Minireview

Photoelectric studies of the transmembrane charge transfer reactions in photosystem I pigment-protein complexes

Alexey Yu. Semenov[a,*], Mahir D. Mamedov[a], Sergey K. Chamorovsky[b]

[a] *A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia*
[b] *Department of Biophysics, Faculty of Biology, Moscow State University, Moscow, Russia*

Received 4 August 2003; revised 21 August 2003; accepted 21 August 2003

First published online 24 September 2003

Edited by P. Brzezinski

Abstract The results of studies of charge transfer in cyanobacterial photosystem I (PS I) using the photoelectric method are reviewed. The electrogenicity in the PS I complex and its interaction with natural donors (plastocyanin, cytochrome c_6), natural acceptors (ferredoxin, flavodoxin), or artificial acceptors and donors (methyl viologen and other redox dyes) were studied. The operating dielectric constant values in the vicinity of the charge transfer carriers in situ were calculated. The profile of distribution of the dielectric constant along the PS I pigment–protein complex (from plastocyanin or cytochrome c_6 through the chlorophyll dimer P700 to the acceptor complex) was estimated, and possible mechanisms of correlation between the local dielectric constant and electron transfer rate constant were discussed.

Key words: Photosynthetic electron transport; Photosystem I; Electrogenic phase; Flash-induced kinetics; Cyanobacteria; Dielectric constant

1. Introduction

Photosystem I (PS I) catalyzes the photoinduced oxidation of plastocyanin (Pc)/cytochrome (cyt) c_6 and reduction of ferredoxin (Fd)/flavodoxin (Fld). The two largest polypeptides (PsaA and PsaB) form the PS I core, which binds a chlorophyll dimer (P700), two molecules of monomeric chlorophyll (A_0), a pair of phylloquinone molecules (A_1), and one molecule of 4Fe–4S center (F_X). The chlorophylls and quinones are almost symmetrically arranged in two branches related by the pseudo-C_2 axis. The terminal acceptors F_A and F_B (4Fe–4S centers) are located on the smaller stroma-exposed PsaC subunit [1].

The light-driven steps within the PS I complex involve electron transfer from P700 to F_A/F_B via A_0, A_1, and F_X. On the lumenal side of PS I, Pc or cyt c_6 are oxidized by $P700^+$, whereas on the stromal side, Fd or Fld are reduced by $(F_A/F_B)^-$. The net electron transfer from P700 to F_A/F_B is electrogenic [2–5]. The experiments with chloroplasts using the light-gradient technique demonstrated the electrogenic phase in PS I shorter than 50 ns [6]. Further upgrade on this method was based on the formation of a multilayer of PS I-containing membrane fragments electrically oriented in a low-impedance capacitor microcoaxial cell. This allowed the rise time of the electrogenic phase to be resolved with picosecond resolution. These measurements revealed the photovoltage generation steps accompanying electron transfer between P700 and A_0 (time constant, 22 ps) and between A_0 and A_1 (time constant, 50 ps) [7]. However, the time window of the low-impedance measurements did not extend longer than 1–2 ns. A variant of this method using a high-input impedance amplifier allowed the time window of detection to be extended up to 50 ns. Further extension of the time window was achieved by calculating an ideal photovoltage as the integral of the displacement current and the removal of decay due to ionic relaxation by a numerical deconvolution procedure. This procedure allowed to reveal an electrogenic phase with a time constant of $\sim$220 ns, which was ascribed to electron transfer from A_1 to F_X and further to F_A/F_B [3]. However, because the kinetic phase with a time constant of $\sim$220 ns was obtained as a decay-cleared trace rather than an actually measured kinetic curve, it was reasonable to use a direct electrometric method of detection of transmembrane electric potential difference ($\Delta\psi$) generation within the time range from 200 ns to $\sim$100 ms. The system based on phospholipid-impregnated collodion film developed in our laboratory [8,9] seems to be well adapted for the measurement of photovoltage kinetics on the microsecond to millisecond time scale.

In a series of our works (1996–2001) [4,10–14] the direct electrometrical method was used for determining relative contributions of electron transfer reactions between Pc/cyt c_6, the PS I complex, and Fd/Fld to the overall electrogenesis mediated by PS I. The results of our photoelectric measurements in combination with independent and virtually concurrently obtained spectroscopic data reported in [15,16] provided a new insight into the sequence of the electron transfer reactions between the terminal electron acceptors F_A and F_B. In addition, comparison of relative electrogenicity of partial electron transfer reactions with the projections of distance vectors between corresponding cofactors onto the membrane normal allows the profile of changes of the effective dielectric constant

*Corresponding author. Fax: (+7)-095-939 3181.
E-mail address: semenov@genebee.msu.su (A.Y. Semenov).

Abbreviations: Pc, plastocyanin; PS I, photosystem I; cyt, cytochrome; P700, chlorophyll dimer; F_X, F_A, F_B, 4Fe–4S clusters; $\Delta\psi$, transmembrane electric potential difference; Fd, ferredoxin; Fld, flavodoxin; A_0, monomeric chlorophyll; A_1, phylloquinone; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine; DCPIP, 2,6-dichlorophenol-indophenol; PMS, phenazine methosulfate

0014-5793/03/$22.00 © 2003 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)01032-9

along the whole PS I complex to be calculated. The goal of this review was to discuss these data in more detail.

2. Electrogenicity accompanying reduction of photooxidized P700 by electron donors

2.1. Electrogenic reduction of P700 by redox dyes

In the absence of soluble electron donors and acceptors, the kinetics of dark reduction of $P700^+$ is characterized by back reactions from one of the bound acceptors [11]. The direct electrometrical method developed in our laboratory [8,9,17,18] was used to monitor the flash-induced photoelectric responses of the proteoliposomes containing PS I complexes from the cyanobacterium *Synechocystis* sp. PCC 6803 adsorbed on the surface of a phospholipid-impregnated collodion film. The formation of $\Delta\psi$ corresponded to the negative charging of the interior of the proteoliposomes. The rise time of the $\Delta\psi$ generation fell within a time interval shorter than the instrument-limited response time constant of 200 ns and was attributed to the charge separation between P700 and the terminal iron–sulfur acceptors F_A/F_B [4]. The main component of the $\Delta\psi$ decay kinetics with the lifetime of $\sim$100 ms corresponded to the charge recombination between $P700^+$ and $(F_A/F_B)^-$, whereas the minor faster components were attributed to back reactions from F_X and A_1 in a fraction of the PS I complexes with impaired forward electron transfer to F_A/F_B [11].

The addition of a donor capable of donating an electron to the photooxidized $P700^+$ more rapidly than the photoreduced acceptor gives rise to competitive substitution of recombination by direct electron transfer. Reduced forms of redox mediators, like *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine (TMPD), 2,6-dichlorophenol-indophenol (DCPIP), and phenazine methosulfate (PMS) were widely used as artificial secondary donors, in particular, for the photooxidized bacteriochlorophyll dimer in bacterial photosynthetic reaction centers and for P700 in PS I complexes [10,19]. The decay kinetics slowed down upon increasing the redox dye concentration. At a certain concentration of the redox dye, the fast generation of the membrane potential related to the electron transfer between P700 and the terminal iron–sulfur acceptor F_B was followed by a new electrogenic phase in the millisecond time domain, which contributed approximately 20% to the overall photoelectric response. The reduced form of PMS was a much more effective electron donor to $P700^+$ than TMPD or DCPIP. This new kinetic phase was attributed to the vectorial transfer of an electron from the reduced form of the redox dye to the protein-embedded Mg-porphyrin ring of $P700^+$ [10].

2.2. Electrogenic reduction of P700 by Pc and cyt c_6

According to the negative sign of the photoelectric response and the effect of non-penetrating oxidizing agent ferricyanide on the amplitude of the flash-induced $\Delta\psi$, the orientation of P700 in the proteoliposome membrane was estimated as $>95\%$ facing external water phase [4]. Thus, accessibility of $P700^+$ from the external water phase makes the PS I-containing proteoliposomes a convenient system for studying the electron donation from non-penetrating hydrophilic donors. As mentioned above, cyt c_6 and Pc were alternative native secondary electron donors to the photooxidized P700 in cyanobacteria.

The addition of reduced spinach Pc in a gradually increasing concentration resulted in slowing the $\Delta\psi$ decay due to competition of Pc with $(F_A/F_B)^-$ for reduction of $P700^+$. At Pc concentration above 50 μM, an additional $\Delta\psi$ rise phase is observed in the kinetics of the photoelectric response. The maximal amplitude makes up $\sim$20% of the amplitude of the fast phase attributed to $P700^+(F_A/F_B)^-$ charge separation, whereas the apparent pseudo first-order rate constant exhibits a saturation profile at increasing Pc concentration, thereby suggesting the formation of a transient complex with PS I. Additionally, a small but detectable fast electrogenic phase was observed at high Pc concentration. The rate constant of this phase was independent of Pc concentration, indicating that it is related to a first-order process. The $\Delta\psi$ rise kinetics at a Pc concentration of 400 μM is clearly biphasic: the small fast component is characterized by the lifetime τ of $\sim$30 μs and the large slow one, by $\tau\sim$3 ms [14].

The addition of cyt c_6 also caused a decrease in the $\Delta\psi$ decay rate and changed the kinetics of the $\Delta\psi$ generation. In addition to the kinetically unresolvable fast phase of the photoelectric response, the slower phases in the submillisecond time scale appeared. These phases can be assigned to the electron transfer from cyt c_6 to $P700^+$. The amplitudes of these phases accounted for 20% of the fast phase amplitude, and kinetics were approximated by two exponents with lifetimes of 25 and 200 μs and an amplitude ratio of 2:3. The kinetics of the former rather than the latter phase did not depend on cyt c_6 concentration, indicating the formation of a specific complex between cyt c_6 and P700 [4].

These results suggest that the electrogenic nature of the electron donation to $P700^+$ is not specific for cyt c_6 and Pc as native electron donors. Moreover, the electrogenic response observed in the presence of artificial donors was approximately equal to the contribution of the phase observed in the presence of Pc or cyt c_6, ($\sim$20% to the overall photoelectric response). Therefore, it may be concluded that the electrogenic reduction of $P700^+$ in vivo occurs as a result of vectorial electron transfer within the PsaA/PsaB heterodimer molecule rather than within the cyt c_6–P700 or Pc–P700 complexes.

The mechanism of interaction of the oxidized PS I dimer with native or artificial donors was studied on the basis of analysis of the donor concentration dependence. Presumably, there are two possible mechanisms of such interaction (see [20] for review): (i) collision mechanism and (ii) formation of a transient complex. Mechanism (i) is dominant in case of artificial donors and at low concentration of native donor. It is characterized by linear dependence of the reaction rate constant and by the lack of saturation. Mechanism (ii) is observed at high concentrations of native donor in reaction medium [14].

3. Electrogenic reactions at the acceptor side of PS I

3.1. Electrogenicity accompanies photoreduction of the iron–sulfur clusters F_A and F_B in PS I

The kinetics of forward electron transfer from A_1^- to the Fe–S clusters in PS I is approximated by two components with half-times $\sim$20 and $\sim$200 ns ([21,22] and references therein). On the basis of photoelectric measurements on electrically oriented PS I-containing membrane fragments it was concluded that the overall electron transfer from A_1^- to F_B was limited by the step from A_1^- to F_X [16]. It was noted

above that in the absence of an external donor to $P700^+$, the photoelectric response associated with the charge separation between P700 and terminal electron acceptors F_A/F_B occurred within the rise time of the instrument (~200 ns) [4,10,11]. Thus, the 20- and 200-ns kinetic phases of forward electron transfer between A_1 and F_A/F_B were not resolved using our instrumentation. Our approach was based on the following assumptions: (i) sodium dithionite was able to reduce F_A and F_B, and (ii) treatment with $HgCl_2$ caused selective extraction of the F_B cluster [23]. The comparison of the dithionite-induced decrease in the magnitude of the total photoelectric responses obtained with control and F_B-less ($HgCl_2$-treated) PS I complexes provides an independent approach to the elucidation of the relative contribution of $F_X \rightarrow F_B$ and $F_X \rightarrow F_A$ electron transfer reactions to the overall electrogenesis within the PS I complex.

The initial amplitude of $\Delta\psi$ can be determined based on extrapolation of the $\Delta\psi$ decay kinetics. The main decay pathway of the flash-induced $\Delta\psi$ built-up across the membrane is the charge recombination between $P700^+$ and the photoreduced terminal acceptors F_A and F_B (lifetime range between 30 and 100 ms [11,22]). To determine the initial amplitude of $\Delta\psi$ we took into account only the charge separation components with the lifetimes longer than 400 μs, i.e. the components related to the electron transfer steps at distances larger than that between P700 and A_1. This excluded the contributions from centers with impaired electron transfer between A_1 and F_X. Based on the room-temperature kinetics of P700 absorption changes, the amount of dithionite used (100 mM) was found to be sufficient to chemically reduce F_A and F_B without any noticeable reduction of F_X in isolated PS I complexes. As expected, the addition of dithionite led to an acceleration of the $\Delta\psi$ decay kinetics, which was mainly due to the back reaction from F_X^- (lifetime ranging between 0.5 and 5 ms). The deconvolution of the kinetic curves shows a ~30% decrease in the amplitude of the photoelectric response in the presence of dithionite, which occurs due to the blockage of the electron transfer between F_X and F_A/F_B.

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 cluster [24]. For the $HgCl_2$-treated (F_B-free) samples we performed similar multiexponential analyses of the decay kinetics in the absence and in the presence of dithionite and determined the initial amplitudes of $\Delta\psi$ after subtracting the fast phases with the lifetimes shorter than 400 μs. The addition of dithionite to both control and $HgCl_2$-treated samples resulted in similar accelerations of the $\Delta\psi$ kinetics, but the decrease of the initial $\Delta\psi$ amplitude associated with the blockage of electron transfer from F_X to F_A was less significant in $HgCl_2$-treated samples (15–20%). It should be noted that Diaz-Quintana et al. [16] observed a decrease in the amplitude of the electrogenic phase corresponding to the $A_1 \rightarrow F_A/F_B$ electron transfer in $HgCl_2$-treated sample compared to control. However, this decrease was attributed in [16] to the treatment-induced destruction of ~30% F_A. Note, that no such decrease was observed in our experiments [12]. The discrepancy could be due to the differences in the PS I preparations, $HgCl_2$-treatment procedure, or techniques used to measure the electrogenic changes (PS I-containing proteoliposomes adsorbed onto the phospholipid collodion membrane [12] versus electrically oriented multilayer membrane fragments adsorbed onto the platinum electrode [16]).

The data obtained unambiguously indicated that the voltage changes on the reducing side of PS I were related to the 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, we concluded that F_B rather than F_A was the acceptor distal to F_X [12].

3.2. *Electron transfer from the terminal cluster F_B to external acceptors is electrically silent*

The electrometrical technique was also used to investigate electron transfer between the terminal iron–sulfur centers F_A/F_B and external electron acceptors in PS I complexes from cyanobacteria *Synechococcus* sp. PCC 6301 and from spinach. The photoelectric responses were monitored in PS I-containing proteoliposomes either in the absence or in the presence of Fd, Fld, and methyl viologen (MV) entrapped in phospholipid vesicles. The significant increase in the relative contribution of the slow components of the $\Delta\psi$ decay kinetics in the presence of both native (Fd, Fld) and artificial (MV) electron acceptors indicates that the interaction between the terminal Fe–S cluster and acceptors is sufficiently effective. The lack of additional electrogenicity under conditions of effective electron transfer from the F_B redox center to soluble acceptors implies that this reaction is electrically silent. The finding that F_A fails to donate electrons to Fld in the F_B-deficient ($HgCl_2$-treated) PS I complexes suggests that F_B is the direct electron donor to Fld [13]. This conclusion is in line with the data obtained by monitoring the kinetics of rereduction of the photooxidized P700 in the presence of Fd and Fld [15] and the kinetics of Fd reduction [16] by control and F_B-deficient PS I complexes.

The finding that F_B is the distal cluster to F_X and serves as the immediate electron donor to Fd/Fld unequivocally indicates that the electron transfer on the acceptor side of PS I is

Table 1
Calculation of effective dielectric constant values in the protein domains between redox cofactors of PS I

PS I protein domains between redox cofactors	Projections of the distance vectors (D) (Å)	Relative photovoltages (ψ)	Effective dielectric constant values (ε).
Pc–P700	~20	0.155[a]	9.7
P700–A_0	15.5	0.38	3
A_0–A_1	7.2	0.095	5.4
A_1–F_X	8.5	0.12	5.4
F_X–F_B	21.5	0.25[a]	6.6
F_B–Fld	11	<0.02[b]	>42

[a]Experimental error in the determination of ψ in different experiments did not exceed ±10%. Because geometrical parameters were estimated from the X-ray model with sufficiently higher accuracy, the total error in the calculation of the effective dielectric constant was ±10%.
[b]The value was taken from the previously estimated lower limit for the detectable relative photovoltage measured by the direct electrometrical technique (see [30] for review).

arranged as the following series: $F_X \rightarrow F_A \rightarrow F_B \rightarrow$ Fd/Fld (see [25] for review).

4. Profile of changes of the effective dielectric constant along the PS I complex

Because the photoelectric signal amplitude is proportional to dielectrically weighted distances, comparison of projections of the distance vectors between redox cofactors onto the membrane normal with the relative photovoltage amplitudes provides a unique opportunity to estimate the profile of distribution of the dielectric constant (ε) values along the whole stretch of the PS I protein complex. The dielectric constants estimated from our experiments are effective values determined with respect to the time scale of corresponding reaction. In terms of dielectric environment, the ε values related to membrane-embedded protein-bound cofactors are presumably determined by local structural properties of the protein matrix, whereas in case of electron carriers exposed to polar aqueous medium, the aqueous phase surrounding the membrane may also contribute to the ε value.

It should be emphasized that the prerequisite for the estimation of ε is the knowledge of the three-dimensional structure of the protein. Although this information was not available at the time of the first photoelectric measurements on PS I [6,7], these experiments gave valuable structural information about the relative transmembrane position of cofactors. To interpret the results of the fast photoelectric measurements on oriented multilayer membrane fragments, it was originally assumed that the dielectric environment is homogeneous, at least for the most part of the PS I complex inside the protein/lipid layer [3,6]. However, this assumption proved to be too simplistic in case of the reaction centers of purple photosynthetic bacteria (BRC) [9]. It is also not supported by the general three-phase model of membrane proteins, which implies the increase of the effective ε value in the direction from the central hydrophobic part of the protein towards the periphery of the protein/lipid layer [26].

Let the electron transfer reactions in the complex be denoted as $A \rightarrow B \rightarrow C$. Given the fact that the electron transfer reactions in the complex are essentially irreversible, the relationship for the ratio between the mean ε values corresponding to $B \rightarrow C$ and $A \rightarrow B$ electron transfer stages ($\varepsilon_{BC}/\varepsilon_{AB}$) with the photovoltage drop between B and C (ψ_{BC}) and between A and B (ψ_{AB}) can be described by the following approximate equation:

$$(\varepsilon_{BC}/\varepsilon_{AB}) = (D_{BC}/D_{AB}) : (\psi_{BC})/(\psi_{AB})$$

where D_{BC} and D_{AB} are the projections for the $B \rightarrow C$ and $A \rightarrow B$ distance vectors onto the membrane normal, respectively. Taking into account the distances between the PS I cofactors [1] and the structural model of the Fd [27] and Pc [27,28] docking sites, as well as relative contributions of the electron transfer reactions $P700 \rightarrow A_0$ and $A_0 \rightarrow A_1$ [7], $A_1 \rightarrow F_X$ [3,16], $F_X \rightarrow F_B$ [12], $Pc \rightarrow P700$ [14] and $F_B \rightarrow$ Fld [13] to the overall electrogenesis provided by the PS I complex, the following suggestions are made:

1. Let the minimal value of the dielectric constant for the $P700 \rightarrow A_0$ protein region be $\varepsilon \sim 3$. Then, the ε value for the $A_0 \rightarrow A_1$ and $A_1 \rightarrow F_X$ domains is estimated to be ~ 5.4.

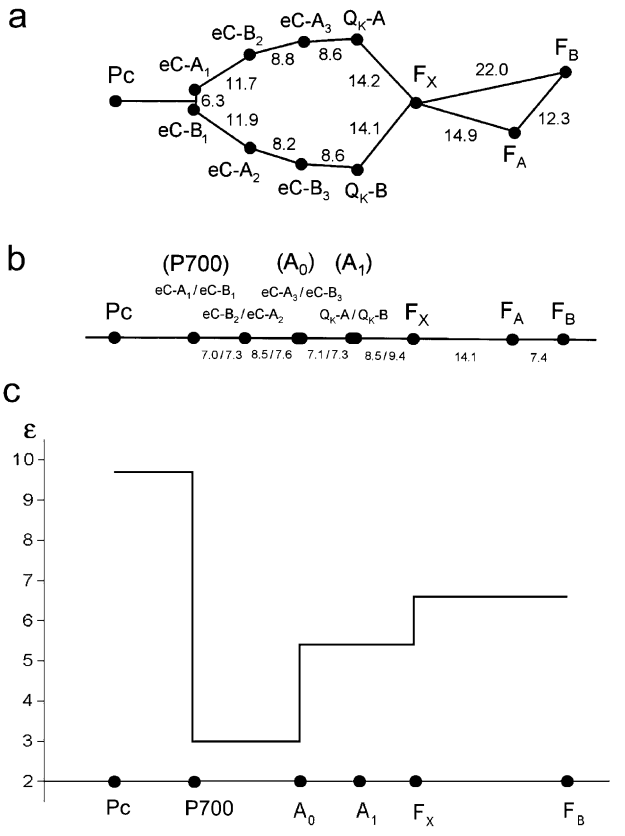

Fig. 1. a: Comparison between the transmembrane locations of redox components of PS I according to the X-ray structure [1]; b: projections of the distance vectors between the redox cofactors onto the membrane normal (adapted from [22]); c: the profile of changes of the effective dielectric constant along the PS I complex. Center-to-center distances are given in Å; eC-A_{1-3} and eC-B_{1-3} denote chlorophyll *a* molecules, and Q_k-A/Q_k-B denote phylloquinone molecules bound to subunits PsaA and PsaB, respectively.

2. On the acceptor side, the ε value further increases up to ~ 6.6 along the PsaC subunit ($F_X \rightarrow F_B$).
3. On the donor side between the Pc binding site and P700 Mg-porphyrin rings embedded into PsaA/PsaB heterodimer ($Pc \rightarrow P700$), the mean ε value is equal to 9.7.

The results of calculation of the effective ε values in the protein domains between redox cofactors are presented in Table 1, whereas the profile of variation of ε along the PS I complex is shown in Fig. 1. The large value of ε on the donor side between the Pc binding site and P700 Mg-porphyrin rings is not very surprising, because, in contrast to the PS I complex, the Pc/cyt subunit is exposed to polar aqueous medium. A gradual increase in the value of ε from ~ 3 at the center of the protein globule to ~ 10 at its periphery was also observed in globular proteins (for review see [29]).

It should be noted that there is a hierarchy of the rate constants of the reactions of charge transfer along the photosynthetic electron transport chain. The fastest primary reactions of charge separation and stabilization of separated charges occur in the photosynthetic reaction center core within the picosecond time range, whereas slower secondary reactions take place at the periphery of the complex within the micro- to millisecond time range. To a first approximation, this hierarchy qualitatively correlates with the hierarchy of the dielectric constant values observed in our experiments (Fig. 1).

Indeed, fastest and slower processes were found to be attributed to regions with low and high values of ε, respectively.

A similar correlation also takes part, at least qualitatively, in the case of BRC. It was shown in our earlier studies that reduction of the photooxidized bacteriochlorophyll dimer P870 by native donor cyt c_2 or by reduced redox dyes in BRC-containing proteoliposomes is electrogenic (see [30] for review). Comparison of the distance vectors to the membrane normal with the relative photovoltage amplitudes for PS I and BRC yields similar ε values, corresponding to the electron transfer from the native donor proteins to P700 and P870, respectively. Note that the electron transport reactions at the donor side of PS I and BRC share the following features of similarity: (i) Gibbs energy differences between Pc/cyt c_6 and P700 and between cyt c_2 and P870 are close to one another; (ii) the corresponding kinetics and proposed reaction mechanisms are very similar in PS I and BRC.

The main electrogenic step on the acceptor side of BRC is due to protonation of the double-reduced secondary quinone Q_B [31]. The ε value in this region increases up to $\sim$20 [32], which is about three times higher compared to the acceptor region of PS I. Note also that the thermodynamic and kinetic properties of the terminal acceptors in two complexes essentially differ. The midpoint potential (E_m) values for the Q_A/Q_A^- and Q_B/Q_B^- redox couples in BRC are in the range of -50 to +100 mV, whereas the E_m values of F_X, F_A and F_B in PS I are much more negative (in the range of -500 to -700 mV). The time constants for the electron and proton transfer reactions on the acceptor side of BRC (submillisecond time range) are at least three orders of magnitude lower than the time constants within the domain of iron–sulfur clusters in PS I (in the range of tens to hundreds of nanoseconds). Perhaps, the three-order-of-magnitude difference between the rate constants of electron transfer on the acceptor side of BRC and PS I, and the three-fold increase in the estimated value of ε reflects the hypothetical correlation between the reaction rates and the dielectric properties of the corresponding protein domains between redox cofactors.

The process of charge transfer proceeds in several stages including medium reorganization and electron tunneling. The efficiency of the process crucially depends on the dielectric reorganization of the medium. Effective relaxation processes (e.g. polarization) of the milieu of the reacting groups are required to provide charge stabilization. Perhaps, the correlation between the dielectric constant values and the rate constants of the electron transfer reactions in the PS I complex is conditioned by the processes of relaxation. According to the Marcus theory [33], the activation energy of the elementary act of charge transfer ΔG^* is determined by two parameters (reorganization energy λ and free energy of elementary act ΔG):

$$\Delta G^* = \frac{(\lambda + \Delta G)^2}{4\lambda}$$

The two parameters can be represented as the sum of the structure-dependent and polarization-dependent components. In the simplest case of charge transfer in a homogeneous medium, the polarization-dependent component of the reorganization energy is proportional to the so-called coupling constant $C = 1/\varepsilon_0 - 1/\varepsilon_S$, where ε_0 and ε_S are the optical and low-frequency dielectric constants, respectively [34,35]. The value of ε_0 for proteins was shown to be constant (about 2.5) [34]. If the effective value of ε deduced from our experiments were proportional to the ε_S, then the variability of the ε evaluated in this work at different sites of the electron transport chain (Pc–P700, P700–A_0, A_0–F_X, and F_X–F_B) would correspond to a considerable (three- to four-fold) change of the coupling constant value. Therefore, this factor might change substantially the activation energy of the charge transfer.

It should be noted that fast charge separation reactions are activationless (at least in the case of BRC). The primary photoproducts are created very close to the free energy level of the excited P870 and relax to the final state on the picosecond and nanosecond time scales [36–38]. These properties require that the reorganization energy should be comparable in magnitude to the free energy change.

A dynamic solvation model of relaxation processes was suggested in [37] to explain the temperature dependence of the complex exponential kinetics of BRC fluorescence decay on the picosecond to nanosecond time scale. This model was later refined by Trissl et al. [39] on the basis of fast photoelectric measurements performed in chromatophores of purple bacteria with nanosecond time resolution. These experiments revealed that under reducing conditions the photovoltage signal decayed significantly faster than the spectroscopically detected recombination of separated charges. This implies the occurrence of considerable dielectric relaxation. It was suggested that these relaxations could be ascribed to protein solvation processes that decrease the Gibbs energy. Various possible molecular candidates were suggested to contribute to the relaxation processes at different stages of charge separation in BRC, including collectively acting ensemble of amino acids [39].

It was also shown in [40] that in the case of very fast reactions the time evolution of the dielectric response of protein should be taken into account. In the case of BRC, the time evolution of the dielectric properties was described phenomenologically as a set of effective dielectric constants operative in different time intervals. On the other hand, the exact compensation of the ε_S-dependent component of reorganization and reaction energies was shown to be inherent only in the fast charge separation process and did not take part in slower charge transfer processes [40]. However, further theoretical and experimental research is required to provide a deeper insight into the problem of correlation between relaxation processes and the kinetics of charge transfer.

Acknowledgements: The authors are grateful to Prof. L.I. Krishtalik for valuable discussion and to Mr. C.S. Chamorovsky for help in preparation of the manuscript. This study was supported by Grant 2296 from the International Science and Technology Center (ISTC) (to A.Y.S.), Grant 01-483 from the INTAS (to A.Y.S.), grants from the Russian Foundation for Basic Research 03-04-49219 (to S.K.C.), 03-04-48983 (to A.Y.S.), and 01-04-49268 (to M.D.M.), and Grant RC1-2400-MO-02 from the Civilian Research and Development Foundation (CRDF) (to A.Y.S.).

References

[1] Jordan, P., Fromme, P., Klukas, O., Witt, H.T., Saenger, W. and Krauss, N. (2001) Nature 411, 909–917.
[2] Vos, M.H. and van Gorkom, H.J. (1990) Biophys. J. 58, 1547–1555.
[3] Leibl, W., Toupance, B. and Breton, J. (1995) Biochemistry 34, 10237–10244.
[4] Mamedov, M.D., Gadjieva, R.M., Gourovskaya, K.N., Drachev,

L.A. and Semenov, A.Yu. (1996) J. Bioenerg. Biomembr. 28, 517–522.
[5] Sigfridsson, K., Hansson, O. and Brzezinski, P. (1995) Proc. Natl. Acad. Sci. USA 92, 3458–3462.
[6] Trissl, H.-W., Leibl, W., Deprez, J., Dobeck, A. and Breton, J. (1987) Biochim. Biophys. Acta 893, 320–332.
[7] Hecks, B., Breton, J., Leibl, W., Wulf, K. and Trissl, H.-W. (1994) Biochemistry 33, 8619–8624.
[8] Drachev, L.A., Semenov, A.Yu., Skulachev, V.P., Smirnova, I.A., Chamorovsky, S.K., Kononenko, A.A., Rubin, A.B. and Uspenskaya, N.Ya. (1981) Eur. J. Biochem. 117, 483–489.
[9] Dracheva, S.M., Drachev, L.A., Konstantinov, A.A., Semenov, A.Yu., Skulachev, V.P., Arutjunian, A.M., Shuvalov, V.A. and Zaberezhnaya, S.M. (1988) Eur. J. Biochem. 171, 253–264.
[10] Gourovskaya, K.N., Mamedov, M.D., Vassiliev, I.R., Golbeck, J.H. and Semenov, A.Yu. (1997) FEBS Lett. 414, 193–196.
[11] Vassiliev, I.R., Jung, Y.-S., Mamedov, M.D., Semenov, A.Yu. and Golbeck, J.H. (1997) Biophys. J. 72, 301–315.
[12] Mamedov, M.D., Gourovskaya, K.N., Vassiliev, I.R., Golbeck, J.H. and Semenov, A.Yu. (1998) FEBS Lett. 431, 219–223.
[13] Mamedova, A.A., Mamedov, M.D., Gourovskaya, K.N., Vassiliev, I.R., Golbeck, J.H. and Semenov, A.Yu. (1999) FEBS Lett. 462, 421–424.
[14] Mamedov, M.D., Mamedova, A.A., Chamorovsky, S.K. and Semenov, A.Yu. (2001) FEBS Lett. 500, 172–176.
[15] Vassiliev, I.R., Jung, Y.-S., Yang, F. and Golbeck, J.H. (1998) Biophys. J. 74, 2029–2035.
[16] Diaz-Quintana, A., Leibl, W., Bottin, H. and Setif, P. (1998) Biochemistry 37, 3429–3439.
[17] Drachev, L.A., Jasaitis, A.A., Kaulen, A.D., Kondrashin, A.A., Liberman, E.A., Nemecek, I.B., Ostroumov, S.A., Semenov, A.Yu. and Skulachev, V.P. (1974) Nature 249, 321–324.
[18] Drachev, L.A., Kaulen, A.D., Semenov, A.Yu., Severina, I.I. and Skulachev, V.P. (1979) Anal. Biochem. 96, 250–262.
[19] Drachev, L.A., Kaminskaya, O.P., Konstantinov, A.A., Semenov, A.Yu. and Skulachev, V.P. (1985) FEBS Lett. 189, 45–49.
[20] Hope, A.B. (2000) Biochim. Biophys. Acta 1456, 5–26.
[21] Joliot, P. and Joliot, A. (1999) Biochemistry 38, 11130–11136.
[22] Brettel, K. and Leibl, W. (2001) Biochim. Biophys. Acta 1507, 100–114.
[23] Kojima, Y., Niinomi, Y., Tsuboi, S., Hiyama, T. and Sakurai, H. (1987) Bot. Mag. 100, 243–253.
[24] Jung, Y.-S., Yu, L. and Golbeck, J.H. (1995) Photosynth. Res. 46, 249–255.
[25] Golbeck, J.H. (1999) Photosynth. Res. 61, 107–144.
[26] Cherepanov, D.A. and Krishtalik, L.I. (1990) Bioelectrochem. Bioenerg. 24, 113–127.
[27] Fromme, P., Schubert, W.D. and Krauss, N. (1994) Biochim. Biophys. Acta 1187, 99–105.
[28] Myshkin, E., Leontis, N.B. and Bullerjahn, S. (2002) Biophys. J. 82, 3305–3313.
[29] Mertz, E.L. (2000) Proc. Natl. Acad. Sci. USA 97, 2081–2086.
[30] Semenov, A.Yu. (1991) in: Soviet Science Review, Section D (Skulachev, V.P., Ed.), Vol. 10, pp. 45–75, Harwood Academic Publishers, New York.
[31] Kaminskaya, O.P., Drachev, L.A., Konstantinov, A.A., Semenov, A.Yu. and Skulachev, V.P. (1986) FEBS Lett. 202, 224–228.
[32] Shinkarev, V.P., Drachev, L.A., Mamedov, M.D., Mulkidjanian, A.Ya., Semenov, A.Yu. and Verkhovsky, M.I. (1993) Biochim. Biophys. Acta 1144, 285–294.
[33] Marcus, R.A. and Sutin, N. (1985) Biochim. Biophys. Acta 811, 265–322.
[34] Krishtalik, L.I. and Topolev, V.V. (2000) Biochim. Biophys. Acta 1459, 88–105.
[35] Parson, W.W., Chu, Z.T. and Warshel, A. (1998) Biophys. J. 74, 182–191.
[36] Woodbury, N.W.T. and Parson, W.W. (1984) Biochim. Biophys. Acta 767, 345–361.
[37] Peloquin, J.M., Williams, J.C., Lin, X., Alden, R.G., Taguchi, A.K.W., Allen, J.P. and Woodbury, N.W.T. (1994) Biochemistry 33, 8089–8100.
[38] Holzwarth, A.R. and Muller, M.G. (1996) Biochemistry 35, 11820–11831.
[39] Trissl, H.-W., Bernhardt, K. and Lapin, M. (2001) Biochemistry 40, 5290–5298.
[40] Krishtalik, L.I. (1996) Biochim. Biophys. Acta 1273, 139–149.