Electrometrical study of electron transfer from the terminal F_A/F_B iron–sulfur clusters to external acceptors in photosystem I

Aygun A. Mamedova[a], Mahir D. Mamedov[a], Kira N. Gourovskaya[a], Ilya R. Vassiliev[b], John H. Golbeck[b], Alexey Yu. Semenov[a,*]

[a] *Department of Photobiochemistry, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia*
[b] *Department of Biochemistry and Molecular Biology, The Pennsylvania State University, S-310 Frear Laboratory, University Park, PA 16802-4500, USA*

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Abstract An electrometrical technique was used to investigate electron transfer between the terminal iron–sulfur centers F_A/F_B and external electron acceptors in photosystem I (PS I) complexes from the cyanobacterium *Synechococcus* sp. PCC 6301 and from spinach. The increase of the relative contribution of the slow components of the membrane potential decay kinetics in the presence of both native (ferredoxin, flavodoxin) and artificial (methyl viologen) electron acceptors indicate the effective interaction between the terminal [4Fe–4S] cluster and acceptors. The finding that F_A fails to donate electrons to flavodoxin in F_B-less ($HgCl_2$-treated) PS I complexes suggests that F_B is the direct electron donor to flavodoxin. The lack of additional electrogenicity under conditions of effective electron transfer from the F_B redox center to soluble acceptors indicates that this reaction is electrically silent.

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1. Introduction

Photosystem I (PS I) catalyzes the light-driven electron transfer from reduced plastocyanin or cytochrome c_6 at the lumenal side of the thylakoid membrane to ferredoxin, a [2Fe–2S] protein at the stromal side via a transmembrane chain of six spectroscopically identified electron carriers [1,2]. In prokaryotes and some eukaryotic algae under iron-deficient conditions, ferredoxin can be replaced by flavodoxin, a low molecular mass flavoprotein [3]. Excitation of the primary donor of the reaction center, P700, results in rapid electron transfer to the terminal F_A/F_B iron–sulfur centers through a series of low-potential electron acceptors, which include the primary chlorophyll acceptor A_0, a phylloquinone A_1, and the F_X, F_A and F_B iron–sulfur clusters. Photooxidized P700 is subsequently reduced by plastocyanin/cytochrome c_6, while reduced F_A/F_B is oxidized by ferredoxin/flavodoxin.

The docking site for ferredoxin/flavodoxin is not yet clearly defined. The terminal electron acceptors F_A and F_B are associated with the extrinsic stromal subunit PsaC. The PsaD subunit was proposed to be a ferredoxin binding subunit [4]. The PsaE subunit was suggested to stimulate the reduction of ferredoxin and/or flavodoxin [5–7]. Kinetic studies of a crosslinked complex of flavodoxin and PS I were compatible with a single binding site for ferredoxin/flavodoxin on the stromal side of PS I [8]. On the basis of X-ray structures of PS I from *Synechococcus elongatus* and ferredoxin from *Spirulina platensis*, a cavity formed by the stromal subunits was proposed to form the ferredoxin binding site [2]. This model is also supported by electron microscopic analyses of crosslinked PS I–ferredoxin [9] and PS I–flavodoxin [8] complexes.

Recent results suggest that F_B is the distal cluster to F_X and serves as an immediate electron donor to ferredoxin/flavodoxin [10–13]. We have demonstrated recently that photoreduction of both F_A and F_B is electrogenic [12], but the electrogenic properties of further electron transfer to soluble acceptors have not been studied. Since the shortest distance from the terminal center F_B to the protein surface was estimated to be ca. 12 Å [2], the intraprotein electron transfer from F_B to soluble electron acceptors may contribute to the overall electrogenesis, as was shown for intraprotein electrogenic reduction of the photooxidized primary donor P700 by water-soluble secondary electron donors [14]. In this work we make an attempt to examine the forward electron transfer from the F_A/F_B centers to flavodoxin, ferredoxin and methyl viologen (MV). Our goal is to gain insight into the nature of this reaction in PS I complexes incorporated into phospholipid vesicles using a direct electrometrical technique.

2. Materials and methods

Soybean lecithin (type IIS), Tris, sodium cholate, 2,6-dichlorophenolindophenol (DCPIP), sodium ascorbate, MV, $CaCl_2$ 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 *Synechococcus* sp. PCC 6301 as described in [15], and extraction of the F_B cluster followed the protocol described in [16]. Preparation of PS I complexes from spinach was carried out as in [17]. Ferredoxin and flavodoxin were purified as described in [18]. Reconstitution of PS I complexes into phospholipid vesicles was described previously [14]. For preparation of proteoliposomes with the PS I complex from *Synechococcus* sp. PCC 6301 with soluble acceptors inside the vesicles, the solution containing 20 mM Tris–HCl, pH 7.8 and 2% sodium cholate was supplemented with

*Corresponding author. Fax: (7)-095-939 0338.
E-mail: semenov@electro.genebee.msu.su

Abbreviations: PS I, photosystem I; F_X, F_A, F_B, iron–sulfur clusters of photosystem I; P700, primary donor of photosystem I; MV, methyl viologen; DCPIP, 2,6-dichlorophenolindophenol; $\Delta\Psi$, transmembrane electric potential difference; τ, characteristic time constant

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certain concentrations of flavodoxin or MV. Transmembrane electric potential difference ($\Delta\Psi$) measurements were performed as described in [19]. The instrument rise time was 200 ns. Saturating light flashes were provided by a frequency-doubled Quantel Nd:YAG laser ($\lambda = 532$ 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 and discussion

In the absence of soluble electron acceptors, charge separation in PS I is followed by a back reaction from one of the bound acceptors. The back reaction is suppressed by addition of an electron acceptor that can withdraw an electron from the reduced terminal electron acceptor more rapidly than does $P700^+$ [20]. Thus, the reactivity of reduced F_A/F_B with flavodoxin/ferredoxin can be evaluated by measuring the degree of inhibition of the back reaction in the presence of various concentrations of the acceptor protein.

As was previously shown, flash excitation of *Synechococcus* sp. PCC 6301 control (intact) PS I complexes induced the formation of $\Delta\Psi$ corresponding to the negative charging of the interior of the proteoliposomes [12]. Formation of $\Delta\Psi$ developed within a time shorter than the instrument-limited response time constant of 200 ns. Fig. 1 (trace 1) shows the kinetics of $\Delta\Psi$ decay on a logarithmic time scale in the absence of soluble acceptors. The main component with a lifetime (τ) of 67 ms corresponds to the back reaction between $(F_A/F_B)^-$ and $P700^+$, while the faster components are likely to correspond to back electron transfer from the earlier acceptors [12,21]. The slowest phase ($\tau = 409$ ms, 22% of the overall amplitude) is attributed to the passive discharge across the membrane (see Table 1) as a result of the oxidation of the terminal acceptor by oxygen in the medium.

The photoelectric response derived from proteoliposomes containing control PS I in the presence of 30 μM flavodoxin entrapped in phospholipid vesicles is shown in Fig. 1 (trace 2). Note that slow components with lifetimes of 234 ms and 1742 ms, corresponding to the passive discharge across the membrane, contribute about 60% to the overall photoresponse amplitude (Table 1). The increase in the contribution of the slow components, compared to the photoresponse in the absence of flavodoxin, reflects the amount of PS I transferring electrons to flavodoxin.

The effect of the artificial electron acceptor MV on the kinetics of the $\Delta\Psi$ decay in the control PS I complexes is shown in Fig. 1 (trace 3). As in the case of added flavodoxin, the slower phases of the $\Delta\Psi$ decay with lifetimes of 181 ms and 1645 ms (65% contribution from both phases) are observed (see Table 1). These data indicate an effective interaction between the terminal Fe–S cluster and external acceptors.

We performed an identical multiexponential analysis of the

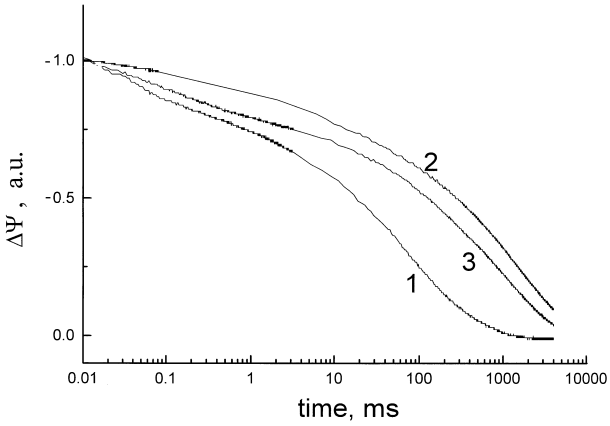

Fig. 1. Decay kinetics of the flash-induced photoelectric responses in proteoliposomes containing PS I complexes from *Synechococcus* sp. PCC 6301 in the absence (trace 1) and in the presence of 30 μM flavodoxin (trace 2) and 100 μM MV (trace 3). Incubation medium contains 25 mM Tris–HCl (pH 8.1), 10 mM sodium ascorbate and 4 μM DCPIP.

$\Delta\Psi$ decay kinetics for the F_B-less PS I samples in the absence and in the presence of added acceptors (Table 2). In the presence of 100 μM flavodoxin the increase of the slowest phase contribution was not observed. This result indicates the lack of electron transfer from PS I to flavodoxin in F_B-less PS I complexes. The finding that F_A fails to donate electrons to flavodoxin when F_B is missing confirms that F_B is the direct electron donor to flavodoxin. This finding is compatible with an orientation of PsaC with F_A as the F_X-proximal iron–sulfur center.

F_B-less PS I complexes, which had almost completely lost the ability to donate electrons to flavodoxin, could still reduce MV but only at a concentration much higher than needed to interact with the intact PS I complex. This is manifest by the presence of the 346 ms component (Table 2). A higher concentration of MV required to accept electrons from F_A could be ascribed to steric hindrance of the F_A cluster relative to the F_B site [11].

The maximal deceleration of the $\Delta\Psi$ decay was observed at 30 μM of flavodoxin and 100 μM of MV entrapped in the PS I-containing proteoliposomes. A further increase in the concentrations of soluble acceptors did not affect the kinetics of the $\Delta\Psi$ decay. Kinetic data for the reduction of ferredoxin by cyanobacterial PS I revealed three components with lifetimes of ~500 ns, 20 μs and 100 μs [22]. The kinetics of flavodoxin reduction studied in a cross-linked flavodoxin–PS I complex [8] was also fitted by three exponential components (lifetimes ~9 μs, 70 μs and 1 ms). However, besides the fast generation of a membrane potential related to electron transfer between P700 and the terminal iron–sulfur cluster F_B, no additional electrogenic phases in the microsecond-to-millisecond time do-

Table 1
Kinetic deconvolution of the $\Delta\Psi$ decay in control PS I complexes

PS I control		PS I+30 μM flavodoxin		PS I+100 μM MV	
lifetime (ms)	$\Delta\Psi$ (%)	lifetime (ms)	$\Delta\Psi$ (%)	lifetime (ms)	$\Delta\Psi$ (%)
409	22	1742	46	1645	42
67	38	234	15	181	23
4	18	33	13	18	13
0.32	9	0.29	18	0.6	10
0.04	13	0.003	7	0.06	12

Table 2
Kinetic deconvolution of the $\Delta\Psi$ decay in F_B-less PS I complexes

PS I control		PS I+100μM flavodoxin		PS I+2 mM MV	
lifetime (ms)	$\Delta\Psi$ (%)	lifetime (ms)	$\Delta\Psi$ (%)	lifetime (ms)	$\Delta\Psi$ (%)
330	42	422	33	346	76
82	36	26	46	49	19
18	14	0.04	13	0.21	5
0.150	4	0.002	9		
0.024	4				

main were detected at any concentrations of both flavodoxin and MV. The latter suggests that electron transfer between the terminal F_B cluster and flavodoxin and/or MV is electrically silent.

One disadvantage of this approach is the orientation of PS I complexes in the proteoliposome membrane. According to the negative sign of the photoelectric response, the terminal iron–sulfur acceptors F_A/F_B are located at or near the internal surface of the membrane and therefore inaccessible to externally added hydrophilic oxidants. In order to observe the interaction of PS I with soluble acceptors, it is necessary to add these acceptors in the medium for proteoliposome preparation. Thus, after formation of vesicles, the acceptors become entrapped in the internal volume of proteoliposomes. However, it cannot be excluded that a very small internal volume of vesicles makes it impossible to reach the saturating concentration of acceptors needed for fast interaction of PS I with flavodoxin and MV. Another disadvantage is the necessity to compare the kinetics of the flash-induced responses of different proteoliposome preparations (either lacking, or containing flavodoxin and MV) in separate experiments. Since the passive discharge in our measuring system is determined by the products of resistances and capacitances of both flat artificial collodion phospholipid membrane and proteoliposomal membrane, it varies to some extent for different experiments.

For further evidence of the non-electrogenic nature of electron transfer reactions between PS I complexes and soluble electron acceptors, we used proteoliposomes containing PS I complexes from spinach. Light excitation of dark-adapted PS I complexes from spinach induced the $\Delta\Psi$ generation due to charge separation between P700 and F_A/F_B and corresponding to the positive charging of the interior of the proteoliposomes. This implies the external location of terminal iron–sulfur clusters and their accessibility for hydrophilic acceptors. The latter circumstance allowed us to add both ferredoxin and MV exogenously, and to monitor the effect of these acceptors on the overall $\Delta\Psi$ kinetics in one and the same experiment.

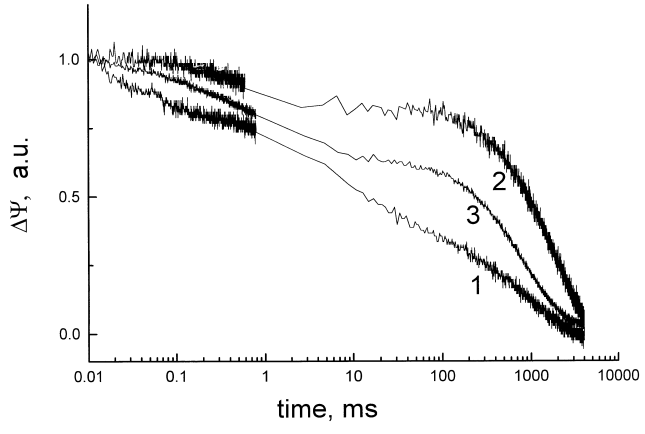

Fig. 2. Kinetics of the photoelectric responses in spinach PS I-containing proteoliposomes in the absence (trace 1) and in the presence of 30 μM ferredoxin (trace 2) and 100 μM MV (trace 3). Experimental conditions as in Fig. 1.

Fig. 2 (trace 1) shows the kinetics of $\Delta\Psi$ decay for the proteoliposomes containing spinach PS I complexes in the absence of soluble acceptors. As in cyanobacterial PS I, the decay kinetics were also complex due to the obvious heterogeneity of the sample. The main component with a τ value of $\sim$30 ms most probably corresponds to the back reaction between $(F_A/F_B)^-$ and $P700^+$, while the faster components are likely to correspond to back electron transfer from the earlier acceptors [12,21]. The slowest phase corresponding to passive discharge (τ=1100 ms) contributed $\sim$30% to the overall amplitude. Addition of MV (trace 2) resulted in a significant increase of the slow phase contribution ($\sim$60%), while the effect of ferredoxin (trace 3) was even more pronounced ($\sim$75% of the overall amplitude). Similar to the experiments with PS I complexes from cyanobacteria, neither ferredoxin nor MV at any concentrations induced the appearance of the additional increase in the membrane potential generation, indicating that in spinach PS I electron transfer between the terminal F_B cluster and ferredoxin and/or MV is electrically silent.

As we have shown earlier, the addition of the protein donor cytochrome c_6 [19] as well as artificial redox dyes, such as DCPIP, phenazine methosulfate and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine [14], resulted in a slowing of the decay kinetics and the appearance of an additional electrogenic phase due to electron transfer inside the PsaA/PsaB heterodimer. This suggests an electro-isolated location of the primary electron donor P700 inside the protein molecule.

The lack of additional electrogenesis in the kinetics of $\Delta\Psi$ generation under conditions of effective electron transfer from the terminal F_B redox center to soluble acceptors in PS I complexes from both cyanobacteria and spinach clearly indicates that this reaction is electrically silent.

According to the structural model, suggested earlier [2], the distance from the center of the distal iron–sulfur cluster (F_B) to the center of the [2Fe–2S] cluster of the ferredoxin is about 14 Å, which is suitable for rapid electron transfer [23]. However, both the location of the ferredoxin binding site and the distance between F_B and ferredoxin is only an estimate, as X-ray structural information on the orientation of ferredoxin is lacking, and also because there may be some structural differences between the ferredoxin of *Spirulina platensis* and that of *Synechococcus* sp. PCC 6301.

Since the $\Delta\Psi$ amplitude is proportional to the sum of the dielectrically weighted distances between the electron carriers, the non-electrogenic nature of the electron transfer between

F_B and soluble acceptors can be explained by the high effective dielectric constant value in the protein region between F_B and the ferredoxin/flavodoxin binding site. In this case the electron transfer between F_B and ferredoxin/flavodoxin would not result in a $\Delta\Psi$ amplitude increase, and the terminal F_B center would be localized on the surface of the PsaC protein dielectric.

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