Electrogenic reduction of *Rhodospirillum rubrum* reaction centre bacteriochlorophyll P870$^+$ by redox dyes

Indication of intraprotein electron transfer

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Reduction of laser flash-oxidized bacteriochlorophyll in *Rhodospirillum rubrum* reaction centre proteoliposomes or in cytochrome c_2-deficient *R. rubrum* chromatophores by artificial redox dyes like *N,N,N',N'*-tetramethyl-*p*-phenylenediamine or phenazine methosulphate gives rise to an electrogenic phase in the millisecond range. This phase contributes as much as 20% to the total photoelectric response and is ascribed to vectorial electron transfer from the reaction centre protein surface to the protein-embedded Mg-porphyrin rings of P870$^+$.

Rhodospirillum rubrum *Membrane potential* *Proteoliposome* *Reaction center bacteriochlorophyll* *Intramolecular electron transfer* *Rapid kinetics*

1. INTRODUCTION

Electron transfer within RC complexes of photosynthetic bacteria like *Rhodospirillum rubrum* or *Rhodopseudomonas sphaeroides* is coupled to transmembrane electric potential difference generation (review [1]). A major contribution to $\Delta\psi$ generation is provided by vectorial e^- transfer from P870 (a special bacteriochlorophyll pair) to the primary acceptor (a tightly bound CoQ_{10}) [2–4] which occurs via a number of intermediates in approx. 200 ps. Indications of the electrogenic nature of P870$^+$ re-reduction by cytochrome c^{2+} [2,4,5] and of the $Q_A \longrightarrow Q_B$ electron transfer [3] can be found in the literature.

Recently we described in detail an electrogenic phase coupled to P870$^+$ reduction by horse heart cytochrome *c* in proteoliposomes with *R. rubrum* RC complexes [6]. Here we show that a similar electrogenic phase can be observed in the absence of cytochrome *c* with high concentrations of reduced TMPD or PMS as electron donors to P870$^+$.

Abbreviations: RC, reaction centre; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; PMS, phenazine methosulphate; $\Delta\psi$, transmembrane electrical potential difference

2. MATERIALS AND METHODS

TMPD, PMS and most of the other chemicals were from Sigma. Cell culture, preparation of chromatophores and of the antenna-free RC complexes from *R. rubrum* and proteoliposome reconstitution were as described [7]. The isolated chromatophores were further subjected to ultrasonic treatment (6 × 20 s) in a high ionic strength medium (150 mM KCl, 50 mM Tris-HCl, pH 7.8) to remove residual cytochrome c_2. 20 ns saturating laser flashes (λ = 694 nm) were used to activate electron transfer. The rapid kinetics of membrane potential generation in proteoliposomes

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and chromatophores was measured by a direct electrometric method as detailed in [3,8]. In short, proteoliposomes were adhered to one side of a collodion film impregnated with asolectin solution in decane; the film was fixed between the 2 compartments of the measuring cell filled with electrolyte solution. $\Delta\psi$ was registered as an electric potential difference across the collodion film with a pair of light-protected Ag/AgCl electrodes connected via an operational amplifier (Burr Brown 3554 BM) with a Datalab DL-922 transient recorder and a NOVA-3D minicomputer.

Spectrophotometric recordings of $P870^+$ reduction kinetics in chromatophores were carried out at 602 or 432 nm in a standard 10 mm rectangular optical cell using a home-built single-beam apparatus consisting of a light source (a 75 W halogen lamp KGM 9V), 2 monochromators (HL Jobin Yvon, UM-2) and an FEU-128 photomultiplier tube linked to the same data collection and storage system as used for the electrometric measurements. Chromatophores were suspended at a concentration corresponding to $\Delta A_{602} = 0.5$ and 40–160 4-s-spaced transients were averaged for each point.

The kinetic traces were decomposed into individual exponents in a NOVA-3D minicomputer; a set of programmes was developed by Dr Alexander L. Drachev in this laboratory.

All experiments were carried out at 23°C.

3. RESULTS

A typical kinetics of laser flash-induced electric responses in proteoliposomes inlaid with *R. rubrum* RC complexes is shown in fig.1. In the absence of additions or in the presence of ascorbate and vitamin K_3 which made no difference (cf. traces a and d), a flash of light gives rise to $\Delta\psi$ generation in less than 200 ns (resolution time of the apparatus) followed by an essentially monophasic exponential decay with $\tau \sim 60$ ms (trace a). (There is a small and variable initial overshoot of the photoelectric response (e.g., see traces b,e,f) as also reported in [3]. So far the nature of this effect is not known; no correlation with any particular experimental conditions has been found.) As discussed in [3,7,10], the effect is typical of the primary dipole $P870^+$-Q_A^- formation and discharge; it is noteworthy that in the collodion film-associated vesicles, Q_B does not function unless special precautions are taken [9,10]. The sign of the response corresponds to the interior of the vesicles being charged negatively. This polarity is opposite to that observed in chromatophores and is specific for *R. rubrum* RC proteoliposomes due to the inverted orientation of RC complexes in these vesicles [6,7].

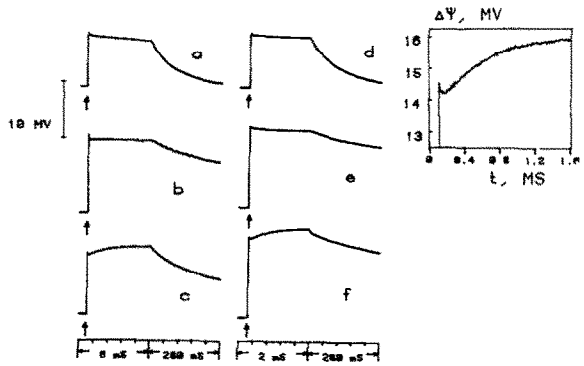

Fig.1. The effect of redox dyes on photoelectric response kinetics in proteoliposomes with RC complexes from *R. rubrum*. The final solution in both compartments of the measuring cell contained 20 mM Hepes-KOH, pH 7.5. Additions: (a) none; (b) 2 mM ascorbate, 0.5 mM TMPD, 100 μM vitamin K_3; (c) 10 mM ascorbate, 4 mM TMPD, 100 μM vitamin K_3; (d) 2 mM ascorbate, 100 μM vitamin K_3; (e) as (d) + 20 μM PMS; (f) as (d) + 150 μM PMS. The inset shows a part of the photoelectric response f as recorded separately under the instrumental conditions optimal for resolution of the slow phase of $\Delta\psi$ generation.

Under these conditions, addition of redox mediators such as TMPD or PMS exerts 2 effects. First, mediators decelerate the decay of $\Delta\psi$ (e.g. fig.1, traces b,e) as they re-reduce rapidly $P870^+$ and thus prevent the 60 ms discharge of the primary dipole; dissipation of $\Delta\psi$ in the $P870_{red}$-Q_A^- state then occurs with $\tau \gtrsim 1$ s characteristic of the proteoliposome membrane passive discharge. The contribution of this slow phase of $\Delta\psi$ decay increases with increasing mediator concentration so that at ~0.5 mM TMPD or ~20 μM PMS virtually all of the laser flash-induced $\Delta\psi$ dissipates slowly with $\tau \sim 1$ s (detailed discussion [3,10]).

Further increase in mediator concentrations has no significant effect on $\Delta\psi$ decay, but elicits an additional phase of membrane potential generation. As shown in fig.1 (traces c,f), in the presence of

either TMPD or PMS the non-resolved initial very rapid electric response is followed by a slower increment of $\Delta\psi$ which contributed in various experiments 15–20% to the total response. A similar electrogenic phase was induced by the mediators in the cytochrome c_2-deficient chromatophores from *R. rubrum* (not shown).

The rate of the second phase of $\Delta\psi$ generation increases with increasing concentrations of TMPD or PMS. Fig.2 shows concentration dependencies of the first-order rate constant of the slow electrogenic phase as induced by TMPD or PMS in RC proteoliposomes (A) and cytochrome c_2-deficient chromatophores (B). In the latter case, spectrophotometrical measurements of $P870^+$ reduction kinetics have also been carried out and the k_v values obtained are given in fig.2 by the filled symbols. One can see that in chromatophores there is a very good correlation between the optical and electrometric measurements with both TMPD and PMS as electron donors. It can also be seen that the kinetics of the electrogenic phase is similar in chromatophores and proteoliposomes. With TMPD there is virtually no difference between the results obtained with the 2 preparations; with PMS, the reaction in chromatophores appears to be ~2-fold faster than in proteoliposomes, which does not seem to be a great discrepancy. It is therefore tempting to identify the slow electrogenic reaction in both chromatophores and proteoliposomes as $P870^+$ reduction by the mediators.

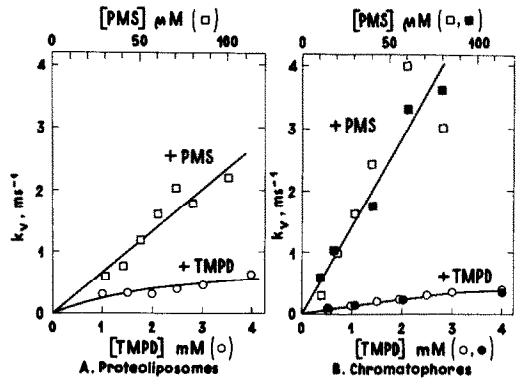

Fig.2. Dependence of the rate constant of the slow electrogenic phase on redox dye concentrations. (A) Experiments with RC proteoliposomes. The reaction medium contained 20 mM Hepes, pH 7.5, 2 (□) or 2–10 (○) mM ascorbate, 100 μM vitamin K_3 and TMPD or PMS as indicated. The kinetic traces of the photoelectric response were computer-analyzed to determine the true values of $\tau_{1/e}$ for the mediator-induced electrogenic phase. Resolution of the curves into individual exponents taking into account the decay of the photoelectric response in the dark (detailed discussion [9]) is essential since $\Delta\psi$ dissipation tends to decrease the apparent τ values especially at slow rates of the electrogenic phase. (B) Experiments with cytochrome c_2-deficient chromatophores. Open symbols, electrometric measurements of $\Delta\psi$ generation; conditions as in (A). Filled symbols, spectrophotometric measurements of $P870^+$ re-reduction kinetics at 602 (■) or 432 (●) nm in the suspension of chromatophores. Reaction medium: as in A except vitamin K_3 was at 200 μM.

PMS has been found to be a much more efficient electron donor for $P870^+$ than TMPD. (Note that the experiments have been carried out aerobically. Under anaerobic conditions PMS proved even more effective.) The highest rates of the reaction observed with it in the given concentration range correspond to τ 250 μs. This is close to the value of 200 μs as reported for the electrogenic phase induced in RC proteoliposomes by cytochrome c^{2+} [6]. At PMS concentrations higher than ~100 μM, a great scatter of data was observed routinely, which is at least partly due to low solubility of the reduced form of the mediator, and at present it is not clear whether the reaction rate saturates in [PMS]. The saturation behaviour is, however, clearly seen in the experiments with TMPD, where the maximal k_v value of 400–500 s^{-1} is reached at ~4 mM mediator.

4. DISCUSSION

Our previous experiments allowed one to identify the electrogenic step of electron transfer between cytochrome c^{2+} and $P870^+$ in proteoliposomes with *R. rubrum* RCs [6]. The present data show that the electrogenic nature of P870 reduction may not be specific for cytochrome *c* as electron donor. This finding has important bearings on the mechanism of electrogenesis. As pointed out earlier [6], cytochrome *c* might form a specific complex with the RC protein(s) in which it would become integrated in the dielectric phase of the protein-lipid membrane of proteoliposomes. In such a complex the electrogenic step of cytochrome c^{2+} oxidation could reside at least partially in the

cytochrome *c* molecule since the haem iron is located within several ångströms of the protein surface around the redox active haem edge [11,12]. However, the amplitude of the electrogenic phase induced by TMPD or PMS (15–20% of the total electrogenic response) is approximately the same as observed previously in the presence of cytochrome *c* [6]. Hence, the main electrogenic event linked to $P870^+$ reduction is likely to be vectorial electron transfer within the RC complex itself (fig.3).

It can be tentatively suggested that P870 (a special bacteriochlorophyll pair) is not exposed at the RC complex surface but is embedded deeply in the protein, which is in keeping with the data in [13–15]. Consequently, reduction of $P870^+$ may not involve direct contact of the pigment with the exogenous electron donors, rather, electrons would have to span a considerable distance from the protein surface site(s), where the donors bind, to the sequestered Mg-porphyrin ring of the pigment. Such an intra-protein transfer of an electron could occur by physical tunneling and/or by a protein-assisted conductance mechanism (reviews [17,18]), as discussed, for instance, for the cytochrome *c* complexes with cytochrome b_5 [18] and cytochrome *c* peroxidase [19,20].

A less likely alternative is that the mediators can penetrate the P870-binding pocket of RC protein and reduce the pigment non-electrogenically. The electrogenic phase could then originate from (i) back-diffusion of the oxidized cationic forms of the mediators to the exterior aqueous phase or (ii) some secondary electrogenic process (e.g., RC protein conformation change) linked to $P870^+$ reduction provided that both processes are fast compared to the $P870^+$ reduction step.

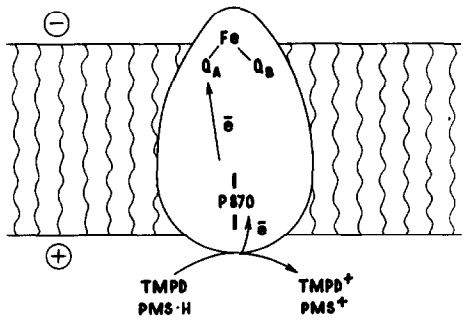

Fig.3. Scheme of the electrogenic electron transfer in reaction centre complex.

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