Electrogenic light reactions in photosystem I: Resolution of electron-transfer rates between the iron–sulfur centers

(electrogenic events/photosynthesis/photovoltage/spinach)

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ABSTRACT Flash-induced voltage changes (electrogenic events) in photosystem I particles from spinach, oriented in a phospholipid layer, have been studied at room temperature on a time scale ranging from 1 μ s to several seconds. A phospholipid layer containing photosystem I particles was adsorbed to a Teflon film separating two aqueous compartments. Voltage changes were measured across electrodes immersed in the compartments. In the absence of added electron donors and acceptors, a multiphasic voltage increase, associated with charge separation, was followed by a decrease, associated with charge recombination. Several kinetic phases were resolved: a rapid (<1 μ s) increase, ascribed to electron transfer from the primary electron donor P700 to the iron–sulfur electron acceptor F_B , was followed by a slower, biphasic increase with time constants of 30 and 200 μ s. The 30- μ s phase is assigned to electron transfer from F_B to the iron–sulfur center F_A . The voltage decrease had a time constant of 90 ms, ascribed to charge recombination from F_A to P700. Upon chemical prereduction of F_A and F_B the 30- and 200- μ s phases disappeared and the decay time constant was accelerated to 330 μ s, assigned to charge recombination from the phyloquinone electron acceptor (A_1) or the iron–sulfur center F_X to P700.

The photosystem I (PSI) reaction center is a membrane-bound protein complex, which converts light energy to redox energy. The spinach complex consists of 2 large (≈ 80 kDa) and at least 10 smaller (5–20 kDa) subunits, which bind a number of cofactors associated with the conversion process. The absorption of light by one of ≈ 200 antenna chlorophyll molecules is followed by a rapid migration of the excitation energy to the chlorophyll primary electron donor, P700, and an electron transfer is then brought about between P700 and the chlorophyll primary electron acceptor A_0 . Subsequent electron transfer to a phyloquinone intermediate electron acceptor, A_1 , and three [4Fe-4S] clusters F_X , F_A , and F_B , stabilizes the charge-separated state for progressively longer times and larger distances across the thylakoid membrane. Further stabilization against charge recombination is achieved by the reduction of $P700^+$ by water-soluble plastocyanin on the lumenal side of the membrane and oxidation of (F_A/F_B) $^-$ by ferredoxin on the stromal side. (See refs. 1–4 for recent reviews of PSI structure and function.)

The sequence and kinetics of the electron-transfer reactions between the different cofactors have been investigated by a number of spectroscopic techniques, including flash-induced optical and electron paramagnetic resonance (EPR) spectroscopy. The details of the electron-transfer reactions between the Fe-S centers are, however, largely unknown. This is so because the optical spectra of the Fe-S centers are nearly identical and, although they can be distinguished with EPR

spectroscopy, such studies can be made only at low temperatures (<40 K) since their EPR spectra are too broad at higher temperatures.

In this study, we have used a different approach. PSI particles were oriented in a phospholipid layer adsorbed to a Teflon film, and light-induced electrogenic events were measured. This technique has previously been used with bacteriorhodopsin (5) and with reaction centers from purple bacteria (6), from PSII (7), and cytochrome *c* oxidase (8). After pulsed illumination of the PSI–lipid layer, in the absence of added electron donors and acceptors, a multiphasic voltage increase was followed by a biphasic decrease. We assign these electrogenic events to charge separation and recombination in the PSI reaction center. The time constants and amplitudes of the measured voltage changes are interpreted in terms of the kinetics of the electron-transfer reactions and the distances between the Fe-S centers that are known from the recently determined 6-Å resolution structure of a cyanobacterial PSI complex (9).

MATERIALS AND METHODS

PSI Particles. Spinach was grown hydroponically and harvested just before preparation. Digitonin-solubilized PSI particles were prepared as described (10) and stored under liquid nitrogen until used. The preparation contains 240 chlorophylls per P700 and is highly competent in plastocyanin photo-oxidation (11). When complemented with plastocyanin, ferredoxin, and ferredoxin-NADP $^+$ oxidoreductase, the rate of NADP $^+$ photoreduction amounts to 240 μ mol of NADPH per h per mg of chlorophyll. The content of PSII is $<5\%$ as determined from studies of EPR signal II (see also *Results*). The preparation contains 10% cytochrome b_6/f complex as determined from an optical quantitation of cytochrome *f*.

PSI-Containing Vesicles. Soybean lecithin lipids (Sigma), washed as described (12), were dispersed to 10 mg/ml in 10 mM Tris-HCl buffer (pH 8.0), supplemented with 10 mM KCl. To prepare lipid vesicles, the lipid solution was Vortex mixed for 5–10 min and then sonicated (Bronson sonicator; model B-12) for ≈ 5 min until the solution was optically clear. The vesicle solution was supplemented with PSI complexes in a total vol of 200 μ l to a final PSI concentration of 2 μ M and then sonicated for ≈ 1 min.

Electrical Measurements. The experimental setup was similar to the one described (5–8); it is shown schematically in Fig. 1. A 12- μ m Teflon film (fluorocarbon) was clamped between two Teflon pieces, forming a cell consisting of two aqueous compartments with a volume of 2 ml each. O rings with a diameter of 5 mm were used to ensure a water-tight contact between the Teflon film and the cell compartments. The Teflon film was illuminated through a window in one of the

FIG. 1. Schematic representation of the experimental setup used to measure electrogenic events in PSI. The Teflon-bound PSI layer is shown on the right. Light-induced voltage changes were measured across electrodes immersed in the cell compartments.

compartments. The illuminated area was 0.3 cm^2 . The resistance between the cell compartments was $\geq 10^{12} \Omega$.

The vesicle solution (see above) was diluted 1:10 in 10 mM Tris-HCl (pH 8.0) or 200 mM glycine (pH 10.0), 10 mM KCl, 10 mM CaCl_2 . In the presence of Ca^{2+} , the vesicles break and a lipid layer is formed at the surface (13). The solution was added to the cell compartment on the right (cf. Fig. 1) to a level just below the Teflon film. The other cell compartment contained only the buffer solution. After ≈ 5 min, the liquid level was raised slowly past the Teflon film allowing the lipid layer to attach to the Teflon. This procedure was necessary to obtain a light-induced voltage change. If the surface was raised too rapidly or if the procedure was done in the absence of Ca^{2+} , no detectable voltage changes were observed. This shows that PSI-containing vesicles in the solution do not contribute to the electrical signal (cf. ref. 14) and that addition of Ca^{2+} in combination with careful, slow raising of the surface is necessary to get light-induced voltage changes. The electrical characteristics of the experimental setup were not affected by changes in the KCl concentration in the range 10–250 mM.

Light-induced electrical signals were measured at $23 \pm 1^\circ\text{C}$ across Ag/AgCl or Pt electrodes (diameter, 1 mm) immersed in the cell compartments but shielded from actinic light. The signals were amplified with an operational amplifier (model OP128, Burr-Brown; model LF356, National Semiconductors), further amplified and filtered with a preamplifier (model SR560, Stanford Research Systems), and recorded on a digital oscilloscope (model Pro 90, Nicolet). The response time of the setup was $1 \mu\text{s}$, limited by the amplifier time constant. An equivalent electrical scheme, which models the experimental setup, has been presented (7).

Optical Measurements. Flash-induced optical absorption transients at 830 nm were acquired with a home-built photodiode detector (15) using a diode laser (model 06DLD203, Melles Griot) for the measuring light. The output from the detector was fed into an analog oscilloscope (model 11302A, Tektronix) equipped with a digital camera (model DCS01, Tektronix). The camera was connected to a personal computer for data storage.

Light Source. Pulsed illumination was provided by a Q-switched Nd:yttrium/aluminum-garnet laser (model YG570, Quantel) at 532 nm. The pulse width was ≈ 10 ns and the pulse energy was $\approx 10 \text{ mJ/cm}^2$.

Data Analysis. Amplitudes and time constants of exponential components in the kinetic traces were determined by using a home-written curve-fitting program based on the Levenberg-Marquardt algorithm (16). The time constants reported, τ , are $1/e$ lifetimes. Note that time constants cited from the work by others in several cases are half-lifetimes, $t_{1/2} (= \tau \times \ln 2)$.

RESULTS

Optical Characterization of PSI Vesicles. The procedure used to prepare lipid layers includes sonication of PSI. To test

the functionality of sonicated PSI we measured time-resolved absorbance changes at 830 nm ($P700^+$ absorption) associated with charge recombination of $P700^+$ (F_A/F_B)⁻ and with electron donation to $P700^+$ by reduced plastocyanin. The time constants of these reactions are known to be sensitive to the subunit composition of PSI (see refs. 2 and 3), which could be affected by sonication. However, no effect of sonication could be detected for the PSI particles even if the sonication time was extended to 20 min.

The degree of PSI incorporation into vesicles was also estimated with optical measurements at 830 nm. For this, the vesicles were subjected to centrifugation at $50,000 \times g$ for 30 min. After this procedure, the PSI particles not incorporated are expected to stay in the supernatant, whereas the vesicles will sediment (pelleting of this type of PSI particles requires an ultracentrifugation at $144,000 \times g$ for 1 h). With the concentrations of lipids and PSI given in *Materials and Methods*, the flash-induced $P700^+$ signal obtained from the supernatant was $<10\%$ of that obtained before the centrifugation, showing an efficient incorporation of PSI into vesicles by the present method.

Electrical Measurements. Fig. 2A shows voltage changes

FIG. 2. Light-induced voltage changes on different time scales measured in PSI complexes oriented in a lipid layer. Buffer solution consisted of 10 mM Tris-HCl (pH 8.0), 10 mM KCl, 10 mM CaCl_2 , except for the lower trace in B, where the buffer contained 200 mM glycine (pH 10.0), 10 mM KCl, 10 mM CaCl_2 , and 40 mM sodium dithionite.

following pulsed illumination of the PSI-lipid layer. An increase in voltage was followed by a biphasic decrease with the dominant phase ($\approx 90\%$ of the amplitude) decaying with a time constant of 90 ± 10 ms. A small fraction of the voltage ($\approx 10\%$) decreased with a time constant of ≥ 1 s, limited by the setup time constant. The decay time constants were similar to those measured optically at 830 nm on PSI particles in solution (data not shown). We ascribe these voltage changes to charge separation from P700 to centers F_A and/or F_B followed by charge recombination (see *Discussion*).

To further elucidate the origin of the observed voltage changes, we added a small amount of ferricyanide to the PSI-containing cell compartment. Ferricyanide oxidizes P700, leaving PSI photochemically inactive, but does not affect PSII. After the addition, no measurable voltage changes were observed, supporting their assignment to PSI and confirming the absence of PSII from the preparation.

To resolve reactions in the charge separation, we measured voltage changes also on shorter time scales (Fig. 2 *B* and *C*). We found that the voltage increase consists of at least three kinetic components with time constants and relative amplitudes (in parentheses) of $<1 \mu\text{s}$ (0.5), $30 \pm 10 \mu\text{s}$ (0.3), and $200 \pm 30 \mu\text{s}$ (0.2). The true lifetime of the fastest phase was obscured by the time resolution of the experimental setup. The lifetimes given for the other phases are the mean $\pm$ SD obtained from two independent measurements on each of three different PSI preparations. The uncertainty in the relative amplitudes is of the order of 30%. This multiphasic voltage increase is ascribed to forward electron-transfer reactions along the chain of electron acceptors in PSI (see *Discussion*).

To test whether the observed voltage changes were associated with electron-transfer reactions to or between F_A and F_B , we made measurements in the presence of dithionite at pH 10. Under these conditions, F_A and F_B are reduced (see ref. 2) so that charge separation proceeds only to F_X (or A_1 ; see *Discussion*), resulting in a faster recombination. In the presence of dithionite, the charge-recombination voltage decayed biphasically with time constants and amplitudes (in parentheses) of $330 \mu\text{s}$ (2/3) and 2.0 ms (1/3) (Fig. 2*B*, lower trace). Similar time constants were measured optically at 830 nm under comparable conditions (with dithionite at pH 10, but with PSI particles in solution and not in lipid vesicles), although the relative amplitude of the 2-ms component was 10%. No 30- or 200- μs components could be discerned in the voltage increase; it all occurred within the 1- μs time resolution of the setup. In addition, this voltage increase had approximately the same amplitude as the $<1\text{-}\mu\text{s}$ component in the voltage increase measured without dithionite at pH 8.0. These results indicate an involvement of F_A and F_B in the 30- and 200- μs phases of the electrical signal as discussed below.

DISCUSSION

The results presented here demonstrate the electrogenic nature of the electron-transfer reactions in PSI. A PSI-containing layer of phospholipids was raised against a Teflon film, and flash illumination of this sample resulted in detectable voltage changes on a time scale ranging from $<1 \mu\text{s}$ to several seconds. A multiphasic voltage increase in the submillisecond range was followed by a slower, biphasic return to the zero level. We ascribe these voltage changes to charge separation and recombination in PSI for the following reasons.

(i) Addition of ferricyanide completely abolishes the signal. This is in line with the ability of ferricyanide to oxidize P700, inhibiting light-induced charge separation.

(ii) In the absence of exogenous reductants or oxidants, the electrical signal decreases biphasically with time constants of ≈ 90 ms (90%) and >1 s (10%). These time constants are similar to those determined with optical kinetic techniques. The dominating phase in the optical signals has a half-life that

varies from 20 to 70 ms in different reports, and it has been assigned to a charge recombination between $P700^+$ and F_A^- (17, 18). A component with a >200 -ms half-time (amplitude, 27%) has been described for a PSI preparation similar to the one used by us (17). It was ascribed to the reaction between reduced Fe-S centers and a diffusible electron acceptor—i.e., one that is not part of PSI. The smaller amplitude of the 1-s phase observed in the present work is consistent with this, since transfer of an electron into a medium with a higher dielectric constant is expected to result in a reduced voltage amplitude.

(iii) When F_A and F_B are chemically prerduced, the major part of the decay of the electrical signal occurs with a 330- μs time constant. A similar time constant is also observed with optical kinetic techniques and has generally been thought to be due to a recombination within the $(P700^+F_X^-)$ pair (see ref. 2). Recently, however, this phase was attributed to a charge recombination between $P700^+$ and A_1^- (refs. 19 and 20; see below).

Thus, it is concluded that electron transfer to F_X (or A_1) as well as further charge separation to F_A is electrogenic. A similar conclusion was drawn from studies of electric field-induced PSI luminescence (18, 21). It is also in line with the recently determined 6-Å resolution electron-density map of PSI from *Synechococcus* sp. (9), which shows that the three Fe-S clusters form an irregular triangle located toward the stromal side of the membrane, and the vectors connecting the clusters all have components perpendicular to the membrane plane. The cluster closest to the middle of the membrane was attributed to F_X , but it was not possible to distinguish between F_A and F_B . Ellipsoidal electron densities located toward the lumenal side of the membrane were tentatively assigned to P700, and additional electron densities between P700 and the Fe-S clusters were attributed to A_0 and A_1 (9). This arrangement suggests that all steps in the electron transfer from P700 to the most remote Fe-S center should be electrogenic.

The present work supplements electrical measurements that are based on the light-gradient method (14, 22). An early study on pea chloroplasts was restricted to the time window between 35 ps and 60 ns (the *RC* discharge time constant of the capacitive measuring cell). Within this time range, all the electrogenicity was found to take place in <50 ps (14). In a study of a PSII-deficient mutant of *Synechocystis* sp. PCC 6803, two phases were resolved in the rise of the photovoltage signal. These were assigned to the formation of the state $P700^+A_0^-$ (22 ps) and to the further electron transfer from A_0 to A_1 (50 ps) (22). The lack of slower electrogenic components in ref. 14 can be understood in the light of recent time-resolved optical and EPR studies. The electron transfer from A_1^- occurs with a half-life of 180–200 ns in the cyanobacterium *Synechococcus* sp. (23–25). In the case of spinach PSI particles, a half-life of 15 ns was first reported for this reaction (26). More recent work shows a biphasic decay of A_1^- (20). The relative proportions of a 25- and a 150-ns component were found to be preparation dependent and it was suggested that PSI in native spinach membranes could behave similarly to the cyanobacterial PSI particles (20)—i.e., that A_1^- is predominantly oxidized with a half-life of 150–200 ns. This would explain why no electrogenic phases in the 50-ps to 60-ns time range were observed by the workers in ref. 14.

From A_1^- , electrons are transferred to an Fe-S center (23). Several lines of evidence suggest that this is F_X : The reduction potential of F_X is intermediate between that of A_1 and F_A/F_B and photoaccumulation of F_X^- at low temperature is possible only after prerduction of F_A/F_B (see ref. 3). Also, F_X was found to be located between A_1 and centers F_A and F_B in the electron-density map (9). However, recently it was observed that chemical prerduction of F_A and F_B blocked the 200-ns forward electron transfer from A_1^- in *Synechococcus* sp. and it was speculated that A_1^- might transfer its electron directly to F_A and/or F_B , without involving F_X (19). An alternative

explanation, also offered in ref. 19, would be that chemical prereduction of F_A and F_B leads to a decrease in the reduction potential of F_X (due to electrostatic interactions) to a value more negative than that of A_1 . This could inhibit forward electron transfer to F_X under these conditions so that recombination occurs from A_1^- . Support for this idea comes from studies of PSI core complexes from the same cyanobacterium. A 180-ns phase in the A_1^- decay could still be observed after removal of F_A and F_B by urea and it was concluded that F_X is an obligatory intermediate in the normal forward electron transfer (25). For spinach PSI particles, the 25-ns phase was assigned to electron transfer from A_1^- to F_X (in part of the centers) while the 150-ns phase was suggested to correspond to reduction of F_A and F_B (20). Thus, there is presently considerable uncertainty about the details of the electron-transfer reactions between the Fe-S centers, and for the reaction between centers F_A and F_B there is a complete lack of kinetic information. In the following, we will try to relate the observed electrical signals to different possible electron-transfer models.

In the absence of exogenous reductants or oxidants, the voltage increase consists of three components: a fast, unresolved phase with a time constant of $\tau_f < 1 \mu s$; an intermediary phase with $\tau_m \approx 30 \mu s$; and a slow phase with $\tau_s \approx 200 \mu s$. If the amplitudes of the components are denoted by ΔV_f , ΔV_m , and ΔV_s , their ratios were $\Delta V_f/\Delta V_m/\Delta V_s \approx 0.5:0.3:0.2$. Addition of dithionite resulted in a disappearance of the intermediary and slow phases, and it is therefore reasonable to ascribe them to forward electron transfer between the Fe-S centers. However, since the photovoltaic technique does not distinguish between different molecular events, it is also possible that the electrical signals could be due to proton transfer or conformational changes.

We will consider the two models that are depicted in Fig. 3. The relative positions of the redox centers have been taken from the x-ray structure analysis but in that work it was not possible to distinguish between F_A and F_B (9). In this paper, we tentatively assign F_B to the center closest to F_X (Fig. 3). This is motivated by the fact that both the intermediary and slow electrogenic phases correspond to a movement of an electron away from P700. The reduction potentials of F_A and F_B are -520 and -580 mV, respectively, as determined by low-temperature EPR spectroscopy under equilibrium conditions (see ref. 4). Assuming that the potentials are not too different during the charge-separation process, an electron transfer

between F_A and F_B , away from P700, can be exergonic only if F_B is closest to P700.

In model I, the fast component contains all the (unresolved) electron-transfer steps from P700 to F_B . The intermediary phase is assigned to electron transfer from F_B to F_A while the remaining, slow phase is associated with electron transfer to another electron acceptor, which possibly is not part of PSI. Alternatively, the slow phase could be due to a proton transfer or a conformational change. In model II, the intermediary and slow phases are assigned to electron transfer from F_X to F_B and from F_B to F_A , respectively (Fig. 3).

The amplitudes of the electrogenic phases in the two models can be estimated from the proposed structural arrangement of the cofactors (9). In general, a voltage change, ΔV_{jk} , associated with a charge transfer from a donor, j , to an acceptor, k , is proportional to d_{jk}/ϵ_{jk} , where d_{jk} is the distance between j and k perpendicular to the membrane surface ($d_{jk} = \mathbf{d}_{jk} \cdot \mathbf{n}$, where $\mathbf{d}_{jk}$ is the distance vector and $\mathbf{n}$ is a unity vector perpendicular to the layer surface) and ϵ_{jk} is the dielectric constant between j and k . Assuming a homogenous dielectricum, model I predicts an amplitude ratio of $\Delta V_f/\Delta V_m = d_{PB}/d_{BA} = 0.85:0.15$ compared with the experimental value $0.62:0.38$. From model II, one gets $\Delta V_f/\Delta V_m/\Delta V_s = d_{PX}/d_{XB}/d_{BA} = 0.60:0.25:0.15$, close to the ratios $0.5:0.3:0.2$ obtained experimentally. Although model II is in better agreement with the data, we consider the uncertainties in the latter to be too large to rule out model I.

The electron transfer from F_B to F_A corresponds to the intermediary phase in model I and to the slow phase in model II. The rate constant k_{ET} in the two models is $3.3 \times 10^4 s^{-1}$ ($=1/30 \mu s$) and $5 \times 10^3 s^{-1}$ ($=1/200 \mu s$), respectively. These values will now be compared with theoretical estimates.

For electron transfer from a donor D to a weakly coupled acceptor A, k_{ET} can be expressed as (27–29):

$$k_{ET} = k_0 |T_{DA}|^2 \exp(-\Delta G^*/k_B T) \\ = k_0 \left(\sum_{i=1}^N t_{i,DA} \right)^2 \exp(-\Delta G^*/k_B T), \quad [1]$$

$$\Delta G^* = \frac{\lambda}{4} \left(1 + \frac{\Delta G^0}{\lambda} \right)^2, \quad [2]$$

where a value of $k_0 = 3 \times 10^{12} s^{-1}$ has been determined from experimental data (28). $|T_{DA}|^2$ is the electronic tunneling matrix element between donor and acceptor localized states along all physical pathways, ΔG^* is the activation barrier, ΔG^0 is the free energy difference between the donor and acceptor, and λ is the reorganization energy. For noninteracting pathways, T_{DA} is a sum over all $t_{i,DA}$ values for all possible N pathways. For the i th pathway $t_{i,DA}$ can be approximated by (28, 29):

$$t_{i,DA}^2 = e^{-\beta \sigma_i l} \quad [3]$$

where $\beta = 0.71 \text{ \AA}^{-1}$. For covalently coupled pathways, σ_i is the number of covalent bonds in the i th pathway and $l = 1.4 \text{ \AA}$ is the average length of one such bond.

The low-resolution three-dimensional PSI structure (9) does not reveal the details of the electron-transfer pathways between the Fe-S centers. However, the PsaC subunit, which contains centers F_A and F_B , is structurally closely related to *Peptococcus aerogenes* ferredoxin (4), which has a known high-resolution three-dimensional structure (30) that can be used to estimate the electron-transfer rate between these centers. In *P. aerogenes* ferredoxin, the main contributions to $|T_{DA}|^2$ are two symmetrical pathways with 18 covalent bonds each between the Fe-S clusters. With Eqs. 1 and 3, this gives $k_0 |T_{DA}|^2 \approx 1.8 \times 10^5 s^{-1}$.

FIG. 3. Two alternative models for the sequence of electron-transfer reactions in the PSI reaction center. Distances were obtained from ref. 9. See text for assignment of centers F_A and F_B . Arrows symbolize the movement of electrons that give rise to the observed electrogenic events. The slow phase in model I could also be due to proton transfer in the opposite direction or to conformational changes. Subscripts f, m, and s denote fast, intermediary, and slow components in the voltage increase.

For electron transfer from F_B to F_A , $\Delta G^0 = -60$ meV. The reorganization energy can be estimated to be 0.26 eV from the expression (20, 31):

$$\lambda = 14.4 \times C_{\text{eff}}(1/r - 1/R), \quad [4]$$

where $C_{\text{eff}} = 0.15$ (31), $r = 4.86$ Å is the effective radius of a [4Fe-4S] cluster (20), and $R = 12$ Å is the center-to-center distance between F_A and F_B (9). Eqs. 1 and 2 then give a rate constant for electron transfer from F_B to F_A of $k_{\text{ET}} = 3.8 \times 10^4$ s⁻¹, corresponding to a time constant of 26 μs. Within experimental error, this is the same as the 30 μs found for the intermediary phase, and it is therefore reasonable to ascribe this phase to electron transfer from F_B to F_A —i.e., accept model I. To achieve a time constant of 200 μs, as in model II, one would have to increase λ to the rather high value of 0.5 eV or decrease the electronic coupling T_{DA} with a factor of 2.6.

So far, we have considered a serial electron transfer through the centers F_X , F_B , and F_A . In principle, a situation with parallel electron flow from F_X to either F_A or F_B is also possible (3). This would give rise to a single phase in the photovoltage signal with a rate constant equal to $k_{\text{XB}} + k_{\text{XA}}$, where k_{XB} and k_{XA} are rate constants of the individual reactions. However, the center-to-center distances between F_X and F_B and between F_X and F_A are 15 and 22 Å, respectively (9); the difference corresponds to approximately five covalent bonds. Using a value of -705 mV for the reduction potential of F_X (see ref. 3), Eqs. 1–4 predict a ratio $k_{\text{XB}}/k_{\text{XA}} \approx 100$, making a model with parallel electron-transfer pathways very unlikely.

In photosynthesis, the charge separation in the PSI reaction center is followed by an electron transfer to soluble ferredoxin. Kinetic data for the reduction of ferredoxin, obtained from studies of cyanobacterial PSI, have recently been published (32). Three different first-order components with half-lifetimes of ≈ 500 ns, 20 μs, and 100 μs were found. The complexity of the kinetics was ascribed to the presence of several types of PSI-ferredoxin complexes. The 500-ns phase was assigned to electron transfer from either F_A or F_B to ferredoxin, and it was suggested that this donor is reduced within a time shorter than 500 ns. Our model I is compatible with this proposal but model II is not, since F_B is reduced with a time constant of 30 μs in this model.

In summary, we have measured electrogenic signals associated with light-induced electron-transfer reactions in PSI. The technique that we have used allows the resolution of kinetic phases in the microseconds to seconds time range. Thus, it supplements the light-gradient method, which is limited to reactions faster than ≈ 50 ns. The charge separation in PSI was found to be associated with a multiphasic voltage increase. A fast, unresolved (< 1 μs) component is suggested to be due to electron transfer from P700 to F_B , while a 30-μs phase is tentatively assigned to electron transfer from F_B to F_A . A slower phase with a time constant of 200 μs was also observed. This could be due to an electron transfer out of PSI, a proton transfer in the opposite direction, or a conformational change. It is interesting to note that an electrical signal with the same time constant was recently ascribed to a conformational change in reaction centers from the purple bacterium *Rhodospirillum rubrum* (33).

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