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Electrogenic reduction of the primary electron donor $P700^+$ in photosystem I by redox dyes

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Abstract The kinetics of reduction of the photo-oxidized primary electron donor $P700^+$ by redox dyes *N,N,N′,N′*-tetramethyl-*p*-phenylendiamine, 2,6-dichlorophenol-indophenol and phenazine methosulfate was studied in proteoliposomes containing Photosystem I complexes from cyanobacteria *Synechocystis sp. PCC* 6803 using direct electrometrical technique. In the presence of high concentrations of redox dyes, the fast generation of a membrane potential related to electron transfer between P700 and the terminal iron–sulfur clusters F_A/F_B was followed by a new electrogenic phase in the millisecond time domain, which contributes approximately 20% to the overall photoelectric response. This phase is ascribed to the vectorial transfer of an electron from the redox dye to the protein-embedded chlorophyll of $P700^+$. Since the contribution of this electrogenic phase in the presence of artificial redox dyes is approximately equal to that of the phase observed earlier in the presence of cytochrome c_6, it is likely that electrogenic reduction of $P700^+$ in vivo occurs due to vectorial electron transfer within RC molecule rather than within the cytochrome c_6–P700 complex.

Key words: Redox dye; Proteoliposome; Photosystem I; Photoelectric response; Electrogenic phase; *Synechocystis sp. PCC* 6803

1. Introduction

Photosystem I (PS I) catalyzes the photo-induced oxidation of plastocyanin and/or cytochrome c_6 and the reduction of ferredoxin. PS I from cyanobacteria and green plants consists of 11–13 polypeptides and 90–100 chlorophyll molecules[1–4]. The two larger polypeptides (PsaA and PsaB) form the PS I core which binds the primary electron donor P700 (a chlorophyll α-dimer), A_0 (a chlorophyll α-monomer), A_1 (a phylloquinone) and the iron–sulfur cluster F_X. The terminal iron–sulfur clusters F_A and F_B are located on the smaller, stroma-exposed polypeptide, PsaC. All iron–sulfur clusters are of the [4Fe–4S] type.

Charge separation between P700 and A_0 is stabilized by the electron transfer through A_1, F_X and F_A/F_B. Photo-oxidized $P700^+$ is reduced by plastocyanin or cytochrome c_6. The kinetics of forward electron transfer from P700 to A_0 takes place in a few picoseconds and that of $A_0 \rightarrow A_1$ with a τ of 20–50 ps. The data on the kinetics of the charge transfer from A_1 to F_X and then to F_A/F_B are not consistent. However, it is estimated that the overall process takes approximately 200 ns [4]. A recently published 4 Å resolution X-ray structure of PS I complexes from the cyanobacterium *Synechococcus elongatus* [5] suggests that an additional chlorophyll α-monomer located near P700 at a distance comparable to that between centers of P700 dimer molecules (~7.5 Å) might take part in the electron transfer. The electron density map also shows two antenna chlorophylls, which are 13 and 15 Å distant from primary acceptors A_0 and A_1. The location of the intermediary acceptor A_1, as well as the succession of electron transfer between F_X and F_A/F_B are not yet clear.

Electron transfer from the primary donor P700 to the terminal iron–sulfur clusters F_A/F_B is electrogenic [6–11]. The generation of a transmembrane potential difference due to the reduction of $P700^+$ by cytochrome c_6 in proteoliposomes containing PS I complexes from *Synechococcus elongatus* has been observed recently [11], its characteristic times estimated as 25 and 200 μs. The model of the architecture of the helices of the PsaA and PsaB polypeptides, based on X-ray structural analysis, suggests that Mg-porphyrin rings of the primary electron donor P700 are located inside the protein dielectric at some distance from the protein surface of the PS I complex donor side. If the primary donor P700 is electro-isolated from the external water phase, then one can consider that an electrogenic character of $P700^+$ reduction may not be specific for cytochrome c_6 as the electron donor, as it is the case with the primary donor (bacteriochlorophyll dimer) in reaction center complex from the photosynthetic purple bacterium *Rhodospirillum rubrum* [12]. Our strategy has been to use different artificial donors for $P700^+$ and to study the kinetics of the modeled reactions. In the present paper we report studies of the reduction of the photo-oxidized $P700^+$ in PS I by redox dyes *N,N,N′,N′*-tetramethyl-*p*-phenylendiamine (TMPD), 2,6-dichlorophenol-indophenol (DCPIP) and phenazine methosulfate (PMS) by means of direct electrometrical technique.

2. Materials and methods

Soybean lecithin (type IIS), Tris-HCl, sodium cholate, DCPIP, PMS, TMPD, sodium ascorbate, potassium ferricyanide, $CaCl_2$ were purchased from Sigma (St. Louis, MO), Sephadex G-50 from Pharmacia. Other reagents were commercial products of the highest purity available.

PS I complexes were prepared from *Synechocystis sp. PCC* 6803 as described earlier [13] with minor modifications [14].

To prepare proteoliposomes, soybean lecithin was dispersed to 40 mg/ml in solution of 20 mM Tris-HCl buffer, pH 7.8, with 2% sodium

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cholate. The lipid solution was sonicated with UZDN-2T disintegrator (22 kHz, 40 mA) for 2 min (six 20-s steps) under argon atmosphere until the solution was optically clear. The lipid vesicle solution was supplemented with PS I complexes at a lipid/chlorophyll ratio of 100:1 (w/w) and loaded on a column of Sephadex G-50, equilibrated with 20 mM Tris-HCl buffer, pH 7.8. The eluate with proteoliposomes containing the PS I complexes was collected and used for measurements. All the procedures were carried out at 4°C. The orientation of PS I complexes in the proteoliposomes was determined as described earlier [11].

Transmembrane electric potential difference ($\Delta\psi$) measurements in PS I-containing proteoliposomes were performed and the kinetic data were measured as described elsewhere [11,15]. The proteoliposome suspension was added to one of the two compartments of a cuvette filled with buffer. PS I-containing proteoliposomes were associated with collodion film impregnated with 10% (w/v) lecithin in *n*-decane, which served as a partition between the compartments. The measuring instrument time constant was 200 ns.

Saturating light flashes were delivered from Quantel Nd:YAG laser operated in doubled frequency mode ($\lambda = 532$ nm; pulse half-width, 15 ns; flash energy, 40 mJ). Computer analysis of the photoelectric response kinetics was accomplished using program DISCRETE [16].

3. Results

Fig. 1A (curve 1) shows laser flash-induced photoelectric response of the proteoliposomes containing PS I complexes from *Synechocystis sp.* PCC 6803. The flash excitation leads to the generation of a transmembrane electric potential difference corresponding to the negative charging of the interior of the proteoliposomes. In each measurement $\Delta\psi$ was built up in less than 200 ns. As we have shown earlier [11], the observed response is due to the charge separation between P700 and F_A/F_B. According to the negative sign of the photoelectric response, we expect the primary electron donor P700 to be located at the external surface of the membrane and therefore accessible for hydrophilic reductants. Thus, accessibility of $P700^+$ from the external water phase makes the proteoliposomes containing PS I complexes a convenient system for studying the electron donation to $P700^+$.

Isolated PS I preparations lack natural electron donors to photo-oxidized P700, plastocyanin and/or cytochrome c_6. In the absence of soluble electron donors and acceptors the kinetics of dark reduction of $P700^+$ is characterized by a back reaction from one of the bound acceptors [14,17]. The decay kinetics can be well deconvoluted into four exponents with characteristic times (τ) of 125 ms (70%), 25 ms (20%), 1.1 ms (5%) and 0.1 ms (5%). Components with $\tau > 20$ ms correspond to the charge recombination between $P700^+$ and $(F_A/F_B)^-$, while the components with $\tau = 1.1$ ms and 0.1 ms must be ascribed to back reactions from F_X and A_1, respectively [14,17]. The results of the electrometrical studies correspond reasonably well to the optical spectroscopic (820 nm) data on the kinetics of $P700^+$ reduction in PS I complexes solubilized using β-dodecyl-maltoside [17].

Increasing TMPD concentration in the presence of excess ascorbate resulted in a slowing of the $\Delta\psi$ decay (Fig. 1A, curve 2) due to competition of reduced TMPD with $(F_A/F_B)^-$ for reduction of $P700^+$. At TMPD concentrations equal to or exceeding 1 mM, an additional $\Delta\psi$ rise phase is observed in the kinetics of the photoelectric response. At TMPD concentration of 4 mM, the multi-exponential fit yields a lifetime of this phase equal to 20 ms, with an amplitude comprising about 20% of that of the fast response attributed to $P700^+(F_A/F_B)^-$ charge separation (Fig. 1B). Similar results were obtained using reduced DCPIP (data not shown, see below).

Note that in the presence of all redox dyes used when added

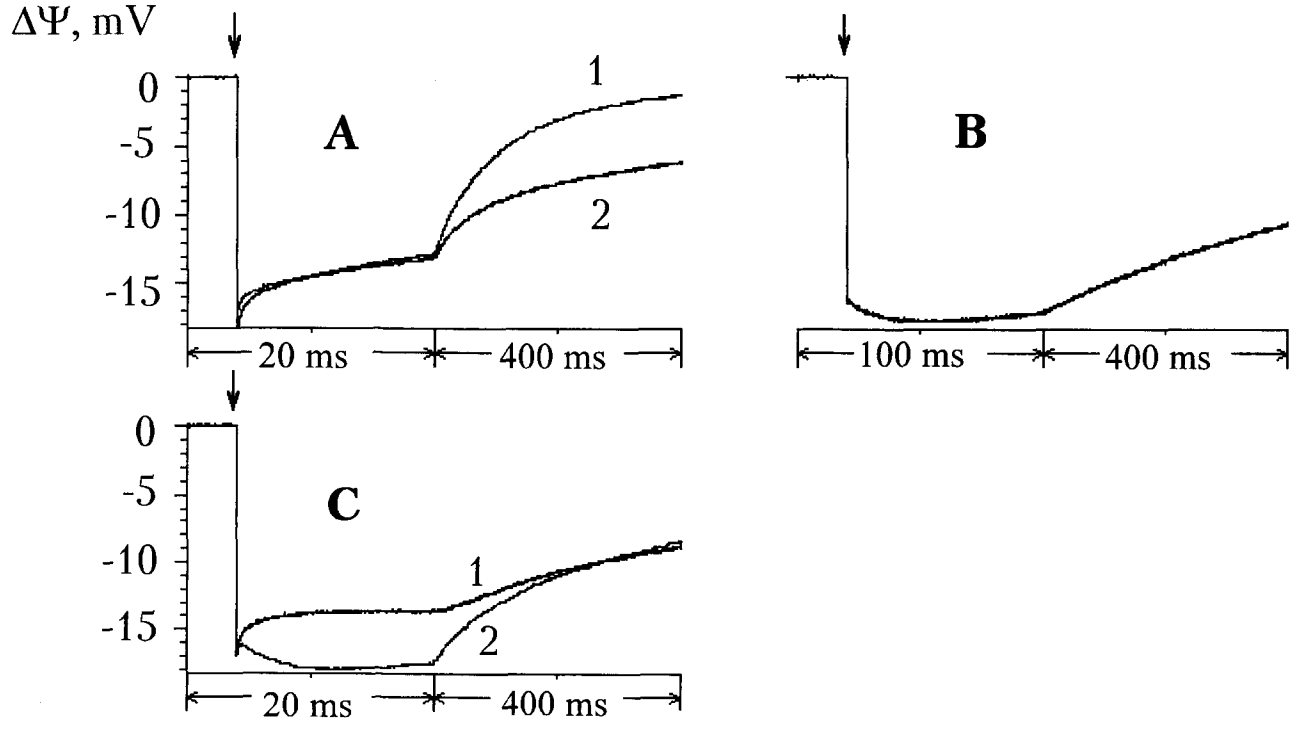

Fig. 1. Effects of TMPD and PMS on the kinetics of the photoelectric responses in proteoliposomes containing PS I complexes from *Synechocystis sp.* PCC 6803. Incubation medium contains 25 mM Tris-HCl (pH 7.8) with the following additions. A: (curve 1) 2 mM sodium ascorbate; (curve 2) 2 mM sodium ascorbate, 500 μM TMPD. B: 10 mM sodium ascorbate, 4 mM TMPD. C: (curve 1) 2 mM sodium ascorbate, 20 μM PMS; (curve 2) 2 mM sodium ascorbate, 100 μM PMS. Arrows indicate laser flashes.

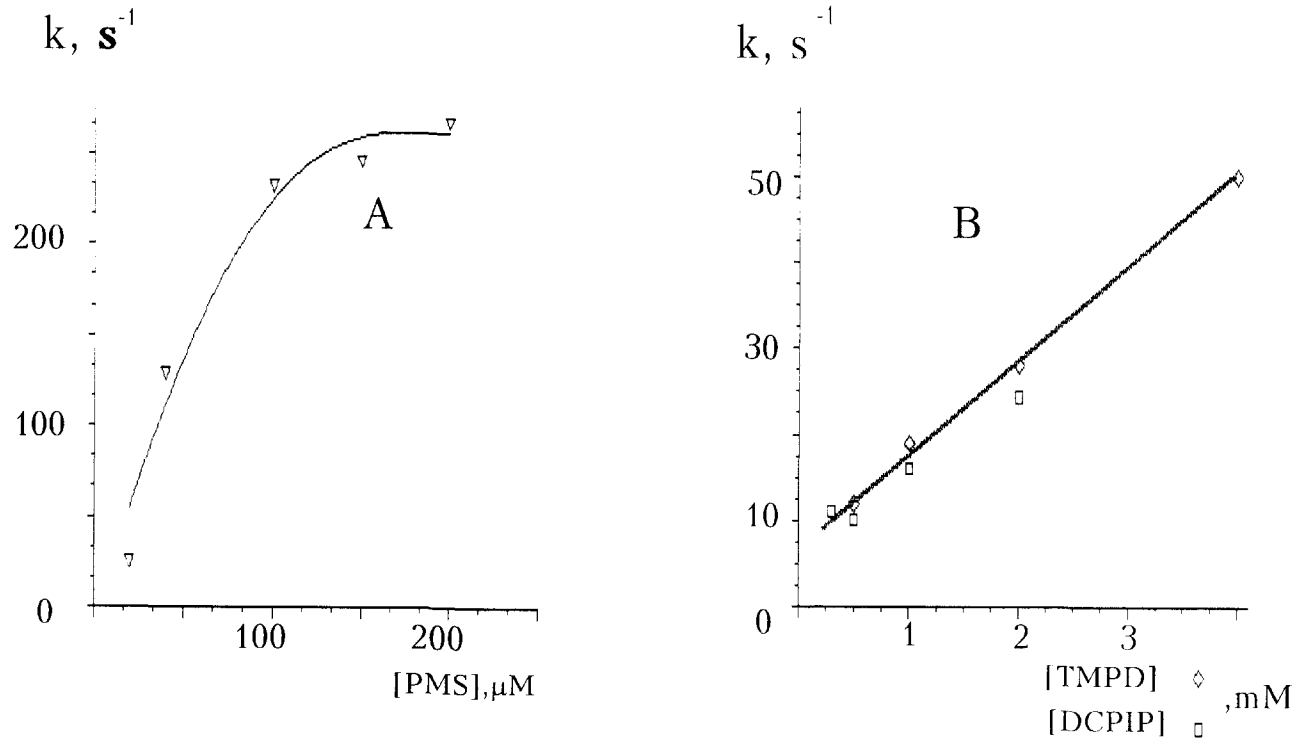

Fig. 2. Rate constant of photo-oxidized P700 reduction by redox dyes dependence on the concentration of redox dyes. A: PMS; B: TMPD (◊), DCPIP (□). Incubation medium contains 25 mM Tris-HCl (pH 7.8), 2 mM sodium ascorbate (for PMS) and 2–10 mM sodium ascorbate (depending on concentration of TMPD and DCPIP).

to both compartments, no photoelectric effects were detected without proteoliposomes.

The reduced form of PMS was much more effective electron donor to photo-oxidized $P700^+$ as compared with TMPD. A considerable slowing of the $\Delta\psi$ decay was already observed in the presence of 20 μM PMS (Fig. 1C, curve 1). At PMS concentration higher than 100 μM, the characteristic time of the new $\Delta\psi$ growth phase was ≈4 ms, and its amplitude was about 20% of the phase due to the charge separation between P700 and F_A/F_B (Fig. 1C, curve 2), as true for TMPD. At high PMS concentrations a slight acceleration of the previously slowed $\Delta\psi$ decay was observed (Fig. 1C, curves 1 and 2). However, this effect was not manifest at high concentrations of TMPD or DCPIP. The likely explanation is that the semireduced radical of PMS formed due to its photo-induced oxidation [18] can act as a weak uncoupler-protonophore and slightly accelerate the passive $\Delta\psi$ discharging through the proteoliposome membrane.

Fig. 2 shows the dependence of $P700^+$ reduction rate constant on PMS (A), TMPD and DCPIP (B) concentrations. The linear dependence of the additional electrogenic phase kinetics on TMPD and DCPIP concentrations (Fig. 2B) shows that the electron transfer from the redox dyes to P700 is a second order reaction. Deviation from the linearity on the graph of the electrogenic reaction rate constant dependence on PMS concentration (Fig. 2A) can be possibly explained by the high speed of auto-oxidation, observed at the high concentrations of this redox dye.

4. Discussion

We have shown earlier that in proteoliposomes containing reaction center complexes from photosynthetic bacteria *Rhodospirillum rubrum* the reduction of the photo-oxidized primary electron donor $P870^+$ (bacteriochlorophyll dimer) by TMPD or PMS is electrogenic [12]. The amplitude of the corresponding electrogenic phase comprised about 20% of the fast phase due to charge separation between P870 and the primary quinone acceptor Q_A. An electrogenic phase with comparable amplitude was observed in the same system in the presence of cytochrome *c* [19]. It was suggested that electrogenic nature of photo-oxidized $P870^+$ reduction is not specific for cytochrome *c* as an electron donor, and the electrogenicity in the presence of high concentrations of redox dyes is due to vectorial electron transfer within the reaction center protein [12,19].

Recently we have reported an electrogenic reaction of the electron transfer from cytochrome c_6 to photo-oxidized $P700^+$ in proteoliposomes containing PS I complexes from cyanobacteria *Synechococcus elongatus* [10,11]. The electrogenic phase observed in the presence of micromolar concentrations of reduced cytochrome c_6 comprised about 20% of the fast phase generated due to the charge separation between P700 and F_A/F_B. This new phase consisted of two exponential components with characteristic times of 25 and 200 μs. However, these results did not resolve the question whether the electrogenic reaction of photo-oxidized P700 is due to the electron transfer within the reaction center protein or within the cytochrome c_6–P700 complex.

The results of the present work suggest that the electrogenic nature of the electron donation to photo-oxidized $P700^+$ is not specific for cytochrome *c* as an electron donor. At the same time the specificity of cytochrome c_6 as a native electron donor to P700 can be supported by the following two facts. First, the maximal amplitude of the electrogenic reaction (~20% of the fast phase) was observed at concentrations of

added cytochrome c_6, which were by 2 or 3 orders less compared to the concentrations of redox dyes (~200 μM of PMS or 2 mM of DCPIP and TMPD) that yield the analogous amplitude. Second, the 25 μs phase in the kinetics of electrogenic $P700^+$ reduction by cytochrome c_6 has a rate constant independent of added cytochrome concentration [11], which suggests the formation of a specific complex between P700 and cytochrome c_6.

Although contributions of electrogenic phases due to $P700^+$ reduction in PS I and $P870^+$ reduction in reaction centers of photosynthetic bacteria by TMPD and PMS are relatively similar, the kinetics of these two reactions at the same concentrations differ considerably. The rate constants of electrogenic $P870^+$ reduction in the presence of 150 μM of PMS or 4 mM TMPD are about 2000 s^{-1} and 1000 s^{-1}, respectively [12], while that of $P700^+$ reduction at the same concentrations of redox dyes are 250 s^{-1} and 50 s^{-1}, respectively (see Fig. 2).

The best resolution of the X-ray structural analysis of PS I crystals obtained at present (4 Å) does not allow one to locate the primary electron donor P700 inside the protein complex unequivocally [3,5]. However, based on the α-helical hydrophobic profile analysis [20], and on the studies of point mutations effects on the spectral and thermodynamic properties of P700 [21], the histidine residues were identified, which serve as additional ligands to Mg atoms in the primary donor P700 [22]. The most probable binding site for plastocyanine on the surface of PS I complex was probed with the aid of computer modeling, and the distance between Cu^{2+} atom and P700 was estimated to be about 20 Å [22]. Taking into consideration comparable sizes of plastocyanine and cytochrome c_6 as well as recently resolved 3-dimensional structure of this cytochrome [23], one can estimate the distance between the central part of protein-embedded primary electron donor P700 and the surface of heterodimer consisting of A and B subunits to be 10–15 Å.

The obtained results are important for understanding of the molecular mechanism of electrogenesis at the donor side of the PS I complex. The electrogenicity observed upon $P700^+$ reduction by low molecular weight artificial donors must be due to the electron transfer inside the PsaA/PsaB heterodimer, which suggests an electro-isolated location of the primary electron donor P700 inside the protein molecule. Under these conditions the photo-oxidized P700 reduction does not require an immediate contact between Mg-porphyrin rings of P700 and the exogenous donors. Since the contribution of this electrogenic phase in the presence of PMS, TMPD or DCPIP is approximately equal to that of the phase observed earlier in the presence of cytochrome c_6, it is likely that the electrogenic reduction of $P700^+$ in vivo occurs due to vectorial electron transfer within PS I complex from the protein surface to the porphyrin ring of P700.

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