Biochimica et Biophysica Acta 893 (1987) 305–319
Elsevier

BBA 42598

Primary electrogenic reactions of Photosystem II as probed by the light-gradient method

H.-W. Trissl [a], J. Breton [b], J. Deprez [b] and W. Leibl [a]

[a] *Universität Osnabrück, Fachbereich Biologie / Chemie, Schwerpunkt Biophysik, Osnabrück (F.R.G.)* and [b] *Service de Biophysique, Centre d'Etudes Nucléaires de Saclay, Département Biologie, Gif-Sur-Yvette (France)*

(Received 30 March 1987)
(Revised manuscript received 16 June 1987)

Key words: Photovoltage; Photosynthesis; Photosystem II; Trapping; Antenna organization; Charge separation

The amplitude and the kinetics of the fast photovoltage from pea chloroplasts, which arise from the light-gradient effect, were reexamined with respect to the relative contributions of the two photosystems. Chloroplasts prepared under stacking conditions displayed no photovoltage due to Photosystem II. The photovoltage observed originated exclusively from Photosystem I. This conclusion was drawn from various experimental assays that have been chosen to separate the two photosystems: (i) reduction of the quinone acceptor, Q_A, of Photosystem II either by 3-(3,4-dichlorophenyl)-1,1-dimethylurea and preillumination or by dithionite; (ii) kinetic analysis of the fast photovoltage elicited by 30-ps flashes; (iii) oxidation of Photosystem I by ferricyanide and continuous far-red light; (iv) the effect of the artificial quencher of the excited state, dinitrobenzene; and (v) the use of Photosystem-I-deficient mutants of the alga *Chlamydomonas reinhardtii* and barley chloroplasts. We ascribe the lack of electrogenicity of Photosystem II to a delocalization of the excitation energy within the grana stacks, such that the anisotropy of the light-gradient is equilibrated before trapping takes place ('excitonic short-circuit' of the light-gradient effect). This conclusion is substantiated by the observation that chloroplasts prepared under destacking conditions ('blebs' or EDTA-vesicles) did display a photovoltage due to Photosystem II. In this case we could show (i) that the primary charge separation proceeds in two kinetically distinct steps, the first occurring in less than 50 ps and the second in 300–450 ps; (ii) that the charge separation between the primary donor, P-680, and the intermediary acceptor, pheophytin, accounts for approx. 1/2 to 2/3 of the total charge separation; (iii) that the trapping kinetics is on the order of 200 ps at 50 $\mu J / cm^2$; (iv) that no charge separation occurs when Q_A is reduced; and (v) that there is no electrogenicity connected with the rereduction of P-680 by the water-splitting enzyme.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNB, dinitrobenzene; PS I, Photosystem I of green plants; PS II, Photosystem II of green plants; P-700, primary donor of PS I; A, primary acceptor(s) of PS I; P-680, primary donor of PS II; Pheo, intermediary pheophytin acceptor of PS II; Q_A, first quinone acceptor of PS II; RC, reaction center.

Correspondence: H.-W. Trissl, Universität Osnabrück, Fachbereich Biologie/Chemie, Schwerpunkt Biophysik, Barbarastraße 11, D-4500 Osnabrück, F.R.G.

Introduction

Current literature on the light-gradient photovoltage from chloroplasts consistently reports a nearly equal contribution of the two photosystems to the photovoltage [1–5]. This finding is in approximate agreement with the reported stoichiometric ratios of PS I/PS II of 1 : 1 as, for instance,

established by measurements of the field-indicating electrochromic bandshift at 515 nm [6] or by other methods [7–9]. However, also lower values than 1 have been reported, and the ratio may vary considerable in different plants and different stages of development [10–12].

In the early light-gradient photovoltage measurements, technical reasons necessitated suspension media of low ionic conductance [1–4]. In some cases distilled water or 2 M sucrose were used [2,4]. In hypo-osmolar media the stacked thylakoid membranes convert into large vesicles [13], called 'blebs' [14], whereas in hyper-osmolar media the overall shape of the chloroplasts is maintained, but the exact structure of the thylakoid membranes is not known, particularly the degree of stacking of the grana membranes. In none of the studies the stacking of the thylakoid membranes was controlled by defined concentrations of divalent ions (e.g., Mg^{2+}). However, one might suspect that the photovoltage may be affected by the structure of the membranous system under investigation.

Methodological developments of the light-gradient method allow now the measurement of the photovoltage under any ionic condition in the time range below 50 ns [15]. This suggests a reinvestigation of the influence of the structure of the thylakoid membranes on the photovoltage as well as of the contributions of the two photosystems. Surprisingly, when we worked with chloroplasts under stacking conditions, we found no electric activity of PS II. Since the light-gradient itself is a physical phenomenon being necessarily present, the reason for this lack of electrogenicity has to be searched in the membrane structure, the only parameter different between this and the former experiments.

It is known that under stacking conditions (the presence of Mg^{2+}-ions) the two photosystems are laterally separated: PS II is located almost exclusively in the appressed membranes of the grana stacks and PS I mainly in the stroma lamellae [16,9,17]. Under destacking conditions, however, the two photosystems mix and distribute over the interconnected thylakoid membranes [18,19]. Hence, the light-gradient photovoltage from the two photosystems originates from different structures under stacking conditions, and from alike structures under destacking conditions (especially in the case of the small vesicles obtained after EDTA incubation).

A further factor that may be intimately related to the generating mechanism of the light-gradient photovoltage, is the extent of connectivity of the antenna system of PS II. The antenna organization of PS II can be described by a lake model, i.e., the excitation energy absorbed in one photosynthetic unit may visit several other units (more than five) before it is trapped (for a review, see Ref. 20). Whether the energy migration occurs only in the planes of the membranes (two-dimensional diffusion) or whether it occurs also perpendicular between appressed membranes (three-dimensional diffusion) is not known. Thus, it is conceivable to assume that the optical asymmetry of the light-gradient, which is necessarily present in the first few picoseconds after the absorption of a photon, might equilibrate perpendicular to the membranes in a grana stack, so that no electrical asymmetry is left at the time the photochemical energy conversion occurs. This would lead to a lack of electrogenicity in the light-gradient experiment.

In the present study we reinvestigate with capacitative metal electrodes and with short laser flashes the ability of the two photosystems to generate a fast photovoltage from pea chloroplasts. The experiments aim at isolating the PS II signal and at measuring the trapping kinetics, as well as the electrogenicity of the various primary reactions of PS II, particularly the possible electrogenicity connected with the reaction

$$\text{P-680}^{*} \cdot \text{Phe} \cdot Q_A^- \rightleftarrows \text{P-680}^{+} \cdot \text{Pheo}^{-} \cdot Q_A^-$$

when all antenna pigments are present (known as Klimov's backreaction [21]).

According to a recent report there was no electrogenicity connected with PS II when Q_A was reduced (closed RCs) [5]. This leaves open two explanations: either the yield of this reaction is very low because the equilibrium is shifted towards the excited state [22–24,34], or both Pheo and P-680 lie at the same level in the membrane. The only chance to distinguish by means of the photoelectric method between the two possibilities arises if (in open RCs) the formation of the two

states ($P\text{-}680^+ \cdot Pheo^- \cdot Q_A$) and ($P\text{-}680^+ \cdot Pheo \cdot Q_A^-$) is electrogenic.

The characterization by the light-gradient photovoltage of the primary processes of trapping and charge separation due to PS I has been described in a preceding article [25].

Materials and Methods

Experimental. Chloroplasts were prepared as described [26]. Destacked chloroplasts were prepared by omitting $MgCl_2$ in all media. Blebs were prepared by suspending stacked chloroplasts in distilled water (100-fold dilution) at 4° C for more than 1 h. EDTA-treated thylakoids (EDTA-vesicles) were obtained by incubating stacked chloroplasts in 100 μM EDTA, 1 mM NaCl, 1 mM Tricine buffer (pH 7.8) at a chlorophyll concentration of 10–20 μM. After 10 min the NaCl concentration was adjusted to 30 mM. After the suspension step both preparations were concentrated by centrifugation. For most experiments the chlorophyll concentration was adjusted to 3.5 mM. Chloroplasts from the barley mutant *viridis-zb*^{63} were prepared analogously. In the following the blebs and the EDTA-vesicles are subsumed under 'destacking conditions'.

The equipment for the photovoltage measurements was the same as described in Ref. 25. Either a 1-GHz or a 7-GHz oscilloscope was used. The distance between the electrodes, i.e., the optical path length, was 0.1 mm.

The excitation source was a frequency-doubled Nd-YAG laser in the Q-switched mode, delivering flashes of 12-ns duration at 532 nm. To achieve 30-ps flashes the laser could be operated under mode-locking conditions [25].

Fluorescence induction curves were measured by d.c. light from a slide projector equipped with a wide band interference filter (WB 550, Ditric Optics, Inc.). The excitation light was adjusted with neutral density filters to 2 mW/cm^2. It extended from 530 to 570 nm. This wavelength regime falls into the absorption minimum of chlorophyll and makes sure that all chloroplasts of the concentrated suspension in the 0.1 mm cuvette were equally exposed to the excitation light. Fluorescence emission was measured at wavelengths longer than 665 nm with a large area photodiode (Oriel, OSD 300). The geometry of this set-up corresponded to the geometry of the light-gradient set-up. The maximum fluorescence level, F_{max}, was reached after approx. 100 ms.

The preillumination conditions used in this study were: 200 ms light as specified above followed by a 200 ms dark period before sending the test flash. After this dark period the fluorescence level was shown to be still F_{max} in the presence of DCMU and ascorbate. After the preillumination and the dark period, the F_0 level in the absence of DCMU but ascorbate present was 20% higher than F_0 without ascorbate after 5 min dark adaptation. By absorption flash spectroscopy it was shown that in the presence of DCMU and 10 mM ascorbate, P-700 was fully rereduced after the dark period.

Kinetic analysis of the photovoltage. The photovoltage in this study was inspected for either consecutive or parallel reactions, depending on the wanted information. The case of consecutive reactions was treated in Ref. 27. The case of parallel reactions, that are needed to account for the two photosystems of higher plants, is treated in Appendix I.

The kinetic analysis of the photovoltage was done by minimizing the residuals between experimental and computer-calculated curves. The parameters used for the calculated curves can be divided into two classes. One class accounts for invariant instrumental parameters, as are the rise time of the apparatus, τ, and the discharge time of the capacitative cell at 50-Ω impedance, τ_d. The other class comprises fit parameters that account for the kinetics of the charge separation, τ_i, and the electrogenicity, K or A.

The rise time of the apparatus was calibrated in flash experiments with purple membranes from *Halobacterium halobium* which were electrically preoriented in the coaxial cell. We assume that the primary charge separation is at least as fast as the formation of the K-intermediate (5 ps) and that this is the only electrogenic reaction in the time range studied [28–30]. The corresponding displacement current in the dielectric of the cell capacitor can then be taken as a δ-function test pulse that probes the response characteristics of the whole apparatus, including the laser flash (30 ps), the measuring cell, the preamplifiers and the

oscilloscope. We found that this photovoltage followed an error function

$$V(t) = V_0 \int_{t=-\infty}^{t} e^{-(t/\tau)^2} dt \quad (1)$$

with $\tau = 330$ ps in the case of the 1-GHz oscilloscope (ten averaged traces) and with $\tau = 80$ ps in the case of the 7-GHz oscilloscope (single shots).

The discharge of the capacitative electrodes via the 50-Ω input resistance of the preamplifier occurred with two exponential phases when millimolar concentrations of mono- and divalent ions were present. The fast decay phase had a τ_{d1} of 1.2 ns, which corresponded to the calculated RC time constant. The slower decay phase had a τ_{d2} of 30 ns. It is tentatively ascribed to electrode/electrolyte relaxations known from electrolyte capacitors [31]. The relative contribution of the slow phase depended on the salt composition of the medium and ranged typically from 0 to 15%. Accordingly, the decay (in the absence of backreactions) is described by the weighting function of the capacitative electrodes which integrate the displacement current:

$$C(t) = C_1 e^{-t/\tau_{d1}} + C_2 e^{-t/\tau_{d2}} \quad (2)$$

where C_1 and C_2 are proportionality constants.

The parameters used to fit a given experimental curve are due to the displacement current, given by Eqn. A-6 in Appendix I. These are the two rate constants k_1 and k_2 and the electrogenicity parameters A_1 and A_2.

Supposing a linear system, the photovoltage, $V(t)$, can then be calculated by the convolution of the displacement current, $I(t)$, with the weighting functions of the subsystems

$$V(t) = I(t) \otimes A(t) \otimes C(t) \quad (3)$$

where $A(t)$ is the weighting function of the whole apparatus which was shown to be a gaussian function with the time constant, τ (see Eqn. 1).

The following two examples shall illustrate how the signal-to-noise ratio determines the achievable time resolution at 1 GHz bandwidth. This can be done by plotting the deviations (residuals) (Fig. 1,

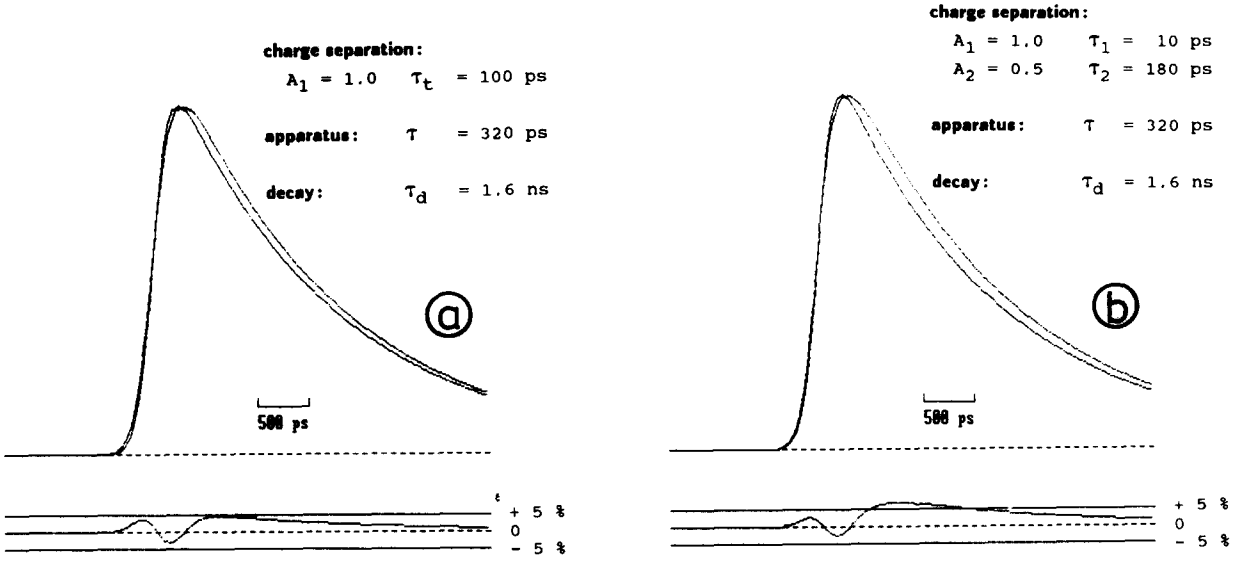

Fig. 1. Determination of the time resolution by the convolution procedure and the residual representation (lower part of the figures) at a given deviation of ±5%. The dashed line in the lower part indicates zero deviation. The rise time of the apparatus is assumed to be an error function with an invariant time constant of $\tau = 320$ ps. (a) Comparison of a calculated response characteristics of the apparatus for a delta-function displacement current (narrow curve) with that for an exponential displacement current, $i(t) = A_1 \exp(-t/\tau_1)$ (broader curve). (b) Comparison of a calculated response characteristics of the apparatus for a delta-function displacement current (narrow curve) with that for two exponential displacement, occurring as parallel reactions according to $i_1(t) = A_1 \exp(-t/\tau_1)$ and $i_2 = A_2 \exp(-t/\tau_2)$ (broader curve).

lower parts) between two computed curves with slightly different parameters. The difference between the parameters was increased until the deviation exceeded a given signal-to-noise ratio (typically 10 in most experiments).

In a first example it was attempted to find the 'worst' fit within ±5% deviations of a photovoltage arising from a displacement current $I(t) = \delta(t)$ with one arising from $I(t) = e^{-t/\tau_t}$ ($K_2 = 0$ in eqn. A-5). The 'worst' fit occurred at $\tau_t = 100$ ps (Fig. 1a), when at the same time a 'best' fit was tried by shifting the two curves on the time axis.

In a second example it was attempted to find the 'worst' fit of a photovoltage arising from a displacement current $i(t) = \delta(t)$ with one arising from two parallel reactions according to Eqn. A-5. Particularly, we chose a case where one reaction was extremely fast, $\tau_1 = 10$ ps, contributing with 2/3 ($A_1 = 67\%$) to the total electrogenicity, and the second reaction was slow (τ_2 = running parameter), contributing with 1/3 ($A_2 = 33\%$) to the total electrogenicity. The ±5% deviations occurred at $\tau_2 = 180$ ps (Fig. 1b).

In summary, a signal-to-noise ratio of 10 allows to deduce kinetics that are up to 3-times faster than the rise time of the apparatus.

Results

Separation of the contribution from Photosystem I and Photosystem II primary reactions

When studying primary electrogenic reactions of Photosystem II (PS II) by the light-gradient method in chloroplasts, one is confronted with the problem of a clear separation of the two photosystems on the level of the electrical signals. At high time resolution this is not a trivial problem, since in the picosecond and nanosecond time ranges several different reactions with unknown electrogenicity may take place [32–34], and different lines may be followed in achieving a separation of the contributions of the two photosystems to the electrogenicity. One possibility would be to eliminate PS I by chemical oxidation of the primary donor of PS I, P-700, in order to observe exclusively PS II. However, with chloroplasts this oxidation is difficult to achieve in a quantitative way [35]. Another possibility to oxidize P-700 would be to apply a saturating preflash a few microseconds prior to the non-saturating measuring flash. This, however, reduces the acceptor site of PS II. Still another possibility is to leave PS I active and to compare the photovoltage with oxidized and reduced acceptor site of PS II (by addition of DCMU and preillumination), in order to extract electrogenic PS II reactions by a differential analysis of the corresponding photovoltages. It would be also feasible to work with PS-I-deficient mutants. We used all of these possibilities to gain a consistent picture of the primary electrogenic reactions in PS II, since each of these procedures alone would allow only ambiguous interpretations.

Photovoltage from stacked chloroplasts in the nanosecond range

To achieve an invariant activity of PS I, 10 mM ascorbate was added to the suspension medium. When stacked chloroplasts were excited in a micro coaxial cell by 12-ns flashes at 532 nm and the photovoltage was measured with a high impedance amplifier (rise time, 1 ns) the signals were a step function rising with the kinetics of the flash (see Fig. 2 in Ref. 25). For consistency the experiment was carried out with a preillumination as described in Mat. and Meth. Under this condition both photosystems are active. The energy dependence of the photovoltage amplitude is shown in Fig. 2 (square symbols). The data could be fit (smooth line) by an exponential saturation law

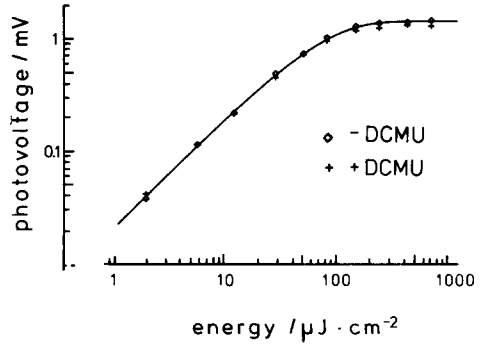

Fig. 2. Dependence of the peak photovoltage from stacked pea chloroplasts with and without DCMU as a function of the energy of 12-ns flashes. Experimental conditions: 10 mM ascorbate; preillumination. The data were fit by exponential functions according to Eqn. 4.

according to

$$V = V_0(1 - e^{-n\kappa\sigma I}) \quad (4)$$

where V_0 is the maximal photovoltage at high energy, n is the number of antenna pigments per trap, κ is the quantum yield for primary photochemistry, and I is the fluence (expressed in photons/cm^2 per pulse). If the quantum yield is taken to be $\kappa = 1$ and $\sigma = 1.6 \cdot 10^{-17}$ cm^2 [25,36], an antenna size of $n = 300$ follows.

The reduction of the first quinone acceptor of PS II, Q_A, by DCMU and preillumination had no effect on the photovoltage, neither on the kinetics (12–50 ns) nor on the amplitude (Fig. 2; crosses). The almost coinciding data without and with DCMU demonstrate that the PS I antenna size is unaffected by closing the PS II reaction centers.

Also the reduction of Q_A by dithionite had no effect on the photovoltage (data not shown). To establish that all reaction centers (RCs) of PS II were closed, we measured the fluorescence induction curve in parallel experiments and found F_{max} at the time the test flash was given (data not shown). The lack of a difference in amplitude and kinetics demonstrates the absence of electrogenicity of PS II in this time range, since the PS I activity was constant. A possible contribution of the photochemical reduction of the intermediary acceptor, Pheo, can be neglected in these experiments because its lifetime of less than 2 ns is short compared to the laser flash duration of 12 ns [21,23,24,33,34].

In further experiments the preillumination was omitted and no changes were observed. Also, when the chloroplasts with DCMU were dark-adapted for 5 min (which restores PS II activity) and then flashed, the photovoltage remained unchanged.

Photovoltage from stacked chloroplasts in the picosecond range

At high time resolution (bandwidth, 1 GHz) the photovoltage from stacked chloroplasts was evoked by 30 ps flashes at 532 nm and measured with 50-Ω impedance (Fig. 3a and b) in the absence and presence of DCMU. For consistency both experiments were carried out with preillumination.

As was the case for 12-ns flashes, the amplitudes without and with DCMU were equal within ±5%. This is indirect evidence that DCMU caused no loss of PS I activity, and further that there is no electrical activity connected with the reduction

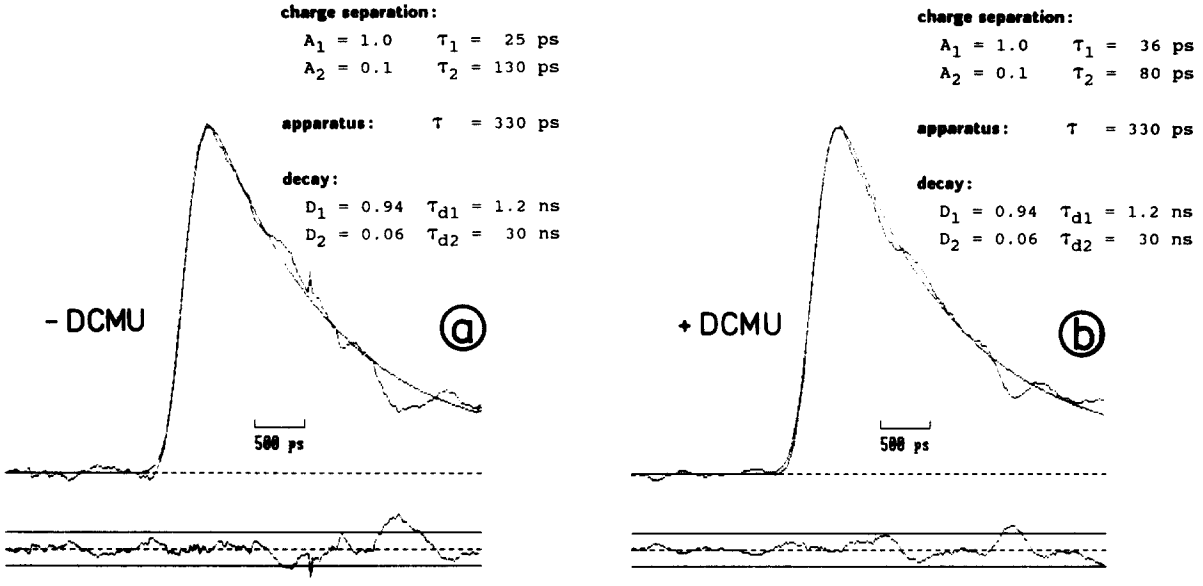

Fig. 3. Photovoltage evoked by 30 ps flashes of 100 μJ/cm^2 from stacked pea chloroplasts, recorded at a bandwidth of 1 GHz. (a) Without and (b) with DCMU (both experiments with preillumination). Number of averaged traces, $N = 10$. The residuals are the deviations of the experimental curve from a calculated one using the convolution parameters labeled in the figure.

of Pheo or Q_A, since in either case the amplitude would be expected to decrease in the presence of DCMU.

Both signals in Fig. 3 could be described with the same set of fit parameters, i.e., a step function convoluted with the rise time of the apparatus and the discharge characteristics of the capacitative cell [15,25]. The signal-to-noise ratio of the traces allowed to estimate that the charge separation occurs to the major part (more than 90%) with a single exponential kinetics of $\tau_1 < 50$ ps. When a second slower parallel reaction was assumed (e.g., $\tau_2 = 130$ ps in Fig. 3a), good fits could be attained only for a less than 10% contribution of such a phase. We conclude from these experiments that both trapping and charge stabilization are faster than 50 ps at the given excitation energy of 100 $\mu J/cm^2$. These are the typical characteristics of PS I [25]. Thus the kinetic analysis of the photovoltage in Fig. 3 delivers indirect evidence for a lack of PS-II electrogenicity, since the trapping kinetics in PS II is expected to be between 180 and 600 ps [37]. However, such slow components in the rising phase were never observed in stacked chloroplasts, even when lower excitation energies were chosen (down to 30 $\mu J/cm^2$; data not shown).

Quenching of the excitation energy by dinitrobenzene

Dinitrobenzene is known to quench excited states (i.e., fluorescence) in photosynthetic organellae [38,39]. The quenching can be described by Stern-Volmer plots. Since the fluorescence at the F_0-level stems predominantly from PS II [20,40], the quenching of F_0 by an artificial quencher probes this photosystem. In contrast, the photovoltage from stacked chloroplasts, if it arises from PS I, could be quenched differently by an artificial quencher, thus allowing for a possible distinction.

In Fig. 4 are plotted the reciprocal yields of F_0, and the reciprocal yields of the photovoltage from stacked pea chloroplasts in the absence and presence of DCMU (ascorbate and preillumination) as a function of the DNB concentration. The yields at a given concentration of the quencher were normalized to the yields where no quencher was present. Straight lines result that meet the ordinate at 1 (Appendix II). The slope of the quenching

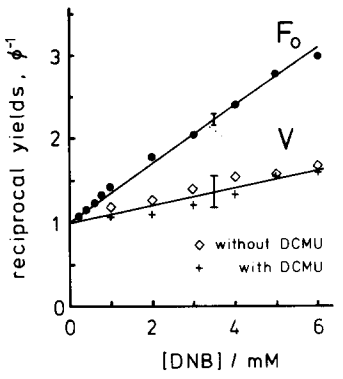

Fig. 4. Reciprocal yields of the fluorescence at F_0 and the photovoltage, V, as a function of the concentration of the fluorescence quencher DNB. The chloroplasts in these two experiments were stacked and the medium contained ascorbate. F_0 was measured with dark-adapted chloroplasts. The photovoltage was measured after a preillumination period in the absence and presence of DCMU. Excitation conditions of the photoelectric measurements: 12-ns flashes of 30 $\mu J/cm^2$.

line for fluorescence data was 3,5-times steeper than for the photoelectric data. Furthermore, the same quenching line for the photovoltage was found whether DCMU was present or not.

As derived in Appendix II, the difference between the slopes, $m = 0.35$ mM^{-1} for F_0 and $m = 0.10$ mM^{-1} for V, allows the conclusion that fluorescence and photovoltage originate from different antenna beds (see Discussion). The inefficiency of DCMU in affecting the photovoltage and the fact that straight lines were observed in the Stern-Volmer plots give strong evidence that PS II does not contribute to the photovoltage from stacked chloroplasts.

Photovoltage from Photosystem-I deficient mutants

The above experiments suggested that the photovoltage from stacked pea chloroplasts was entirely due to PS I. To support this finding further, we studied mutants that are deficient in PS I, like barley chloroplasts (*viridis-zb*^{63}) and broken cells from the alga *Chlamydomonas reinhardtii* (mutant strain M19). Thin-section electron micrographs of barley chloroplasts show an internal membrane structure similar to the one commonly known, displaying grana stacks and stroma lamellae [41].

In the presence of $MgCl_2$ (without and with artificial acceptors), neither of the two membrane preparations displayed ever a photovoltage in the

time range between 100 ps and 50 ns. The acceptors used were either ferricyanide, 2,6-dimethyl-*p*-benzoquinone or no additions.

Photovoltage from destacked chloroplasts

In contrast to the experiments with stacked pea chloroplasts (Fig. 2), there was a difference between the photovoltage amplitudes under destacking conditions in the absence or presence of DCMU (Fig. 5). These experiments were carried out with 12-ns flashes. When the acceptor site of PS II was reduced by DCMU and preillumination the photovoltage vs. energy curve was fit by a V_0 (see Eqn. 4) that was approx. 40% smaller than the one obtained when both photosystems were active, and also $n\sigma$ was 25% larger. We ascribe the difference to the electrogenic contribution of PS II, particularly to the charge separation between P-680 and Q_A. An explanation for the appearance of a PS-II photovoltage under destacking conditions will be given below.

To substantiate this PS-II photovoltage, we studied PS-I-deficient barley chloroplasts (*viridis-zb*^{63}) prepared as blebs. The photovoltage evoked by 30-ps flashes at an energy of 80 ± 15 $\mu J/cm^2$ recorded at 1 GHz bandwidth is shown in Fig. 6a. The trace displayed an obviously broader summit than the ones in Fig. 3. Good fits could be obtained only with two consecutive electrogenic reactions. The range of the parameters which allowed reasonable fits of this and other traces ($N = 8$) were: first reaction, $\tau_1 = 60$–190 ps and $A_1 = 1$ (normalized to one); second reaction, $\tau_2 =$ 300–450 ps and $A_2 = 0.3$–0.6. We ascribe tentatively the first phase as being due to the trapping kinetics at this energy as revealed by the initial P-680$^+$ · Pheo$^-$ charge separation, and the second phase as being due to the forward electron transfer from Pheo to Q_A (see following section and Discussion).

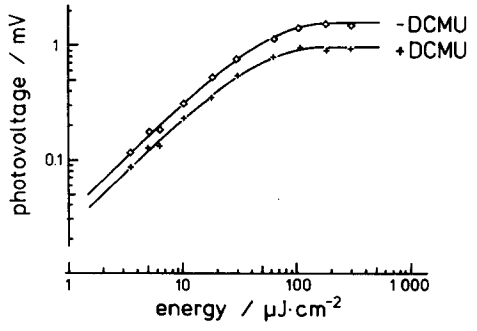

Fig. 5. Dependence of the peak photovoltage from EDTA-vesicles (pea) with (+) and without (△) DCMU as a function of the energy of a 12-ns flash. Experimental conditions: 10 mM ascorbate; preillumination. The data were fit by an exponential saturation law (see text).

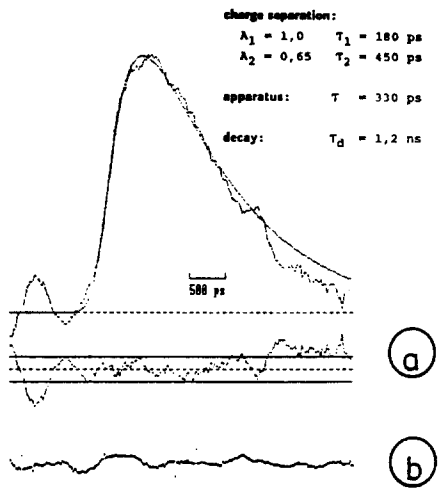

Fig. 6. (a) Upper trace: normalized photovoltage evoked by 30-ps flashes from blebs (PS-I-deficient barley mutant, *viridis zb*^{63}), recorded at a bandwidth of 1 GHz. Number of averaged traces, $N = 10$. Middle trace: residuals between the upper trace and a calculated fit using the convolution parameters labeled in the figure. Excitation energy: 80 ± 15 $\mu J/cm^2$. (b) The same blebs after addition of sodium dithionite (final concentration, 10 mM).

It might be conceivable that the slower kinetics observed for PS II could be due to spurious relaxations occurring only in media of low ionic strength. This possibility was excluded by control experiments in which we measured the PS-I photovoltage of destacked chloroplasts. These traces obtained at low ionic strength were indistinguishable from those obtained at high ionic strength [25].

Since this barley mutant has no detectable levels of P-700 chlorophyll *a*-protein 1 (CP1) [42], the signal in Fig. 6a can be taken as a pure PS-II photovoltage. When the quinone, Q_A, was reduced by addition of dithionite, the photovoltage disappeared (Fig. 6b). There was also no photovoltage when a saturating preflash from a Q-switched ruby laser was given 5–15 μs prior to the probe flash (data not shown). This indicates the absence of an electrogenic reaction in PS II with reduced

Q_A and fully connected antenna system in the time range between 300 ps and 5 ns (see Discussion).

Trapping time in Photosystem II

In Fig. 7a is shown at our highest time resolution the photovoltage from PS II in a destacked pea chloroplast preparation (EDTA-vesicles) elicited by 30-ps flashes of high energy (600 $\mu J/cm^2$). To shut down PS-I activity 2 mM ferricyanide was added and far-red background light (longer than 700 nm) was given. The trace could not be fit by a single exponential electrogenic reaction alone and its convolution with the response characteristics of the apparatus (Fig. 7a; faster decay). However, a satisfying fit was obtained when, in addition to a fast rise with $\tau_1 = 20 \pm 15$ ps ($A_1 = 1$), a second consecutive electrogenic reaction with $\tau_2 = 450$ ps and $A_2 = 0.8$ was assumed. The second rising phase would correspond to the second step in the forward charge separation in open PS II reaction centers

$$P\text{-}680^+ \cdot Phe^- Q_A \rightarrow P\text{-}680^+ \cdot Phe \cdot Q_A^-$$

The time constant for this reaction of 450 ps, which was used in the fit of Fig. 7, was taken from recent spectroscopic measurements of PS-II particles from *Synechococcus* sp. [23]. A time constant of 250–300 ps of PS-II particles from spinach has been reported by Nuijs et al. [43]. From the analysis of five analogous traces as in Fig. 7a ($N = 5$) obtained at energies between 300 and 1000 $\mu J/cm^2$, we can give the following estimates for the first reaction: $\tau_1 < 35$ ps with $A_1 = 1$ (normalized to one) and for the second reaction: $\tau_2 = 300$–450 ps with $A_2 = 0.6$–1.0.

The fast kinetics of the first step and the relatively slow kinetics of the second electrogenic step give the basis for an attempt to measure the trapping time in PS II in the low-energy limit. When the excitation energy was reduced to 50 $\mu J/cm^2$ (lower energies gave photovoltages with a too poor signal to noise ratio), the photovoltage rose significantly slower. The photovoltage rise shown in Fig. 7b was fit by an electrogenic reaction with a first phase of $\tau_1 < 180$ ps. (The second rising phase had very little influence on the quality of the fits.) Repetition of such single-shot traces yielded 160 ps $< \tau_1 <$ 220 ps.

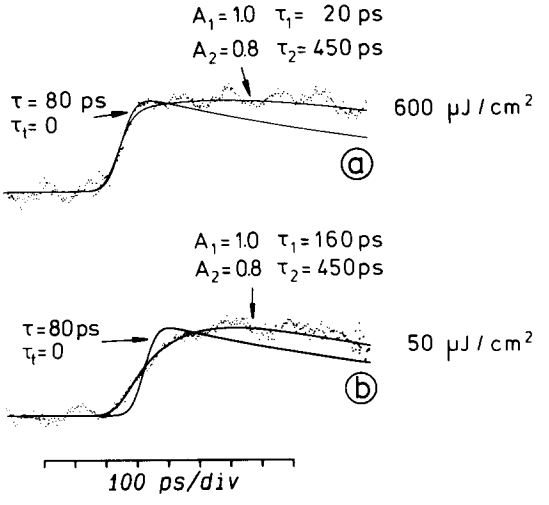

Fig. 7. Photovoltages evoked by 30-ps flashes of high (a) and low energy (b) from EDTA-vesicles (pea) recorded at a bandwidth of 7 GHz. The suspension medium contained 2 mM ferricyanide and far-red background light was given. The drawn lines represent computer curves resulting by convolution of charge displacements in a capacitor with assumed exponential kinetics.

Is the rereduction of P-680 electrogenic?

The reduction of $P680^+$ by Z, an electron donor located between the water-splitting enzyme and P-680, is known to occur in 20–30 ns [44,45]. To study the location of Z with respect to the membrane dielectric we measured at high impedance the photovoltage in this time domain.

The experiment was carried out with blebs from pea chloroplasts and 30-ps flashes. With both

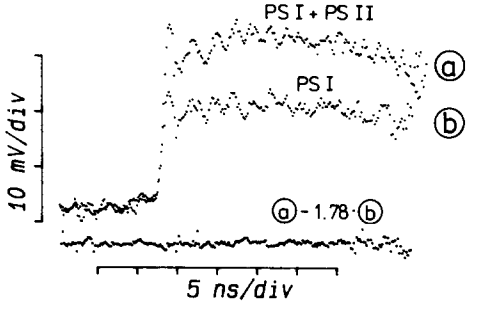

Fig. 8. Photovoltage in the 50 ns range evoked by 30-ps flashes from blebs (pea). The photovoltage was measured with a amplifier of high impedance to avoid the *RC*-decay in this time range. (a) With ascorbate only; (b) with ascorbate and DCMU; (c) difference between the above traces, normalized to each other by multiplication of (b) by a factor of 1.78.

photosystems active (absence of DCMU) the photovoltage was 1.8-times bigger than with only PS I active (presence of DCMU) (Fig. 8, upper and middle traces). Within the experimental error this amplitude ratio corresponds to the one found with 12-ns flashes (Fig. 5). It indicates a smaller electrogenicity arising from PS II than from PS I. The difference between the curves can be ascribed to PS II.

The ringing and the curvature seen after the steep rise are due to imperfect impedance matching between the coaxial cell and the high impedance amplifier. Nevertheless, one can see that the shape of the two traces are alike. To extract the wanted information on the electrogenicity in this time range we applied a differential method: the middle trace was digitally amplified by a factor of 1.78 and substracted from the upper trace. The result of this operation was a straight line (Fig. 8, lower trace). Hence, within the limit of less than 10% there was no electrogenic phase around 30 ns. This is in very good agreement with the conclusions derived from electrochromic measurements [46].

Discussion

Two steps in the primary charge separation of Photosystem II

The fast photoelectric measurements monitor the first steps of the primary photosynthetic charge separation. These may appear monophasic like in PS I [25] or biphasic like in purple bacteria [27,47]. In general, if kinetically different rising phases of the photovoltage are found, it is not obvious how to attribute them to the physical event: a slow rising phase may either mean a slow charge separation kinetics or a fast charge separation preceded by a slow trapping. These two possibilities can be distinguished by the approach of high and low excitation energy [25].

In the low energy limit there is less than one exciton in a domain at the same time (in vivo condition). Using the random-walk description of the trapping process, an exciton has to jump statistically from pigment to pigment as many times as the mean number of pigments per RC [20]. The time it takes to find an open trap and to get trapped is the trapping time, usually determined under the low-energy limit (fluorescence measurements). In contrast to fluorescence methods, the fast photoelectric method allows principally to measure also the trapping time at high energy when several excitatons reside in a domain at the same time. Then the trapping time may be expected to be shortened (to values beyond the present time resolution), because the number of jumps necessary to reach an open trap decreases drastically.

The acceleration of the trapping time in PS II with increasing energy is documented in Fig. 7a, b. At high energy the trapping kinetics could not be time-resolved ($\tau_1 < 35$ ps). Therefore, this condition could be used to analyse for a second step of the primary charge separation, which was found to contribute with an electrogenicity of A_2 of 0.6–1.0 to the total charge separation. This yields an estimation of the location of the intermediary acceptor, Pheo, with the neighbouring electron carriers P-680 and Q_A: if the total charge separation is assumed to be 1, the dielectrically weighted distance between P-680 and Pheo is 1/2 to 2/3, and between Pheo and Q_A it is 1/3 to 1/2. This relative distance is similar to the corresponding one found for RCs of purple bacteria [27,47,48]. Both the similar relative distance and the existence of a second step in the forward electron transfer of several 100 ps suggests that the structure and function of PS II reaction centers resemble the RCs of bacteria rather than the RC of PS I [25,47]. This may be considered as an indication for a possible common evolutionary origin of PS II and purple bacteria. Our result is at variance with the conclusions of Meiburg et al. [49], who suggest that the electron transfer between Pheo and Q_A spans 90% of the potential difference across the membrane. At present we cannot offer an explanation for the discrepancy.

The Klimov hypothesis

According to the well-known hypothesis of Duysens and Sweers [50], the increase in the yield of fluorescence of chloroplasts observed upon illumination (so-called variable fluorescence) is due to the reduction of Q_A (i.e., the closing of PS II reaction centers). Furthermore, ample evidence exists that establishes Pheo to act as the intermediary electron acceptor between P-680 and Q_A

[21,33,34]. A reversible photoaccumulation of $Pheo^-$ under reducing conditions has been investigated by Klimov and coworkers [21,33]. In addition, these authors have proposed that the variable fluorescence in closed RCs was in fact luminescence produced by the charge recombination between $P\text{-}680^+$ and $Pheo^-$ by opposition to prompt fluorescence. This proposal, generally referred to as the 'Klimov hypothesis', has been derived from simultaneous measurements of fluorescence lifetimes and flash-induced absorbance change (ΔA) measurements on small PS II particles with reduced Q_A.

More specifically, Shuvalov et al. [51] observed a ΔA signal decaying in approx. 4 ns which paralleled the fluorescence decay. The ΔA spectrum could be assigned to $P\text{-}680^+ \cdot Pheo^-$. This observation has been recently confirmed and extended to the time range of hundreds of picoseconds by Nuijs et al. [43] on PS II particles containing about 80 chlorophylls per P-680. However, the Klimov hypothesis has also been questioned by several researchers involved in the measurements of the fluorescence lifetimes of chlorophyll in vivo as reviewed in Ref. 52.

Going beyond the controversy on the validity of the Klimov hypothesis, one of the problems which should be solved in order to understand the physical processes involved in the trapping of excitation by PS II is the question, already addressed by Van Gorkom [34], of the partition of the excitation energy between the excited antenna chlorophylls and the charge-separated state in closed RCs

$$Chl^* \cdot P\text{-}680 \cdot Pheo \cdot Q_A^- \rightleftharpoons Chl \cdot P\text{-}680^+ \cdot Pheo^- \cdot Q_A^-$$

and the dependence of this equilibrium on the antenna size.

The lack of PS-II electrogenicity in chloroplasts with fully connected antenna system when Q_A is reduced together with our finding that in open RCs the formation of the state ($P\text{-}680^+ \cdot Pheo^-$) is electrogenic, demonstrates that in chloroplasts with intact membranes the equilibrium between the excited state in the antenna pigments and the charge-separated state of the RC rests on the excited state. Thus our results do not support an extension of the Klimov hypothesis to chloroplasts, but rather support the view proposed by Holzwarth and Schatz [22–24], namely that the equilibrium is progressively shifted to the excited state the larger the number of connected antenna pigments. This latter explanation has been substantiated recently by picosecond absorption spectroscopy of PS-II particles (80 Chl a/PS II) where a more than two-fold lower yield of the state ($P\text{-}680^+ \cdot Pheo^-$) was found with closed RCs (reducing conditions) as compared to the yield with open RCs [23]. Concomitantly, the lifetime of the excitation energy in the antenna increased, as indicated by the appearance of slower phases in the fluorescence decay [22–24,37].

Trapping kinetics

The photovoltage from chloroplasts with open PS II reaction centers at the lowest feasible energy of 50 $\mu J/cm^2$ rose significantly slower than the one evoked by higher energy (Fig. 7a and b). At this low energy there is approx. one exciton/PS II. We attribute the kinetics of approx. 200 ± 40 ps to the time needed for the exciton to reach the trap and to get photochemically converted into the state ($P\text{-}680^+ \cdot Pheo^-$) (= trapping kinetics). The conclusion is based on the fact that the first step of charge separation is electrogenic and is faster than 50 ps (see preceeding subsections).

Unfortunately, this excitation energy lies above the low-energy limit, where less than one exciton resides at the same time in a domain. Therefore, the trapping time determined photoelectrically may not be directly compared to the corresponding kinetics obtained from single-photon-counting fluorescence [37]. However, our trapping time is just at the lower limit of the values determined by the fluorescence method for the major population of PS II, the α-centers [37]. It should be mentioned here that the trapping kinetics as measured by the primary-charge separation may not be as sensitive to annihilation effects as is the fluorescence method. The signal-to-noise ratio of the present photoelectric data is not sufficient to make a statement on the electrogenicity and the trapping time of β-centers, which would be expected to contribute to only about 20% [37].

Connectivity between Photosystem-II units

There is general agreement that the antenna organization of PS II can be described by a matrix

(or lake) model [20]. At least five PS-II units share a common pool of antenna pigments forming a so-called 'domain'. Commonly a domain is viewed as interconnected antenna systems between which an exciton can move as to visit several PS II reaction centers, may they be open or closed. Particularly, when a fraction of RCs is closed, an exciton that has visited a closed trap has an increased probability to escape and to find another open trap as compared to the probability when all traps are open. However, this latter case was recently called in question by Ley and Mauzerall who found that the absorption cross section of PS II did not increase with increasing fractions of closed traps [53]. The authors conclude that either the probability for escape from closed traps is small, or the probabilities for escape from open and closed traps are nearly equal.

The photoelectric data reported here support a matrix organization of PS-II units. We suggest that the lack of PS-II electrogenicity in stacked chloroplasts in the light-gradient experiment is due to an 'excitonic short circuit' of the anisotropy caused by the gradient of light within the stacked thylakoid membranes. The excitonic short circuit may be viewed as a migration of excitons either over at least one thylakoid or across the partition region between at least two appressed membranes. The excitonic equilibration of the light-gradient anisotropy must take place before trapping (i.e., charge stabilization) in the RCs occurs.

The present signal-to-noise ratio of the photoelectric data is not sufficient to study trapping times and trapping efficiencies in chloroplasts with partially closed PS-II traps. Such experiments (that are envisaged by the authors) would allow to gain more insight into the probabilities of an exciton to escape from open or closed traps and to migrate to neighbouring PS-II units.

The excitonic short circuit could be canceled by destacking the thylakoid membranes. This was the condition to observe a light-gradient photovoltage from PS II (Figs. 5–8). However, we cannot exclude that even under destacking conditions there would be still partial energy transfer around the small EDTA-treated vesicles (mean diameter, 600 nm [54]) that would diminish the actual photovoltage. Within this context it may be mentioned that inside-out vesicles from chloroplasts (enriched in PS II) displayed only a small photovoltage [55], and that chromatophores from the purple bacterium *Rhodobacter sphaeroides* displayed significantly smaller photovoltage than whole cells [56].

Spill-over from closed Photosystem II

It had been considered that excitation energy might migrate directly from PS II to PS I [57–60]. The process, termed spill-over, depends on various physiological parameters which regulate the energy distribution between the two photosystems [57,61]. We examined our data for a possible spill-over due to a closure of PS II. The presence of such an effect would be expected to result in an increase of the apparent antenna size of PS I. The experiment shown in Fig. 2 excludes spill-over due to PS II closing for chloroplasts with stacked thylakoid membranes. First, the finding of the same maximal photovoltage with and without DCMU demonstrates that only PS I contributes to the photovoltage, in agreement with the other experiments that show the lack of PS-II electrogenicity. Second, the identical exponentials (= antenna sizes) needed to fit the energy dependence of the photovoltage under F_0 (without DCMU) and F_{max} (with DCMU) conditions restrict an additional energy supply of PS I to less than 6%. This is in agreement with other photoelectric measurements of PS I [25].

In line with this conclusion is the finding that the effect of the artificial quencher of the excited state, DNB, on the PS-I photovoltage is independent of whether the RCs of PS II are open or closed (Fig. 4; ±DCMU).

In contrast, in chloroplasts with destacked thylakoid membranes we do have evidence for an increase of the effective antenna size of PS I when the RCs of PS II are closed. This became manifest by the different exponents in Eqn. 4 needed to fit the two saturation curves in Fig. 5. A more detailed investigation of this spill-over will be subject of another study.

Appendix I. Displacement current from two parallel reactions

Let us assume two parallel reactions as they occur in chloroplasts of higher plants from PS I

and PS II. To treat the simplest cases, each reaction shall be assumed to proceed with one rate-limiting step. It may be either the first step of the primary charge separation

$$\text{P-700}^{*}\cdot\text{A}\xrightarrow{k_1}\text{P-700}^{+}\cdot\text{A}^{-} \tag{A-1}$$

$$\text{P-680}^{*}\cdot\text{Pheo}\xrightarrow{k_2}\text{P-680}^{+}\cdot\text{Pheo}^{-} \tag{A-2}$$

or the trapping process

$$(\text{antenna}^{*})\cdot\text{P-700}\cdot\text{A}\xrightarrow{k_1}(\text{antenna})\cdot\text{P-700}^{+}\cdot\text{A}^{-} \tag{A-1a}$$

$$(\text{antenna}^{*})\cdot\text{P-680}\cdot\text{Pheo}\xrightarrow{k_2}(\text{antenna})\cdot\text{P-680}^{+}\cdot\text{Pheo}^{-} \tag{A-2b}$$

where k_1 and k_2 are the corresponding rate constants. Then the time-dependent concentrations of the states $\text{P-700}^{+}\cdot\text{A}^{-}$ and $\text{P-680}^{+}\cdot\text{Pheo}^{+}$, denoted $n_1(t)$ and $n_2(t)$ respectively, are given by:

$$n_1(t) = N_0'\left(1-\mathrm{e}^{-k_1 t}\right) \tag{A-3}$$

$$n_2(t) = N_0''(1-\mathrm{e}^{-k_2 t}) \tag{A-4}$$

The formation of the states $\text{P-700}^{+}\cdot\text{A}^{-}$ and $\text{P-680}^{+}\cdot\text{Pheo}^{-}$ is connected with a displacement current given by

$$I(t) = K_1\frac{\mathrm{d}n_1}{\mathrm{d}t} + K_2\frac{\mathrm{d}n_2}{\mathrm{d}t} \tag{A-5}$$

where K_1 and K_2 are electrogenicity parameters that represent the change of the dipole strength associated with the charge transfer steps in Eqns. A-1 and A-2. Introducing Eqns. A-3 and A-4 into Eqn. A-5 yields the analytical expression for the displacement current

$$I(t) = A_1k_1\,\mathrm{e}^{-k_1 t} + A_2k_2\,\mathrm{e}^{-k_2 t} \tag{A-6}$$

where $A_1 = K_1N_0'$ and $A_2 = K_2N_0''$. Note, that in the case of two parallel reactions, photovoltage measurements alone do not allow the separation between the electrogenicity parameters K and the initial concentrations N_0, unless the N_0 are determined independently. In the case of a two-step consecutive reaction, however, it is possible to determine different K's as relative ratio [27].

Appendix II. Quenching of fluorescence and trapping by an artificial quencher

Several possible decay paths exist for an excited singlet state in a given antenna system with open traps, one of them being the loss by fluorescence. To correlate the effect of an artificial quencher on the fluorescence path and on the trapping path, the following statistical average rate constants are needed: k_f = fluorescence; k_t = trapping; k_1 = all loss processes, including fluorescence; k_q = quenching by the artificial quencher.
The fluorescence yield is then given by

$$\phi_f = \frac{k_f}{k_1 + k_t + k_qD} \tag{B-1}$$

where D is the average number of quenching molecules per RC. The trapping yield is given by

$$\phi_t = \frac{k_t}{k_1 + k_t + k_qD} \tag{B-2}$$

When the reciprocal yields are taken and when the yields are normalized to the yields at $D = 0$, these equations become

$$\phi_{f,n}^{-1} = 1 + \frac{k_q}{k_1 + k_t}D \tag{B-1a}$$

$$\phi_{t,n}^{-1} = 1 + \frac{k_q}{k_1 + k_t}D \tag{B-2b}$$

The last two equations show that the slopes are necessarily equal when the artificial quencher acts in the identical antenna system, in which fluorescence and trapping takes place. A reverse conclusion can also be drawn: when different slopes are found, the processes cannot originate from one and the same antenna system.

Acknowledgements

We are greatly indebted to Dr. A. Dobek for his contribution to the measurements with highest time resolution, to Dr. D. von Wettstein (Carlsberg Laboratories) for the supply with seeds of barley mutants, to Dr. U. Hinz for teaching to H.-W.T. the selection of mutants and for the supply with mutant chloroplasts at the early stage

of the experiments, to Dr. B. Diner for the supply with membranes from *Chlamydomonas rheinhardtii* (mutant M19), to Dipl. Biol. C. Kischkoweit for fluorescence quenching measurements, and to Dr. H. von Gorkom for stimulating discussions. W.L. and H.-W.T. acknowledge the financial support of the Deutsche Forschungsgemeinschaft (SFB 171).

References

1 Fowler, C.F. and Kok, B. (1972) Sixth International Congress on Photobiology, Bochum, Abstr. no. 417
2 Witt, H.T. and Zickler, A. (1973) FEBS Lett. 37, 307–310
3 Fowler, C.F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 308–318
4 Gräber, P. and Trissl, H.-W. (1981) FEBS Lett. 123, 95–99
5 Trissl, H.-W. and Kunze, U. (1985) Biochim. Biophys. Acta 806, 136–144
6 Schliephake, W., Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 1571–1578
7 Haehnel, W. (1976) Biochim. Biophys. Acta 423, 499–509
8 Melis, A. and Brown, J.S. (1980) Proc. Natl. Acad. Sci. USA 77, 4712–4716
9 Andersson, B. and Haehnel, W. (1982) FEBS Lett. 146, 13–17
10 Melis, A., Spangfort, M. and Andersson, B. (1987) Photochem. Photobiol. 45, 129–136
11 Melis, A. and Harvey, G.W. (1981) Biochim. Biophys. Acta 637, 138–145
12 Melis, A. and Anderson, J.M. (1983) Biochim. Biophys. Acta 724, 473–484
13 Isawa, S. and Good, N.E. (1966) Plant Physiol. 41, 544–552
14 Arnold, W.R. and Azzi, J.R. (1977) Plant Physiol. 60, 449–451
15 Trissl, H.-W. (1985) Biochim. Biophys. Acta 608, 124–135
16 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 427–440
17 Anderson, J.M. and Melis, A. (1983) Proc. Natl. Acad. Sci. 80, 745–749
18 Ojakian, G. and Satir, P. (1974) Proc. Natl. Acad. Sci. USA 71, 2052–2056
19 Staehelin, L.A. (1976) J. Cell Biol. 71, 136–158
20 Van Grondelle, R. (1985) Biochim. Biophys. Acta 811, 147– 195
21 Klimov, V.V., Klevanik, A.V. and Shuvalov, V.A. (1977) FEBS Lett. 82, 183–186
22 Schatz, G.H. and Holzwarth, A.R. (1986) Photosynthesis Res. 10, 309–318
23 Holzwarth, A.R., Brock, H. and Schatz, G.H. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. I, pp. 61–65, Martinus Nijhoff, Dordrecht
24 Schatz, G.H. and Holzwarth, A.R. (1987) Proc. Natl. Acad. Sci. USA, in the press
25 Trissl, H.-W., Leibl, W., Deprez, J., Dobek, A. and Breton, J. (1987) Biochim. Biophys. Acta 893, 319–331
26 Steinback, K.E., Burke, J.J. and Arntzen, C.J. (1979) Arch. Biochem. 195, 546–557
27 Deprez, J., Trissl, H.-W. and Breton, J. (1986) Proc. Nat. Acad. Sci. USA 83, 1699–1703
28 Trissl, H.-W. (1983) Biochim. Biophys. Acta 723, 327–331
29 Groma, G.I., Szabo, G. and Varo, Gy (1984) Nature (London) 308, 557–558
30 Nuss, M.C., Zinth, W., Kaiser, W., Kölling, E. and Oesterhelt, D. (1985) Chem. Phys. Lett. 117, 1–7
31 Boone, S. and Bügel, R.D. (1963) Bull. ASE 54, 313–322
32 Rutherford, A.W. and Heathcote, P. (1985) Photosynth. Res. 6, 295–316
33 Klimov, V.V. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 131–138, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht
34 Van Gorkom, H.J. (1985) Photosynth. Res. 6, 97–112
35 Setif, P. and Mathis, P. (1980) Arch. Biochem. Biophys. 204, 477–485
36 Geacintov, N.E. and Breton, J. (1982) in Trends in Photobiology (Helene, C., Charlier, M., Montenay-Garestier, T. and Laustriat, G., eds.), pp. 549–559, Plenum Press, New York
37 Holzwarth, A.R. (1986) Photochem. Photobiol. 43, 707–725
38 Etienne, A.-L., Lemasson, C. and Lavorel, J. (1974) Biochim. Biophys. Acta 333, 288–300
39 Sonneveld, A., Rademaker, H. and Duysens, L.M.M. (1980) Biochim. Biophys. Acta 593, 272–289
40 Boardman, N.K., Thorne, S.W. and Anderson, S.W. (1966) Proc. Natl. Acad. Sci. USA 56, 586–593
41 Simpson, D.J. and Von Wettstein, D. (1980) Carlsberg Res. Commun. 45, 283–314
42 Hiller, R.G., Møller, B.L. and Høyer-Hansen, G. (1980) Carlsberg Res. Commun. 45, 315–328
43 Nuijs, A.M., Van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 848, 167–175
44 Deprez, J., Dobek, A., Geacintov, N.E., Paillotin, G. and Breton, J. (1983) Biochim. Biophys. Acta 725, 444–454
45 Brettel, K., Schlodder, E., and Witt, H.T. (1984) Biochim. Biophys. Acta 766, 403–415
46 Conjeaud, H., Mathis, P. and Paillotin, G. (1979) Biochim. Biophys. Acta 546, 280–291
47 Trissl, H.-W. (1983) Proc. Natl. Acad. Sci. USA 80, 7173–7177
48 Jortner, J. and Michel-Beyerle, M.E. (1985) Chem. Phys. 42, 345–365
49 Meiburg, R.F., Van Gorkom, H.J. and Van Dorssen, R.J. (1983) Biochim. Biophys. Acta 724, 352–358
50 Duysens, L.N.M. and Sweers, H. (1963) in Studies on Microalgae and Photosynthetic Bacteria, pp. 353–372, University of Tokyo Press, Tokio
51 Shuvalov, V.A., Klimov, V.V., Dolan, E., Parson, W.W. and Ke, B. (1980) FEBS Lett. 118, 279–282
52 Geacintov, N.E. and Breton, J. (1987) Critical Rev. Plant Sci. 5, 1–44
53 Ley, A.C. and Mauzerall, D. (1986) Biochim. Biophys. Acta 850, 234–248
54 Lill, H., Althoff, G. and Junge, W. (1987) J. Membrane Biol., in the press
55 Gräber, P., Zickler, A. and Åkerlund, H.-E. (1978) FEBS Lett. 96, 233–237

56 Trissl, H.-W., Kunze, U. and Junge, W. (1982) Biochim. Biophys. Acta 682, 362–377
57 Butler, W.L. and Kitajima, M. (1975) Biochim. Biophys. Acta 396, 72–85
58 Arntzen, C.J. (1978) Curr. Top. Bioenerg. 7, 111–160
59 Butler, W.L. (1980) Proc. Natl. Acad. Sci. USA 77, 4697–4701
60 Barber, J. (1980) FEBS Lett. 118, 1–10
61 Haworth, P., Kyle, D.J. and Arntzen, C.J. (1982) Biochim. Biophys. Acta 680, 343–351