Electrogenicity accompanies photoreduction of the iron-sulfur clusters F\textsubscript{A} and F\textsubscript{B} in photosystem I

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Abstract  Photovoltage responses accompanying electron transfer on the acceptor side of photosystem I (PS I) were investigated in proteoliposomes containing PS I complexes from the cyanobacterium \textit{Synechococcus} sp. PCC 6301 using a direct electrometical technique. The relative contributions of the F\textsubscript{X}→F\textsubscript{B} and the F\textsubscript{X}→F\textsubscript{A} electron transfer reactions to the overall electrogeneracy were elucidated by comparing the sodium dithionite-induced decrease in the magnitude of the total photoelectric responses in control and in F\textsubscript{B}-less (HgCl\textsubscript{2}-treated) PS I complexes. The results obtained suggest that the electronegeness on the acceptor side of PS I is related to electron transfers between both F\textsubscript{X} and F\textsubscript{A} and F\textsubscript{B}. Based on the electrogenenic nature of the latter reaction in PS I complexes, we conclude that F\textsubscript{A} rather than F\textsubscript{B} is the acceptor proximal to F\textsubscript{X}.

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Key words: Proteoliposome; Photosystem I; Photoelectric response; Electrogenicity; \textit{Synechococcus} sp. PCC 6301

1. Introduction

Photosystem I (PS I) catalyzes the reduction of soluble ferredoxin or flavodoxin and the oxidation of plastocyanin or cytochrome \textit{c} \textsubscript{6} by a multi-step electron transfer process initiated by light. The initial step involves charge separation between the primary donor P700, a chlorophyll \textit{a} dimer, and the primary acceptor A\textsubscript{0}, a chlorophyll \textit{a} monomer. This step is followed by subsequent electron transfer through \textit{A} \textsubscript{1} (phylloquinone), and three iron-sulfur clusters, F\textsubscript{X}, F\textsubscript{A} and F\textsubscript{B}. P700, A\textsubscript{0}, A\textsubscript{1} and F\textsubscript{X} are bound to the PsaA/PsaB heterodimer, while F\textsubscript{A} and F\textsubscript{B} are located on the stromal PsaC subunit. The kinetics of forward electron transfer from P700 to A\textsubscript{0} takes place in a few picoseconds and further electron transfer from A\textsubscript{1} takes place in times shorter than 50 ps. The electron is then transferred from A\textsubscript{1} to F\textsubscript{X} and in the following step to F\textsubscript{A}/F\textsubscript{B} in the submicrosecond time range. One of the most challenging questions regarding the arrangement of the electron cofactors within the PS I complex concerns the sequence of electron transfer between the two terminal iron-sulfur clusters, F\textsubscript{A} and F\textsubscript{B} (for discussion see [1]). One promising strategy used to approach the acceptor sequence problem involves chemical extraction of one of the clusters, F\textsubscript{B} [2–6]. Our recent results based on single-turnover laser flash excitation to promote step-by-step electron transfer in control and F\textsubscript{B}-less PS I complexes in the presence of the efficient electron donor for P700\textsuperscript{+}, phenazine methosulfate, also argue for the assignment of F\textsubscript{B} as the F\textsubscript{X}-distal cluster [5]. The same conclusion was reached by analysis of the reduction kinetics of ferredoxin externally added to control and F\textsubscript{B}-less PS I complexes [6].

Electron transfer from P700 to the terminal iron-sulfur clusters F\textsubscript{A}/F\textsubscript{B} is electrogenic [7–10]. Given that the vector between the two terminal clusters is tilted from the membrane normal [11], electrogenicity developed from electron transfer between these clusters is expected. However, the relative magnitudes of the electrogenic steps accompanying electron transfer between F\textsubscript{X} and F\textsubscript{A}, and between F\textsubscript{A} and F\textsubscript{B} are not yet estimated. The goal of the present work is to investigate the photovoltage changes due to electron transfer between the clusters F\textsubscript{X}, F\textsubscript{A} and F\textsubscript{B} in control and in F\textsubscript{B}-less (HgCl\textsubscript{2}-treated) PS I complexes. We use this information in an attempt to provide insight into the sequence of electron transfer between the terminal acceptors F\textsubscript{A} and F\textsubscript{B}.

2. Materials and methods

Soybean lecithin (type IIS), Tris, sodium cholate, 2,6-dichlorophenol-indophenol (DCPIP), sodium ascorbate, sodium dithionite, potassium ferricyanide, CaCl\textsubscript{2} and Sephadex G-50 were purchased from Sigma (St. Louis, MO, USA). Other reagents were commercial products of the highest purity available. PS I complexes were prepared from \textit{Synechococcus} sp. PCC 6301 as described in [12], and extraction of the F\textsubscript{B} cluster followed the protocol described in [4]. Proteoliposomes with PS I complexes were prepared as described in [13]. Transmembrane electric potential difference (ΔΨ) measurements in PS I-containing proteoliposomes were performed in anaerobic conditions as described in [9]. The instrument rise time was 200 ns. Saturating light flashes were provided by a frequency doubled Quantel Nd:YAG laser (λ= 532 nm; pulse half-width, 15 ns; flash energy, 40 mJ). Multiexponential analysis of the photoelectric response kinetics was performed using Igor Pro v. 3.11 (WaveMetrics, Inc., Lake Oswego, OR, USA).

3. Results

Flash excitation of PS I complexes incorporated into liposomes adsorbed on the surface of a phospholipid-impregnated collodion film leads to a ΔΨ formation corresponding to the negative charging of the interior of the proteoliposomes (Fig. 1). The ΔΨ amplitude is proportional to the sum of the dielectrically weighted distances between the electron carriers in the PS I complex. Although ΔΨ is developed within a time shorter than the instrument-limited response time constant of 200 ns, the initial amplitude of ΔΨ can be determined based on extrapolation of the ΔΨ decay kinetics. The main decay
pathway of the $\Delta \Psi$ built across the membrane is the charge recombination between P700$^+$ and the photoreduced terminal acceptor, but in a fraction of complexes this pathway may be substituted by a slower passive discharge. This may occur as a result of the interaction of P700$^+$ with an artificial donor or due to oxidation of the terminal acceptor (for instance, by a trace amount of oxygen present in the medium).

Taking into account that $F_A$ can be selectively extracted by HgCl$_2$ treatment, we consider two possible sequences of the iron-sulfur clusters: $F_A$ is proximal to $F_X$ and $F_B$ is proximal to $F_X$. (1) If $F_A$ is the terminal cluster, the amplitude of the electrogenic response should be the same for the control and HgCl$_2$-treated samples. Under these conditions, we assume that $F_A$ is capable of photoreduction in the absence of $F_B$. (2) If $F_B$ is the terminal cluster, the amplitude of the electrogenic response should be lower in the HgCl$_2$-treated sample. The ability of $F_A$ to be reduced in either instance follows from the tens of milliseconds decay kinetics of P700$^+$ reduction in HgCl$_2$-treated PS I complexes [5,14]. The amplitudes of the photoelectric responses can be compared for different samples only if the number of PS I complexes per unit membrane surface is constant between the PS I samples. This is a difficult condition to meet in the estimation of a relatively small amplitude change. To overcome this limitation, we normalized the $\Delta \Psi$ amplitudes corresponding to the intrinsic charge recombination in the PS I complexes by using the $\Delta \Psi$ amplitudes obtained after chemical reduction of the PsaC-bound cluster(s), $F_A$ and $F_B$. Based on the room temperature kinetics of P700 absorbance change, the amount of dithionite used (100 mM) was found to be sufficient to chemically reduce $F_A$ and $F_B$ without any apparent reduction of $F_X$ in the isolated PS I complexes (not shown). Thus, all $\Delta \Psi$ amplitudes are normalized to the dithionite-independent amplitude of the P700 to $F_X$ charge separation.

In the presence of a slow electron donor, DCPIP, the kinetics of $\Delta \Psi$ decay can be decomposed into five exponents with lifetimes ($\tau$) of 7.9 $\mu$s, 415 $\mu$s, 3.5 ms, 31 ms, 138 ms and 924 ms (Fig. 2, left). Components with $\tau$ values of 31 ms (21.3% of amplitude) and 138 ms (31.5%) correspond to the charge recombination between P700$^+$ and ($F_A/F_B^{-}$), while the 924 ms component (20.2%) corresponds to a passive discharge across the membrane. The minor components with $\tau$ $=$ 415 $\mu$s and 3.5 ms correspond to back reactions from $F_X^-$ in a fraction of centers with impaired forward electron transfer to $F_A$ and $F_B$. The lifetime values derived from the photovoltage measurements are in good agreement with optical spectroscopic measurements of the kinetics of P700$^+$ reduction in the near IR [5,14]. The fast decay phases with the lifetimes of ca. 5–10 $\mu$s and 70–200 $\mu$s and variable amplitudes could be observed in most of measurements. Although these lifetimes correspond to the lifetimes of $A_1^-$ back-derived from optical data [14,15], we cannot rigorously exclude that the 5–10 $\mu$s decay phase is due to some electrical artifact [14]. To determine the initial amplitude of $\Delta \Psi$ we took into account only those charge separation components with lifetimes longer than 400 $\mu$s, i.e. those related to electron transfer at distances greater than that between P700 and $A_1$. This excluded contributions from centers with impaired electron transfer between $A_1$ and $F_X$ and the possible above mentioned electrical artifact.

Addition of 100 mM dithionite to the electrometric cell compartment containing one and the same sample leads to the expected acceleration of the $\Delta \Psi$ decay kinetics, which now is due to the backreaction of $F_X^-$. Multi-exponential analysis of the kinetics yield components with lifetimes of 11 $\mu$s, 97 $\mu$s, 963 $\mu$s, 5.1 ms and 18 ms and relative contributions of 13.6, 8.2, 46.1 and 10.3%, respectively.

The deconvolution shows a decrease in the amplitude of the photoelectric response in the presence of dithionite, which occurs due to blockage of electron transfer between $F_X$ and $F_A/F_B$ (Fig. 2, left). This alone indicates that electron transfer between the iron-sulfur clusters $F_A$ and $F_B$ is electrogenic. Although the time constant of our experimental setup did not allow measurement of the forward kinetics of electron transfer between $F_X$ and $F_A/F_B$, our data is consistent with electron transfer occurring between the iron-sulfur centers in PS I in the submicrosecond time range.

Treatment of cyanobacterial PS I complexes with HgCl$_2$ results in $>95\%$ destruction of the $F_B$ iron-sulfur cluster and in the retention of $>90\%$ of the $F_A$ [5]. For the HgCl$_2$-treated samples we performed identical multi-exponen-
Fig. 2. Decay kinetics of the flash-induced photoelectric responses in proteoliposomes containing control and F$_B$-less PS I complexes in the absence (top curves) and in the presence of 100 mM sodium dithionite. The pH value after incubation in the presence of dithionite was controlled before registration. Experimental conditions as in Fig. 1. Dashed curves indicate the multieponential data fits; solid lines are fit curves with the fast components ($t < 400$ ms) being subtracted. Horizontal lines indicate the initial amplitudes of the photoelectric responses. The kinetics are normalized to the initial amplitudes obtained after subtraction of the fast ($t < 400$ ms) components.

4. Discussion

Although the F$_A$ and F$_B$ clusters were among the first acceptors to be discovered in PS I, the issue of the sequence of electron transfer among them is still controversial. Although time-resolved optical spectroscopy can in principle resolve the rise times of the acceptors, the difference spectra of F$_A$, F$_B$ and F$_X$ are nearly identical, with a broad and relatively weak bleaching due to S$\rightarrow$Fe charge transfer bands centered around 430 nm [15]. Conversely, F$_A$, F$_B$ and F$_X$ can be distinguished by low-temperature EPR spectroscopy, but the instrument response time is insufficient to determine rates of electron transfer between the iron-sulfur clusters. Thus application of photovoltage measurements, which are indicative of directionality of electron transport in membrane-oriented complexes, is a promising approach to resolve the sequence of electron transfer among the iron-sulfur clusters in PS I.

Photovoltage measurements have been applied in several laboratories in an attempt to resolve the forward electron transfer kinetics in PS I. Sigfridsson et al. [8] resolved several kinetic phases in the flash-induced $\Delta \Psi$ increase of spinach PS I particles oriented in a phospholipid layer adsorbed on Teflon film. The kinetic phase with time constant of 30 ms was ascribed to electron transfer from F$_{550}$ to F$_A$, while the 200 ms phase was assigned to electron transfer out of PS I, a proton transfer in the opposite direction, or a conformational change. Leibl et al. [7] studied the flash-induced kinetics of $\Delta \Psi$ generation of oriented PS I membranes from Synechocystis sp. PCC 6803 which formed a multilayer on a platinum electrode. Besides an unresolved rapidly rising phase, an additional positive electrogenic phase was observed with a time constant of 220 ns. Assuming that the reduction of F$_X$ is the rate limiting process for the reduction of F$_A$/F$_B$, this phase can be attributed to electron transfer from A$_{700}$ via F$_X$ to F$_{550}$. It is likely that the different photovoltage kinetics reported by Leibl et al. [7] and by Sigfridsson et al. [8] reflect different capabilities of methods used in these laboratories. However, one cannot also exclude the possibility of differences between PS I complexes prepared from higher plants and cyanobacteria. Our experimental results are more compatible with those obtained by Leibl et al. [7] than by Sigfridsson et al. [8], since we did not observe any additional phases of $\Delta \Psi$ increase in the microsecond time scale. A comparison of the dithionite-induced decrease of the magnitude of the total photoelectric

<table>
<thead>
<tr>
<th>Sample</th>
<th>Membrane potential ratio $\Psi_{X\Delta}/\Psi_{FX}$</th>
<th>Average $\Psi_{X\Delta}/\Psi_{FX}$</th>
<th>Distance ratio $D_{X\Delta}/D_{FX}$</th>
<th>Dielectric constant ratio $\varepsilon_{X\Delta}/\varepsilon_{FX}$</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.19 ± 0.01</td>
<td>0.42</td>
<td>2.19</td>
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<tr>
<td>2</td>
<td>0.18</td>
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The ratio between the dielectric constant values corresponding to electron transfer from F$_X$ to F$_A$ and from P700 to F$_X$ yields the following equation: $\varepsilon_{X\Delta}/\varepsilon_{FX} = (D_{X\Delta}/D_{FX})/(1/L_{X\Delta}+1); (\Psi_{X\Delta}/\Psi_{FX}) = 0.42:1.01:0.19 = 2.19$, where $D_{X\Delta}$ is the projection for the F$_X$ $\rightarrow$ F$_A$ distance vector to the membrane normal; $\Psi_{X\Delta}$ is the photovoltage dropped between F$_X$ and F$_A$.  

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responses obtained with control and HgCl$_2$-treated PS I complexes provides an independent approach for elucidation of the relative contribution of F$_X$→F$_B$ and F$_X$→F$_A$ electron transfer reactions to the overall electrogenesis within the PS I complex.

Given the close midpoint potentials of F$_A$ and F$_B$ as well as the high (>10$^3$ s$^{-1}$) electron exchange rate between the clusters (as inferred from broadening of $^3$H NMR resonances of partially reduced clusters in unbound PsAc [16]), it is reasonable to assume that the $\Delta\psi$ amplitude in the native PS I complex corresponds to the dipole with the negative charge located between F$_A$ and F$_B$ rather than on F$_B$ itself. Based on the analysis of kinetics of P700$^+$ dark relaxation, the microscopic equilibrium constants between F$_X$ and F$_A$ as well as between F$_A$ and F$_B$ were recently estimated as 70 and 0.5–1.15, respectively (V.P. Shinkarev, I.R. Vassiliev and J.H. Golbeck, in preparation). Based on the known X-ray structure of PS I complex [11], the sum of projections of the center-to-center vectors from P700 to F$_X$ to the membrane normal is equal to 33 Å, the projection for the F$_X$→F$_A$ vector is 14 Å, while that for the F$_X$→F$_B$ vector is 20 Å. We can recalculate the photovoltage amplitudes as normalized to the amplitude of the P700 to F$_X$ electron transfer derived in the presence of dithionite. This will yield values of 1, 0.41 and 0.19 relative units for the P700→F$_X$, F$_X$→F$_B$ and F$_X$→F$_A$ components of $\Delta\psi$, respectively (see Table 1 and 2). Note that in the recent paper by Diaz-Quintana et al. [6], the corresponding $\Delta\psi$ values were estimated as 1, 0.24 and 0.23 relative units, respectively. Thus the contribution of F$_A$ component ascribed to F$_X$→F$_A$ electron transfer is close to our value, while that for F$_X$→F$_B$ is significantly lower. The reason for this discrepancy is not clear; however, there are differences in the PS I preparations, in the technique used to measure the electrogenic changes, and in the criterion used to normalize the $\Delta\psi$.

The ratio between the dielectric $\varepsilon_{F_{X}A}/\varepsilon_{F_{X}B}$ is equal to 1.47 (see Table 1), while that for electron transfer from F$_X$ to F$_A$ and from P700 to F$_X$ constants corresponding to electron transfer from F$_X$ to F$_B$ and from P700 to F$_X$ ($\varepsilon_{F_{X}A}/\varepsilon_{F_{X}B}$) is 2.19 (Table 2). The lower $\varepsilon_{F_{X}A}/\varepsilon_{F_{X}B}$ value for the whole F$_X$→F$_B$ region compared to $\varepsilon_{F_{X}A}/\varepsilon_{F_{X}B}$ for the F$_X$→F$_A$ region assumes that the average effective dielectric constant value in the F$_X$→F$_B$ region is lower relative to the F$_X$→F$_A$ region. This result seems to be somewhat surprising, since it is not in line with the general three-phase model for the membrane proteins [17]. This model is mainly based on the comparison of the relative magnitudes of the electrogenic steps [18] and the distances between the corresponding cofactors in Rhodopseudomonas viridis reaction centers [19]. Accordingly, the redox centers more remote from the hydrophobic inner part of membrane protein are located in the protein region with the dielectric constant similar or higher to that of the inner part of the membrane. There are several notable exceptions to this general rule. For instance, the primary (Q$_A$) and the secondary (Q$_B$) quinone acceptors in the photosynthetic bacterial reaction centers are located at the same distance from the border of the protein-pigment complex [20,21]. However, Q$_B$ is surrounded by more charged amino acid residues and water molecules than Q$_A$, which leads to a higher dielectric constant in its vicinity compared to Q$_A$ [22,23]. The substantially higher effective dielectric constants were suggested in the vicinity of the redox cofactors of the functional branch than that for the non-functional branch of Rhodobacter sphaeroides reaction centers [24]. Taking into account these considerations, one may speculate that the observed decrease of the effective dielectric constant in the F$_X$→F$_B$ region compared to the F$_X$→F$_A$ region can be due to a more polar surrounding of the F$_X$-proximal F$_A$ compared to the F$_X$-distal F$_B$ cluster. Note also that the effective dielectric constant values are usually considered under static conditions where the variations of the electric field proceeds so slowly that protein polarization modes have enough time to respond to the changes of the field. Due to a wide range of atom and group mobilities in proteins, there exists a wide range of dielectric relaxation times, making the effective static constant dependent on the characteristic time of the process [25].

Our data therefore indicate that the voltage changes on the reducing side of PS I are related to electron transfer between F$_X$ and F$_A$ as well as between F$_A$ and F$_B$. Based on the electrogenic nature of the latter reaction in PS I complexes, we conclude that F$_B$ rather than F$_A$ is the acceptor distal to F$_X$.

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