photovoltage at 1 mM KCl was significantly slower than the rise-time of the apparatus (Fig. 2e). When the resistance of the sample was decreased ($R = 25$ kΩ at 10 mM KCl), the photovoltage rose faster (Fig. 2f). Hence, this experimental observation indicates that also the rise-time is determined by an RC time-constant. The behaviour is analogous to the photovoltage signals from chloroplasts in a light gradient where also high ionic strengths were needed to achieve a time resolution of picoseconds [30].

A systematic inspection of the dependence of the peak amplitudes of the early photovoltage from both experimental systems revealed that they were inversely related. When the reciprocal peak amplitudes were plotted vs. the external capacitance, straight lines resulted which extrapolated close to the origin of the axes (Fig. 3a and b). This means that the source capacitance from which the signal arises is close to zero. It also means that the photovoltages would approach infinite values as the measuring conditions become ideal ($C_a = 0$; no stray capacitance).

A similar behaviour was found for the fast photovoltage from thylakoid vesicles and chromatophores of photosynthetic bacteria in a light gradient [30]. Also in these systems, a flash of given energy is expected to evoke a given charge (or charge separation). The results in Fig. 3 and the corresponding ones in Ref. 30 can be empirically described by a capacitative voltage divider which is formed by the capacitance between the electrodes in the medium (smaller than 1 pF) and the external stray capacitance. We suggest that the electrodes in the measuring cuvette themselves represent the source capacitance, C_e (compare Ref. 30). Due to the small value of the latter, a quanti-

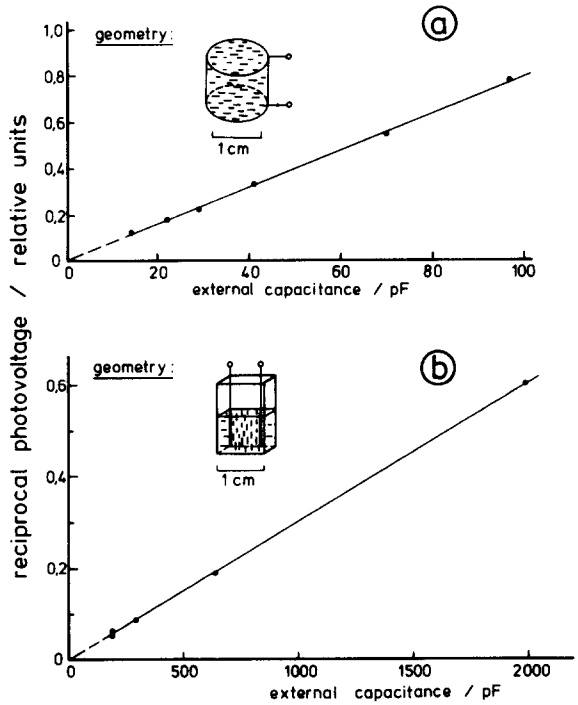

Fig. 3. Plot of the reciprocal early peak photovoltage from the two experimental systems studied vs. the external capacitance. (a) Oriented purple membranes in gel (10 mM KCl) at room temperature; (b) oriented purple membranes in glycerol/water at 80 K.

tative evaluation of the photovoltage under ideal measuring conditions ($C_e \gg C_a$) is experimentally not feasible. To avoid this particular problem, we suggest for future experiments of this type to form the electrodes as a planar capacitor. Its capacitance should be considerably higher than all stray capacitances, in order to attain proper impedance matching conditions and undistorted pulse transmission.

Conclusions

The above experiments demonstrated the influence of external measuring parameters on (a) the kinetics and (b) the absolute size of the photovoltage from oriented purple membranes in two macroscopic dielectrically polarized systems.

(a) In addition to real molecular relaxations, different *RC*-relaxations may contribute to the shape of the photovoltage, depending on the sample resistance and the time-domain: (i) if the sample resistance is high (Fig. 1), the voltage decays with an *RC* time-constant formed by the load resistance and the stray capacitance; (ii) if the sample resistance is less (room temperature data in Fig. 2), the fast negative voltage decays with a *RC* time-constant, τ_i, being on the order of that formed by the sample resistance and the stray capacitance.

Transients occurring in times less than τ_i may be considered as 'true' voltage signals, whereas transients occuring in times larger than τ_i may be interpreted as current signals (i.e., voltage drop across the resistance of the medium). Relaxations due to the kinetics of molecular charge displacements occurring around τ_i are shaped by this *RC* time-constant. To evaluate molecular-reaction kinetics in this time-domain requires either complete-signal analysis or shifting τ_i far from the molecular relaxation. This is reliazable by a variation of the ionic strength.

(b) The absolute magnitude of the early phase of the photovoltage was dependent in an inverse manner on the external capacitance (Fig. 3). This is typical for a capacitative voltage divider (with constant charge) [26]. The data reported here and in Ref. 30 point to a negligible small source capacitance which is, most likely, the capacitance of the electrodes (smaller than 1 pF). In the high-frequency range the small source capacitance may cause signal-shaping due to impedance mismatching with the larger stray capacitance (approx. 15 pF or approx. 190 pF, respectively). For these types of measurements we suggest to construct the electrodes in form of a planar capacitor ($C_e > 100$ pF).

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