Light-Induced Voltage Changes Associated with Electron and Proton Transfer in Photosystem II Core Complexes Reconstituted in Phospholipid Monolayers

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ABSTRACT We have measured light-induced voltage changes (electrogenic events) in photosystem II (PSII) core complexes oriented in phospholipid monolayers. These events are compared to those measured in the functionally and structurally closely related reaction centers from the photosynthetic bacterium *Rhodobacter sphaeroides*. In both systems we observed a rapid (<100 ns) light-induced increase in voltage associated with charge separation. In PSII reaction centers it was followed by a decrease (decay) of ~14% of the charge-separation voltage and a time constant of ~500 μs. In bacterial reaction centers this decay was ~9% of the charge-separation voltage, and the time constant was ~200 μs. The decay was presumably associated with a structural change. In bacterial reaction centers, in the presence of excess water-soluble cytochrome c^{2+}, it was followed by a slower increase of ~30% of the charge-separation voltage, associated with electron transfer from the cytochrome to the oxidized donor, P^+. In PSII reaction centers, after the decay the voltage remained on the same level for ≥0.5 s. In PSII reaction centers the electron transfer $Q_A^- Q_B \rightarrow Q_A Q_B^-$ contributed with an electrogenicity of ≤5% of that of the charge separation. In bacterial reaction centers this electrogenicity was ≤2% of the charge-separation electrogenicity. Proton transfer to Q_B^{2-} in PSII reaction centers contributed with ~5% of the charge-separation voltage, which is approximately a factor of three smaller than that observed in bacterial reaction centers.

INTRODUCTION

Photosystem II (PSII) from plants converts light energy into electrochemical energy. In the conversion process a light quantum is first absorbed by a chlorophyll-antenna complex. The excitation energy is then transferred to a thylakoid membrane-bound protein complex, the reaction center, where the energy conversion takes place (reviewed by van Gorkom, 1985; Hansson and Wydrzynski, 1990). The reaction center accommodates a number of cofactors involved in this process. The initial excitation of the primary donor, P680, is followed by a sequential electron transfer from P680 to a pheophytin, a primary plastoquinone acceptor, Q_A, and then to a secondary plastoquinone acceptor, Q_B. Re-reduction of $P680^+$ is generally associated with stepwise oxidation of water in the oxygen-evolving complex (OEC) of PSII. After the absorption of a second photon, Q_B becomes doubly reduced and two protons are transferred from the surrounding solution to Q_B^{2-}. The dihydroquinone leaves the reaction center and is replaced by a quinone from a quinone pool in the membrane.

In the reaction cycle described above electrons are transferred across the thylakoid membrane dielectrics. As a result, protons are transferred into the membrane in the opposite direction. When these events have components perpendicular to the membrane surface, they give rise to voltage changes, defined as electrogenic events. The electrogenicities associated with electron-transfer reactions in PSII reaction centers have been measured in PSII-containing membranes using the light-gradient method (Witt and Zickler, 1973; Fowler and Kok, 1974; Gräber and Trissl, 1981; Trissl et al., 1987; Leibl et al., 1989; Trissl and Leibl, 1989) and recently also using oriented PSII membranes (Pokorny et al., 1994). In this study we have reconstituted PSII core complexes in phospholipid monolayers adsorbed to a Teflon support (Trissl et al., 1977; Feher and Okamura, 1984). This allowed measurements of light-induced electrogenic events in PSII reaction centers on the time scale of ~100 ns to ~1 s. These events are compared to those measured in reaction centers from the photosynthetic bacterium *Rhodobacter sphaeroides* R-26 (see, e.g., Feher and Okamura, 1984; Gopher et al., 1985; Brzezinski et al., 1993; Packham et al., 1982; Apell et al., 1983; Drachev et al., 1989). These two systems have many structural and functional similarities (Michel and Deisenhofer, 1988), and because a high-resolution three-dimensional structure of the bacterial reaction center is available (reviewed by Feher et al., 1989), important structural and functional information about PSII can be obtained from a comparison of light-induced electrogenic events measured in these two systems.

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Abbreviations used: PSII, Photosystem II; OEC, oxygen-evolving complex; P680, primary donor of PSII; Y_Z, electron donor to P680; P, primary donor of bacterial reaction centers; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCBQ, 2,6-dichloro-*p*-benzoquinone; DPQ, decyl-plastoquinone; UQ-10, ubiquinone-50; CAPS, 3-(cyclohexylamino) propanesulfonic acid; CHES, 2-(cyclohexylaminoethanesulfonic acid); HEPES, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid.

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MATERIALS AND METHODS

Sample preparation

PSII core complexes were prepared from spinach grown under controlled conditions, harvested just before preparation. Oxygen-evolving PSII particles were prepared as described (Franzén et al., 1985). The PSII core complexes were purified from these particles as described (Ghanotakis and Yocum, 1986; Ghanotakis et al., 1987) with dodecyl β-D-maltoside (Sigma Chemical Co., St. Louis, MO) as a solubilizing detergent. The rate of oxygen evolution was 400 μmol·(mg chlorophyll)$^{-1}$·h^{-1} measured with excess DCBQ. Core complexes and the PSII particles were stored under liquid nitrogen until used.

Reaction centers from *Rhodobacter sphaeroides* R-26 were purified as described (Feher and Okamura, 1978) with lauryl dimethylamine N-oxide (LDAO, Calbiochem, La Jolla, CA) as a solubilizing detergent. Cytochrome *c* (horse heart type VI, Sigma) was more than 95% reduced with hydrogen gas, using platinum black (Aldrich, Milwaukee, WI) as a catalyst (Rosen and Pecht, 1976).

To prepare lipid vesicles, soybean lecithin (Sigma), washed as described in (Kagawa and Racker, 1971), was dispersed to 10 mg/ml in the buffer solution composed of 2.5 mM sodium citrate, 2.5 mM PIPES, 2.5 mM HEPES, 2.5 mM CHES, and 2.5 mM CAPS (all buffers except sodium citrate were from Calbiochem-Behring) at pH 6.0 or 8.0, supplemented with 10 mM KCl. The buffer mixture allowed measurements at different pH values without changing the buffer conditions. The lipid solution (1 ml) was vortexed for ~5 min and then sonicated (Bronson Sonicator, model B-12, CT) for ~10 min until the solution was optically clear. The vesicle solution was supplemented with PSII core complexes at 50 μM or bacterial reaction centers at 100 μM to a final concentration of 1.5 μM and then sonicated for ~1 min.

To prepare vesicles with excess decyl-plastoquinone (DPQ, Sigma) or ubiquinone (UQ-10, Sigma), lipids and quinones were dried together from a hexane solution before dispersion in the above solution. 2,6-dichloro-*p*-benzoquinone (DCBQ, Pfaltz & Baur, Germany) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, Sigma) were dissolved in ethanol before use. All other chemicals were of the purest grade available.

Electrical measurements

The experimental set-up is shown schematically in Fig. 1. It was similar to the one described previously (Trissl et al., 1977; Feher and Okamura, 1984; Gopher et al., 1985; Brzezinski et al., 1993). Two aqueous compartments of a Teflon cell were separated by a 12 μm Teflon film (Saunders, Los Angeles, CA). The vesicle solution was diluted 1:10, supplemented with $CaCl_2$ to 10 mM, and added to one of the cell compartments to a level just below the Teflon film. In the presence of Ca^{2+} the vesicles break and a monolayer is formed at the surface (Schindler, 1980). The liquid level was raised slowly past the Teflon film allowing the monolayer to attach to the film. The other cell compartment contained an electrically conducting solution (the buffer solution above). In all experiments 10 mM $CaCl_2$ was present in both cell compartments.

Light-induced voltage changes were measured across Ag/AgCl electrodes immersed in the cell compartments. Care was exercised to prevent the actinic light from illuminating the electrodes. Voltage changes were measured using an operational amplifier (OP128, Burr-Brown or LF356, National Semiconductors), further amplified using a preamplifier with a variable time constant (Stanford Research Systems, model SR560, Stanford, CA) and recorded on a digital oscilloscope (Nicolet, model 490, Madison, WI). Time constants of the light-induced changes (1/*e*) were determined using a Nelder-Mead simplex algorithm in the Matlab software (MathWorks, S. Natick, MA) and using a program based on the Levenberg-Marquardt algorithm, written by Dr. Örjan Hansson in this Department.

The light-induced voltage changes were found to be stable for about 3 h after monolayer preparation. The temperature was 22 ± 1°C in all experiments.

The orientation of bacterial reaction centers in the lipid layer was determined using a cytochrome *c*-based assay (Schönfeld et al., 1979) and was found to be ~14% (corresponding to a distribution of 57/43%) with the largest number of reaction centers with the donor facing the aqueous solution. We were not able to determine the orientation of PSII reaction centers. The sign of the charge-separation voltage (see below) showed that the majority of PSII reaction centers were oriented with P680 facing the aqueous solution.

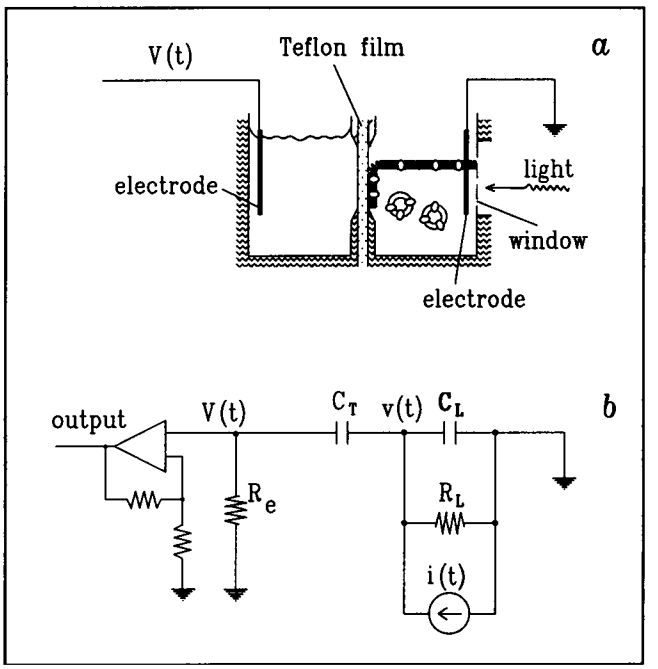

FIGURE 1 (*a*) Experimental set-up. The Teflon chamber was assembled of two compartments separated by a ~12 μm Teflon film. The lipid monolayer containing reaction centers was illuminated through a window as indicated (illuminated area was ~0.3 cm^2). (*b*) Equivalent electrical circuit for the experimental set-up. The light-induced displacement current in the reaction centers is $i(t)$; $R_L \cong 10^8\ \Omega$ and $C_L \cong 0.5\ \mu F$ are the electrical characteristics of the lipid layer (values estimated from those of a lipid bilayer; see, e.g., Montal and Mueller, 1972). The Teflon film capacitance, C_T, was ~50 pF. R_e prevented C_T from charging by leak currents from the amplifier. Typically, R_e was 10^9–$10^{10}\ \Omega$ so that the discharge time constant of C_T, R_eC_T, was 50–500 ms. $v(t)$ is the voltage induced across the interfacial layer, and $V(t)$ is the measured voltage. Under these conditions $V(t) \cong v(t)$.

Light source

Actinic illumination was provided by an Nd-YAG pulsed laser (Quantel, model YG570, Orsay, France) at 532 nm with a pulse width of ~10 ns and energy variable in the range 10–200 mJ or a flash-lamp pumped dye laser (Phase-R, model 2100A, New Durham, NH) using Rhodamine 101 (Lambda Physik, Goettingen, Germany) at 610 nm with a pulse with of ~0.5 μs and energy of ~0.5 J. Care was exercised to adjust the light energy so that the flashes saturated all reaction centers.

THEORETICAL MODELS

Voltage changes

The measured voltage change, ΔV_{jk}, associated with charge transfer from a donor *j* to an acceptor *k*, across the lipid layer is given by the total charge transferred, divided by the capacitance of the protein-lipid layer (first factor in Eq. 1 below). If the charge is transferred within the layer, a distance

that is smaller than the thickness of the layer, ΔV_{jk} must be weighted with the distance component perpendicular to the layer surface. In addition, the measured voltage must be weighted with the dielectric constant of the protein medium in which the reaction takes place:

$$\Delta V_{jk} = \frac{Nq_{jk}}{C_L} \cdot \frac{\vec{d}_{jk} \cdot \vec{n}/\epsilon_{jk}}{D_L/\epsilon_L} = \Delta V_0 \frac{d_{jk}}{\epsilon_{jk}} \frac{\epsilon_L}{D_L} \quad (1)$$

where N is the number of reaction centers in the lipid layer, q_{jk} is the charge transferred in each reaction center, $\vec{d}_{jk}$ is the charge-transfer distance vector, $\vec{n}$ is a unity vector perpendicular to the lipid layer surface ($d_{jk} \equiv \vec{d}_{jk} \cdot \vec{n}$), ϵ_{jk} is the effective protein dielectric constant in a region around the donor j and acceptor k, and ΔV_0 is the voltage associated with the transfer of one charge across the entire layer in each reaction center. C_L and ϵ_L are the capacitance and the average dielectric constant of the monolayer, respectively (cf. legend of Fig. 1). D_L is the thickness of the reaction center lipid monolayer. Note that D_L must be chosen so that the maximal voltage change associated with a charge transferred across the entire layer, i.e., a distance $d_{jk} = D_L$, is ΔV_0.

Charge stabilization

After a light-induced electron transfer from a donor D to an acceptor A in the reaction centers, $DA \rightarrow D^+A^-$, charge stabilization (indicated by the index "s") competes with charge recombination:[1]

$$DA \underset{k_{-1}}{\overset{h\nu}{\rightleftarrows}} D^+A^- \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} (D^+A^-)_s. \quad (2)$$

Before the first flash all reaction centers are in state DA at a concentration of $[DA]_0$. Immediately after a flash all reaction centers in state DA shift to D^+A^-. Assuming that

$$k_{-2} \ll k_2, k_{-1} \quad (3)$$

at time $t \ll 1/k_{-2}$ after a light flash, the concentration of reaction centers in state DA, $[DA](t)$ is

$$[DA](t) = [DA]_0 \frac{k_{-1}}{k_2 + k_{-1}} (1 - e^{-(k_2 + k_{-1})t}). \quad (4)$$

In the context of this scheme we define the fraction, α, of reaction centers that have recombined at time τ after a flash

$$\frac{1}{k_2 + k_{-1}} \ll \tau \ll \frac{1}{k_{-2}}, \quad (5)$$

$$\alpha = \frac{k_{-1}}{k_2 + k_{-1}}. \quad (6)$$

[1] In state $(D^+A^-)_s$ the donor and acceptor, respectively, may be different than in state D^+A^-. For simplicity, only one donor and acceptor are indicated in Eq. 2. For example, if a secondary donor, D_2, and acceptor, A_2, are present, $(D^+A^-)_s$ corresponds to $D_2^+DAA_2^-$.

Assuming that the time between two flashes is much shorter than $1/k_{-2}$, the concentration of reaction centers in the state DA at the nth flash is

$$[DA]_n = [DA]_1 \alpha^{n-1} \qquad n \in 1, 2, 3, \cdots. \quad (7)$$

Assuming the measured charge-separation voltage on the nth flash, $\Delta V_{DA,n}$, to be proportional to the concentration of reaction centers in state DA

$$\Delta V_{DA,n} = \Delta V_{DA,1} \alpha^{n-1} \qquad n \in 1, 2, 3, \cdots. \quad (8)$$

RESULTS

Fig. 2 *a* shows voltage changes after pulsed illumination of PSII core complexes reconstituted in a lipid monolayer. Immediately after the flash the voltage increased due to charge separation with a time constant <100 ns. The sign of this voltage change was consistent with a negative charge moving in the direction from the aqueous solution towards the Teflon film, which shows that PSII reaction centers were preferentially oriented with P680 facing the aqueous compartment. The initial voltage increase was followed by a 14 ± 2% decrease (decay) with a time constant of 500 ± 100 μs and a slower decrease with a time constant ≥0.5 s. The latter was

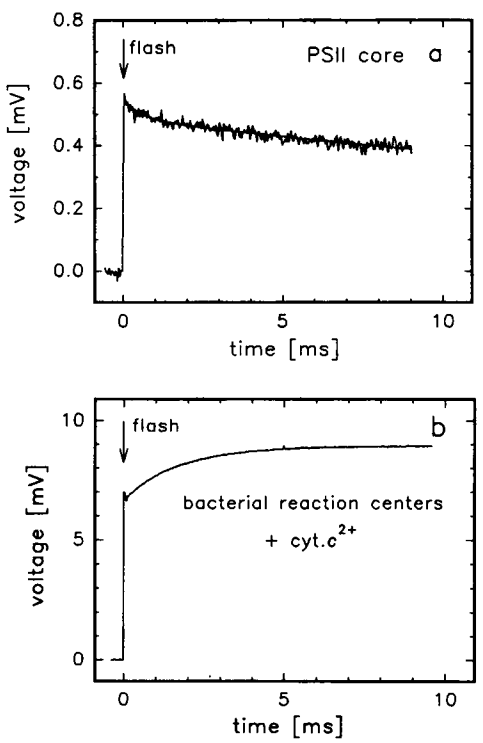

FIGURE 2 Voltage changes after pulsed illumination of PSII and bacterial reaction centers incorporated in phospholipid monolayers. (*a*) PSII reaction centers. In this experiment, R_e was 10^9 Ω, which yields a decay time constant of the experimental set-up, R_eC_T, of ~50 ms (see Fig. 1 legend). With $R_e = 10^{11}$ Ω, this time constant was ~0.5 s. (*b*) Bacterial reaction centers in the presence of excess cytochrome *c* (30 μM). The charge-separation voltage measured in the bacterial reaction centers was corrected (multiplied by 6.7) for the partial orientation of reaction centers in the layer (see Materials and Methods).

determined by the discharge time constant of the experimental set-up (see legend of Fig. 1), which shows that the charge-separated state was stabilized for at least 0.5 s.

Fig. 2 *b* shows light-induced voltage changes in bacterial reaction centers in the presence of excess cytochrome c^{2+} in the aqueous solution. The cytochrome was added to provide an external electron donor to P^+, analogous to the OEC in PSII reaction centers. The observed voltage changes were similar to those observed in PSII but with an approximately 10 times larger charge-separation voltage. An increase in voltage with a time constant of <100 ns, associated with charge separation, was followed by a 9 ± 2% decay with a time constant of 200 ± 50 μs, previously ascribed to a structural change (Brzezinski et al., 1993). It was followed by a slower further increase in voltage associated with electron transfer from cytochrome c^{2+} to P^+. Its time constant increased with decreasing cytochrome *c* concentration.

Upon pulsed illumination of the PSII reaction centers with several consecutive laser flashes the voltage change associated with charge separation decreased to reach a charge-separation voltage 10–20% of that on the first flash after ~10 flashes (Fig. 3). The voltage decrease was independent of the time between flashes in the range 1–30 s. The original charge-separation voltage was restored when the sample was left in the dark for at least 30 min. After that time the voltage behavior after an illumination sequence was the same as described above.

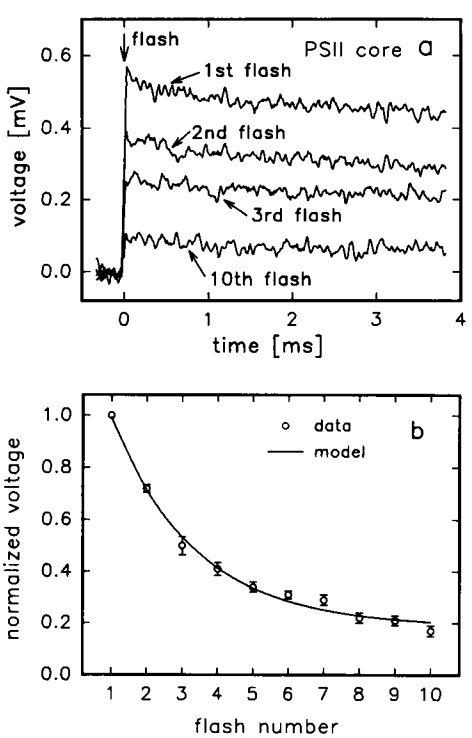

FIGURE 3 (*a*) Voltage changes in PSII reaction centers after consecutive laser flashes (~2 s between flashes). (*b*) Charge-separation voltage (at $t \cong 0$) in *a* as a function of the flash number. Voltage changes are normalized to the charge-separation voltage on the first flash. The error bars represent the SDs of the mean based on two to five experiments. Time between flashes was 1 or 10 s.

Addition of 75 μM ferricyanide to the cell compartment containing PSII reaction centers immediately after an illumination sequence restored ~60% of the original charge-separation voltage (not shown), and this voltage was constant during illumination with several flashes (10–30 s between flashes). This shows that ferricyanide was able to accept electrons from PSII reaction centers although the acceptor side was preferentially oriented towards the Teflon film. Assuming that ferricyanide cannot penetrate the lipid monolayer, this implies that electrons were transferred through the reaction center lipid layer to reach ferricyanide in solution. In addition, it shows that before addition of ferricyanide, reducing equivalents were accumulated in the reaction centers, which impeded further charge separation.

Similarly, after an illumination sequence upon addition of excess DCBQ (200 quinones per reaction center), which binds in the Q_B site, ~75% of the original charge-separation voltage was restored (Fig. 4), and this voltage was constant during illumination with several flashes. This shows that DCBQ was able to accept electrons from Q_A^- in the Teflon-bound layer of core complexes. In the presence of excess quinone the amplitude of the 500 μs voltage change was 20 ± 2% of the charge-separation voltage (Fig. 4), slightly larger than without excess quinone. After further addition of 100 μM DCMU, which competes with quinones for the Q_B site, the same behavior was observed as before addition of excess quinone; the charge-separation voltage decreased after each consecutive flash (cf. Fig. 3).

When measured in the presence of excess DPQ in the lipid phase (200 quinones per reaction center) the charge-separation voltage remained at the same level for ~5 consecutive flashes. During further illumination the charge-separation voltage decreased similarly to the decrease observed without exogenous quinone. This shows that the amount of DPQ in the lipid membrane accessible to the PSII reaction centers corresponded to 2–3 quinones per reaction center.

To identify possible electrogenic events associated with transitions in the OEC, we compared the voltage changes measured with excess DCBQ after four consecutive flashes.

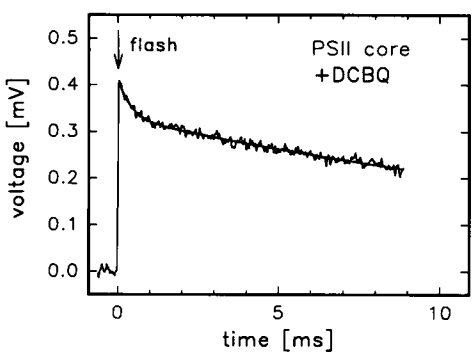

FIGURE 4 Voltage change in PSII reaction centers after addition of excess DCBQ (200 quinones per reaction center) after an illumination sequence of 10 flashes as shown in Fig. 3.

We did not observe any differences or periodicity consistent with electron transfer from OEC to $P680^+$.

Electrogenic events associated with $Q_A^-Q_B^- + 2H^+ \rightarrow Q_AQ_BH_2$ in bacterial reaction centers were studied after two consecutive laser flashes (cf. Feher and Okamura, 1984; Brzezinski et al., 1991). As shown in Fig. 2 *b* the initial voltage change after the first flash associated with the charge separation was followed by a slower increase in voltage associated with the reduction of P^+ by cytochrome c^{2+}. Thus, the final state of the reaction centers was $PQ_AQ_B^-$. After a second flash, 10 s after the first, a rapid increase in voltage associated with the charge separation was followed by a slower increase in voltage associated with proton uptake by Q_B^{2-} followed by the reduction of P^+ by cytochrome c^{2+}. To calculate the voltage changes associated with the proton-uptake reaction alone, the voltage changes after the first flash were subtracted from the voltage changes after the second flash. The difference is shown in Fig. 5 *b*: it was ~15% of the charge-separation voltage, consistent with previous observations (e.g., Feher and Okamura, 1984; Drachev et al., 1989).

Similarly, the electrogenicity associated with proton uptake by Q_B^{2-} in PSII reaction centers was measured after two consecutive laser flashes (10 s between flashes) in dark-adapted reaction centers in the presence of excess quinone. Fig. 5 *a* shows the voltage change on the second flash minus that on the first flash. This voltage was typically ~5% of the charge-separation voltage.

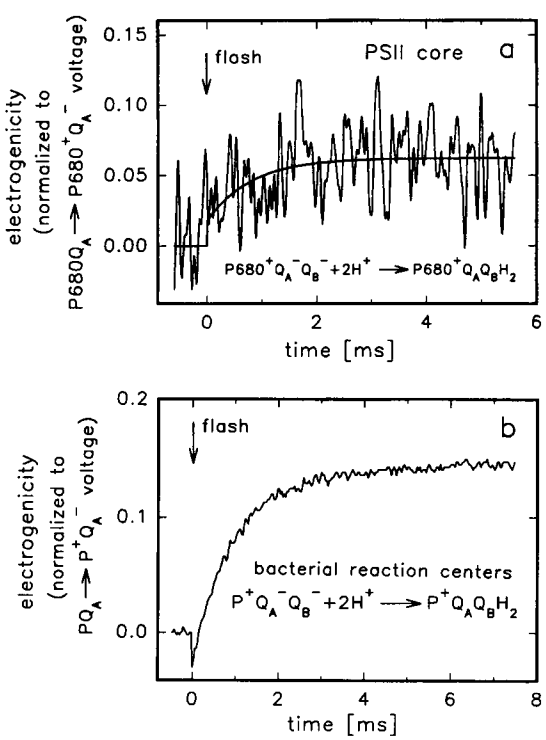

FIGURE 5 Voltage difference second minus first flash in dark-adapted reaction centers. Voltage changes are normalized to the charge-separation voltage. (*a*) PSII reaction centers with 200 DCBQ per reaction center. (*b*) bacterial reaction centers with 50 UQ-10 per reaction center.

DISCUSSION

We have measured flash-induced voltage changes in PSII core complexes oriented in phospholipid monolayers. These changes were compared with those measured in bacterial reaction centers from *Rb. sphaeroides* R-26. The charge-separation voltage in PSII reaction centers was approximately 10 times smaller than that observed in the bacterial reaction centers (Fig. 2). Assuming a similar distance for the charge separation and a similar dielectric constant (see Theoretical Models) in these two systems, the difference may be due to either a smaller degree of orientation in PSII reaction centers or a smaller number of active reaction centers in the membrane. Because the PSII complexes are larger and associated with an antenna complex, the density of PSII reaction centers in the membrane was most likely smaller than that of bacterial reaction centers.

In PSII reaction centers the charge-separation voltage decreased after each consecutive flash (see Fig. 3). This decrease may be explained in terms of accumulation of a stable (>30 s, the longest time between two consecutive flashes) photochemically inactive charge-separated state (see Theoretical Models, Eq. 2); after each flash a fraction of reaction centers remains in the charge-separated state, whereas the remaining fraction (α in Eqs. 6–8) recombines and can undergo charge separation on the next flash. From a fit of the data in Fig. 3 *b* to Eq. 8, α was estimated to be 0.65 ± 0.04. This model explains phenomenologically the observed behavior but does not explain the molecular mechanism. One possible such mechanism is that after each flash, in a fraction of reaction centers, the electron on Q_A^- is stabilized against charge recombination by a structural change.

Upon addition of excess DCBQ to PSII reaction centers subjected to an illumination sequence, i.e., with Q_A reduced, ~75% of the original charge-separation voltage was restored. The smaller amplitude compared to that on the first flash of the illumination sequence may be due to a fraction of reaction centers with Q_A still reduced after addition of excess DCBQ due to an equilibrium between states $Q_A^-Q_B$ and $Q_AQ_B^-$ or a fraction of reaction centers lacking DCBQ in the Q_B site.

After addition of DCMU to PSII reaction centers in the presence of excess exogenous quinones, a decreasing charge-separation voltage was observed after several consecutive flashes, similar to that observed in the absence of exogenous quinones. This indicates that DCMU was able to displace DCBQ from the Q_B site. Previously, DCMU at 5 μM was found to have a small effect on the PSII core preparation used (Ghanotakis et al., 1987). The altered effect of DCMU in our experiments may be due to the larger concentration used or to the presence of a lipid membrane around the PSII core complex, which accentuates the activity of DCMU compared to that of DCBQ.

In bacterial reaction centers the electron transfer $Q_A^-Q_B \rightarrow Q_AQ_B^-$ was found not to be electrogenic (Feher and Okamura, 1984; Drachev et al., 1989). The ~10% voltage decay with a time constant of ~200 μs, the same as that of

the electron transfer, after charge separation was attributed to a structural change that precedes electron transfer from Q_A^- to Q_B and thereby determines the time constant of this electron transfer (Brzezinski et al., 1993). In PSII reaction centers a similar decay was found to follow charge separation. The amplitude was ~15% of the charge-separation voltage, and the time constant was ~500 μs. The sign of this decay was not consistent with electron transfer from the OEC to P680, assuming that the OEC is closer than P680 to the lumen membrane surface. The time constant of the decay was similar to that of the charge recombination $P680^+Q_A^- \rightarrow P680Q_A$ as well as to that of the electron transfer $Q_A^-Q_B \rightarrow Q_AQ_B^-$ (see Thompson et al., 1988). Because the decay amplitude increased from ~15 to ~20% when measured with an excess of DCBQ, i.e., with Q_B present, it is unlikely that the decay in PSII was associated with charge recombination. Because the decay was observed also in the absence of excess quinone and with DCMU present, it cannot be associated with electron transfer from Q_A^- to Q_B. Consequently, it is quite possible that the 500-μs decay in PSII reaction centers was due to a structural change similar to that observed in bacterial reaction centers. In PSII reaction centers such a structural change may not only control electron-transfer rates but could also trigger other structural changes such as those associated with photoinhibition (reviewed by Aro et al., 1993).

The increase in the decay amplitude from ~15 to ~20% of the charge-separation voltage after addition of DCBQ indicates that a part of the decay (~5% of the charge-separation electrogenicity) may be contributed by the electron transfer $Q_A^-Q_B \rightarrow Q_AQ_B^-$. This would imply that during the electron transfer Q_B is slightly further away from the stroma membrane surface than Q_A.

In PSII reaction centers the voltage change associated with $Q_A^-Q_B^- + 2H^+ \rightarrow Q_AQ_BH_2$ was ~5% of the charge-separation voltage, whereas in the bacterial reaction centers this voltage was ~15% of the charge-separation voltage (see Fig. 5). According to Eq. 1, the ratio of the proton-transfer voltage and the charge separation voltage is

$$\frac{\Delta V_{SQ_B}}{\Delta V_{PQ_A}} = \frac{q_{SQ_B}}{q_{PQ_A}} \frac{d_{SQ_B}/\epsilon_{SQ_B}}{d_{PQ_A}/\epsilon_{PQ_A}} \qquad (9)$$

where the index S indicates the cytoplasmic surface of the bacterial reaction center. Note that D_L does not enter Eq. 9. In *Rb. sphaeroides* reaction centers d_{PQ_A} is ~25 Å and the shortest distance from the reaction center surface to Q_B, d_{SQ_B}, is ~10 Å (Allen et al., 1987a, b). With $\Delta V_{SQ_B} \cong 0.15 V_{PQ_A}$ and $q_{SQ_B} = 2$ (two protons), $q_{PQ_A} = 1$ (one electron), $\epsilon_{SQ_B}/\epsilon_{PQ_A} \cong 6$. Assuming the dielectric constant within the membrane-spanning part of the reaction center protein, ϵ_{PQ_A}, to be ~4, ϵ_{SQ_B} becomes ~24. This value is consistent with a large number of charged amino-acid residues in the vicinity of the Q_B site (see Okamura and Feher, 1992) and with dielectric constants found in some water-soluble proteins (see Warshel et al., 1984). In the bacterial reaction centers there is no channel leading all the way from the surface to the Q_B site. Instead, protons are transferred ~10 Å through a chain of protonable amino-acid residues leading from a cavity near Asp-L210 to the Q_B site (Okamura and Feher, 1992). The smaller electrogenicity associated with proton transfer in PSII compared to the bacterial reaction centers corresponds to a dielectrically weighted distance (defined as d/ϵ) between the Q_B site and the reaction center surface at least 3 times smaller than in the bacterial reaction centers. For example, assuming the same distance for the proton transfer in PSII as in the bacterial reaction centers, the measured voltage would yield a dielectric constant in the Q_B region of the PSII reaction centers of ~70, which is close to that of water. This situation may prevail if, for example, the proton-transfer pathway from solution to the Q_B site includes a water channel (c.f. Blubaugh and Govindjee, 1988).

Trissl et al. (1987) originally reported that electron transfer from the OEC to $P680^+$ was not electrogenic. Recently, using a new set-up with a larger signal-to-noise ratio, the same research group found that electron transfer from Y_Z to $P680^+$ has an electrogenicity of ~16% of the charge-separation electrogenicity (Pokorny et al., 1994). The time constant of our experimental set-up did not allow measurements of the electron transfer from Y_Z to $P680^+$. Electron transfer from the OEC to Y_Z^+ takes place on the time scale of our set-up, but we did not observe any electrogenicity associated with this reaction. However, it is difficult to draw any definitive conclusions from this because the oxygen-evolution measurements (see Materials and Methods) suggest that reaction centers with inactive OEC are present in the layer. In these reaction centers electrons can be transferred to $P680^+$ from other electron donors (see Thompson et al., 1988).

As discussed above, in the PSII reaction centers, proton transfer to Q_B^{2-} was associated with an electrogenicity of ~5% of the charge-separation voltage. Consequently, together with the results of Trissl et al. (1987) and Pokorny et al. (1994), this shows that in PSII reaction centers the charge-separation voltage was ~85% of the total electrogenicity that contributed to the creation of an electrical potential across the PSII reaction center membrane after pulsed illumination. In bacterial reaction centers this number was ~70% and the remaining electrogenicity was produced by electron transfer from cytochrome c^{2+} to the oxidized donor, P^+, and by proton transfer from solution to Q_B^{2-}. This shows that the dielectrically weighted distances between P680 and the surface, and between the quinones and the surface, are smaller in PSII than in the bacterial reaction centers. This may be due to either smaller distances or larger dielectric constants in PSII than in the bacterial reaction centers, for example, due to water channels or a more hydrophilic protein medium.

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