ON THE RISE TIME AND POLARITY OF THE PHOTOVOLTAGE GENERATED BY LIGHT GRADIENTS IN CHLOROPLAST SUSPENSIONS

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1. Introduction

The primary process in photosynthesis is a redox reaction within 2 different reaction centers. These reaction centers are asymmetrically embedded in the membranes of the thylakoids of the chloroplasts. The oxidizing sites are located at the internal aqueous phase and the reducing sites at the external aqueous phase. Thus, the transmembrane redox process is coupled with the generation of an electrical field across the thylakoid membrane, such that the inner phase becomes positive with respect to the outer phase. The generation of the transmembrane field has been detected first by measurements of electrochromic absorption changes of intrinsic pigments [1]. This method, however, does not demonstrate the polarity of the field.

Two electrical methods have been used to reveal the polarity of the transmembrane field:

(i) The microelectrode technique indicates directly the above-stated polarity [2];

(ii) The polarity of the photovoltage arising from a suspension of chloroplasts being illuminated by flashes of non-saturating intensity indicates the same direction of the transmembrane field [3–5].

In (ii) the origin of the photovoltage was ascribed to an asymmetry in the chloroplast suspension which is caused by the intensity gradient of the exciting light in the measuring cell: That membrane side of an individual spherical thylakoid vesicle which is located closer to the light source absorbs more quanta than the opposite membrane side. Consequently, more charge separations occur within the membrane exposed closer to the light source and less occur within the membrane on the opposite side. In this way, an array of oriented dipoles is generated in the measuring cell resulting in a measurable photovoltage. The photovoltage can be detected with macroscopic electrodes spatially separated on the axis of the exciting light path. This explanation of the origin of the photovoltage allows a definite relation between the measured polarity and the direction of the transmembrane charge separation. Since in the [3–5] the electrode closest to the light source became negative, it was concluded that the photosynthetic charge separation makes the outside of the vesicles negative with respect to the inside.

The decay kinetics of the photovoltage has been explained by ion fluxes around the thylakoid vesicles which neutralize the charge imbalance [3–5].

The original aim of this work was to measure electrically the photosynthetic charge separation with high time resolution. It was found that the polarity of the photovoltage obtained by excitation with a laser flash (excitation wavelength of 530 nm) was opposite to that in [3–6]. Since the only difference between the literature data and our observation was the excitation wavelength we measured the polarity of the photovoltage as a function of the excitation wavelength. Furthermore, to examine whether the structural state of the chloroplasts influences the polarity we compared chloroplasts suspended in hypertonic medium with swollen chloroplasts (blebs) in hypotonic medium.

2. Materials and methods

Spinach chloroplasts were prepared as in [7]. The preparation solution for the grinding step contained

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium-methosulfate; BV, benzylviologen; P_i, inorganic phosphate; chl, chlorophyll

in addition 10 mM ascorbate. The chloroplasts were stored in liquid nitrogen until use. All light-gradient experiments were carried out at room temperature. The concentration of chloroplasts corresponded to 5×10^{-5} M chl. Osmotically swollen chloroplasts (blebs) were prepared as follows: The chloroplasts were resuspended in distilled water ($\sim 10^{-5}$ M chl), centrifuged at $5000 \times g$, again resuspended in distilled water and then stored in the dark for 1 h. After another centrifugation step the chloroplasts were resuspended in the reaction medium which contained 10^{-3} M tricine buffer (pH 7.0) and 10^{-4} M benzylviologen. Except the last step, all steps were carried out at 4°C.

The measuring cell consisted of a black-stained Kel F cylinder with an inner diameter of 12 mm and a length of 25 mm. The photovoltage was measured with 2 Ag/AgCl electrodes located at the top and the bottom of the cell.

Monochromatic light flashes were delivered via a light guide from the top of the measuring cell. The flash source was either a Q-switched and frequency doubled Nd-Yag laser (Laser Assoc., model 252) or a flash lamp of ~50 μs duration. The light of the flash lamp passed through interference filters (Schott, DAL) of half bandwidths of 5–10 nm.

For experiments with high time resolution the photovoltage was amplified with a wide band pulse amplifier (Keithley, model 105) and for the experiments with low time resolution with a high impedance amplifier (Ithaco, model 167). The signals were stored in a transient recorder (Biomation, model 6500) and were then transferred to an averager (Nicolet, model 1170).

3. Results

Fig.1, bottom, shows the photovoltage of a chloroplast suspension excited by non-saturating laser flashes ($\lambda = 530$ nm). The chloroplasts were suspended in a medium containing a high salt concentration in order to obtain the small access resistance necessary for high time resolution. At the top of fig.1 the integrated time course of the laser flash is depicted as measured with a fast photodiode (Motorola MRD 500; response time $\leqslant$1 ns). Since the same recording device was used, this procedure yields the rise time of the apparatus including the flash duration. The rise time of the photovoltage itself, τ_{photo}, can be calculated from the measured rise time of the signal, τ_{sig}, and the rise time of the apparatus, τ_{app}, according to [8]:

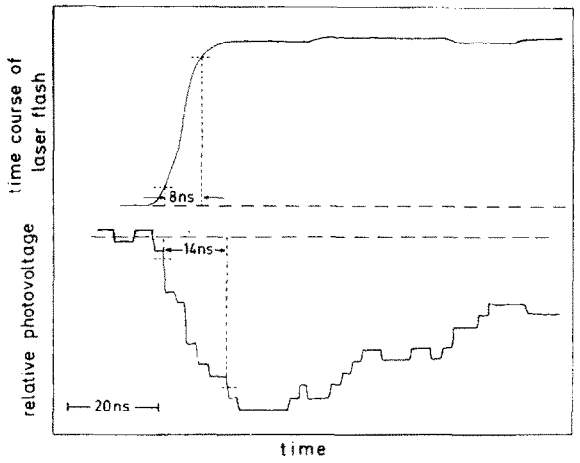

Fig.1. Time course of the photovoltage from a chloroplast suspension (bottom trace). Excitation with a laser flash of 530 nm. Reaction medium: 1 M NaCl; 10^{-3} M P_i buffer (pH 7.0); 10^{-4} M benzylviologen. Five signals were sampled. The time between successive flashes was 1 s. Intergrated time course of the laser flash as measured with a photodiode (top trace).

$$\overline{\tau}^2_{photo} = \overline{\tau}^2_{sig} - \overline{\tau}^2_{app}$$

As an average of 10 different measurements, a 10–90% rise time of $\overline{\tau}_{sig} = 14 \pm 2$ ns and $\overline{\tau}_{app} = 9 \pm 1$ ns was measured. From these values the mean rise time of the photovoltage can be estimated to be:

$$\overline{\tau}_{photo} \leqslant 10.8 \pm 3.5 \text{ nm}$$

The half rise time of the photovoltage would then be:

$$\tau_{1/2,photo} \leqslant 5.4 \pm 1.8 \text{ ns}$$

In contrast to [3–6] the electrode positioned at the light exit of the cell became negative with respect to the other one. Therefore, it was checked whether this fast photovoltage represents the photosynthetic charge separation. At the bottom of fig.2 the photovoltage is shown when both photosystems are operating. After addition of the electron transport inhibitor DCMU almost no photovoltage was observed (fig.2, middle trace). Addition of PMS and ascorbate (in the presence of DCMU) reactivates the electron transport

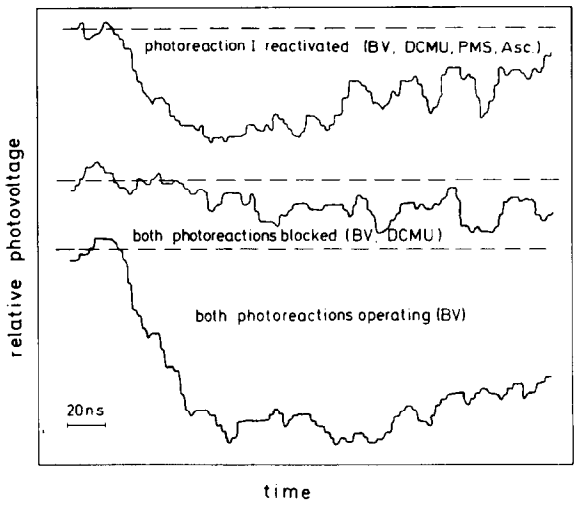

Fig.2. Influence of different reaction conditions on the photovoltage. Excitation with a laser flash of 530 nm. Reaction medium common to the three experiments: 10^{-3} M P_i buffer pH 7.0, 10^{-4} M benzylviologen. Bottom trace: Both photosystems operating. Middle trace: both photosystems blocked by addition of 10^{-5} M DCMU. Upper trace: photosystem I operating by addition of 10^{-4} M PMS, 10^{-2} M ascorbate and 10^{-5} M DCMU. Nine signals were sampled. The time between successive flashes was 1 s.

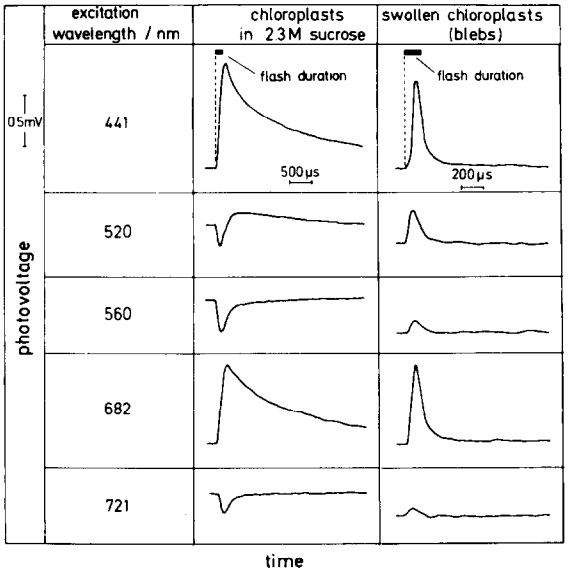

Fig.3. Influence of different excitation wavelengths on the photovoltage. Left: Chloroplasts suspended in 2.3 M sucrose, 10^{-3} M P_i buffer (pH 7.0), 10^{-4} M benzylviologen. Right: Swollen chloroplasts (blebs) suspended in 10^{-3} M tricine buffer (pH 7.0), 10^{-4} M benzylviologen. For further details see section 2. Excitation with a 50 µs flash at different wavelengths.

of photosystem I and ~1/2 the photovoltage measured in the first experiment is generated (fig.2, upper trace). Similar experiments have already been reported, however, using red flashes for excitation [3–6,9]. In these experiments the same functional characteristics of the photovoltage were found; however with an opposite polarity. These observations suggest a dependence of the polarity on the excitation wavelength.

Fig.3 (left) shows the photovoltages from chloroplasts suspended in hypertonic sucrose medium. The different traces result from excitation with flashes of different wavelengths. As seen, the polarity and the kinetics of the signals depend strongly on the excitation wavelength. Excitation in the absorption bands of chlorophyll, at 441 nm and 682 nm, causes a positive polarity of the electrode farther from the light source which is in accordance with [3–6]. The half decay time is ~1 ms. It depends linearly on the conductivity of the medium [4,5]. At 560 nm and at 721 nm the electrode farther from the light source became negative. In contrast to the positive signal the negative signal decays significantly faster. Upon excitation at 520 nm the signal was first positive and then negative. This bipolar signal can be described as a superposition of the 2 signals described before.

The signals at 441 nm and 682 nm show a dependence on light intensity as in [5,9], i.e., with increasing light intensity they first increase, reach a maximum, and then decrease. The signals shown in fig.3 are obtained near the maximum of the light intensity curve. The shape of the signals does not change with light intensity, at least in a range where their amplitudes are decreased by a factor of 10 by going to lower light intensities.

In order to check whether the structural state of the chloroplasts affects the observed changes of polarity, the experiment described before was repeated with osmotically swollen chloroplasts (blebs) which were suspended in a medium of low salt concentration. Here, the electrode farther from the light source became always positive, independent of the wavelength of the flash (fig.3, right). At all wavelengths the decay kinetics were faster than those belonging to the medium containing sucrose.

In all experiments reported here the polarity of the signals was reversed when the flash was delivered from the bottom of the measuring cell instead of from the top.

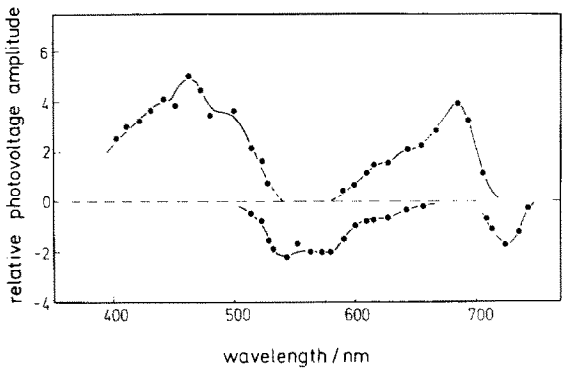

Fig.4. Action spectrum of the photovoltage. Reaction medium: 0.4 M sucrose; 10^{-3} M P_i buffer (pH 7.0); 10^{-4} M benzylviologen. The data reflect the positive or negative amplitudes of the photovoltage.

The complete action spectrum of the photovoltage is shown in fig.4, where the maximal photovoltage of the positive and negative components are plotted. Obviously, the positive signal matches the blue and red absorption peaks of chlorophyll in accordance with earlier measurements [9]. Negative signals were observed in the region between 510 nm and 560 nm and between 700 nm and 740 nm. Both wavelength regions of positive polarity overlap with regions of negative polarity. In the overlapping regions the photovoltage signals possess a bipolar shape (e.g., fig.3, 520 nm). It should be mentioned that the data for the action spectrum were not corrected for equal number of absorbed quanta and that the kinetics of the bipolar signals have not been taken into account for a further analysis. Therefore, these data give only an approximate action spectrum.

4. Discussion

The kinetics of the primary charge separation in chloroplasts is still not time resolved. From measurements of the electrochromic absorption changes it had been calculated that the half rise time of the charge separation is <20 ns [8] and later it was measured that the rise time is <10 ns [10], respectively. The low signal-to-noise ratio of the optical signals from chloroplasts makes faster measurements difficult. In contrast, the photovoltage generated by a light gradient in a chloroplast suspension shows a much better signal-to-noise ratio (fig.1) and may therefore allow a better time resolution. In this study a half rise time of $\lesssim$5 ns was found.

Although the polarity of the photovoltage elicited by the green laser flash was opposite to that predicted by the simple vesicle model outlined above, we assume that the signal reflects the primary charge separation of photosynthesis for the following reasons:

(i) The rise time of the photovoltage is as fast as the rise time reported for the primary reactions measured spectroscopically; i.e., the chlorophyll oxidation [11] and the field generation [8,10];

(ii) The photovoltage is sensitive to DCMU and PMS (fig.2) in the same way as the photovoltage elicited by red flashes [3,9] and as the field-indicating absorption changes [12].

In this study it was shown that excitation with flashes of different wavelengths cause different polarities of the photovoltages (fig.3). This finding cannot be understood on the basis of the simple vesicle model described above.

Another interpretation of this type of photovoltage has been suggested [6]. Based on light-gradient experiments with magnetically preoriented chloroplasts it was concluded that the photovoltage originates from differences in the mobilities of anions and cations at the membrane surfaces. Depending on which ion species is more mobile, a negative or a positive polarity of the photovoltage could easily be explained. However, since in our experiments the ionic composition of the suspension medium was constant (fig.3a) the assumption of different ion mobilities cannot explain the opposite polarities observed.

At present we are not able to give a plausible physical interpretation of this effect. Nevertheless, two experimental findings might provide a basis for an understanding of the reversed polarity:

(i) If the decay kinetics is interpreted to reflect an ion flux around individual vesicles the faster decay would indicate a smaller structural unit from which the photovoltage arises and the slower decay would indicate a larger unit;

(ii) In suspensions of osmotically swollen chloroplasts, where the shape of the chloroplasts is essentially spherical [13,14] we found that the polarity of the photovoltage was independent of the excitation wavelength. The structural state may also influence the polarity observed.

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