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II. Primary electrogenic reactions in chloroplasts probed by picosecond flash-induced dielectric polarization

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Excitation of chloroplasts by non-saturating flashes is known to generate an electric polarization of the suspension which can be measured as a photovoltage with electrodes positioned alongside the propagation axis of the light. The polarization originates from the light-gradient in the suspension and the anisotropic excitation of the primary charge separation in the reaction centers of both photosystems. By using a capacitative measuring cuvette and light pulses of 30 ps duration we could achieve a time resolution on the order of 200 ps, even at low ionic strengths. At the excitation wavelengths of 532 and 694 nm, a photovoltage developed with the rise-time of the apparatus when the reaction centers of both photosystems were open. Closing of the reaction centers of Photosystem I by oxidizing P-700 resulted in a photovoltage with the same fast rise, but of half amplitude. This shows that (i) the primary charge separation in both types of reaction centers and (ii) the mean trapping time of excitons from the antenna system by P-680 and P-700 are shorter or equal to 175 ps. When the traps of Photosystem I were closed and the primary quinone acceptor Q in the reaction centers of Photosystem II was reduced, there was almost no photovoltage response around 1 ns. The only primary reaction known under these conditions is the charge separation in Photosystem II between the primary donor P-680 and the intermediary pheophytin acceptor. Assuming this reaction, the observed lack of a photovoltage indicates that the intermediary acceptor Phe lies on the same membrane side as P-680.

Introduction

Primary events of photosynthesis are commonly investigated by fast spectroscopical methods [1–5]. Photoelectric methods have contributed little to our knowledge of the very primary reactions, because their time resolution could not compete with spectroscopical techniques; e.g., time-resolved fluorescence-decay kinetics [6–8] or picosecond-flash spectroscopy [9–11]. The electrical interfacial system described by Trissl and Graeber [12,13] in which thylakoid membranes are spread at a heptane/water interface and the photosynthetic charge separation is measured as a change of the interface potential by means of a capacitative electrode shows an intrinsic limitation of time resolution of about 1 ns. The limitation is of principal nature and is due to the finite resistance of the electrolyte and the finite stray capacitance which form an *RC*-time constant on this order of magnitude. On the other hand, this experimental system offers a wide frequency window for 'open circuit' photovoltage. This means that the photosynthetic charge separation which occurs presumably within 100 ps and which is stable for milliseconds is

Abbreviations: PS I, Photosystem I of green plants; PS II, Photosystem II of green plants; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; P-700, primary donor of Photosystem I; P-680, primary donor of Photosystem II; Q_A, first quinone acceptor of Photosystem II; LHC, light-harvesting complex; Mes, 4-morpholineethanesulphonic acid.

reflected by a photovoltage in the form of a step function. In this capacitative system the kinetics of the photovoltage follows the kinetics of the molecular charge displacements and the amplitude of the photovoltage is proportional to the distance between separated charges [14,15].

A higher time resolution was achieved with another photoelectrical method in which a light gradient within a suspension of chloroplasts produces a dielectric polarization [16–20]. As argued in Refs. 16,17,19 and 20, the flash-induced photovoltage is a direct measure for the photosynthetic transmembrane charge separation. A real time resolution of 350 ps was reached only at very high ionic strengths [20]. The experiments were carried out in a measuring cell of large geometrical dimension (on the order of 1 cm) and small electrode capacitance (= macroscopic dielectrically polarized system). This small capacitance gave rise to only photovoltage transients: the fall times were approx. 5-times slower than the rise-times, although the generating process has the quality of a step function [19,20]. As discussed in Refs. 20 and 21, this narrow frequency window results from the properties of the measuring system and the instrumentation (e.g. finite source resistance and finite stray capacitance).

Generally in photoelectric measurements, the difficulty appears to be in distinguishing between relaxations originating from electrogenic processes in the proteins and those originating from the measuring system. In the case of oriented purple membranes embedded in a gel of macroscopic dimension, this problem was experimentally pursued [21]. A significant influence of the stray capacitance and the sample resistance on the kinetics of fast photovoltages was found. In order to eliminate this influence, it was suggested to construct the metal electrodes as a planar capacitor so that the electrode capacitance exceeds the stray capacitance.

In a preceding article a capacitative measuring cuvette was described and electrically characterized which combines a macroscopic cell size with a high capacitance [22]. The cuvette was especially designed for an electrical preorientation of purple membranes. In contrast to the macroscopic cells with a small electrode capacitance, this capacitative cuvette showed a rather broad frequency window for open-circuit photovoltages and an unprecedented high time resolution of picoseconds, even at low ionic strengths [22]. In the present study a similar capacitative cuvette is described which was especially designed for experiments of the light gradient type. This experiment requires transparent or semitransparent electrodes to allow illumination through them. The electrodes used here consisted of two thin planar sheets of Pt-mesh separated by some tens of millimeters. The electrical properties of this modified cuvette are similar to the one described before [22].

Using this capacitative cuvette, picosecond light gradient experiments with pea chloroplasts were performed. Such photoelectric measurements give information on the rate of the primary photosynthetic charge separation and on the mean trapping time of excitons from the antenna system by the reaction centers of Photosystem I (PS I) and Photosystem II (PS II). Furthermore, the measurements allow an estimation of the location of the intermediary pheophytin acceptor in the reaction centers of PS II relative to the primary donor, P-680, and to the first quinone acceptor, Q_A, in an analogous way as reported for the RC of the purple bacterium *Rhodopseudomonas sphaeroides* [15].

Materials and Methods

Experimental

The specific requirements for a fast responding capacitative measuring cuvette which is suited for light-gradient experiments are: (i) the electrodes must be made of metal in order to achieve the high conductivity necessary for picosecond time resolution; (ii) the electrodes must be transparent or semitransparent in order to shine light through them, and (iii) the gap between the electrodes must be so narrow that the capacitance formed between them exceeds that of the stray capacitance. Trials with semitransparent copper-coated mica or glass plates showed large electric flash artifacts. The artifacts were negligibly small when the electrodes were made of a Pt-Rh-net (used as catalyst in chemistry) and when they were insulated from the reaction volume by thin teflon foils (6.5 μm thick) or by mica sheets (approx. 100 μm thick).

The construction and dimension of the capaci-

tative measuring cell used in the present experiments are shown in Fig. 1. Most experimental data were obtained with teflon film insulation, since this yielded a higher cell capacitance as compared to insulation by mica. As a consequence this 'teflon cell' showed less high-frequency ringing than the 'mica cell'. The spacer which determined the effective volume for the chloroplast suspension was 0.3 mm thick. The cell was mounted in a metal housing equipped with metal-mesh-covered windows to allow for illumination of the interior.

In all experiments, the photovoltage was amplified by two cascaded GHz-preamplifiers, each having a gain of 12. Details of the detection and recording electronics have been described in Ref. 22.

The excitation source was either a ruby laser or a frequency-doubled Nd-Yag laser purchased from J.K. Lasers Inc. Both lasers were operated mode-locked and a single pulse was selected with a Pockel cell. A split fraction of the flash light was used to trigger externally the oscilloscope by a picosecond photodiode. Care was taken to illuminate homogeneously the measuring cuvette. Strong ringing resulted when this precaution was not observed.

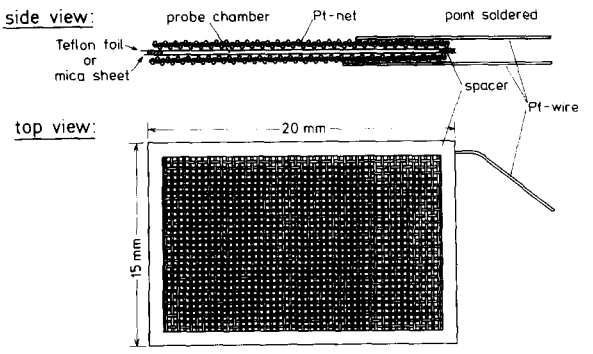

Fig. 1. Construction of the capacitative cuvette for measurements of flash-induced photovoltages of the light-gradient type. The two planar electrodes consist of platinum/rhodium mesh which was point-welded along the verges to achieve good metallic contact. Those sides of the mesh sheets which face the cell interior were covered with 6.3 μm thick teflon film in order to avoid electrode artifacts due to photochemical reactions of chlorophyll with the electrodes. The spacer adjusted an optical length of the cell of 0.3 mm.

Chloroplasts and media

Chloroplasts were prepared from peas (*Pisum sativum*) grown under artificial illumination. The young seedlings were ground in a medium containing 400 mM sorbitol/10 mM NaCl/4 g per l bovine serum albumin/50 mM Mes (pH 6.5). The pulp was squeezed through two layers of nylon tissue and then centrifuged for 12 min at 10000 × *g*. The supernatant was discarded and the pellet was homogenized with a medium containing 100 mM sorbitol/25 mM NaCl/20 mM tricine (pH 7.5), diluted with ethylene glycol (30%, v/v). The chloroplasts were freeze-thawed before use. The chlorophyll concentration was adjusted to 1.2 mM with the freezing medium. This concentration yielded an optical density on the order of 0.5 in the measuring cuvette at the two wavelengths tested. The experiments were carried out at room temperature.

Numerical evaluation of the data

Signal shapes at the maximal time resolution of the apparatus were Gaussian like in the case of a δ-function input signal (obtained from a 100 ps photodiode and a 30 ps laser flash), and error function like in the case of a 70 ps step function input signal (obtained from a pulse generator, Tektronix, model 284). This was shown by comparing the measured curves with the corresponding calculated curves. The deviation of shape between measured and calculated rising phases lay within the resolution of the detection system. Under this condition the 10–90% rise-times of processes with different rising phases, τ_a and τ_r, (of the Gaussian type) add geometrically [23]:

$$\tau^2 = \tau_a^2 + \tau_r^2 \qquad (1)$$

For the calculation of reaction time constants, τ_r, which lie near the time resolution of the apparatus, the exact rise-time τ_a of the apparatus itself at the final recording level is needed. The rise-time was determined in two ways. Firstly, it was calculated from the limiting frequencies of the electronic components, f_1, involved, using the relation [23]:

$$\tau_{10-90\%} = \frac{0.35}{f_1} \qquad (2)$$

The two preamplifiers of 3.15 GHz (corresponding rise-time 111 ps) and one 1 GHz oscilloscope (corresponding rise-time, 350 ps) yield a calculated rise time of:

$$\tau_a\,(\text{calculated}) = \sqrt{111^2 + 111^2 + 350^2} = 384\ \text{ps} \qquad (3)$$

Secondly, the rise-time was measured by giving a step function (rise-time of 70 ps) to the input of the cascaded amplfiers and recording the response function. The thus determined 10–90% rise-time was $\tau_a = 360 \pm 10$ ps, when the 10–90% values of 12 single-shot experiments were analyzed. If this rise-time is calculated analogously to Eqn. 3 and the 70 ps rise-time of the pulse generator is taken into account, a τ_a value of 390 ps resulted. The faster rise measured can be attributed to a small overshoot (ringing) present in this control experiment (Fig. 2 in Ref. 22).

In order to determine the rise-time of the apparatus under conditions approximating the real photoelectric experiments as close as possible, a decaying phase was simulated by connecting the step function generator via a capacitor to the amplifiers (see Fig. 2a and Results and Discussion). If the 10–90% rise-time were read from this curve, taking the maximum of the curve as 100%, $\tau_a = 370 \pm 10$ ps resulted (12 single-shot experiments). This number is used later as reference to estimate the limits of the photosynthetic reaction times by means of Eqn. 1.

Results and Discussion

Electrical test

The maximal rise-time of the apparatus was determined by substituting the capacitative measuring cuvette by a step-function generator whose output was connected by a 50 pF capacitor to the 50 Ω input of the preamplifiers. The voltage step was attenuated to 1.2 mV in order to allow for the same amplification as used for the experiments with chloroplasts. Photovoltages from chloroplasts were of this order of magnitude. The coupling capacitor was introduced to simulate the source capacitance (capacitance of the measuring cuvette) of the photovoltage. In connection with the 50 Ω input impedance of the first preamplifier, the coupling capacitor formed an *RC* high-pass element causing a decay of the step-function signal with a time constant of 2.5 ns. This decay influenced slightly the shape of the upper part of the rising phase when compared to the step-function transmitted without the coupling capacitor (not shonw). The 10–90% rise-time of such control signals (Fig. 2a) as described in Materials and Methods was 370 ± 10 ps.

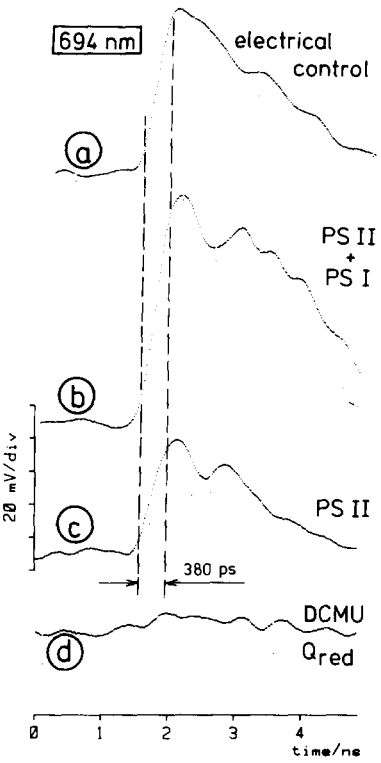

Fig. 2. Electrical control signal and photovoltages from pea chloroplasts evoked by 30 ps flashes from a mode locked ruby laser at 694 nm (energy: 600 ± 100 μJ/cm^2). (a) Simulation of the photovoltage signals by a step function generator connected by a series capacitor to the 50 Ω-preamplifier. The capacitor served to mimic the decaying phase. The trace is the mean of 5 single shots. (b) Photovoltage from chloroplasts with 500 μM phenazine methosulphate and 4 mM ascorbate as acceptor. The trace is the average of 3 single shots. (c) Photovoltage from chloroplasts with 20 mM ferricyanide to oxidize P-700. Single-shot experiment. (d) Photovoltage from chloroplasts with 20 mM ferricyanide and 200 μM DCMU to reduce Q_A of Photosystem II. The trace is the average of 3 single shots. The chlorophyll concentration of the chloroplasts was 1.3 mM. In all three cases the chloroplasts were preilluminated for 3 s with white light of 3 mW/cm^2. The picosecond flashes were given 100 ms after the end of the preillumination.

Excitation of chloroplasts at 694 nm

The following experiments were carried out with freeze-thawed pea chloroplasts under isoosmolaric conditions. In all experiments the chloroplasts were preilluminated with white light of an intensity of 3 mW/cm^2 for 3 s. Single picosecond flashes were given 100 ms after switching off the preillumination.

Excitation of the chloroplasts with 30 ps flashes of a wavelength of 694 nm under the above conditions evoked a photovoltage with a fast rising phase and a slow decaying phase (Fig. 2b). The energy of a single flash was on the order of 600 $\mu J/cm^2$. At this energy the amplitude of the photovoltage is only slightly dependent on variations of the energy [20]. The signal shape was very similar to that of the electrical test (compare traces a and b of Fig. 2). The 2.5 ns decay of the photovoltage can be ascribed to the *RC*-discharge of the capacitance of the measuring cuvette through the load resistance of the 50 Ω input resistance of the preamplifier [22].

This interpretation is supported by two further experiments. In one experiment a custom-built impedance converter (input resistance, 18 kΩ; gain, 1; limiting frequency, approx. 5 GHz) was interconnected between the cuvette and the first amplifier. As seen in Fig. 3 the decay was slowed down. However, in this case the impedance of the measuring cell is much smaller than the impedance of the converter. This mismatching causes the high-frequency ringing seen in Fig. 3. In the other experiment the measuring cuvette of smaller capacitance (mica plates as insulating material instead of the thinner teflon film) was combined with the 50 Ω amplifier. Here, the decay was accelerated to about 1 ns (curve not shown).

These experiments with preamplifiers of different input impedance and cells of different capacitances show that the decay of the photovoltage can be attributed to an electronic *RC*-decay, where *R* is the input resistance of the first amplifier and *C* is the capacitance of the measuring cell. The same explanation was given in Ref. 22 for a differently designed capacitative metal cuvette suited for the orientation of purple membranes.

A remarkable feature of the capacitative measuring cuvette used for the present experiments is the observation of a photovoltage despite an electric insulation of the metal electrodes from the photoelectrically active medium. Obviously, no ohmic contact is needed to transmit the polarization change of the chloroplast suspension to the metal plates. This can be understood by considering a capacitor with several layers of different dielectrics from which one changes its polarization. The potential at the two surfaces of this subdielectric is the same at the metal plates positioned at some distance, because the other dielectric layers remain equipotential. The time resolution of such a capacitor stack is expected to be unprecedentedly fast, since the field lines of the dielectric polarization of the reaction medium reaches the metal plates with the velocity of electromagnetic radiation. Therefore, such types of measuring cuvettes may not possess an intrinsic high-frequency limitation (compare Ref. 22).

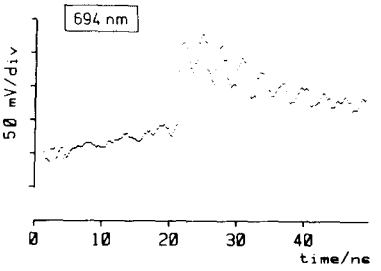

Fig. 3. Photovoltage from pea chloroplasts in the 10 ns time range recorded with high impedance. Excitation by 30 ps flashes at 694 nm. Between the measuring cuvette and the first amplifier an impedance converter with an input resistance of 18 kΩ was introduced. The waves seen are ringing phenomena due to an unmatched impedance between the measuring cell and the impedance converter.

The rise-times of different single-shot photovoltages from chloroplasts with both photosystems operating were evaluated as described in Materials and Methods. The mean value of the exponential time constant from ten experiments was $\tau = 380 \pm 10$ ps. This time constant is not very different from the one found for the electrical test pulses. The reaction time constant of the charge separations in the reaction centers of PS I and PS II, τ_r, can be estimated by Eqn. 1 taking into account the mean deviation of the rise times:

$$\tau_r(\text{PS I} + \text{PS II}) = \sqrt{390^2 + 360^2} \approx 150 \text{ ps}$$

In order to test the rise-time of PS II alone, the traps of PS I were closed by oxidation of P-700 with ferricyanide. This resulted in a photovoltage of about half the amplitude but the same fast rise. The halved amplitude, in comparison to chloroplasts with both photosystems operating, demonstrates that PS I and PS II contribute about 50% each to the generation of the membrane potential also in the subnanosecond range. This result agrees with similar measurements at lower time resolution [13] and with spectroscopic measurements [24,25]. However, it is in contrast to other measurements made with microelectrodes in the millisecond range [26,27].

Analyzing the rising phase according to the above procedure yielded again a mean rise time of 380 ± 10 ps. Therefore, the 10–90% rise-time of the charge separation in reaction centers of PS II is also shorter than or equal to 150 ps. The result also means that the energy transfer from the antenna pigments to the traps is as fast or faster.

When the acceptor site of PS II was reduced (DMCU and 3 s preillumination) and P-700 was oxidized (ferricyanide), there was almost no photovoltage (Fig. 2d). The small signal left is likely due to an incomplete oxidation of P-700 [28] (Förster, V., unpublished results). Under these conditions a primary charge separation between P-680 and the intermediary pheophytin acceptor Phe is expected to occur as the only photosynthetic reaction [29,30] (for review, see Ref. 31). This state recombines within approx. 4 ns:

$$\begin{array}{l} \text{P*-680 pheophytin } Q_A^- \longrightarrow \text{P}^+\text{-680 pheophytin}^- \; Q_A^- \\ \qquad\qquad\qquad\qquad \swarrow 4\text{ ns} \\ \text{P-680 pheophytin } Q_A^- \end{array} \qquad (4)$$

The lack of a significant photovoltage shows that the corresponding charge separation contributes only very little to the transmembrane potential. Within the scope of this reaction scheme, the result indicates that Phe is located on the same membrane side as P-680.

This conclusion is in agreement with recent literature. Meiburg et al. [32], who studied the recombination fluorescence induced by electrical field pulses in osmotically swollen chloroplasts, came to the same conclusion. They found that the electron transfer between pheophytin and Q_A spans 90% of the potential difference across the membrane. From EPR studies, Rutherford and Thurnauer [33] concluded that the distance between the radicals of the pair D^+P-680 pheophytin$^-$ is 0.6–0.8 nm, which implies the near neighbourhood of P-680 and pheophytin.

Excitation of chloroplasts at 532 nm

The excitation in the foregoing experiments was done at a wavelength of 694 nm which is rather at the red end of the π-π^+ absorption band of chlorophyll. Since the core antenna pigments of PS I and PS II are red-shifted compared to the bulk antenna pigments, a preferential excitation of core pigments prevails. Energy transfer from the core pigments to the traps might possibly be faster than energy transfer from the light-harvesting complex (LHC). Therefore, the fast rise-time found with excitation at 694 nm does not yield an estimation for the trapping time from a statistical absorption over the whole antenna system. In order to get this information, the above experiments were repeated at the excitation wavelength of 532 nm where the antenna pigments are almost equally excited.

Fig. 4 shows the photovoltage from pea chloroplasts again under preillumination condition. The amplitude and the shape of the photovoltage were

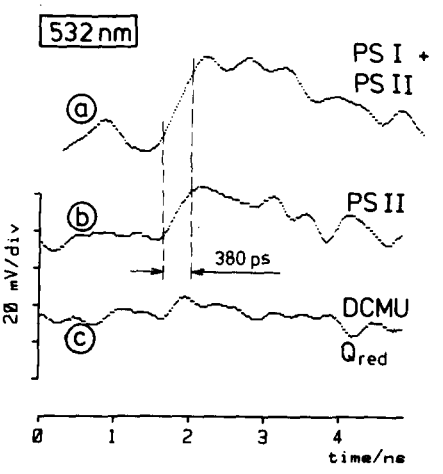

Fig. 4. Photovoltages from pea chloroplasts evoked by 30 ps flashes from a mode locked and frequency doubled Nd-Yag laser at 532 nm (energy: 30 ± 10 μJ/cm^2). All traces represent single shot experiments. (a) Chloroplasts with 500 μM phenazine methosulphate and 4 mM ascorbate; (b) chloroplasts with 20 mM ferricyanide to oxidize P-700; (c) chloroplasts with 20 mM ferricyanide and 200 μM DCMU.

comparable with and without preillumination. Since the maximal available excitation energy was only on the order of 30 $\mu J/cm^2$ at this wavelength, the signals at 532 nm were smaller than the ones obtained at 694 nm.

As seen in Fig. 4 the photovoltage from chloroplasts with both photosystems active was about twice as large as that from chloroplasts with only the reaction centers of PS II active. This shows that also at this excitation wavelength, both photosystems contribute about 50% to the electrogenicity in this early time domain. When P-700 was oxidized by ferricyanide and the quinone acceptor Q_A was reduced by DMCU, the photovoltage was very small (Fig. 4). Based on the reaction scheme of Eqn. 4, this result shows again that the intermediary pheophytin acceptor lies on the same membrane side as P-680. This result is different to the one found for the reaction center of purple bacteria where the intermediary acceptor bacteriopheophytin is located close to the middle between the primary donor P and Q_A [15].

We ascribe the remaining small photovoltage seen in Fig. 4c as due to a small fraction of reaction centers of PS I which are not oxidized [28] (Förster, V., unpublished results). This interpretation is also supported by the same kinetics of the residual photovoltage when the signal-to-noise ratio was improved by averaging 16 traces (data not shown). If the photovoltage were to be due to a 2 or 4 ns back reaction from Phe, the decay kinetics should be accelerated.

The rise-times of the photovoltage with both photosystems active or with only Photosystem II active showed the minimal rise-time of the instrumentation $\tau = 380 \pm 20$ ps. Because the signal amplitudes at 532 nm excitation were smaller, the accuracy for the determination of rise-times was less than at 694 nm excitation. Therefore, at 532 nm, the trapping of excitons from the whole antenna system by the reaction centers of PS I and PS II as well as the primary charge separation can be estimated to be faster than about 175 ps, according to:

$$\tau_r(\text{PS I} + \text{PS II}) \approx \sqrt{400^2 - 360^2} \approx 175 \text{ ps}$$

The rise-time of the photovoltage could not be time resolved in the present experiments. The two estimates of the mean trapping time of 150 and 175 ps given here represent an upper limit for the rate of the primary photosynthetic charge separation under conditions of a fully connected antenna system. The data allow the conclusion that in chloroplasts, the mean trapping time as well as the time needed to complete the transmembrane charge separation is faster than the given numbers. The primary charge separation seems to be substantially completed within this short time, since the amplitudes of the photovoltage observed at 400 ps (Fig. 2b) and in the range of 1–10 ns (Fig. 3) were comparable. This indicates that except for the primary charge separation, no further electrogenic reaction takes place in this time range.

The shape of the photovoltage signals at both excitation wavelengths showed a fast rise and a slower decay which is consistent with a dielectric polarization of the chloroplast suspension in a manner of a step function. The fast rise is determined by the limiting frequency of the detection electronics, whereas the decay is determined by the *RC*-decay of the cell capacitance and the load resistance. At the given signal-to-noise ratio (approx. 5 : 1) there was no evidence for an intermediate rising phase of the photovoltage in the time range of 400 ps to 4 ns which could correspond to a 500 ps phase found in fluorescence decay experiments [6,7].

Improvements of the measuring cuvette are conceivable which may lead to higher cell capacitances as to record photovoltages with a shape closer to a step function. This would give the chance for a more accurate examination of the early electrogenic steps in photosynthesis.

Photoelectric versus fluorescence measurements

Much of our present knowledge on the energy transfer from the antenna system to the traps of the two photosystems stems from the analysis of fluorescence decay kinetics [6,7,34–37]. Single-photon timing with picosecond resolution showed a fluorescence decay which is governed by essentially three components: 100, 500 and 1100 ps. In a recent work on gree algae, Haehnel et al. [34] found a strong decrease of the yield of the fast component when the reaction centers of PS II were progressively closed. This indicates that the fast component represents the mean trapping time

for open PS II reaction centers. The estimation of this time by the present photoelectric measurements is in agreement with the fluorescence data since a trapping time shorter than 150 ps is below our present time resolution.

The middle fluorescence component contributes with a high yield to the total fluorescence and is only slightly influenced by the redox state of PS II reaction centers. It is tentatively ascribed to the energy transfer from light-harvesting Chl *a/b* antennae to Chl *a* antennae. The exact meaning of the middle component is not yet understood. We conclude from the photoelectric experiments where no 500 ps component could be detected that such a component contributes less than about 20% to the total trapping efficiency.

Based on the decrease of the fluorescence yield of the fast component with increasing concentration of the state P-680 pheophytin Q_A^-, the widely accepted reaction written in Eqn. 4 was questioned [34]. It was argued that if the state P-680 pheophytin Q_A^- would be able to trap excitons and to hold the energy for some nanoseconds (the proposed life time of the state P-680$^+$ pheophytin$^-$ Q_A^-), then the 100 ps decay component should be independent of the redox state of the quinone acceptor. This was experimentally not found. However, the argument would fail if a non-trapping state of PS II reaction center would accumulate under the repetitive excitation (82 kHz) of the fluorescence measurements. Note, that the photoelectric data in this study resulted from no more than 2 shots per min. It would also fail if the primary photosynthetic reaction produces a quenching substance [38].

In contrast to fluorescence measurements, photoelectric measurements do not respond to quenching processes other than the photosynthetic charge separation. Herein lies the potential power of the photoelectrical methods, especially if the time resolution can be increased which should be possible simply by using faster detection electronics. The fundamental difference between both methods can be summarized by the following: whereas the time-resolved fluorescence technique probes all excitons that are not quenched, the photoelectric technique probes only those excitons that are successfully utilized for photosynthesis.

It may be worth mentioning, that the localization of the intermediary acceptor Phe given above depends totally upon the charge separation and backreaction concept of Eqn. 4. If this reaction scheme would be doubted, our conclusion on the localization of Phe could not be maintained. Rather, it would support the doubts raised by Haehnel et al. [34].

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References

1 Rueppel, H. and Witt, H.T. (1969) Methods Enzymol. 16, 316–379
2 Clayton, R.K. (1970) in Light and Living Matter. The Physical Part, Vol. I, McGraw-Hill, New York
3 Witt, H.T. (1971) Q. Rev. Biophys. 4, 365–477
4 Junge, W. (1976) in Chemistry and Biochemistry of Plant Pigments, Vol. 2, pp. 233–333 (Goodwin, T.W., ed.) Academic Press, London, New York
5 Govindjee and Jursinic, P.A. (1979) Photochem. Photobiol. Rev. 4, 125–205
6 Haehnel, W., Nairn, J.A., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 680, 161–173
7 Nairn, J.A., Haehnel, W., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 682, 420–429
8 Deprez, J., Dobek, A., Geacintov, N.E. and Breton, J. (1983) Biochim. Biophys. Acta 725, 444–454
9 Netzel, T.L., Rentzepis, P.M. and Leigh, J.S. (1973) Science 182, 238–341
10 Kaufman, K.J., Dutton, P.L., Netzel, T.L., Leigh, J.S. and Rentzepis, P.M. (1975) Science 188, 1301–1304
11 Rockley, M.G., Windsor, M.W., Cogdell, J.R. and Parson, W.W. (1975) Proc. Natl. Acad. Sci. USA 72, 2251–2255
12 Trissl, H.-W. (1980) Biochim. Biophys. Acta 595, 82–95
13 Trissl, H.-W. and Graeber, P. (1980) Biochim. Biophys. Acta 595, 86–108
14 Trissl, H.-W. (1983) Biochim. Biophys. Acta 723, 327–331
15 Trissl, H.-W. (1983) Proc. Natl. Acad. Sci. USA 80, 7173–7177
16 Witt, H.T. and Zickler, A. (1973) FEBS Lett. 37, 307–310
17 Fowler, C.F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 308–310
18 Becker, J.F., Geacintov, N.E. and Swenberg, C.E. (1978) Biochim. Biophys. Acta 503, 545–554
19 Graeber, P. and Trissl, H.-W. (1981) FEBS Lett. 123, 95–99
20 Trissl, H.-W., Kunze, U. and Junge, W. (1982) Biochim. Biophys. Acta 682, 364–377

21 Trissl, H.-W., Der, A., Ormos, P. and Keszthelyi, L. (1984) Biochim. Biophys. Acta 765, 288–294
22 Trissl, H.-W. (1985) Biochim. Biophys. Acta 806, 124–135
23 Lipinski, K. (1978) Das Oszilloskop. Funktion und Anwendung, p. 28, VDE-Verlag GmbH, Berlin
24 Schliephake, W., Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 1571–1578
25 Renger, G. and Wolff, C. (1975) Z. Naturforsch. 30c, 161–171
27 Kulandaivelu, G. and Senger, H. (1976) Biochim. Biophys. Acta 430, 94–104
27 Satoh, K. and Katoh, S. (1977) Plant Cell Physiol. 18, 1077–1087
28 Hong, Y.-Q., Förster, V. and Junge, W. (1981) FEBS Lett. 132, 247–251
29 Klimov, V.V., Klevanik, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186
30 Jursinic, P. and Govindjee (1977) Biochim. Biophys. Acta 461, 253–267
31 Vermaas, W.F.J. and Govindjee (1981) Photochem. Photobiol. 34, 775–793
32 Meiburg, R.F., Van Gorkom, H.J. and Van Dorssen, R.J. (1983) Biochim. Biophys. Acta 724, 352–358
33 Rutherford, A.W. and Thurnauer, M.C. (1982) Proc. Natl. Acad. Sci. USA 79, 7283–7287
34 Haehnel, W., Holzwarth, A.R. and Wendler, J. (1983) Photochem. Photobiol. 37, 435–443
35 Breton, J. and Geacintov, N.E. (1980) Biochim. Biophys. Acta 594, 1–32
36 Bose, S. (1982) Photochem. Photobiol. 36, 725–731
37 Karukstis, K.K. and Sauer, K. (1983) J. Cell. Biochem. 23, 131–158
38 Shuvalov, V.A., Klimov, V.V., Dolan, E., Parsan, W.W. and Ke, B. (1980) FEBS Lett. 118, 279–282