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Partial reversion of the electrogenic reaction in the ubiquinol

Cytochrome c_2-oxidoreductase of *Rhodobacter sphaeroides* chromatophores under neutral and alkaline conditions

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The interaction of the photosynthetic reaction center (RC)-generated ubiquinol with the ubiquinone-reducing center *C* of ubiquinol:cytochrome c_2-oxidoreductase (bc_1-complex) has been studied electrometrically in *Rhodobacter sphaeroides* chromatophores. The addition of myxothiazol inhibited the ubiquinol-oxidizing center *Z*, suppressing the phases of membrane potential generation by the bc_1-complex, but at the same time induced an electrogenic phase of opposite polarity, sensitive to antimycin A, the inhibitor of center *C*. The rise time of this reverse phase varied from 3 ms at pH 6.0 to 1 ms at pH 9.5. At pH > 9.5 the reverse phase was limited by the rate of ubiquinol formation in RC. The magnitude of the reverse phase was constant within the pH range 7.5–10.0. It is assumed that the reverse phase is due to the electrogenic deprotonation reaction which takes place after the binding of the RC-generated ubiquinol to center *C*.

bc_1-Complex; Electrogenesis; Ubiquinone; *Rhodobacter sphaeroides*

1. INTRODUCTION

In chromatophores of *Rhodobacter sphaeroides* ubiquinol molecules formed by the photosynthetic reaction center (RC) are oxidized in the quinol-oxidizing center *Z* of the ubiquinol: cytochrome c_2-oxidoreductase (bc_1-complex), the process being accompanied by transmembrane electric potential difference ($\Delta\Psi$) generation [1]. According to the Q-cycle concept [2,3], one of every two electrons released returns via water-soluble cytochrome c_2 to the primary donor *P*. The other electron moves via the low-potential heme b_l and the high-potential heme b_h to the ubiquinone-reducing center *C* which reduces ubiquinone molecules from the membrane pool. The study of the interaction between RC-formed ubiquinol and center *C*, when center *Z* is blocked by myxothiazol, is an approach for partial reversion of the electrogenic reaction in center *C* and thus for revealing the contribution of the center *C* supported electrogenic reaction to the overall electrogenesis [4,5]. A component of the carotenoid bandshift that reflects the reverse electrogenic reaction in center *C* has been observed only under alkaline conditions (pH above 8.5–9.0) [4,5]. The authors concluded that the reduction of heme b_h by RC-formed ubiquinol [6,7] was responsible for this reverse phase. Under investigation of $\Delta\Psi$ generation by chromatophores adsorbed on the surface of a phospholipid-impregnated collodion film, we observed the reverse electrogenic phase at neutral pH values, when heme b_h could not be reduced by RC-formed ubiquinol because of thermodynamic constraints [8]. In the present work we have investigated the interaction between RC-formed ubiquinol and the ubiquinone-reducing center *C* of the bc_1-complex in *Rb. sphaeroides* chromatophores over a wide pH range.

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2. MATERIALS AND METHODS

Cells of *Rhodobacter sphaeroides* (wild type, strain R1) were grown and chromatophores were isolated by French-press treatment as described in [8,9]. Photoelectric measurements were made and the kinetic curves were subtracted and analyzed as in [8].

3. RESULTS

Under oxidizing conditions ubiquinol formation in RC takes place only after even-numbered flashes [1,9]. Thus after illumination of the dark adapted *Rb. sphaeroides* chromatophores adsorbed on the phospholipid and uniquinone-impregnated collodion film by the first flash (Fig. 1a), only electrogenic phases due to the primary charge separation were observed (the rise time (τ) < 100 ns,) and due to the reduction of oxidized *P* by cytochrome c_2 (τ about 500 μs at a given buffer concentration). After the second flash (Fig. 1a) two

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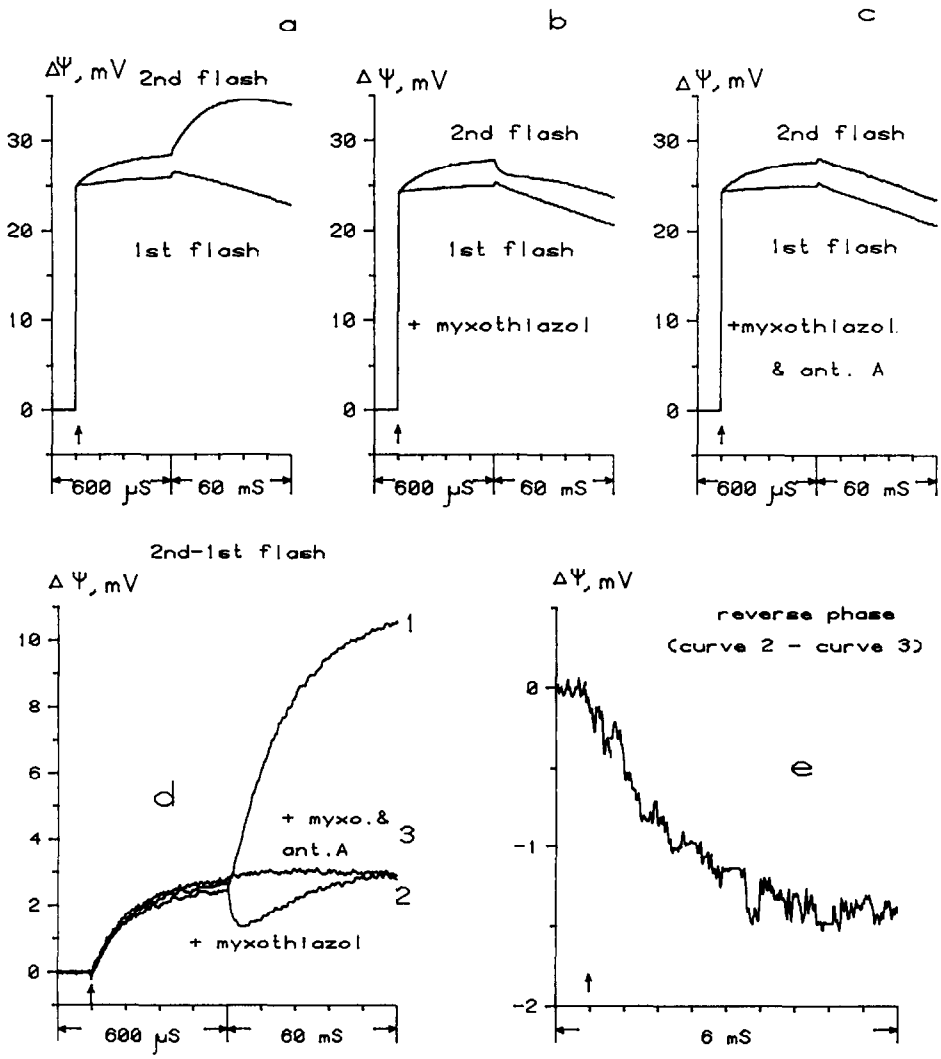

Fig. 1. Flash-induced photoelectric responses of *Rb. sphaeroides* chromatophores (see the text for details). Incubation medium: 30 mM Hepes (pH 7.5), 50 μM TMPD (*N,N,N',N'*-tetramethyl-*p*-phenylenediamine), 2 mM potassium ferrocyanide, E_h = 300 mV. Additions: 4 μM antimycin A, 5 μM myxothiazol. Arrows indicate laser flashes. Dark adaptation time between the flash cycles: 5 min.

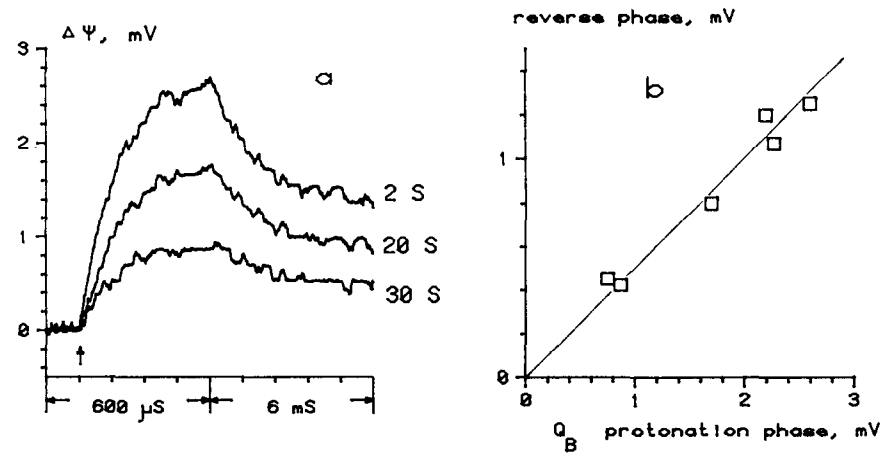

Fig. 2. Dependence of the reverse phase amplitude on the amount of RC-formed ubiquinol. Differences between the 2nd and the 1st flash-induced electric responses in the presence of myxothiazol at different time intervals between the flashes (a); dependence of the reverse phase amplitude on the Q_B^{2-} protonation phase amplitude (b). Conditions as in Fig. 1.

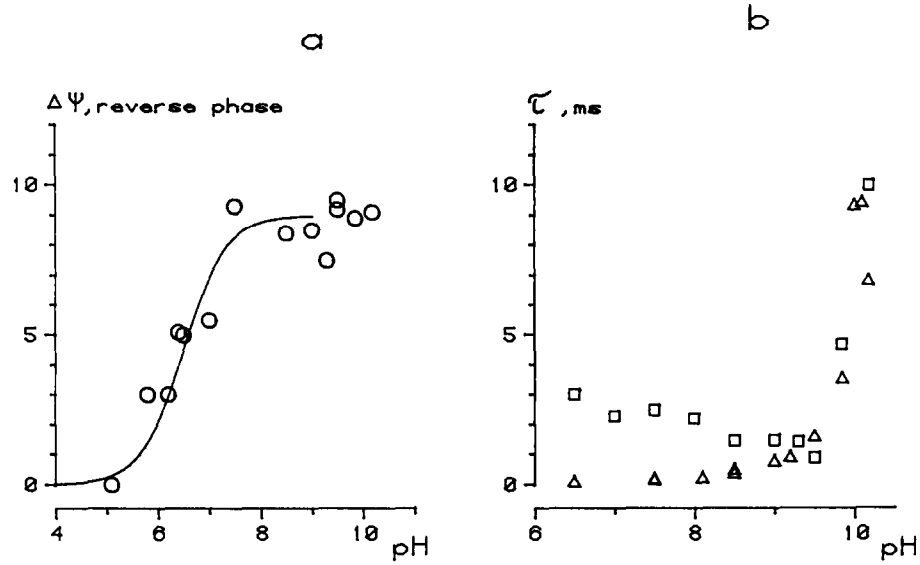

Fig. 3. pH-dependences of the reverse phase characteristics. pH-dependence of the reverse phase amplitude (in % of the primary charge separation phase amplitude; the titration curve for a single protonatable group with pK 6.5 is plotted via the experimental points) (a); pH-dependence of the τ values of the reverse phase (squares) and the Q_B^{2-} protonation phase (triangles) (b). Buffers used: 20 mM Hepes, 20 mM CHES, 20 mM Bis-Tris Propane and 20 mM Caps. Other conditions as in Fig. 1.

new phases appear in addition: a fast phase due to secondary acceptor (Q_B) protonation [10], and a slow phase due to ubiquinol oxidation by the bc_1-complex [8] (τ values 150 μs and 20 ms at pH 7.5, respectively; the difference between the second and the first flash is presented in Fig. 1d, curve 1). Myxothiazol suppresses the electrogenic reactions associated with center *Z* and causes the appearance of a 'reverse' phase after the second flash (Fig. 1b; Fig. 1d, curve 2), sensitive to antimycin A (Fig. 1c; Fig. 1d, curve 3). As seen from Fig. 1e, derived by subtracting curve 3 from curve 2 (Fig. 1d) the τ of the reverse phase is approx. 2.5 ms at pH 7.5.

We have found earlier [8,11] that the increase in the time between the flashes causes the amount of first flash-generated Q^-_B and consequently, the amount of RCs capable of forming uniquinol after the second flash to decrease because of the oxidation of Q^-_B by the redox-mediator. As is shown in Fig. 2 the amplitude of the reverse phase decreases in proportion to the amplitude decrease in the fast, Q_B^{2-} protonation-associated phase, that is in proportion to the amount of RC-formed ubiquinol.

As seen from Fig. 3a, the amplitude of the reverse phase does not vary over a wide pH range. When correlated for relaxation, its amplitude constitutes about 10% of the amplitude of the primary charge separation phase. With pH lowering, the reverse phase decreases and then disappears at approx. pH 5.0.

The τ value of the reverse phase decreases from nearly 3 ms at pH 6.0 to approx. 1 ms at pH 9.5 (Fig. 3b). At pH 9.5 the τ values of the reverse phase (squares) and Q_B^{2-} protonation-associated phase (triangles, data from [12]) became comparable Fig. 3b). With further alkalinization the reverse phase drastically slows down, because of the sharp slowing down of the rate of uniquinol removal out of RC caused by the slowing down of the electron transfer from Q_A^- to Q_B^- [13] and of Q_B^{2-} protonation rate (Fig. 3b) [12].

4. DISCUSSION

The data confirm the view that the observed reverse electrogenic phase arises from the interaction of RC-formed uniquinol with uniquinone-reducing center *C* of the bc_1-complex. As the amplitude of the reverse phase is pH-dependent under neutral and alkaline conditions this phase apparently is not associated with the reduction of heme b_h by RC-formed ubiquinol and thus differs from the phase kinetically coupled to heme b_h reduction via center *C* described previously [4,5]. Presumably the phase arises as a consequence of proton release out of center *C* upon uniquinol binding, and also as a result of the transfer of this proton from center *C* to the outer surface of the chromatophore. The proton is most likely to be released in a reaction $QH_2 \Leftrightarrow QH^- + H^+$ (1), that is supported by center *C* after ubiquinol binding. As was shown by Rich [14] this reaction precedes the ubiquinol oxidation reaction in different quinol:oxidoreductases. The ubiquinol is oxidized in center *C* according to reaction $QH^+ + b_h^{ox} \Leftrightarrow QH^\bullet + b_h^{red}$ (2) only under alkaline conditions when E_m of the ubiquinol/ubiquinone pair becomes lower than E_m of heme b_h [6,7], but there is no reason to exclude that the reaction (1) can occur under neutral conditions. According to our data on the non-electrogenic character of the electron transfer from heme b_h to center *C* ubiquinone [8], reaction (2) proceeds along the membrane plane.

The distance between center C and the outer chromatophore surface is estimated by us as $\geq$ 5 Å (considering the distance between P and Q_A to be about 27 Å [15], one bc_1-complex to serve two RCs [1], and proposing that the dielectric constant does not change dramatically in the hydrophobic region of the membrane).

The fact that the reverse phase rise time is limited at pH values above 9.5 by the rate of Q_B^{2-} protonation (Fig. 3a) suggests that (i) ubiquinol removal out of RC, (ii) its transfer from RC to the bc_1-complex and (iii) its binding to the bc_1-complex are not rate-limiting reactions. Hence, the time of each reaction is much smaller than 1 ms, since the reverse phase has a τ value of about 1 ms at pH 9.5. The estimates for reactions (i)–(iii) kinetics obtained from the literature give somewhat slower values [1,3].

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