Electrogenic reduction of the secondary quinone acceptor in chromatophores of *Rhodospirillum rubrum*

Rapid kinetics measurements

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Electron transfer $Q_A \rightarrow Q_B$ has been reconstituted with added Q-10 in *Rhodospirillum rubrum* chromatophores associated with a phospholipid-impregnated collodion film. Rapid kinetics measurements of laser flash-induced $\Delta\psi$ generated in the chromatophores show that whereas electron transfer from Q_A^- to Q_B upon the first flash is not electrogenic in dark-adapted chromatophores, reduction of Q_B^- to Q_BH_2 induced by the second flash gives rise to an electrogenic phase with $\tau = 250$ μs at pH 7.5 which contributes about 10% to the total $\Delta\psi$ generated upon the flash. The electrogenic phase is ascribed to vectorial protonation of Q_B^{2-}.

(Rhodospirillum rubrum) *Chromatophore* *Membrane potential* *Quinone acceptor* *Kinetics* *Binary oscillation*

1. INTRODUCTION

Electron transfer in the reaction centre complexes of photosynthetic purple non-sulfur bacteria, such as *Rhodospirillum rubrum* or *Rhodopseudomonas sphaeroides*, comprises 3 major steps (e.g. [1–3]): (i) very rapid oxidation of the photoexcited P-870 (a special bacteriochlorophyll molecule pair) by the primary ubiquinone acceptor Q_A which occurs via a number of intermediates with $\tau \sim 0.2$ ns; (ii) re-reduction of the photooxidized $P\text{-}870^+$ by a *c*-type cytochrome (varying from 10^{-5} to 10^{-3} s, depending on the exact conditions); (iii) oxidation of the photoreduced primary quinone by a secondary quinone acceptor Q_B.

Rapid kinetics measurements of membrane potential generation have shown that reactions (i) and (ii) are electrogenic [1,4–10]. As to reaction (iii), it was proposed to be electrogenic in [7], but reported as non-electrogenic in [8,9].

Reduction of the secondary acceptor is known to occur in 2 different sequential reaction steps. Single turnover experiments have shown that, under appropriate conditions, Q_B is reduced upon one first flash to a stable tightly bound semiquinone-anion Q_B^- which after the second flash is converted to hydroquinone, receiving a second e^- and binding 2 H^+ from the chromatophore exterior aqueous phase (review [2,3]).

Here we show with the aid of the direct electrometric method that the first step of Q_B reduction is not electrogenic whereas the second one is associated with the electrogenic phase contributing ~10% to the photopotential generated upon e^- transfer from P-870 to Q_B.

2. METHODS

Isolation of chromatophores from *R. rubrum*,

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their association with the phospholipid-impregnated collodion film and rapid kinetics measurements of $\Delta\psi$ by the direct electrometric method were as described earlier [7,10–12].

Saturating light pulses were delivered from a LOMO OGM-40 ruby laser (λ = 694 nm; pulse half-width, 20 ns; 50 mJ output) and a Quantel neodim laser operated in a doubled frequency mode (λ = 530 nm; pulse half-width, 15 ns; 50 mJ output), linked to a home-built programmed pulse generator. The data storage and processing system consisted of a transient recorder DL-1080 (Datalab) interfaced to a NOVA-3D minicomputer (Data General).

Reconstitution of the secondary acceptor function in the collodion film-associated chromatophores was achieved by adding 30 mg/ml of Q-10 (Sigma) to the solution of asolectin in decane used to impregnate the collodion film or 4 μM Q-2 (Ferak, West Berlin) directly to the measuring cell as described [11].

3. RESULTS

As described elsewhere, the secondary quinone acceptor is lost from chromatophores of *R. rubrum* upon their association with a phospholipid-impregnated collodion film, but $Q_A \longrightarrow Q_B$ electron transfer can be reconstituted to more than 70% by excess Q-10 [11].

Disappearance of the rapid ($\tau \sim 70$ ms) phase of the flash-induced $\Delta\psi$ decay corresponding to the back-reaction $Q_A^- \longrightarrow P\text{-}870^+$ is diagnostic of the reconstitution [8,11]. For instance, under the conditions of the experiment shown in fig.1, the contribution of the 70 ms decay of the laser flash-induced photopotential is virtually 100% in the absence of additions (trace 1) but becomes only 25% in the case of Q-10 supplemented collodion film (trace 2), the effect of Q-10 being reversed by *o*-phenanthroline (trace 3).

The electron transfer from Q_A to Q_B reconstituted, we examined the kinetics of $\Delta\psi$ generation upon the 1st and 2nd laser flashes in *R. rubrum* chromatophores. In dark-adapted samples, the 1st flash would give rise to a relatively stable $Q_AQ_B^-$ state of the acceptor side of reaction centre and the second one would entail reduction of Q_B^- to Q_B^{2-} and its protonation to Q_BH_2. Since the *R. rubrum* chromatophores obtained were

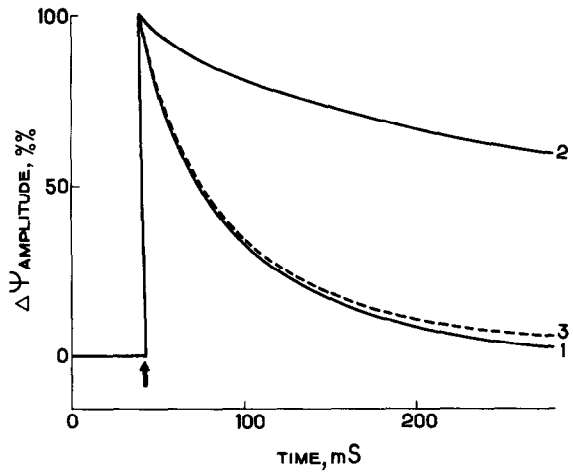

Fig.1. Reconstitution of $Q_A \longrightarrow Q_B$ electron transfer in the collodion film-associated chromatophores of *R. rubrum* with ubiquinone-10. *R. rubrum* chromatophores were adhered to one side of a phospholipid-impregnated collodion film insulating the 2 compartments of the measuring cell filled with the basic medium containing 20 mM Hepes, pH 7.5. A laser-flash generated electric potential difference between the 2 compartments was measured. Curves: 1, no additions; 2, ubiquinone-10 (30 mg/ml) was added to the decane solution of phospholipids employed for impregnation of the collodion film; 3, as 2 but in the presence of 1 mM *o*-phenanthroline.

deficient in cytochrome c_2, excess ascorbate and 100 μM TMPD were added to provide for sufficiently rapid (τ = 25 ms as measured spectrophotometrically, see also [13]) re-reduction of $P\text{-}870^+$ between the 1st and 2nd flashes. 2–4 μM methylene blue was included in most experiments to ensure a fully oxidized state of the Q_AQ_B couple before the flashes, following the observation of Shinkarev et al. [14] that methylene blue is a very efficient oxidant for $Q_B^{\cdot-}$.

Some typical results are given in fig.2. Upon the 1st flash rapid generation of $\Delta\psi$ is observed which is complete in less than 0.2 μs (resolution time of the apparatus), and there are no additional electrogenic events during the subsequent several milliseconds except for a small relaxation of the response in the first ~10 μs which was discussed earlier [7,11] (trace A). The 2nd flash delivered in 0.5 s gives rise to the rapid phase of $\Delta\psi$ generation of approximately the same amplitude as the first one, followed by a second slower phase of $\Delta\psi$ in-

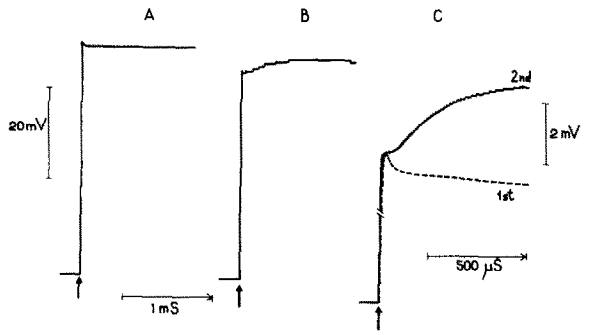

Fig.2. Rapid kinetics of membrane potential generation by *R. rubrum* chromatophores upon the 1st and the 2nd flashes. The incubation medium contained 50 mM Hepes pH 7.5, .2 mM ascorbate, 100 μM *N,N,N',N'*-tetramethyl-*p*-phenylene diamine and 4 μM methylene blue. Other conditions were as in fig.1, curve 2. Prior to the 1st flash, the chromatophore/collodion film-system was incubated for several minutes in the dark. Curves: A, electrical response induced by the 1st flash; B, electrical response induced by the 2nd flash fired in 0.5 s after the first one; C, expanded recording of the upper part of curves A and B.

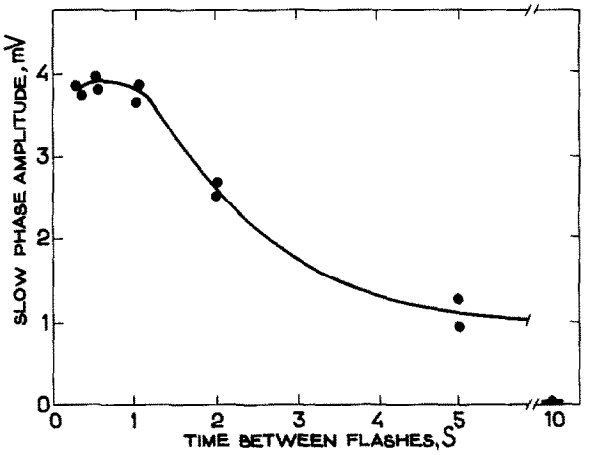

Fig.3. Effect of the time interval between the 2 flashes on the amplitude of the 250 μs phase of photopotential generation observed on the 2nd flash. The experiment shown in fig.2 was repeated; the interval between the 2 flashes varied as indicated. The pairs of flashes were separated by 60 s.

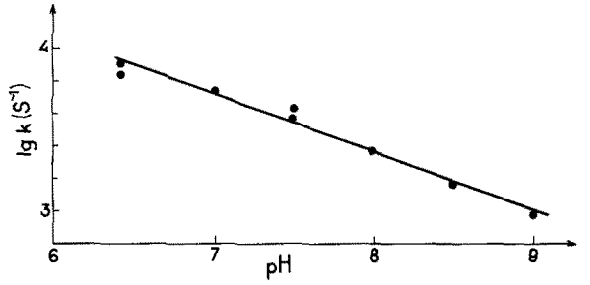

Fig.4. pH dependence of the rate constant ($k = 1/\tau$) of the additional phase of $\Delta\psi$ generation elicited by the 2nd flash. The experiment shown in fig.2 was repeated at different pH values. The buffers used at pH values indicated in parentheses are: Mes (6.4), Mops (7.0), Hepes (7.5), Tris (8.0–8.5), Ches (9.0), 50 mM each.

crease (trace B). This second phase has a τ value of 250 μs at pH 7.5 (see the expanded recording in trace C) which is similar to characteristics of the Q_A to Q_B electron transfer in *R. rubrum* chromatophores [15] and *Rps. sphaeroides* reaction centres [15,16]. Notably $\tau_{1/e}$ of 220 μs at pH 7.0 was reported also for the rapid flash-induced H^+ uptake by *Rps. sphaeroides* chromatophores [17] (and see [18–21] for related observations).

At pH 6–8 (at pH > 8 a significant decrease of the second phase amplitude was observed, cf. [16,17]), the maximal amplitude of the 250 μs phase of $\Delta\psi$ generation is ~1/10 that of the initial rapid phase but it decreases as the time interval between the two flashes is increased beyond 1 s (fig.3). This is presumably due to $Q_B^{\cdot-}$ being reoxidized by methylene blue before the 2nd flash. At 4 μM of the dye, the reoxidation half-time can be evaluated as ~3 s (fig.3), which is in reasonable agreement with the results of Shinkarev et al. [14] on *R. rubrum*.

The additional phase of $\Delta\psi$ generation upon the 2nd flash could also be observed if 50–100 μM duroquinone was substituted for methylene blue or even in the absence of added electron acceptors for $Q_B^{\cdot-}$ under the conditions of a sufficiently long dark adaptation. In the latter case, however, a small 250 μs phase of $\Delta\psi$ generation was usually revealed even upon the 1st flash; presumably, some part of the reaction centres was still in the $Q_AQ_B^{\cdot-}$ state before the flashes. The even flash-induced second electrogenic phase was not specific to Q-10 as the Q_B and could be observed in Q-2 reconstituted chromatophores as well (not shown).

As mentioned above, a τ value of 200–500 μs is typical of electron transfer between Q_A and Q_B at neutral pH and of proton uptake associated with secondary quinone acceptor reduction. The rate of both processes is known to decrease upon alkalinization [15–17]. As shown in fig.4, this also

holds for the rate constant of the additional phase of $\Delta\psi$ generation observed upon the 2nd flash. Actually, the pH dependence in fig.4 is in very good agreement with the pH profiles both of $Q_A \longrightarrow Q_B$ electron transfer in *R. rubrum* and *Rps. sphaeroides* chromatophores [15] and of H^+-binding kinetics in *Rps. sphaeroides* chromatophores [17].

4. DISCUSSION

It was suggested earlier that electron transfer between the primary and secondary quinone acceptors in reaction centres of photosynthetic bacteria could be coupled to $\Delta\psi$ generation via vectorial e^- or H^+ transfer [7]. However, this reaction was reported to be non-electrogenic in [8,9].

The results given in this paper show for the first time that reduction of the secondary acceptor semiquinone to quinol is indeed linked to vectorial charge movement across the chromatophore membrane.

This electrogenic reaction can hardly be electron transfer from Q_A to Q_B per se, since in this case Q_B reduction to $Q_B^{\cdot -}$ upon the 1st flash would be electrogenic as well, which is not the case. A plausible explanation is that $Q_A \longrightarrow Q_B$ electron transport occurs non-electrogenically along the membrane, the quinone acceptor molecules being embedded in the dielectric phase of the reaction centre complex (fig.5). Indeed, the quinone acceptors of the reaction centre complex are not readily accessible to membrane-impermeable electron acceptors in *R. rubrum* chromatophores [22,23] and have been reported to be localized ~10 Å from the reaction centre surface [24,25]. Consequently, protonation of the doubly reduced Q_B^{2-} will require vectorial electrogenic H^+ uptake from the external aqueous phase. Conceivably, this protonation could be mediated by the protein acid-base groups and these latter are more likely to serve as immediate H^+ donors to Q_B^{2-}.

Another possibility which cannot be ruled out at present is that $Q_B^{\cdot -}$ reduction to Q_BH_2 is associated with a protein structure change involving charged group(s) dislocation across the membrane.

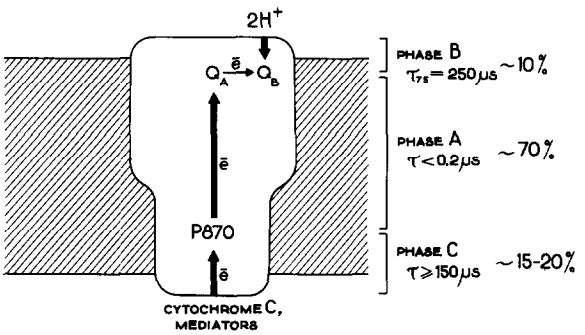

Fig.5. Electrogenic events in the reaction centre complex of *R. rubrum* revealed by the direct electrometric assay. The diagram summarizes results of the present and previous [7,10,13] works with the collodion film-associated photosynthetic bacteria membranes. The 3 principal electrogenic reactions within the centre are depicted by thick lines. These include: (A) electron transfer from P-870 to Q_A [7]; (C) P-870$^+$ re-reduction by a *c*-type cytochrome [10] or redox dyes [13]; (B) Q_B^{2-} protonation, as tentatively identified here.

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