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Electrogenicity at the Donor/Acceptor Sides of Cyanobacterial Photosystem I

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To study electrogenesis the photosystem I particles from *Synechococcus elongatus* were incorporated into asolectin liposomes, and fast kinetics of laser flash-induced electric potential difference generation has been measured by a direct electrometric method in proteoliposomes adsorbed on a phospholipid-impregnated collodion film. The photoelectric response has been found to involve three electrogenic stages associated with (i) iron-sulfur center F_X reduction by the primary electron donor P700, (ii) electron transfer between iron-sulfur centers F_X and F_A/F_B, and (iii) reduction of photo-oxidized $P700^+$ by reduced cytochrome c_{553}. The relative magnitudes of phases (ii) and (iii) comprised about 20% of phase (i).

KEY WORDS: Photosystem I; proteoliposomes; electrogenicity; electron transfer; cytochrome c_{553}; iron-sulfur centers; *Synechococcus elongatus*.

INTRODUCTION

The photosystem I (PS I) complex of cyanobacteria is an integral membrane pigment–protein complex, consisting of at least 12 individual polypeptides (for review, see Golbeck and Bryant, 1991; Chitnis and Nelson, 1991; Setif, 1992; Bryant, 1992). The primary electron donor P700 and early electron acceptors A_0 (a monomeric chlorophyll), A_1 (vitamin K_1, a phylloquinone), and F_X (iron-sulfur center, 4Fe-4S) are coordinated by two related polypeptides of approximately 83 kDa while F_A and F_B (iron-sulfur centers, 4 Fe-4S) reside on the polypeptide of 9 kDa.

PS I catalyzes the light-dependent electron transfer from reduced plastocyanin or cytochrome c_{553} to ferredoxin. Following flash excitation of reaction center complexes, an electron is transferred from P700 to A_1 via A_0. Subsequent charge stabilization is achieved by electron transfer from A_1 to F_A and F_B through the F_X (Brettel, 1988; Luneberg *et al.*, 1995; Vassiliev *et al.*, 1995). Additional electron transfer events involve reactions associated with the reduction of $NADP^+$ through a soluble ferredoxin and ferredoxin:$NADP^+$ oxidoreductase (Schluchter and Bryant, 1992). Re-reduction of the photo-oxidized $P700^+$ requires transfer of electrons from reduced plastocyanin or cytochrome c_{553}.

Although the electron transfer sequence in the PS I complex has been clarified somewhat of late, the mechanism whereby electron transport gives rise to a transmembrane electric potential difference is not yet clear.

Our knowledge of electrogenic events within the PS I complex is essentially derived from photovoltage (Hecks *et al.*, 1994; Sigfridsson *et al.*, 1995; Mamedov *et al.*, 1995; Leibl *et al.*, 1995), flash-induced carotenoid electrochromic bandshift (Schliephake *et al.*, 1968), as well as electroluminescence (Vos and van Gorkom, 1988, 1990) measurements. According to electroluminescence data (Vos and van Gorkom, 1988), the primary charge separation $P700\ A_0 \rightarrow P700^+A_0^-$ as well as subsequent electron transport to F_A and F_B

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are electrogenic. It has been concluded that P700 A_0 spans 30%, A_0 A_1, 50%, and A_1 F_A, 20% of the membrane dielectric (Vos and van Gorkom, 1990). The results obtained in PS I membrane preparation of *Synechocystis sp. PCC*6803 demonstrate a two-step primary charge separation connected with $P700^+$ A_0^- (22 ± 4 ps) and $P700^+$ A_1^- (50 ± 15 ps) formation (Hecks *et al.*, 1994). Recently, a further electrogenic reaction occurring on a nanosecond time scale was measured in oriented PS I membranes (Leibl *et al.*, 1995) and it was concluded that electron transfer from A_1 to F_X is electrogenic and it is the rate-limiting process for reduction of F_A and F_B. According to these authors, the time constant for electron transfer from F_X to F_A/F_B is faster than 220 ns. The electrogenic nature of all steps in the electron transfer from P700 to the most remote Fe-S center was reported by flash-induced voltage changes in PS I particles from spinach, oriented in a phospholipid monolayer (Sigfridsson *et al.*, 1995).

The purpose of the present work was to investigate the electron transfer reactions at the donor and acceptor sides in photosystem I complexes of *Synechococcus elongatus* by a direct electrometric method.

MATERIALS AND METHODS

PS I particles from *Synechococcus elongatus* were prepared as described earlier (Bottin and Setif, 1991) and stored in liquid nitrogen until used. The chlorophyll a/P700 ratio was determined from the maximal absorption change of $P700^+$, using the differential molar absorption coefficients of 64 mM^{-1} cm^{-1} (Hiyama and Ke, 1972). The preparation contains 110 chlorophyll molecules per P700.

To prepare lipid vesicles, soybean lecithin (type II-S, Sigma) was dispersed to 10 mg/ml in a buffer solution of 20 mM Tris/HCl, pH 8.1, supplemented with 2 mM $MgCl_2$. The lipid solution was vortexed for 1 min and then sonicated in a UZDN-2T ultrasonic disintegrator for 5 min (50 mA, 22 kHz) under argon until the solution was optically clear. The vesicle solution was supplemented with photosystem I particles at lipid/chlorophyll ratio 80:1 (w/w) and sonicated for 1 min. All the procedures were carried out at 4°C.

The accessibility of the cytochrome c_{553} binding site of the PSI from the external aqueous phase can be exploited for an orientation assay. Flash-induced oxidation of the primary electron donor, P700, will be followed by re-reduction either from cytochrome c_{553} or, when this electron donor is not available, from the terminal iron-sulfur clusters, F_A/F_B (data not shown). These two modes of re-reduction display kinetic differences. Therefore, a kinetic analysis of the rate of re-reduction of P700 allows a quantitation of the fraction of the PSI to which reduced cytochrome c_{553} is available as a reductant. The results obtained suggest that about 80% of the PSI have their cytochrome c_{553} binding site accessible to the external aqueous phase.

Cytochrome c_{553} was extracted from the cyanobacterium *Synechococcus elongatus* by a minor modifications of the procedure as described previously (Ho *et al.*, 1979).

Flash-induced absorbance measurements at 700 nm were performed using a home-made single-beam spectrophotometer (Drachev *et al.*, 1989).

Measurements of transmembrane electric potential difference ($\Delta\psi$) generation by PS I-containing proteoliposomes were done and the kinetic data were processed as in Drachev *et al.* (1989). The proteoliposome suspension was added to one of the two compartments of a Teflon cuvette filled with buffer. A collodion film impregnated with 10% (w/v) asolectin in *n*-decane was used as a partition between the compartments. Association of vesicles with the collodion film was achieved upon 1–1.5 h incubation in the presence of 20 mM $CaCl_2$ at room temperature. Subsequently both compartments were washed with a tenfold volume of the same buffer to remove excess proteoliposomes and $CaCl_2$. The electric potential difference generated across the collodion film was measured with two light-protected Ag/AgCl electrodes connected via an operational amplifier (Burr-Brown 3554 BM) with a transient recorder DL-1080 linked to an IBM computer. The instrument time constant was 200 ns.

For measurements of the redox potential, a platinum electrode and Ag/AgCl reference electrode (saturated KCl) were used.

Saturating light flashes were delivered from a Quantel Nd laser operated in doubled frequency mode (λ = 530 nm; pulse half-width, 15 ns; 40 mJ output).

All the measurements were carried out at 24°C.

RESULTS AND DISCUSSION

Reduction of the Photooxidized Primary Electron Donor P700

Figure 1A shows typical kinetics of the flash-induced absorption changes at 700 nm obtained in a

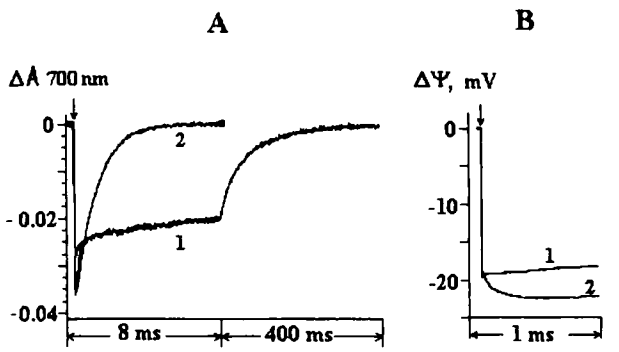

Fig. 1. (A) Flash-induced absorption changes in *Synechococcus elongatus* photosystem I particles at 700 nm in the absence (trace 1) and in the presence of 2 μM cytochrome c_{553} (trace 2). All measurements were performed at 5 μg Chl/ml in 20 mM HEPES/NaOH buffer (pH 7.4) with 0.03% β-dodecylmaltoside and 2 mM sodium ascorbate. Traces are averages of 16 experiments. (B) Flash-induced photoelectric responses of proteoliposomes with *Synechococcus elongatus* photosystem I particles, adsorbed onto the phospholipid-impregnated collodion film in the absence (trace 1) and in the presence of 2 μM cytochrome c_{553} (trace 2). The reaction mixture contained 20 mM HEPES/NaOH (pH 7.4) and 2 mM sodium ascorbate. Arrows here and further indicate laser flashes.

detergent/PS I dispersion. Addition of hexacynoferrate (III) abolishes about 90% of the signal, as expected from signals originating from PS I. In the absence of an added electron acceptor or a rapid donor for $P700^+$, the major part of the flash-oxidized $P700^+$ decay has a characteristic time (τ) of 60 ms (trace 1). This is interpreted as charge recombination between $P700^+$ and the iron-sulfur centers F_A/F_B (Rutherford and Heathcote, 1985). Subsequent addition of reduced cytochrome c_{553} causes acceleration of the flash-induced absorption change decay (curve 2). Evidently, cytochrome c_{553}^{2+} rapidly reduces photo-oxidized $P700^+$ and hence prevents discharge of $P700^+$ $(F_A/F_B)^-$. Under these conditions, the decay kinetics becomes clearly biphasic. A rapid initial phase ($\tau \sim 30$ μs) is followed by a second slower process with $\tau \sim 700$ μs.

Figure 1B demonstrates the typical laser flash-induced photoelectric response of proteoliposomes with *Synechococcus elongatus* PS I particles associated with the phospholipid-impregnated collodion film. The flash generates $\Delta\psi$ which proved to be too fast to be measured with our technique (time resolution of 200 ns). The sign of the photoelectric response corresponds to negative charging of the interior of the vesicles. Thus the PS I particles incorporated into liposomes provide an attractive system for studying the electrogenicity due to electron transfer between cytochrome c_{553}^{2+} and $P700^+$. In particular, this reaction is difficult to investigate in the thylakoid membranes of *Synechococcus elongatus* which lose most of the endogenous cytochrome c_{553} during the isolation procedure.

In the absence of exogenous reductants, the decay of the photoelectric response is dominated by a phase with $\tau \sim 50$ ms closely corresponding to the $(F_AF_B)^- \rightarrow P700^+$ back reaction (not shown). This τ value is similar to those determined by flash-absorption measurements in PS I particles in solution (Fig. 1A, trace 1).

As one can see from Fig. 1B (trace 2), cytochrome c_{553}^{2+} affects the kinetics of the $\Delta\psi$ generation. In addition to the very fast stage of the photoelectric response, a slower phase appears on a submillisecond time scale. Oxidized cytochrome c_{553} did not elicit such an effect (not shown). This new phase of $\Delta\psi$ generation can be assigned to electron transfer from cytochrome c_{553}^{2+} to photo-oxidized $P700^+$ in the reaction centers of photosystem I.

Analysis of the kinetics of $\Delta\psi$ generation shows that the amplitude of the additional phase of the photoelectric response comprises up to approximately 20% of the amplitude of the fast response. As to the kinetics of the additional electrogenic phase, it is approximated well by two exponents with τ values of 25 and 200 μs and relative amplitudes of 40 and 60%, respectively.

We have no explanation for this, other than the possibility that two cytochrome molecules can be simultaneously bound to the reaction center complex, the distant one replacing or re-reducing the close one after it has been oxidized (Mathis, 1985). The multiphasic reduction kinetics of $P700^+$ by cytochrome c_{553}^{2+} in cyanobacterial PS I has been reported earlier (Hiayama and Ke, 1972; Bengis and Nelson, 1977; Nanba and Katoh, 1983; Hervas *et al.*, 1992; Hatanaka *et al.*, 1993; Xu *et al.*, 1994).

In general, the mechanism of electron transfer from the donor proteins to photo-oxidized $P700^+$ is still controversial. Under steady-state and fast kinetic conditions, the electrostatic nature of the process, the specific role played by divalent cations, the effect of pH (Lien and San Pietro, 1979; Tamura *et al.*, 1983a,b; Hervas *et al.*, 1992), the effect of peripheral antenna polypeptides (Takabe *et al.*, 1989), as well as the effect of lipid compositions (Ishikawa *et al.*, 1984) have clearly been shown.

Note that the kinetics of the slow phase of $P700^+$ rereduction by cytochrome c_{553}^{2+} was different in purified PS I preparations solubilized in detergent suspensions and in proteoliposomes. The difference in the kinetics of electron transfer from cytochrome c_{553}^{2+} to

$P700^+$ can be explained by the modification of the PS I complexes in a detergent/PS I dispersion and will have to be studied in detail.

Figure 2 shows the results of redox titration of the fast phase of the photoelectric response of photosystem I incorporated into liposomes. It is evident that the amplitude of the $\Delta\psi$ falls under oxidizing conditions. The experimental points fit the theoretical Nernst curve for a one-electron transfer with midpoint potential E_m = + 460 mV, which agrees with the value of E_m for $P700/P700^+$ (Evans and Nugent, 1993).

Electron Transfer on the Acceptor Side of PS I

An important question concerning the functioning of PS I reaction center complexes is the elucidation of the nature of the reactions associated with the electron transfer between F_X and F_A/F_B, which are difficult to study by absorption spectroscopy due to the similarity of their optical spectra.

Note that it is difficult to obtain a preparation that keeps all F_X when F_A and F_B are quantitatively removed. The fact is that all biochemical procedures bear the risk of leading to heterogeneity of the sample in terms of acceptor population (Leibl *et al.*, 1995).

In contrast to higher plants, F_A and F_B of the cyanobacteria are chemically reduced with difficulty in the dark at an alkaline pH (Evans and Heathcote, 1980). In order to reduce completely the terminal iron-sulfur centers, it was necessary to illuminate the samples in the presence of the reductant at pH 10 (Sonoike *et al.*, 1990).

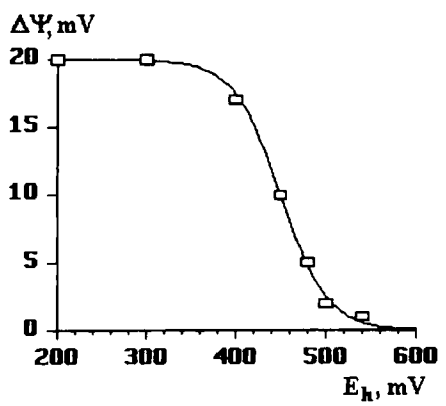

Fig. 2. Redox titration of the photoelectric responses of the photosystem I-containing proteoliposomes. The redox titrations were carried out in the presence of 50 μM N,N,N,N-tetramethyl-*p*-phenylenediamine, 2,3,5,6-tetramethyl-*p*-phenylenediamine and phenazine methosulfate. Hexacyanoferrate (III) was used as an oxidant in the titration.

Laser-induced absorption changes of P700 in PS I particles under strongly reducing conditions without (trace 1) and with (trace 2) background illumination are shown in Fig. 3A. The acceleration of decay observed after pre-reduction of both F_A and F_B was generally ascribed to a recombination reaction between $P700^+$ and F_X^- (Zhao *et al.*, 1992).

Figure 3B shows the photoelectric responses of PS I particles incorporated into liposomes adsorbed on the surface of the phospholipid-impregnated collodion film under strongly reducing conditions without (trace 1) and with (trace 2) background illumination. The decrease in amplitude of photoelectric response (trace 2) is most probably due to prevention of electron transfer between F_X and F_A/F_B and suggests that electron transfer between iron-sulfur centers F_X and F_A/F_B is electrogenic. The amplitude of this phase comprises approximately 20% of the fast phase ascribed to formation of $P700^+F_X^-$. Unfortunately, the time constant of our experimental setup did not allow measurements of the real kinetics of the electron transfer between F_X and F_A/F_B. However, the data obtained suggest that electron transfer between iron-sulfur centers of PSI of *Synechococcus elongatus* takes place in the submicrosecond time range, which is not consistent with the data obtained in PS I particles from spinach (Sigfridsson *et al.*, 1995).

Thus, isolated PS I particles reconstituted in phospholipid vesicles proved to be a useful model for studying $\Delta\psi$ generation by these pigment–protein complexes, and the data presented here clearly demonstrate that there are at least three electrogenic reactions: (i) charge separation between the primary electron donor P700 and iron-sulfur center F_X, (ii) electron transfer between F_X and F_A/F_B, and (iii) re-reduction of photo-oxidized $P700^+$ by cytochrome c_{553}^{2+}. This conclusion is generally in line with the X-ray structure of PS I from thermophilic cyanobacteria *Synechococcus sp.* (Krauss *et al.*, 1993).

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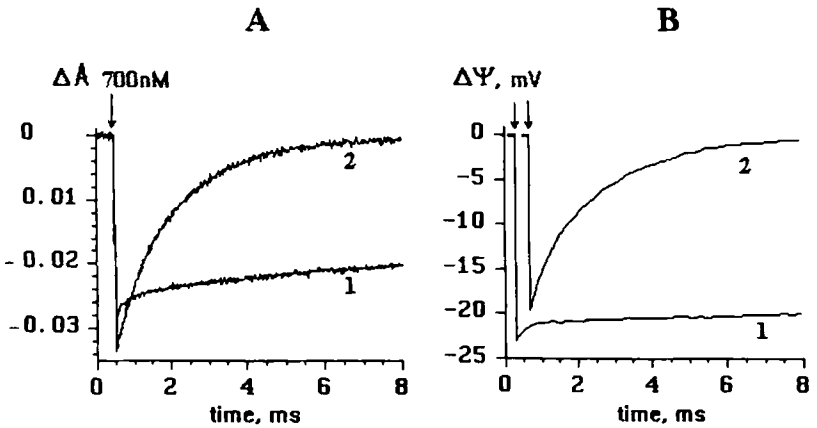

Fig. 3. (A) Flash-induced absorption changes in *Synechococcus elongatus* photosystem I particles at 700 nm under strongly reducing conditions without (trace 1) and with (trace 2) illumination with white light for 2 min at room temperature. The reaction mixture contained 200 mM Glycine (pH 10.0), 10 mM NaCl, 0.5 mM benzyl viologen, and 40 mM sodium dithionite. (B) Flash-induced photoelectric responses of proteoliposomes with *Synechococcus elongatus* photosystem I particles, adsorbed onto the phospholipid-impregnated collodion film under strongly reducing conditions without (trace 1) and with (trace 2) background illumination. Conditions as in Fig. 3A.

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