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Effects of electron donors and acceptors on the kinetics of the photoelectric responses in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* chromatophores

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Chromatophores of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* were adhered to one side of a collodion film impregnated with a phospholipid solution in decane and 20 ns laser flashes were delivered to produce an electrical potential difference generated across the collodion film in less than 0.2 μs (resolution time of the apparatus). The kinetics of $\Delta\psi$ decay in the dark was studied. In the absence of additions there occurs a 'rapid' decay of photoelectric potential ($\tau \approx 70$ ms) corresponding to charge recombination within the primary dipole $P\text{-}870^+\text{-}Q_A^-$. The rapid decay of $\Delta\psi$ is prevented by ascorbate in the presence of permeable redox dyes which can reduce the photooxidized $P\text{-}870^+$ rapidly. Under these conditions, $\Delta\psi$ dissipates with $\tau > 0.5$ s typical of a passive discharge of the chromatophore membrane. Prevention of the rapid decay of $\Delta\psi$ by 70–75% can be observed upon addition of excess ubiquinone-10 to the solution of phospholipids used to impregnate the collodion film, and to a lesser extent by addition of some other quinones. The effect of quinones is inhibited by *o*-phenanthroline. The data obtained show that upon association of chromatophores with the collodion film, the secondary quinone acceptor is extracted from its binding site into a hydrophobic volume of the macroscopic membrane, and this effect can be reversed by exogenous ubiquinone. About 4-times less Q-10 is required to reconstitute Q_B function in chromatophores from *Rps. sphaeroides* than in those from *R. rubrum*, which points to a tighter binding of the secondary acceptor in the former. No evidence for electrogenic nature of $Q_A^- \rightarrow Q_B$ electron transfer could be obtained in experiments with Q_B-replenished chromatophores.

Introduction

The electron-transfer chain of purple non-sulfur photosynthetic bacteria catalyzes a light-driven cyclic electron flow coupled to generation of the proton-motive force, the latter being largely comprised of electric potential difference ($\Delta\psi$) across the coupling membrane of chromatophores (for reviews, see Refs. 1 and 2). It is important to

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Abbreviations: $\Delta\psi$, transmembrane electric potential difference; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylene diamine; PMS, phenazine methosulphate; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; Ches, 2-[*N*-cyclohexylamino]ethanesulphonic acid; P-870, bacteriochlorophyll dimer (special pair); DCIP, 2,6-dichlorophenolindophenol; RC, reaction centre complex; Q-10, ubiquinone-10; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid.

know which of the electron-transfer steps are electrogenic and what are the mechanisms of electrogenesis. To this end, rapid kinetics measurements of $\Delta\psi$ generation are most useful.

For many years $\Delta\psi$ could have been monitored in chromatophores only indirectly, e.g., by a carotenoid or bacteriochlorophyll absorption bandshift method [3–8]. More recently, association of chromatophores with several types of macroscopic artificial planar membrane [9–11] and reconstitution of the isolated reaction centres into planar membrane [12–14] was achieved which made it possible to apply an electrometric method of $\Delta\psi$ assay (see also Refs. 15 and 16 for other related approaches).

Combined with the use of thin phospholipid-impregnated collodion films as artificial membranes, the electrometric method proved particularly useful for rapid kinetics measurements due to its extreme sensitivity; as a rule, a photovoltage due to electrogenic reactions of collodion film-associated chromatophores can be easily monitored on a microsecond scale with a signal-to-noise ratio which eliminates any need for data averaging (see, e.g., Refs. 17 and 18). A significantly inferior sensitivity was reported for the photocurrent measurements with the planar membrane-associated isolated reaction centres [14].

Previously, general characteristics of $\Delta\psi$ photogeneration by the collodion film-adhered chromatophores of *Rhodospirillum rubrum*, *Rhodopseudomonas sphaeroides* and *Ectothiorhodospira shaposhnikovii* were described [17–20]. These studies showed that the cyclic electron flow is impaired upon association of chromatophores with artificial membranes so that in the absence of added redox cofactors the laser flash-induced events may be confined to electron redistribution within the bacteriochlorophyll reaction centre complex [9,19]. However, the details of the photo-induced electron transfer within this pigment-iron-ubiquinone-protein complex in the planar membrane-associated chromatophore vesicles remained obscure. In particular it was not clear whether electron transfer from the primary to the secondary quinone acceptor ($Q_A \rightarrow Q_B$) did occur in those experiments. Paradoxically, this uncertainty was due at least partially to the extreme sensitivity of the direct electrometric assay which measures electrogenic activity of such a minute amount of chromatophores (actually, of a monolayer adhered to the collodion film) that spectral routine controls cannot be run in this experimental system.

Accordingly, it is desirable to extract maximum of information on electron transfer in the collodion film-associated chromatophores from the electrometric measurements themselves. Earlier studies in this group focused mainly on the rapid kinetics of $\Delta\psi$ generation of the laser flash-activated bacterial photo-redox chains [17,18,20]. Here we have carried out analysis of the kinetics of $\Delta\psi$ dissipation which provides meaningful complementary information and may be helpful for better understanding of peculiarities of electron transfer in the collodion film-associated chromatophores. The usefulness of this approach was demonstrated earlier by Packham et al. [14] in experiments with the planar-membrane-incorporated isolated reaction centres from *Rps. sphaeroides.*

Materials and Methods

Asolectin (phosphatidylcholine type II S), TMPD, PMS, DAD, Q-10 and artificial quinones were from Sigma, Q-1 and Q-2 were from Ferak (Berlin, F.R.G.) and Hepes, Tris, Mes, Ches from Serva.

Chromatophores from *R. rubrum* cells were isolated by an ultrasonic treatment procedure as described earlier [21]. *Rps. sphaeroides* chromatophores were prepared according to Ref. 22.

Photoelectric activity of collodion film associated chromatophores was measured as described in detail previously [11,18]. In brief, a chromatophore suspension ($A_{870} = 1.5–1.9\ cm^{-1}$) was added to one of the two compartments of the teflon cuvette filled with buffer and insulated by a collodion film impregnated with asolectin solution in *n*-decane. Association of chromatophores with the collodion film was achieved upon 4–5 h incubation in the presence of 30 mM $CaCl_2$. Subsequently, the solution in both compartments of the cuvette was replaced by an appropriate incubation medium (typically, 20 mM Hepes-KOH, pH = 7.5) without $CaCl_2$. Saturating light flashes were fired from a LOMO ruby laser ($\lambda = 694$ nm; half-width

of the flash, 20 ns; energy, 20 mJ). In double-flash experiments the second saturating flash was delivered by a neodimium laser (Quantel, France) operated at a double-frequency mode ($\lambda = 530$ nm). The lasers and the registrating system were synchronized with the aid of a programmed pulse generator constructed in this laboratory.

Electric-potential difference generated across the collodion film was measured with a pair of light-protected Ag | AgCl electrodes connected via an operational amplifier (Burr Brown 3554BM) with a transient recorder Data Lab DL-922 linked to a computer NOVA-3D (Data General). The monitoring system did not distort electric pulses longer than 200 ns, the resolution time being limited by DL 922 parameters.

The kinetics analysis of $\Delta\psi$ decay recordings was carried out in the NOVA-3D minicomputer, using a set of programmes developed by A.L. Drachev.

Results

Fig. 1 shows several typical laser flash-induced electric responses of *R. rubrum* chromatophores associated with the phospholipid-impregnated collodion film. The responses consist of a non-resolved very rapid phase of $\Delta\psi$ generation (less than 200 ns, which is a rise-time of the monitoring system) followed by dissipation of $\Delta\psi$.

In the absence of additions (Fig. 1, curve 1), $\Delta\psi$ dissipation is virtually complete in 300 ms (the 'rapid decay'). The kinetics of this decay is approximated reasonably well by a single exponent with τ of 66 ms; however, a much better fit is obtained for 2-exponent approximation with τ values of 14 and 78 ms and amplitudes of 21 and 79%, respectively. These two phases with an approx. 1 : 4 amplitude ratio were observed throughout pH range 6–9 studied (Table I). The parameters of the $\Delta\psi$ rapid decay kinetics are similar to the well-known characteristics of the primary dipole recombination ($P\text{-}870^+\text{-}Q_A^- \rightarrow P\text{-}870\text{-}Q_A$) in reaction centres of *R. rubrum* chromatophores as measured spectrophotometrically [23,24] and are in reasonable agreement with the electrometric measurements of Packham et al. [14] on the membrane-incorporated *Rps. sphaeroides* reaction centres.

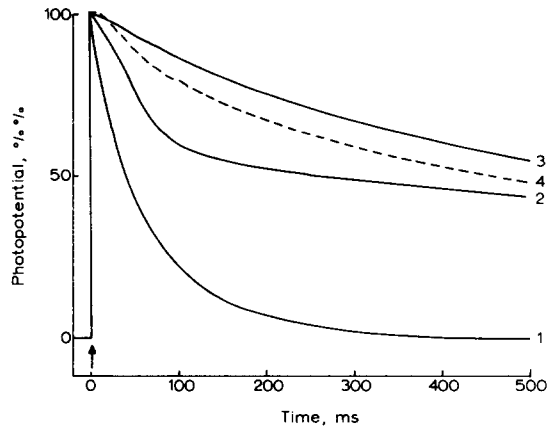

Fig. 1. Effect of redox-mediators on the kinetics of chromatophore photoelectric response decay. The basic incubation medium contained 20 mM Hepes-KOH (pH 7.5). (1) No additions; (2) 2 mM ascorbate + 100 μM TMPD; (3) 2 mM ascorbate + 500 μM DAD; (4) as (3) + 1 mM *o*-phenanthroline. Ascorbate alone (up to 10 mM) had no effect on the kinetics of $\Delta\psi$ decay.

Correlation between the kinetics of the rapid $\Delta\psi$ decay and primary dipole recombination was further tested right in the collodion film-associated chromatophores using a double-flash technique. In these experiments two saturating laser flashes 1–500 ms apart were fired and the ampli-

TABLE I

COMPUTER RESOLUTION OF THE KINETICS OF THE 'RAPID' DECAY OF PHOTOELECTRIC RESPONSE OF THE COLLODION FILM ASSOCIATED *R. RUBRUM* CHROMATOPHORES INTO TWO COMPONENTS

Conditions as in Fig. 1, line 1; but the incubation medium contained 20 mM of each Mes, Hepes, Tris and Ches buffers. The data are given as mean values ± mean deviations for 2–5 typical kinetics traces analysed at each pH. The contribution is given for 2 exponent analysis.

pH	τ (ms)		Contribution (%)
	1 exponent	2 exponents	
6.2	65 ± 5	21 ± 2	19 ± 2
		75 ± 2	81 ± 2
7.5	61 ± 4	14 ± 7	24 ± 9
		70 ± 11	76 ± 10
8.6	51 ± 1	13 ± 2	14 ± 0.5
		58 ± 1	86 ± 0.5
9.5	35 ± 3	10 ± 2	22 ± 7
		46 ± 2	78 ± 7

tude of $\Delta\psi$ generated upon the second flash was plotted as a function of time interval between the flashes. Conceivably, in the absence of exogenous electron donors to P-870$^+$, the plot would give the kinetics of P-870$^+$ repopulation by the electrons coming back from Q_A^- reduced by the first flash (cf. Ref. 14).

The results of a typical experiment are given in Fig. 2. One can see that the time-course of P-870$^+$ re-reduction by Q_A^- as measured by the double-flash method (circles) is fairly close to, although not identical with, the kinetics of $\Delta\psi$ discharge (solid line). A minor but reproducible difference between the two curves may indicate that besides the current associated with electron return from Q_A^- to P-870$^+$, there are other charge movements contributing to $\Delta\psi$ dissipation.

The above data show that the rapid decay of the photoelectric response of the collodion film-associated chromatophores corresponds essentially to electron return from Q_A^- to P-870$^+$. This conclusion implies that, in the absence of additions, neither the electron transfer from Q_A to Q_B, nor the P-870$^+$ re-reduction by the cyclic electron-transfer chain are operative in the collodion-film-associated chromatophores. Accordingly, exogenous redox compounds reducing P-870$^+$ or oxidizing Q_A^- rapidly enough to compete with the primary dipole recombination can be envisaged to prevent the rapid $\Delta\psi$ decay.

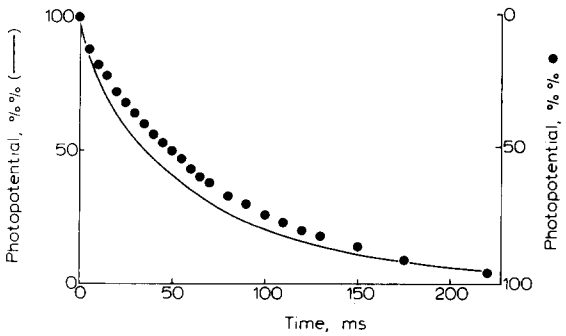

Fig. 2. Kinetics of the rapid decay of photoelectric response and of the primary dipole charge recombination in the collodion-film-associated chromatophores from *R. rubrum*. Basic experimental conditions as in Fig. 1, line 1. The circles show the amplitude of the photoelectric response induced by a second saturating laser flash fired at indicated time intervals after the first one; the response was measured 100 μs after the second flash. Note the inverted ordinate scale for the circles (right-hand axis). The curve gives a typical kinetic trace of the 'rapid' decay of photoelectric rsponse after a single flash (left-hand ordinate scale).

Effect of P-870 reductants

We found that addition of PMS or some other membrane-permeable redox-dyes like TMPD, DAD or DCIP in the presence of ascorbate greatly impedes dissipation of laser flash-induced $\Delta\psi$ (Fig. 1, curves 2–4). In the presence of moderately high concentrations of the ascorbate-reduced mediators, the decay of $\Delta\psi$ becomes clearly biphasic (e.g., Fig. 1, trace 2). A rapid initial phase similar to that observed in the absence of additions ($\tau <$ 100 ms) * is followed by a second slower process with τ which varied in a range of 0.5–2.2 s in various experiments and apparent life-time of the entire process depends on the relative amplitudes of the two components.

A contribution of the slow phase increases up to 100% with an increased concentration of the mediator, a 50% contribution of the slow phase achieved at 70 μM of TMPD or DAD and at approx. 0.3 μM PMS.

The deceleration of $\Delta\psi$ decay by the redox mediators is virtually insensitive to *o*-phenanthroline (e.g., see Fig. 1, curve 4). A small acceleration of the decay induced by this inhibitor originates in a slight decrease of the τ of the slow and rapid phases rather than in an increased contribution of the rapid phase [27] and is probably due to some deterioration of the dielectric properties of the chromatophore membrane.

Evidently, the reduced forms of the membrane-permeable mediators slow down dissipation of $\Delta\psi$ as they reduce rapidly the photooxidized P-870$^+$ and thus prevent the rapid discharge of the primary

* It is sufficient for the purposes of the present work to treat the 'rapid' (approx. 70 ms) decay of $\Delta\psi$ as a single phase. We would however, indicate some complications inherent in the reaction. First, besides the two components of the rapid decay mentioned above (Table I), a small initial very rapid (less than 10 μs) relaxation of photopotential was sometimes observed; the origin of this effect is unknown. Second, in the presence of redox mediators, the rapid phase of $\Delta\psi$ decay shows sigmoidicity (cf. Fig. 1, traces 2–4); this is due to a contribution of a second phase of $\Delta\psi$ generation associated with P-870$^+$ re-reduction by the dyes [25,26].

dipole. Under these conditions the slow phase of $\Delta\psi$ decay with $\tau = 0.5–2.5$ s is most probably due to passive discharge of a chromatophore membrane which is known to occur in this time-scale [17,18]. Importantly, ascorbate-reduced membrane-impermeable electron donors such as $Ru(NH_3)_6^{2+}$, cobalt(phenanthroline)$_3^{2+}$ or cytochrome c^{2+} did not show any effect on the kinetics of $\Delta\psi$ dissipation in chromatophores, where P-870 is localized inside the vesicles, whereas they impeded the decay kinetics in the inside-out RC-proteoliposomes (data not shown, see Refs. 28 and 29).

Effect of Q_A^- oxidants

In the intact chromatophores the photoreduced Q_A^- is reoxidised in less than 1 ms by a secondary quinone acceptor. However, according to the present observations the $Q_A^- \rightarrow Q_B$ transfer is not operative in chromatophores, associated with a phospholipid-impregnated collodion film. This might be due to extraction of endogenous CoQ into a hydrophobic milieu of the collodion film [9,19] or some other damage to the electron-transfer mechanism during the association procedure.

We attempted to reconstitute the secondary quinone function in the collodion film-associated chromatophores. To this end Q-10 was added to the asolectin solution in decane used for the collodion film impregnation. As shown in Fig. 3, such a treatment greatly decelerates the photoelectric response decay (cf. curves 2 and 1) and this effect is completely reversed by *o*-phenanthroline (curve 3).

As in the case of the above described experiments with the ascorbate-reduced redox dyes, the decay of the photovoltage as decelerated by Q-10 (trace 2 in Fig. 3) is biphasic. A rapid phase with $\tau \approx 60$ ms contributes about 25%; a second slow phase has τ of 1.3 s and contributes 75%. Presumably, in approx. 25% of RCs the Q_B function is not restored, whereas in the rest 75% the photogenerated Q_A^- is reoxidised effectively by added CoQ in an *o*-phenanthroline-sensitive way (cf. [14]).

Contribution of the slow phase of $\Delta\psi$ decay increased with an increased addition of Q-10. A typical titration curve is shown in Fig. 4 by curve 1. It can be seen that in *R. rubrum* chromatophores, the effect of added ubiquinone saturates at approx. 10 mg/ml (11 mM) with a half-maximal effect at 6 mg/ml. The maximal contribution of the slow phase of $\Delta\psi$ decay under these conditions reached approx. 70–75%.

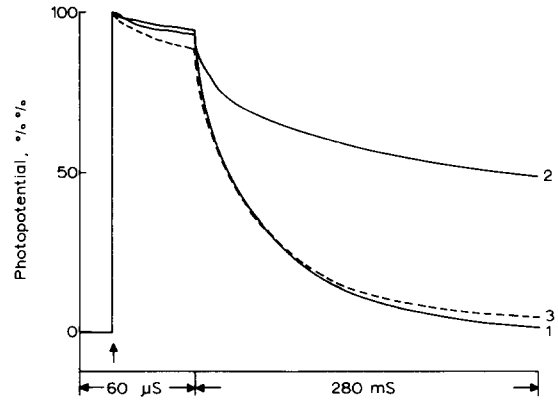

Fig. 3. Effect of ubiquinone on the kinetics of the *R. rubrum* chromatophore photoelectric response decay. (1) Control; (2) 20 mg of Q-10 was added per 1 ml of the phospholipid solution in decane used to impregnate the collodion film; (3) as (2), but after 1 mM *o*-phenanthroline addition.

A very similar effect of Q-10 on the decay of the laser flash-induced photoelectric response was also observed with chromatophores from *Rps. sphaeroides* (kinetic traces not shown, see titration curve in Fig. 4, 2).

Significantly, whereas the maximal amplitude of the Q-10-induced slow phase of $\Delta\psi$ decay for

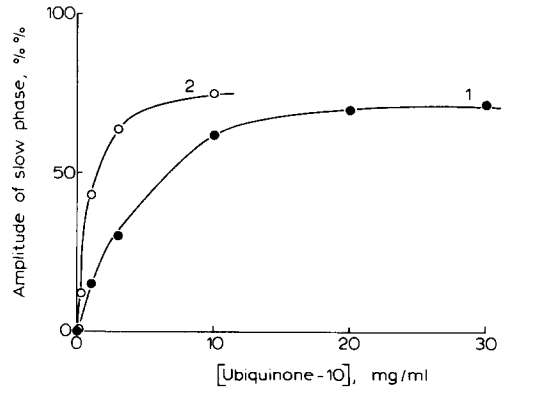

Fig. 4. Dependence of the slow phase of $\Delta\psi$ decay on the concentration of Q-10. Experiment shown in Fig. 3, line 2 was carried out with chromatophores from *R. rubrum* (●———●) or *Rps. sphaeroides* (○———○) at indicated concentrations of Q-10 added to the asolectin solution in decane.

Rps. sphaeroides is virtually the same as for *R. rubrum,* the concentration dependencies of the effect are markedly different for the two bacteria (Fig. 4). Thus, the half-maximal effect in *Rps. sphaeroides* is observed at 1.6 mg/ml of Q-10 which is approx. 1/4 of the concentration needed in the case of *R. rubrum.* Presumably, reaction centres from *Rps. sphaeroides* are characterized by higher affinity for CoQ at the Q_B site than *R. rubrum* centres. We found that short-chain homologues of ubiquinone like Q-1 and Q-2 can substitute for Q-10 but in contrast to the latter can be added merely to the aqueous solution in the measuring cell, which is more convenient. Restoration of the slow phase of $\Delta\psi$ decay (data not shown) was saturated at approx. 50 μM Q-1 and approx. 2 μM Q-2 in agreement with the higher partition of the latter into the hydrophobic membrane phase.

Several artificial quinones (2-methyl-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, 2,3,5,6-tetramethyl-1,4-benzoquinone, 1,4-benzoquinone and some other compounds) were assayed for their ability to bring about the slow phase of $\Delta\psi$ decay. Among the compounds tested only 1,4-benzoquinone induced considerable deceleration of $\Delta\psi$ dissipation (e.g., see Fig.5B) so that at 0.3 mM of the quinone a contribution of the slow phase attained approx. 50%, the effect being reversed by *o*-phenanthroline. The relatively high effectiveness of 1,4-benzoquinone as compared with the other quinones tested could be due to its high midpoint potential which makes it a good oxidant for Q_A^-.

Effect of exogenous redox compounds on the amplitude of photoelectric response in a series of flashes

Interaction of the flash-induced primary dipole P-870$^+$-Q_A^- with electron donors or acceptors would yield photoinactive states P-870-Q_A^- and P-870$^+$-Q_A, respectively. Experiments with multiple flashes show that this is indeed the case (Figs. 5 and 6).

In the absence of added redox compounds, repetitive flashes bring about a series of identical electrical responses of *R. rubrum* chromatophores, associated with the collodion film (Figs. 5a and 6a). The decay of $\Delta\psi$ is rapid and essentially monophasic with a kinetics typical of P-870$^+$-Q_A^- recombination.

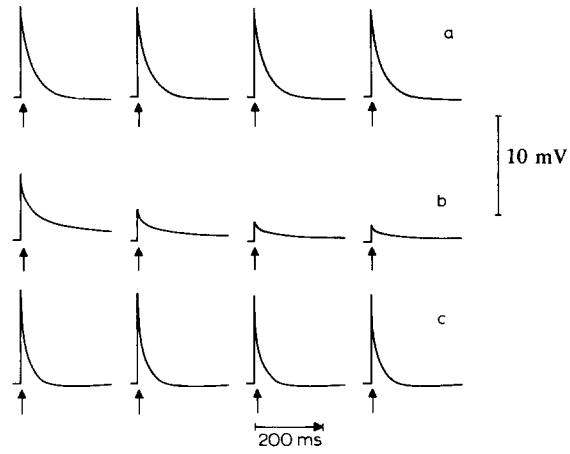

Fig. 5. Effect of multiple flashes on the amplitude of the chromatophore photoelectric response. (a) No additions; (b) in the presence of 100 μM 1,4-benzoquinone; (c) as (b), but after addition of 1 mM *o*-phenanthroline. The flashes were given 20 s apart.

In the presence of 1,4-benzoquinone, there occurs a rapid diminution of the amplitude of the response in a series of flashes (Fig. 5b) which is prevented by *o*-phenanthroline (Fig. 5c). A smaller amplitude of the response as observed already on the first flash in the presence of 1,4-benzoquinone (or DAD, see Fig. 6b) is due to preillumination of the sample during the addition of the compound.

Attenuation of the photoelectric response amplitude in the course of a series of flashes is also

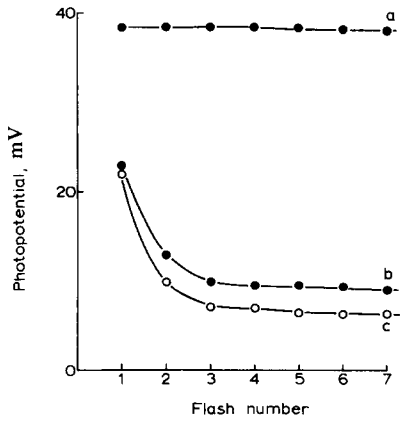

Fig. 6. Effect of electron donor to P-870$^+$ on the amplitude of photoelectric response of chromatophores upon multiple flash conditions. (a) No additions; (b) in the presence of 2 mM ascorbate and 200 μM DAD; (c) as (b)+1 mM *o*-phenanthroline. The flashes were given 20 s apart.

observed in the presence of DAD as illustrated by Fig. 6b. In this case *o*-phenanthroline does not release the inhibition but rather slightly augments the inhibitory effect (Fig. 6c), which can be due to prevention of some reoxidation of Q_A^- by trace amounts of the oxidized DAD.

Complete recovery of the photoresponse after illumination of the sample in the presence of ascorbate + DAD required approx. 20 min dark incubation. This time could be reduced to less than 1 min by adding 0.1 mM vitamin K_3 or duroquinone, the effect of the latter two being prevented by *o*-phenanthroline (data not shown). Apparently, the relatively low-potential quinones like vitamin K_3, although unable to re-oxidize Q_A^- rapidly enough to compete with the 70 ms back reaction as discussed above, can operate as electron acceptors for the primary quinone on a tens of seconds time scale in the stabilized P-870-Q_A^- state; notably, this reaction appears to proceed via the same *o*-phenanthroline-sensitive mechanism as the rapid $Q_A^- \rightarrow Q_B$ electron transfer. These results are in agreement with the ability of vitamin K_3, duroquinone and a number of other quinones with E_m values in the range from -100 to $+100$ mV to support a steady-state electron flow in the teflon filter-associated *R. rubrum* chromatophores supplemented with ascorbate + DAD (or TMPD) in continuous light [19].

Discussion

It is important to relate the light-induced transient events measured electrometrically in the system chromatophores/collodion film with the electron-transfer reactions in the photosynthetic redox chain known from the independent spectroscopic studies. Earlier we have identified the very rapid (less than 0.2 μs) photoelectric response of the collodion film-associated chromatophores from *R. rubrum* and *Rps. sphaeroides* as electron transfer from P-870 to Q_A [18]. The present work provides a basis for a better understanding of the processes following this rapid charge separation under various experimental conditions.

According to the results obtained, the 'rapid' decay of $\Delta\psi$ observed in the chromatophores/collodion film-model in the absence of added redox cofactors can be identified to a first approximation as electron return from Q_A^- to P-870$^+$. As the nature of the rapid discharge of $\Delta\psi$ has been established, analysis of the photopotential decay kinetics can be used subsequently for elucidation of details of electron transfer in chromatophores associated with the collodion film.

Earlier studies with continuous illumination established that cyclic electron transfer is impaired upon chromatophore incorporation into phospholipid-impregnated teflon filters or collodion films [9,19]. The present data allow to conclude that light-induced electron transfer in these conditions is confined to charge separation P-870 $\overset{e}{\rightarrow} Q_A$ within the reaction centre and that the $Q_A^- \rightarrow Q_B$ step does not take place. Appearance of the *o*-phenanthroline-sensitive slow phase of $\Delta\psi$ decay upon addition of Q-10 or certain other quinones shows that the Q_B function can be reconstituted; this supports the hypothesis [9,19] that it is ubiquinone extraction into the hydrophobic milieu of the collodion film, which leads to inhibition of electron transfer in the film-adhered chromatophores. Accordingly, inhibition of all CoQ-dependent redox reactions but not of cytochrome oxidase or transhydrogenase was observed upon association of submitochondrial particles with phospholipid-impregnated teflon filters [30, 31]. However, the restoration of Q_A reoxidation by Q_B was not complete in either *R. rubrum* or *Rps. sphaeroides* despite the saturation of the reconstitution with the quinone concentration. It is possible that in addition to reversible CoQ extraction an irreversible inactivation of $Q_A \rightarrow Q_B$ electron transfer can take place in approx. 25% of the reaction centres. Incidentally, the same 25% of Q_B could not be reconstituted under the experimental conditions of Packham et al. [14].

In the previous publication [18] the appearance of a second approx. 200 μs phase of $\Delta\psi$ generation was observed in collodion film-associated chromatophores upon addition of ascorbate, vitamin K_3 and PMS. This phase was tentatively ascribed to electron transfer from Q_A to K_3, the latter substituting for Q_B, and a hypothesis was put forward on the electrogenic nature of the $Q_A \rightarrow Q_B$ reaction. At the same time, according to Refs. 14 and 32, the $Q_A \rightarrow Q_B$ reaction is not electrogenic. Our present data show that electron transfer from Q_A to vitamin K_3 (or to a number of

other artificial quinones with $E_m^7 \approx 0$–0.1 V) does not occur on a fast time scale and requires seconds or tens of seconds. Therefore the slower electrogenic phase as observed in Fig. 2 of Ref. 18 was most probably due to PMS present which at the concentration used in Ref. 18 can reduce P-870$^+$ electrogenically with τ of less than 0.3 ms [25,26]. Moreover, in experiments with the collodion-film associated chromatophores in which approx. 70% of $Q_A \rightarrow Q_B$ electron transfer had been reconstituted with Q-10, we failed to obtain any evidence for a second phase of $\Delta\psi$ generation. Therefore it is likely that the first electron is transferred from Q_A to Q_B non-electrogenically. On the other hand, H^+ uptake associated with Q_BH_2 formation renders the reaction electrogenic upon a second flash as shown recently in this group [33].

Finally, the results obtained with multiple flashes show that in the presence of redox cofactors, illumination of the collodion film-associated chromatophores can easily yield photoinactive states of the chromatophore reaction centres P-870-Q_A^- or P-870$^+$-Q_A and a long dark adaptation of the samples may be required for recovery of the maximal photoelectric response. These observations bear on the choice of optimal conditions for the studies of the photoelectric responses and, in particular, provide an explanation for the beneficial effect of the mixture of ascorbate, PMS (or TMPD) and vitamin K_3 on the amplitude and stability of the rapid phase of laser flash-induced $\Delta\psi$ generation in collodion film-associated chromatophores reported earlier [18,19].

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