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Electrogenic Protonation of the Secondary Quinone Acceptor Q_B in Spinach Photosystem II Complexes Incorporated into Lipid Vesicles

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Abstract—The generation of transmembrane electric potential difference ($\Delta\Psi$) in quinone acceptor complex of proteoliposomes containing core complexes of photosystem II from spinach was studied using for the measurements a direct electrometric technique. Besides the fast increase in the membrane potential associated with the electron transfer between the redox-active tyrosine 161 residue (Y_Z) in D1 polypeptide and the primary quinone acceptor Q_A, an additional electrogenic phase with $\tau \sim 0.85$ msec at pH 7.3 and the maximal relative amplitude of ~11% of the $Y_Z^{ox}Q_A^-$ phase was observed after the second light flash. The sensitivity of this phase to diuron (an inhibitor of electron transfer between Q_A and the secondary quinone acceptor Q_B), the dependence of its amplitude on the light flash parity, and also a decrease in its rate constant with increase in pH indicated that it was due to dismutation of Q_A^- and Q_B^- with the subsequent protonation of a doubly reduced plastoquinone molecule: $Q_A^-Q_B^- + 2H^+ \rightarrow Q_AQ_BH_2$.

Key words: core complex of photosystem II, oxygen evolving complex, secondary quinone acceptor, proteoliposomes, membrane potential, electrogenic phase, direct electrometric technique

The pigment–protein photosystem II (PSII) complex is one of the most important elements of the photosynthetic apparatus of plants, algae, and cyanobacteria. This complex joins the one-electron charge transfer in the reaction center (RC), two-electron reduction of the secondary quinone acceptor Q_B, and four-electron reaction in the complex of water oxidation.

Photochemical events preceding the oxidation of water occur in a heterodimer consisting of two homologous polypeptides, D1 and D2. Excitation of the primary electron donor (the chlorophyll dimer P680) results in the state $P680^+Q_A^-$, where Q_A is the primary acceptor quinone. The cation radical $P680^+$ rapidly oxidizes Y_Z, the redox-active tyrosine 161 amino acid residue of the D1 subunit of PSII [1, 2], producing a neutral radical Y_Z. This radical, in turn, oxidizes the manganese (Mn) cluster of the oxygen evolving complex (OEC) [3-6], and Q_A^- reduces Q_B. Subsequent light flashes result in the further oxidation of the Mn-cluster of the OEC [1, 5, 7, 8]. During the transfer of four electrons from two H_2O molecules onto the photooxidized $P680^+$, the redox-state (S) of the OEC successively changes from S_0 to S_4 (the subscripts 0-4 show value of the positive charge which is accumulated in the water oxidation complex).

Elucidation of the physicochemical mechanism of conjugation of the electron and proton transfer in the quinone acceptor complex during reduction of the secondary quinone acceptor Q_B is an important problem in studies on the functioning of PSII complexes. Significant progress in this field was achieved due to determination of the three-dimensional structure of the PSII complex with the resolution of 3.8 [9], 3.7 [10], and 3.5 Å [11], development of a site-specific mutagenesis of PSII [12], and kinetic analysis of individual stages of the charge transfer in the RC in response to single and successive light flashes [13, 14].

Reduction of Q_B in the course of the two-electron catalytic cycle is associated with its protonation. Amino acid residues adjacent to Q_B play a key role in this process [15]. The transfer of the first electron onto Q_B ($Q_A^-Q_B \rightarrow Q_AQ_B^-$) is not followed by its protonation, whereas the transfer of the second electron is accompanied by a suc-

Abbreviations: PSII) pigment–protein complex of photosystem II; RC) reaction center; Q_A and Q_B) primary and secondary quinone acceptor, respectively; P680) primary electron donor of PSII RC; Y_Z) redox-active tyrosine 161 residue of PSII D1 subunit; $\Delta\Psi$) transmembrane electric potential difference; τ) time of response amplitude changing e-fold.

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cessive uptake of two protons to Q_B, and the transfer mechanisms of these electrons are significantly different [13].

It was earlier shown electrometrically using proteoliposomes containing PSII particles from spinach [16] and the cyanobacterium *Anacystis nidulans* [17] that protonation of the secondary quinone acceptor Q_B after the second light flash was accompanied by appearance of an additional phase in the kinetics of generation of the transmembrane electric potential difference ($\Delta\Psi$). The amplitude of this additional phase in these works was ~5% of the phase corresponding to the generation of $Y_Z^{ox}Q_A^-$. But it was unclear why the electrogenic "quinone" phase in the PSII complexes was so small compared with chromatophores of photosynthesizing purple bacteria (~15-20% of $P^+Q_A^-$) [18, 19].

Therefore, this work was designed to study the mechanism of the membrane potential generation associated with functioning of the quinone acceptor complex in the spinach PSII core complexes with an active water oxidation system.

MATERIALS AND METHODS

Spinach core complexes of PSII with an active water oxidation system were prepared from membrane fragments enriched with PSII (BBY preparations) [20] as described in [21].

Proteoliposomes were prepared by mixing the liposomes resulting on sonication of azolectin (40 mg/ml) in 25 mM HEPES-NaOH buffer (pH 7.3) containing 0.8% octylglucoside with PSII complexes at the lipid/protein ratio of 30 : 1 (w/w) and subsequent removal of the detergent by dialysis of the suspension against 2 liters of 25 mM HEPES-NaOH (pH 7.3) for 24 h, with two changes of the buffer [22]. All procedures were performed at 4°C.

Generation of the transmembrane potential difference was recorded using a direct electrometric technique with resolution of 100 nsec as described in [23]. The technique includes an arrangement of specimens on the surface of a collodion phospholipid-impregnated film separating two partitions of the measuring cell, and recording $\Delta\Psi$ with light-protected Ag/AgCl macroelectrodes. To reconstruct the function of Q_B, the phospholipid solution in decane was supplemented with decylplastoquinone (30 mg/ml). Before recording the signals, the specimens were adapted to dark for 10 min. A pulsed Nd-YAG laser (YG-481, Quantel, $\lambda = 532$ nm, pulse half-width 15 nsec, flash energy 40 mJ/cm^2) was used as a source of light flashes.

The kinetic curves were processed and decomposed into exponentials using Graph editor of curves (4.09.00 version) [24].

The rate of oxygen release (~910 μmol O_2/h per mg chlorophyll) was measured polarographically with a closed Clark-type electrode in medium containing 25 mM Mes-NaOH (pH 6.5), 10 mM $CaCl_2$, 1 mM potassium ferricyanide, and 100 μM phenyl-*p*-benzoquinone.

To remove Mn from OEC, core complexes of PSII were incubated in the dark for 2 h in the presence of 0.8 M Tris-HCl (pH 9.1) and 5 mM EDTA, as described in [25].

Azolectin (type II S phosphatidylcholine), octylglucoside, Triton X-100, potassium ferricyanide, phenyl-*p*-benzoquinone, NaCl, $CaCl_2$, $MgCl_2$, EDTA, Mes, HEPES, MOPS, Tris, CAPS were from Sigma (USA); some recrystallized reagents of domestic production of special purity were also used.

RESULTS AND DISCUSSION

Figure 1a presents a typical photoelectric response of PSII complexes incorporated into phospholipid vesicles associated with a collodion film. The light flash generated a transmembrane potential difference ($\Delta\Psi$) with the negative potential inside the proteoliposomes and faster kinetics than the time resolution of our apparatus (100 nsec). The induction of the negative photoelectric response by the laser flash suggests that the water oxidation system is located near the outer surface of the proteoliposomal membrane. The lack of influence of sodium dithionite on the amplitude of the photoelectric responses indicates a unidirectional orientation of PSII complexes in the phospholipid vesicles (data not presented). If the recorded photoelectric activity of the proteoliposomes was caused by functioning of PSII, this would be manifested by a decrease in the amplitude of the photoelectric response to the second flash in the presence of diuron which inhibits the electron transfer between the primary Q_A and the secondary quinone acceptor Q_B. However, this effect was observed even without addition of diuron into the medium, which was associated with the absence of Q_B on its specific binding site in the protein in our system for measurement of photopotential (Fig. 1b). This result can be explained by the presence of Q_A^- in the PSII complexes before the second flash.

A decline in the photoelectric response is approximated by three exponentials with the characteristic times τ of ~1.55 sec, ~445 msec, and ~35 msec and relative contributions of ~65, 36, and 11%, respectively. The phases with characteristic times of 1.55 sec and ~445 msec seem to be due to reactions of electron recombination between S_2 (the transient state of the OEC) and Q_A^-, whereas the phase with τ ~ 35 msec probably represents the electron recombination between Q_A^- and the tyrosine residue Y_Z^+ in the PSII complexes with inactive OEC [26, 27].

Figure 1c shows the photoelectric response of proteoliposomes containing PSII core complexes with inactive OEC. A rapid decline in the photopotential in these

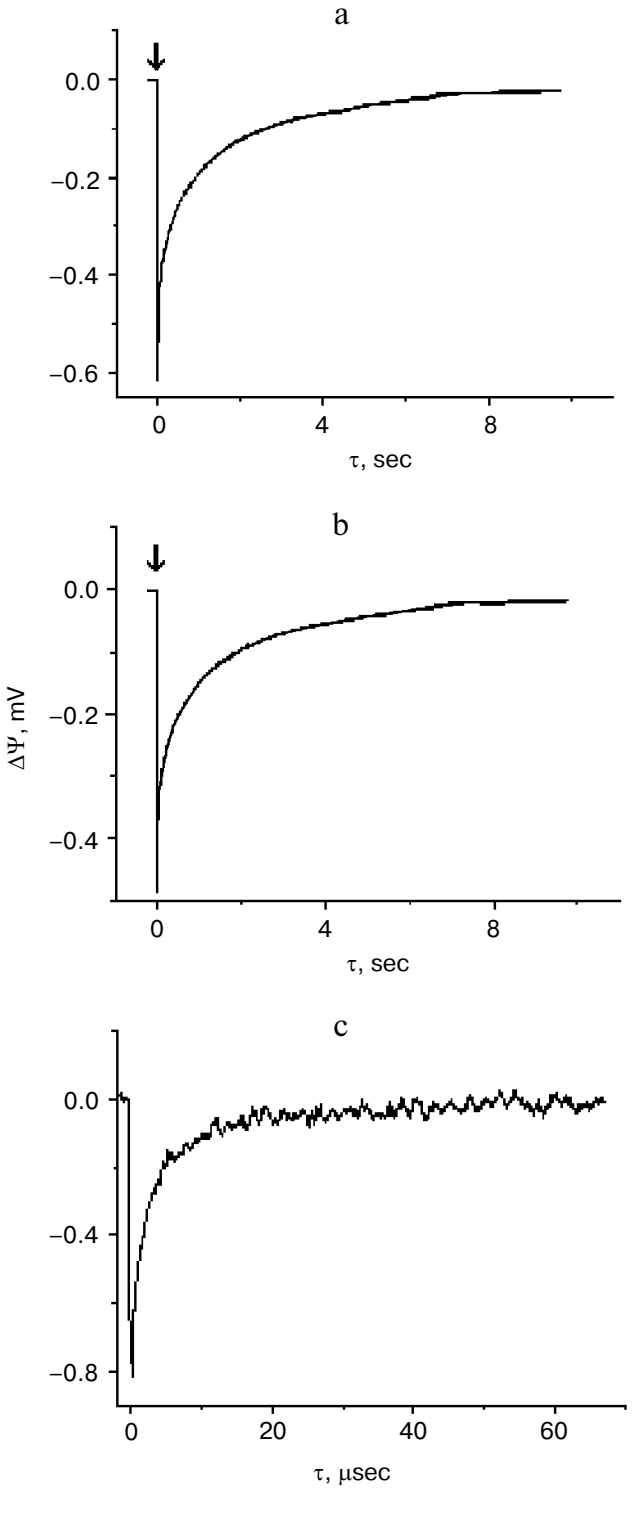

Fig. 1. Generation of electric potential transmembrane difference in dark-adapted proteoliposomes containing PSII core complexes with active water oxidation complex in response to the first (a) and second (b) light flash; c) the same in proteoliposomes containing PSII core complexes with inactive water oxidation complex. The interval between flashes was 5 sec. The incubation medium: 25 mM HEPES/NaOH (pH 7.3), 10 mM $CaCl_2$. Here and further the arrows indicate moments of laser flashes.

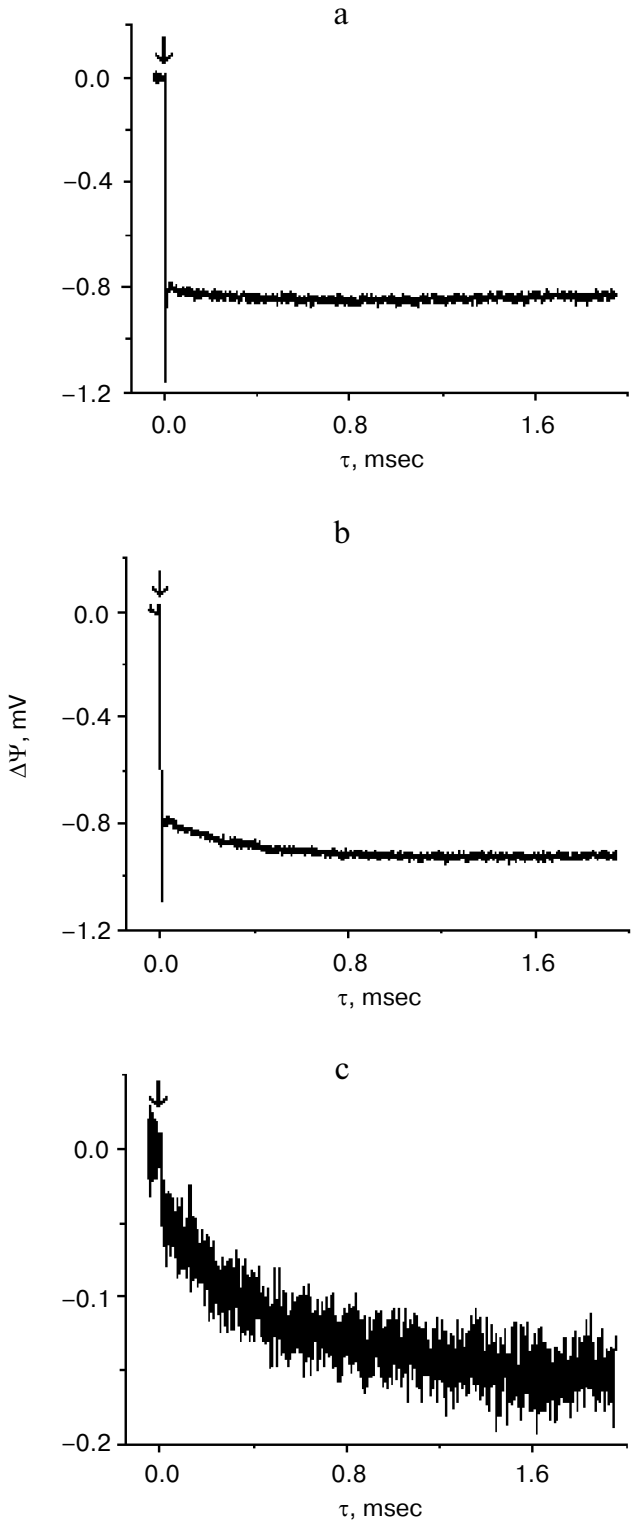

Fig. 2. Generation of transmembrane electric potential difference in dark-adapted proteoliposomes containing PSII core complexes with active water oxidation complex in response to the first (a) and second (b) laser flash; c) the difference of photoresponses (a – b). The incubation medium contained 25 mM HEPES/NaOH (pH 7.3), 10 mM $CaCl_2$, and decylplastoquinone (30 mg/ml).

preparations seems to be caused by the electron recombination between P680 and Q_A [28]. Thus, the oxygen-evolving activity is probably retained in ~81% of the PSII complexes incorporated into the liposomes.

The possibility of reconstructing functions of the secondary quinone Q_B in the proteoliposomes with PSII core complexes allowed us to study electron transfer reactions in the acceptor region using the direct electrometric technique. The function of Q_B was reconstructed by addition of plastoquinone into the phospholipid solution in decane. Plastoquinone and decylplastoquinone have similar values of midpoint potentials (E_m) and p*K*, but decylplastoquinone is more hydrophilic and has a higher distribution coefficient in the water/lipid system [29].

Figure 2 presents photoelectric responses induced by the first (a) and second (b) laser flashes on dark-adapted proteoliposomes containing PSII core complexes upon the reconstruction of electron transfer from Q_A to Q_B. The rapid phase maximal in amplitude and with growth time shorter than the resolution time of our apparatus was observed under the influence of both the first and second flash and corresponded to separation of charges between the redox-active tyrosine 161 residue (Y_Z) and quinone acceptor Q_A [22]. An additional electrogenic stage is recorded in the kinetic curve of the photoresponse to the second flash. The difference between the photoresponses induced by the second and first flashes is shown in Fig. 2c. The amplitude of this electrogenic phase is constant and maximal in the range of intervals to 5 sec between the two flashes. With a further increase in the interval, the amplitude of this phase decreases and the difference in amplitudes of the responses to the first and second flash disappears (data not shown).

The maximal amplitude of the additional "quinone" phase with $\tau \sim 0.85$ msec at pH 7.3 is ~11% of the amplitude of the rapid photoelectric response to the charge separation between Y_Z and Q_A. This phase disappears on addition of 5 μM diuron, which inhibits electron transfer from Q_A to Q_B (not shown).

Figure 3 presents the dependence of the additional electrogenic phase amplitude on the concentration of decylplastoquinone. As differentiated from chromatophores of photosynthesizing bacteria [18], a higher concentration of quinone is required to completely restore the function of the secondary quinone acceptor in PSII. This difference can be caused by: (i) higher concentration of the complexes in the proteoliposomes and (ii) difference between the binding constants of ubiquinone-10 and plastoquinone, which is the secondary quinone acceptor for PSII. The amplitude of the electrogenic "quinone" phase is maximal at decylplastoquinone concentration higher than 25 mg/ml.

Figure 4a presents the pH dependence of the additional electrogenic phase amplitude. The amplitude value is ~11% of the stage due to generation of $Y_Z^{ox}Q_A^-$ at pH from 6.0 to 8.1 and decreases with further increase in pH.

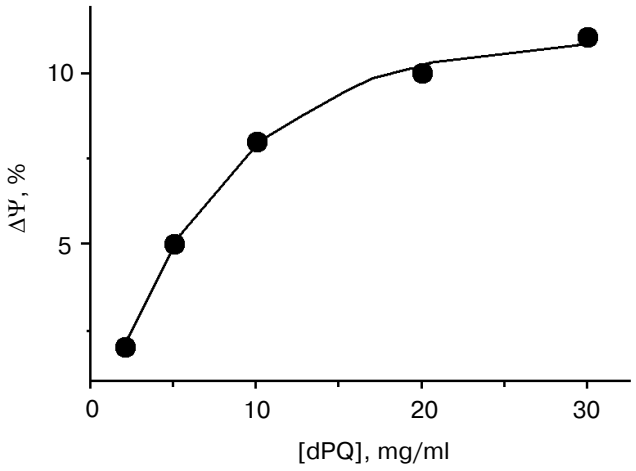

Fig. 3. Dependence of electrogenic "quinone" phase amplitude on the concentration of added decylplastoquinone (dPQ). The incubation medium: 25 mM HEPES/NaOH (pH 7.3), 10 mM $CaCl_2$.

The pH dependence of logarithm of the rate constant ($k = 1/\tau$) of the electrogenic protonation of Q_B^{2-} is shown in Fig. 4b. At pH values from 6.0 to 8.3, the rate constant of the reaction weakly depends on pH, whereas further increase in pH is accompanied by a significant fall. The appearance of such dependence under alkaline conditions suggests that at high pH values the proton transfer from the aqueous phase is a limiting stage in the generation of the additional phase.

Thus, the sensitivity of the additional electrogenic phase to diuron, the dependence of its amplitude on the flash number, as well as the dependence on pH of the amplitude and rate constant indicate that the phase recorded in response to the second flash is caused by protonation of the doubly reduced form of Q_B in the PSII complexes.

Note that the reaction of electron transfer between quinones Q_A^- and Q_B seems to be not electrogenic, although the production of Q_B^- can be associated with uptake of protons by amino acid residues located near the surface of PSII RC. Generation of membrane potential in response to the first flash in the presence and absence of diuron in the submillisecond time range can be also caused by $S_1 \rightarrow S_2$ transitions (the electron transfer between the Mn cluster and Y_Z) of OEC, although kinetics of this reaction are about 30 μsec [22]. As to the amplitude of the quinone electrogenic phase in response to the second flash, it can be also contributed to by electrogenesis caused by $S_2 \rightarrow S_3$ transitions of OEC [22, 30]. The lack of electrogenesis at the $S_2 \rightarrow S_3$ transition in the presence of diuron after incubation of the samples for 1 h seems to be due to the absence in the incubation medium of glycine betaine, which can prevent structural rearrangements in the water-splitting PSII complex [22, 30].

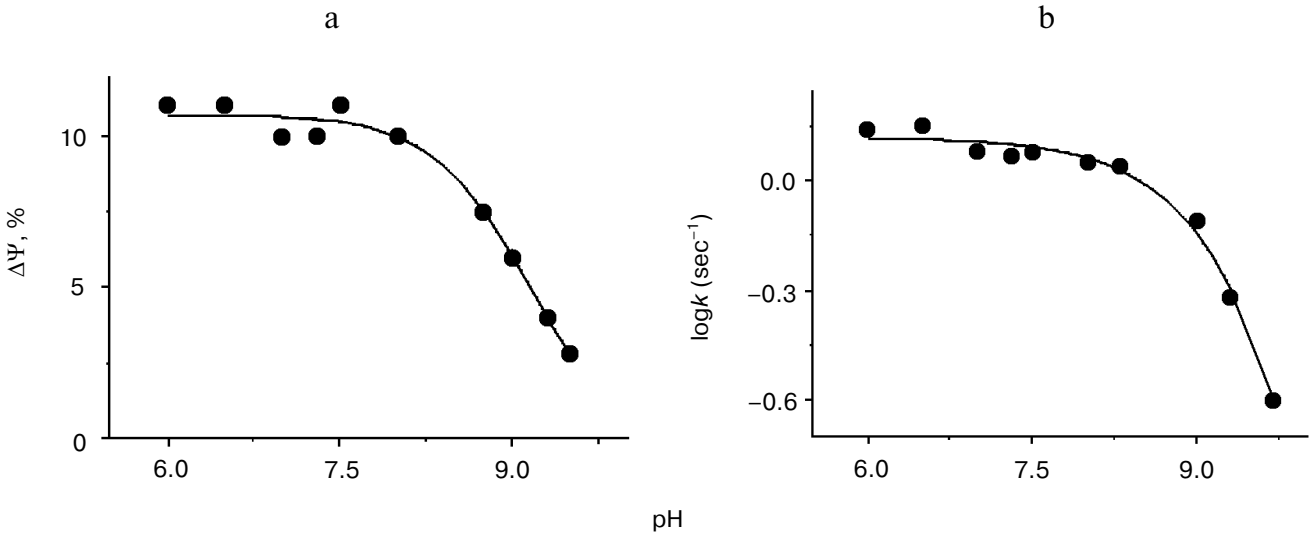

Fig. 4. The pH dependence of the amplitude (a) and logarithm of the rate constant ($k = 1/\tau$) (b) of $\Delta\Psi$ generation in the complex of quinone acceptors in response to the second light flash. The amplitude is given in percent of the amplitude of the phase corresponding to the charge distribution between Y_Z and Q_A in response to the first flash. The incubation medium: 10 mM $CaCl_2$, decylplastoquinone (30 mg/ml), and corresponding buffers with p*K* in the region of pH given.

The higher amplitude of the electrogenic "quinone" stage measured in the present work (as compared to the earlier recorded values [18, 19]) is probably associated with the presence of proteoliposomes containing PSII with active water oxidation system.

Thus, based on our findings, the electron transfer in the region of $Q_A \rightarrow Q_B$ is suggested to be not electrogenic (lengthwise of the membrane) and the quinone acceptor molecules seem to be submerged into the dielectric phase of the PSII RC. On such an imbedding into the protein, the protonation of the reduced secondary quinone Q_B (~11% of $Y_Z^{ox}Q_A^-$) needs the binding of H^+ from the external aqueous phase. This proton transfer can occur with involvement of acidic-basic groups of the protein, which are likely to be immediate donors of H^+ for Q_B^{2-}. Comparison of the amplitudes of electrogenic reactions in the quinone-acceptor complex in PSII from spinach and photosynthesizing bacteria [18, 19] shows that the observed difference is more likely to be associated with the lesser distance between Q_B and the surface of the membrane (or the PSII protein) [11] and also with the lower degree of reconstruction of the Q_B function.

Note that the quinone-binding site of Q_B in the RC from *Rb. sphaeroides* is mainly formed by amino acid residues of the subunit L of the reaction center and located at a depth of ~15-20 Å beneath the cytoplasmic surface of the protein [31].

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