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Functioning of quinone acceptors in the reaction center of the green photosynthetic bacterium *Chloroflexus aurantiacus*

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The photosynthetic reaction centers (RC) of the green bacterium *Chloroflexus aurantiacus* have been investigated by spectral and electrometrical methods. In these reaction centers, the secondary quinone was found to be reconstituted by the addition of ubiquinone-10. The equilibrium constant of electron transfer between primary (Q_A) and secondary (Q_B) quinones was much higher than that in RC of purple bacteria. The Q_B binding to the protein decreased under alkalinization with apparent pK 8.8. The single flash-induced electric responses were about 200 mV. An additional electrogenic phase due to the Q_B protonation was observed after the second flash in the presence of exogenous electron donors. The magnitude of this phase was 18% of that related to the primary dipole ($P^+Q_A^-$) formation. Since the *C. aurantiacus* RC lacks H-subunit, this subunit was not an obligatory component for electrogenic Q_B protonation.

RC-complex; Electrogenesis; Ubiquinone; *Chloroflexus aurantiacus*

1. INTRODUCTION

The structure of the reaction center (RC) complexes of the green photosynthetic bacterium *Chloroflexus aurantiacus* is rather similar to that of purple bacteria [1–3]. However, the former contain only two protein subunits, which resemble the L and M subunits of purple bacteria RCs, and lack the third H-subunit [2]. They differ also by their co-factor composition, containing 3 molecules of bacteriochlorophyll, 3 molecules of bacteriopheophytin, 2 menaquinone molecules and a manganese atom [1].

We have analyzed the photoinduced electron transfer reactions in the quinone acceptor complex over a wide pH range. In addition, we have studied the parameters of the second flash-induced electrogenic phase associated with the secondary quinone Q_B protonation in order to establish whether the two subunit-containing RC-complex is competent in electrogenic H^+ transfer from water to Q_B.

2. MATERIALS AND METHODS

Cells of *C. aurantiacus* were cultivated and RCs were isolated as described in [2]. Kinetics of laser flash-induced absorption changes were measured using a single-beam spectrophotometer [4]. The RC-containing proteoliposomes were prepared by sonication with subsequent filtration through a Sephadex G-50 column. The transmembrane electric potential difference ($\Delta\psi$) generation was measured as described in [4].

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3. RESULTS

The isolated preparation of *C. aurantiacus* RCs does not contain Q_B. Data presented in Fig. 1A (curve 1) show that the kinetics of the photo-oxidized primary donor (P^+) dark reduction are mono-exponential. The time constant (τ) equal to 65 ms is characteristic of the primary dipole recombination: $(P^+Q_A^- \rightarrow PQ_A$ [5].

The secondary quinone Q_B function could be restored almost completely by addition of ubiquinone Q-10. (Fig. 1A, curve 2). The kinetics of the P^+ reduction were much slower and reflect on the whole the $P^+Q_B^- \rightarrow PQ_B$ back reaction. Under these conditions, we observed oscillations of the absorption changes at 450 nm, which reflect semiquinone Q_B^- formation after odd-numbered flashes and its disappearance after even numbered flashes (Fig. 2).

According to Fig. 1B, the time constant of the flash-induced $P^+Q_B^-$ relaxation is: (i) much slower than that of purple bacteria; (ii) pH-dependent at pH <8.5. Since the back electron flow from Q_B^- to P^+ occurs only through Q_A[1], one can estimate the L_{AB} equilibrium constant of the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ transition: $L_{AB} = \tau_{BP}/\tau_{AP}$, where τ_{BP} and τ_{AP} are the time constants of $P^+Q_B^- \rightarrow PQ_B$ and $P^+Q_A^- \rightarrow PQ_A$ back reactions, respectively. Fig. 1B shows that L_{AB} values, which are equal to 45 and stable at pH >8.5, increase approximately twice under acidification. Kinetics of the $P^+Q_B^-$ discharge is rather slow in

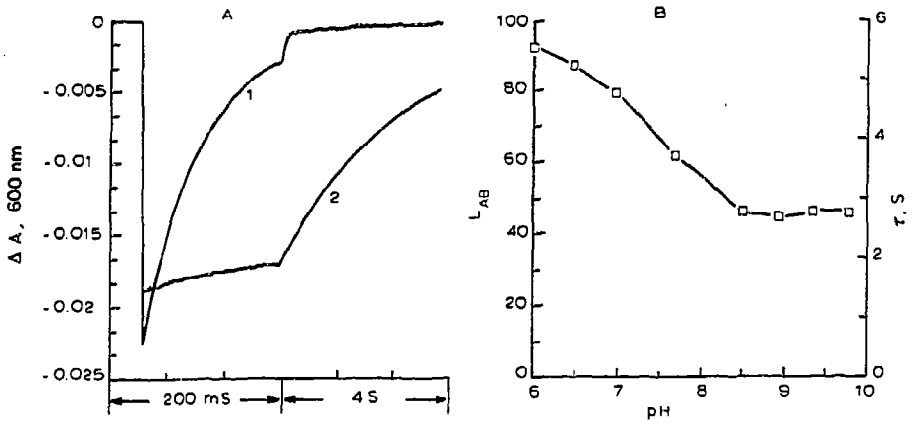

Fig. 1. Flash-induced absorption changes of a *C. aurantiacus* RC suspension at 600 nm. A. The kinetic curves without additions (1) and with 5 μM ubiquinone (Q-6) (2). B. pH dependence of the P^+ reduction characteristic time (right y-axis) and L_{AB} equilibrium constant (left y-axis) in the presence of ubiquinone. Incubation medium: (A) 50 mM Tris-HCl, pH 8.3; (B) 20 mM MES, HEPES, bis-tris-propane buffer each. RC concentration, 1.3 μM. Here and below arrows indicate laser flashes.

comparison with that for *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* RCs and pH-dependent. The equilibrium constant L_{AB}-values are markedly higher than those for *Rb. sphaeroides* RCs, probably, due to the lower midpoint redox potential value of the primary quinone (menaquinone for *C. aurantiacus*) and ubiquinone for *Rb. sphaeroides*.

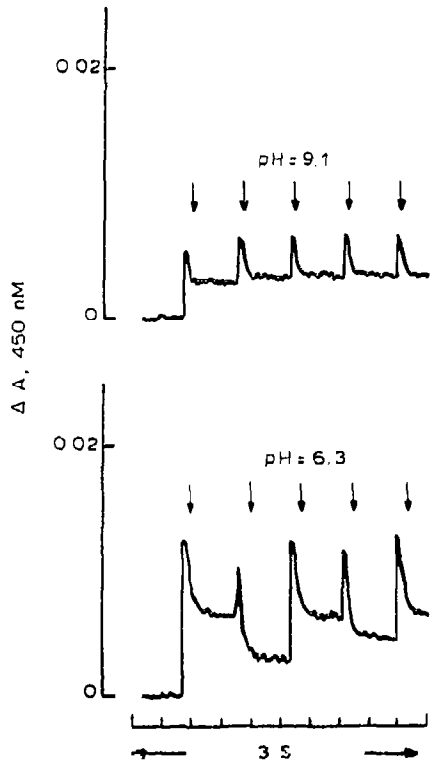

Fig. 2. Absorption changes of a *C. aurantiacus* RC preparation at 450 nm induced by a series of flashes at pH 9.1 (A); and pH 6.3 (B). The incubation medium contained 20 mM MES, HEPES, bis-tris-propane buffer; 5 μM Q-6, 0.1 mM TMPD. RC concentration, 1.2 μM. To eliminate the absorption changes due to TMPD oxidation, the absorbance at 480 nm was subtracted from that at 450 nm with coefficient 0.42.

Fig. 2 presents the pH dependence of the flash-induced absorption change oscillations at 450 nm on the flash number. At pH >8 the oscillations essentially diminish.

Extremely high magnitudes of the flash-induced electric responses (up to 200 mV) were observed in *C. aurantiacus* RC-containing proteoliposomes. The reason for high amplitudes of $\Delta\psi$ is a higher protein–phospholipid ratio in this proteoliposome preparation. The kinetic curves of the 1st and 2nd flash-induced $\Delta\psi$ generation are presented in Fig. 3. The main phase of $\Delta\psi$ generation was extremely fast (<0.1 μs, resolution limit of the apparatus) and ascribed to the primary dipole ($P^+Q_A^-$ formation. There are no additional phases after a single flash, while the second flash induces a new electrogenic phase in the millisecond time-scale. As shown earlier [6,7], this phase (so called 'quinone electrogenic phase') is the result of the electrogenic protonation of the double-reduced Q_B^2: $Q_A^-Q_B^- \xrightarrow{2H^+} Q_AQ_BH_2$. The pH dependence of the quinone electrogenic phase amplitude is presented in Fig. 4A. The amplitude of the quinone phase at 6 <pH <7.5 is 14% of the fast electrogenic phase, due to P^+Q_A formation (Fig. 4A, squares) and sharply diminishes under alkalinization. This effect could be related to decrease of the Q_B^2: $Q_AQ_B \xrightarrow{+2H^+} Q_A Q_bH_2$ equilibrium constant [8]. Alternatively, it could be explained as a result of pH-dependent decrease of Q_B binding constant. This explanation seems to be preferable, since the decrease in the first flash-induced amount of Q_B formed (Fig. 4A, diamonds) has the same pH

[1]Notice that, according to the data provided by Dr G. Venturoli (personal communication), the direct tunneling from Q_B to P^+ could be observed under appropriate conditions.

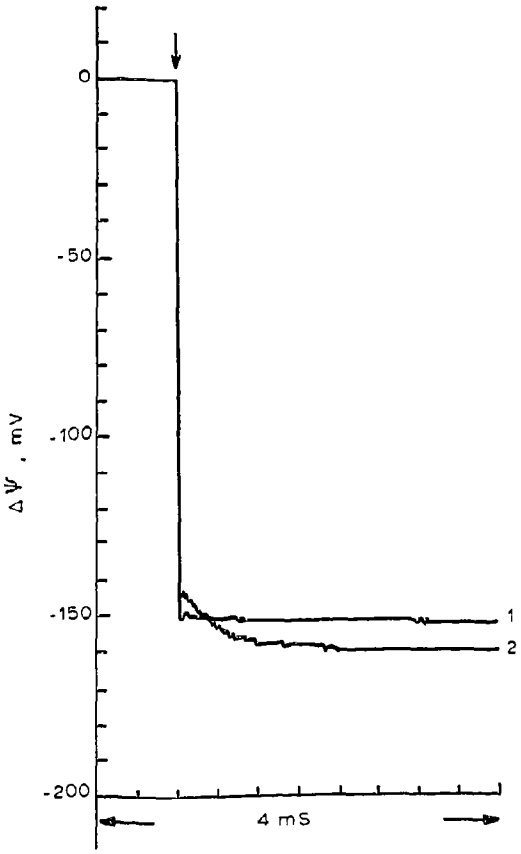

Fig. 3. $\Delta\psi$ generation by RC-containing proteoliposomes induced by the first (curve 1) and the second (curve 2) flashes. Incubation medium: 25 mM HEPES, pH 7.5, 0.1 mM TMPD. 15 mg of Q-10 was added per ml of phospholipid in *n*-decane used for impregnation of collodion film.

dependence as the quinone electrogenic phase amplitude. The p*K* values for both dependencies are about 8.8. Note that the same effect has a p*K* 9.8 for *Rb. sphaeroides* [8].

Fig. 4B shows the dependence of the quinone phase magnitude on the concentration of ubiquinone added to reconstruct Q_B. The concentration of Q-10 (15 mg/ml of phospholipid solution in *n*-decane), which was shown to be sufficient for the complete reconstruction of Q_B function in *Rb. sphaeroides* [9], proved to be too low for *C. aurantiacus* RC-containing proteoliposomes. The possible reasons are: (i) a very high concentration of RC-complexes in the proteoliposomes, and (ii) a difference between the binding constants of ubiquinone-10 and the native menaquinone, which operates as Q_B in this species. The maximum amplitude of the quinone phase is achieved at a Q-10 concentration above 40 mg/ml. This value (18% of the fast electrogenic phase) is close to that for *R. rubrum* and *Rb. sphaeroides* chromatophores [4,6].

It is interesting to compare the electrogenesis in the quinone-acceptor complex of the purple bacteria and *C. aurantiacus* since the latter lacks the H-subunit which covers the quinone-binding site of *Rb. sphaeroides* and *Rhodopseudomonas viridis*. The proton-conducting pathway from the protein surface to the Q_B-binding site possibly includes some residues belonging to the H-subunit of purple bacteria. The very fact that the magnitude of the quinone electrogenic phase is the same for *C. aurantiacus*, *Rb. sphaeroides* and *Rps. viridis* indicates that the H-subunit is not necessary for the electrogenesis.

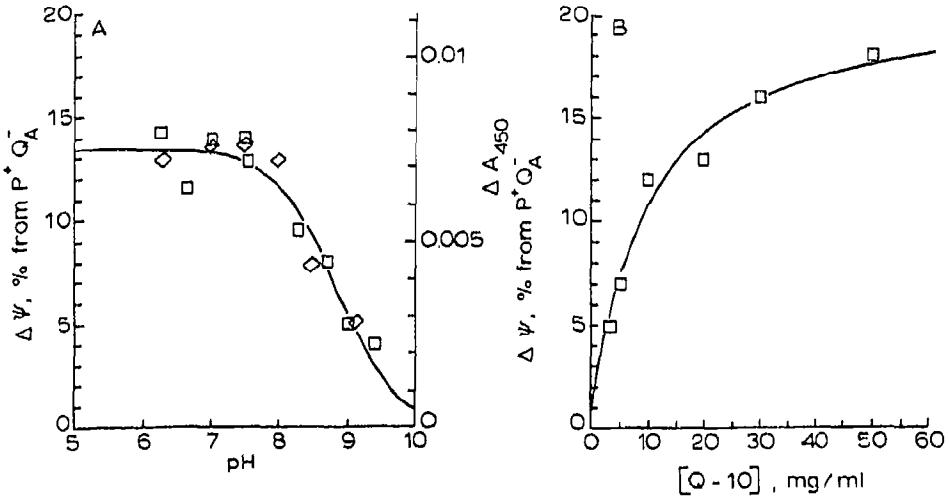

Fig. 4. Factors affecting the quinone electrogenic phase amplitude. A, pH dependence of this amplitude presented in % from the amplitude of the phase related to the P^+Q_A formation (squares). The single group titration curve with p*K* 8.8 obtained by the non-linear regression method was drawn through experimental points. B, The influence of Q-10 concentration of the quinone phase amplitude. For (B) the incubation medium was as in Fig. 3 and for (A) 25 mM of MES, HEPES and bis-tris-propane each were added. Diamonds (A) show the pH dependence of the first flash-induced ΔA_{450} absorption changes, reflecting the amount of stable semiquinone formed.

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