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GENERATION OF MEMBRANE POTENTIAL DURING PHOTOSYNTHETIC ELECTRON FLOW IN CHROMATOPHORES FROM *RHODOPSEUDOMONAS CAPSULATA*

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Summary

1. When cytochrome c_2 is available for oxidation by the photosynthetic reaction centre, the decay of the carotenoid absorption band shift generated by a short flash excitation of *Rhodopseudomonas capsulata* chromatophores is very slow (half-time approximately 10 s). Otherwise the decay is fast (half-time approximately 1 s in the absence and 0.05 s in the presence of 1,10-*ortho*-phenanthroline) and coincides with the photosynthetic back reaction.

2. In each of these situations the carotenoid shift decay, but not electron transport, may be accelerated by ioniophores. The ionophore concentration dependence suggests that in each case the carotenoid response is due to a delocalised membrane potential which may be dissipated either by the electronic back reaction or by electrophoretic ion flux.

3. At high redox potentials, where cytochrome c_2 is unavailable for photooxidation, electron transport is believed to proceed only across part of the membrane dielectric. Under such conditions it is shown that the driving force for carbonyl cyanide trifluoromethoxyphenyl hydrazone-mediated H^+ efflux is nevertheless decreased by valinomycin/K^+; demonstrating that the $[BChl]_2 \rightarrow Q$ electron transfer generates a delocalised membrane potential.

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Abbreviations: FCCP, carbonyl cyanide trifluoromethoxyphenyl hydrazone; $E_{m7.0}$, the midpoint redox potential at pH 7.0; E_h, the ambient redox potential measured with platinum and calomel electrodes.

Introduction

In the original form of the chemiosmotic hypothesis of oxidative and photophosphorylation, Mitchell [1] proposed that reducing equivalents flow down electron transport chains as electrons in one direction across the membrane dielectric, and as hydrogen atoms in the other direction. Using short flash illumination of photosynthetic systems it has been possible kinetically to resolve individual oxidation and reduction events, and tentatively to assign electrogenic and electrically neutral reactions with respect to the membrane topology [2]. In chromatophores from *Rhodopseudomonas sphaeroides* the reactions cytochrome $c_2 \rightarrow [BChl]_2 \rightarrow Q_I \rightarrow Q_{II}$ are believed to take place across the membrane since the cytochrome is located on the inside of the chromatophore vesicle [3] and H^+ binding, associated with the reduction of Q_{II}, occurs on the outer face of the membrane [4].

The net electron transfer from cytochrome c_2 to Q_{II} would therefore be expected to give rise to an electrostatic potential between the inside and outside of the chromatophore membrane. It is believed that this potential is responsible for the shift of the absorption spectrum of a small fraction of the endogenous carotenoid pigments to longer wavelengths [5]. In the presence of antimycin A the carotenoid red shift is generated in two phases. Phase I appears to accompany the reaction $[BChl]_2 \rightarrow Q_I$ and phase II, cytochrome $c_2 \rightarrow [BChl]_2$. To account for these observations, it was proposed that each of these reactions contributes to the development of membrane potential and that $[BChl]_2$ is embedded towards the centre of the membrane dielectric [5]. Supporting evidence for this proposal has come from an analysis of the redox poise between cytochrome c_2 and $[BChl]_2$ after a series of short flashes [6].

Crucial to the development of this model was the finding that exceedingly low concentrations of uncoupling agents and ionophores were effective in promoting an accelerated decay of the carotenoid shift after a flash [7]. The inference was that the electrostatic potential, although generated by electron transport, may rapidly delocalise after the flash and be dissipated by electrophoretic ion transport across the membrane.

In the present experiments we have sought to test this hypothesis by restricting the electron transport reactions through and adjacent to the photosynthetic reaction centre by suitably poising the redox potential or by treating the chromatophores with 1,10-*ortho*-phenanthroline. If we recognise the contribution of dissipative back reactions to photo-oxidised $[BChl]_2^+$, analysis of the decay of the carotenoid shift in the presence of ionophores suggests that the reaction $[BChl]_2 \rightarrow Q_I$ is accompanied by the generation of a delocalised membrane potential even though it may not entirely span the membrane. We have also performed a series of experiments which, independently of carotenoid shift measurements, were designed to discover whether $[BChl]_2 \rightarrow Q$ is accompanied by the generation of a delocalised membrane potential.

Methods

Rps. capsulata was grown in the light in completely filled, screw-cap bottles in the malate, NH_4^+, RCV medium [8]. Strain N22 (kindly supplied by Dr.

O.T.G. Jones, University of Bristol) with a single species of carotenoid was used in these studies. Cells were harvested after 24 h growth of a 5% innoculum. Chromatophores were prepared immediately either by sonication or French-press treatment in a medium containing 50 mM KCl, 8 mM $MgCl_2$, 50 mM Tricine in 10% sucrose at pH 7.4. The membrane fraction sedimenting between 30 000 × *g* for 30 min and 100 000 × *g* for 120 min was either resuspended in a small volume of the above medium for carotenoid shift experiments or was washed once in Tricine-free medium for pH indicator experiments. Bacteriochlorophyll was extracted in acetone/methanol (7 : 2, v/v) and determined spectrophotometrically [9].

Flash-induced carotenoid shift and cresol red absorption changes were monitored essentially as described in Ref. 10. Experiments were carried out in the medium described above (minus Tricine for cresol red measurements) or as otherwise noted. In some experiments, 1 mM succinate was added to the chromatophores suspension to maintain cytochrome c_2 in its reduced state before flash excitation, or the redox potential was poised at a higher value by addition of ferri- and ferrocyanide (the precise potential in this case was measured with platinum and calomel electrodes). All experiments were carried out aerobically at 20° C. Antimycin A was routinely added to the sample to inhibit electron flow through the cytochrome b/c_2 oxidoreductase.

Carotenoid absorption band shifts were measured at 503 nm, the red-most carotenoid peak in the light minus difference spectrum for chromatophores isolated from strain N22 of *Rps. capsulata*. Control experiments showed that the spectra of the different phases of the generation and decay of the carotenoid signal were identical within the resolution of our instrument (±1.0 nm). Cresol red absorption changes were measured at 583 nm where background changes in chromatophore absorption were minimal. Control experiments in the presence of 10 mM Tricine were always carried out.

FCCP, valinomycin and 1,10-*ortho*-phenanthroline were bought from Sigma and all other reagents were of analytical grade.

Results

The carotenoid shift and protolytic reactions associated with the photosynthetic back reaction in chromatophores from Rps. capsulata

In the experiment shown in Fig. 1 the ambient redox potential of the chromatophore suspension was poised at 440 mV. At this potential the reaction centre $[BChl]_2$ ($E_{m7.0}$ = 440 mV) is 50% reduced, but the other components of the photosynthetic electron transport chain, including cytochrome c_2 ($E_{m7.0}$ = 340 mV) are present in their oxidised state [11].

Following flash excitation, electrons are driven from the $[BChl]_2$, measured as an absorption decrease at 605 nm, through the reaction centre complex to the primary ubiquinone (Q_I). Within 200 μs subsequent electron transfer reduces the secondary ubiquinone (Q_{II}), accompanied by H^+ binding on the outer face of the chromatophore vesicle [12]. The H^+ binding is sensed by an absorption change of the added cresol red. Further, but incomplete, electron transfer to a *b*-type cytochrome follows on the millisecond time scale. No attempt has been made to resolve the reported oscillations between Q_{II} and

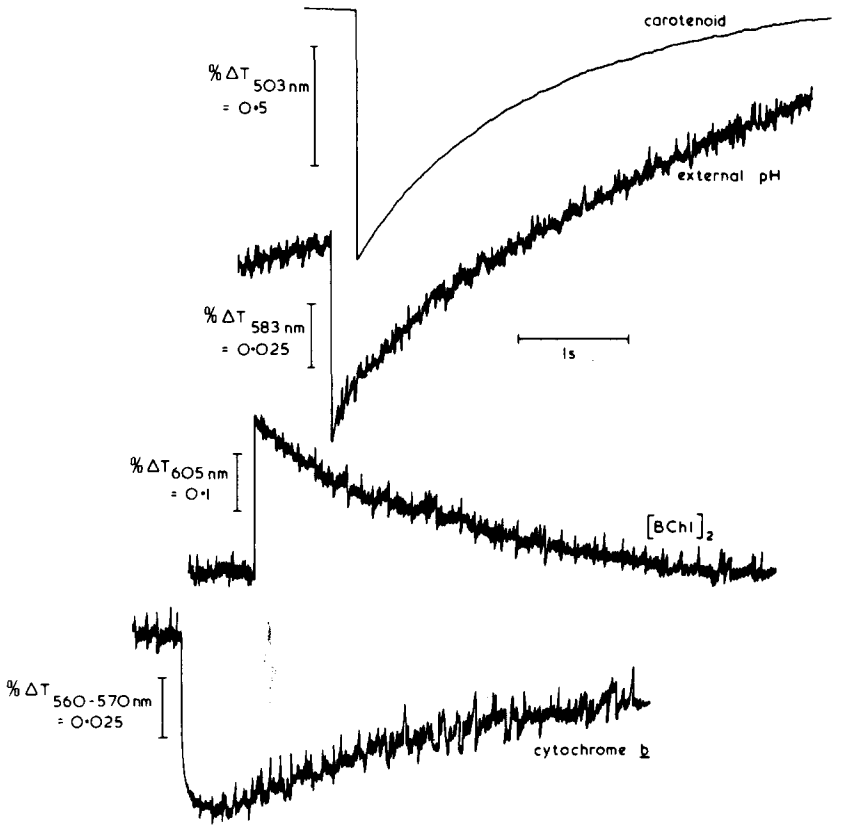

Fig. 1. Decay kinetics of the carotenoid shift, cresol red absorption change and reaction centre bacteriochlorophyll re-reduction and cytochrome *b* re-oxidation after short flash excitation of *Rps. capsulata* chromatophores poised at high redox potential. The upper two traces were recorded simultaneously (90° measuring beams) in a medium containing 10% sucrose, 50 mM KCl, 0.36 mM potassium ferrocyanide, 0.44 mM potassium ferricyanide, 40 μM cresol red, 5 μM antimycin A and chromatophores at a bacteriochlorophyll concentration of 20 μM (final pH = 7.5, E_h = 440 mV). The traces are an average of 256 flashes spaced 50 s apart (each sample was replaced after 32 flashes). A control experiment in the presence of 50 mM Tricine yielded an unaffected carotenoid response and the interfering absorption change at 583 nm was less than 10% of that recorded in the absence of buffer. The third trace was recorded under similar conditions except that the cresol red was omitted and the medium was supplemented with 50 mM Tricine (32 signals were averaged). The lower trace was also recorded in this medium but the bacteriochlorophyll in this case was 31 μM (32 signals were averaged). In all cases the initial rapid absorption change signifies the instant of the flash.

cytochrome *b* [13,14]; two flashes were fired at the chromatophore suspension prior to recording.

The charge separation between $[BChl]_2$ and Q_I is also registered by a red shift of the chromatophore carotenoid absorption spectrum. This process is virtually complete before the H^+ binding reaction commences [5]. In the absence of ferrocytochrome c_2, the $[BChl]_2$ is re-reduced from the secondary acceptors with a half-time of 1 s. The rate of this back reaction compares favourably with that measured in *Rps. sphaeroides* chromatophore and reaction centre preparations [15,16]. Important to the present discussion, the kinetics of the relaxation of both the cresol red and carotenoid absorption changes are similar to those of the $[BChl]_2$ re-reduction.

These observations on the decay processes could be explained by a direct reversal of the forward reactions with rate-limiting H^+ release accompanying $Q_{II}H \rightarrow Q_I$ and the carotenoid shift decaying with $Q_I \rightarrow [BChl]_2$. There is, however, some evidence for the back reaction from Q_{II} proceeding directly to $[BChl]_2^+$ without Q_I behaving as intermediary [16]. This does not materially affect our argument. For the present we are concerned only that the back reaction results in H^+ re-release and dissipation of the carotenoid-sensitive field (see also Ref. 17).

Cytochrome *b* re-oxidation, whether through a back-reaction or through an antimycin leak, is substantially slower than these processes.

The effect of 1,10-ortho-phenanthroline on the back reaction

ortho-Phenanthroline has been shown to inhibit electron transport and the accompanying H^+ binding reaction between the primary and secondary ubiquinone [18,19]. As a consequence of this inhibition, *o*-phenanthroline promotes an accelerated back reaction ($Q_I \rightarrow [BChl]_2$) [16]. Representative recordings from a titration of *o*-phenanthroline on the re-reduction of $[BChl]_2$ and the carotenoid shift decay after short flash activation of *Rps. capsulata* chromatophores poised at high redox potential are shown in Fig. 2. At complete inhibition of the $Q_I \rightarrow Q_{II}$ electron transfer the half-time for the back reaction is about 60 ms, in fair agreement with the values obtained for *Rps. sphaeroides* reaction centres [16]. With lower concentrations of *o*-phenanthroline, the back reaction is distinctly biphasic and semi-logarithmic plots of the decay reveal a mixed population of inhibited and completely uninhibited reaction centres. The apparent dissociation constant for *o*-phenanthroline from the data of Fig. 2 is approximately 2.5 mM.

o-Phenanthroline accelerates the decay of the carotenoid shift completely in parallel with its stimulatory effect on the rate of $[BChl]_2$ re-reduction. Again

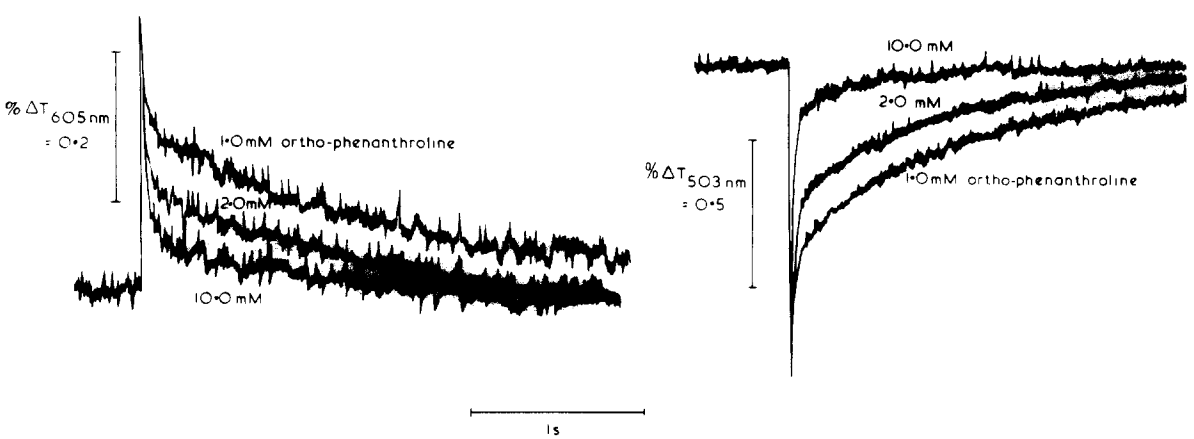

Fig. 2. The effect of 1,10-*ortho*-phenanthroline on the re-reduction of reaction centre bacteriochlorophyll and the decay of the carotenoid shift after short flash excitation of chromatophores poised at high redox potential. The experiments were carried out in the same medium as in Fig. 1 (absence of cresol red, presence of Tricine) and the bacteriochlorophyll concentration was 26 μM. The traces are an average of 32 flashes spaced 50 s apart.

it appears that the back reaction may dissipate the field to which the carotenoids respond. The amplitude of the carotenoid shift immediately after the flash is not affected even by high concentrations of the inhibitor (20 mM).

The effect of ionophores on the back reaction and carotenoid shift decay

In chromatophore suspensions poised at high redox potential (+440 mV) both FCCP and valinomycin/K^+ are capable of accelerating the rate of decay of the carotenoid shift without affecting the rate of $[BChl]_2$ re-reduction in either the presence or absence of *ortho*-phenanthroline (Fig. 3). Since high ionophore concentrations were required to accelerate the decay of the carotenoid shift, particularly in the presence of 1,10-*ortho*-phenanthroline, (0.43 valinomycin/reaction centre for a 2-fold, and 3.14 valinomycin/reaction centre for a 10-fold decrease in half decay time, Fig. 4), it becomes difficult to distinguish between two possible interpretations. First that the carotenoid sensitive field may be localised between redox centres of individual electron transport chains, in which case at least one ionophore per reaction centre would be required to dissipate the field. Alternatively the carotenoid-sensitive field could be delocalised across the chromatophore membrane and the high ionophore concentration would be required to effectively compete with the fast back reaction. Below we present evidence consistent with the second interpretation.

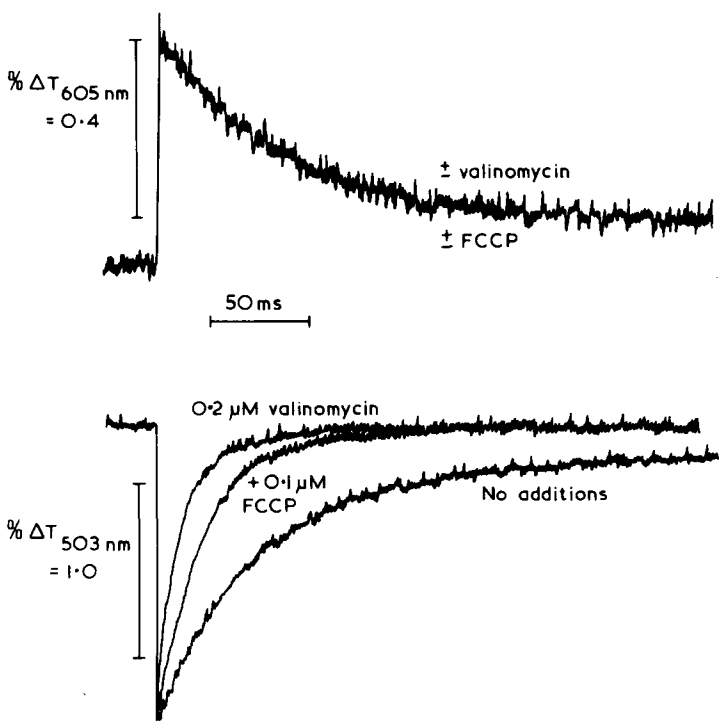

Fig. 3. The influence of ionophores on the re-reduction of bacteriochlorophyll and the decay of the carotenoid shift after short flash excitation of chromatophores treated with *ortho*-phenanthroline and poised at high redox potential. The experiments were carried out in a similar medium to that described in Fig. 1 (absence of cresol red, presence of Tricine), and the bacteriochlorophyll concentration was 27 μM. 32 signals were averaged.

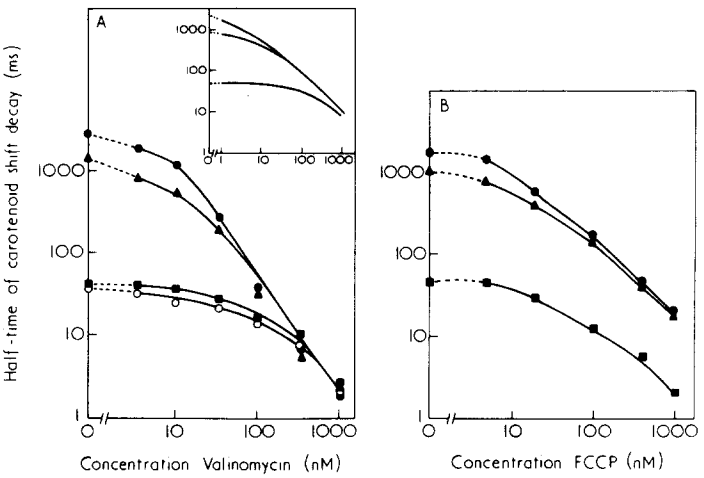

Fig. 4. The effect of ionophores on the decay of the carotenoid shift after short flash excitation of chromatophores. The experiments were carried out in a medium containing 10% sucrose, 50 mM KCl, 50 mM Tricine, 5 μM antimycin A (final pH 7.4) and the bacteriochlorophyll concentration was 21 μM in A and 26 μM in B. ●, plus 1 mM sodium succinate; ▲, plus 0.4 mM potassium ferrocyanide and 0.4 mM ferricyanide, E_h = 436 mV; ■, plus 0.4 mM ferro- and ferricyanide and 12 mM 1,10-*ortho*-phenanthroline; ○, plus 1 mM sodium succinate and 1 μM FCCP. The inset in A is a graphical solution to the model described in the text. The ordinate and abscissa are as in the main figure.

The back reaction and electrophoretic ionic flux

At lower ambient redox potentials, with cytochrome c_2 reduced before flash activation, the extent of the carotenoid shift produced by a flash is enhanced (a second phase in the generation of the shift with the same kinetics as cytochrome c_2 oxidation may be detected) [5]. Two major components can be resolved from the decay of the carotenoid shift; a component of $t_{1/2} \simeq 1$ s, which is due to the back reaction in those reaction centres lacking cytochrome c_2 (an unavoidable preparative artifact in our chromatophores), and a component of $t_{1/2}$ between 5 and 15 s, depending on the preparation [17]. This second component is slower than any measured electron transport reaction; at pH 7.4 manifests similar kinetics to re-release of bound H^+ and is extremely sensitive to ionophores: about 0.04 valinomycin/reaction centre and about 0.24 valinomycin/reaction centre are required to decrease the half-time of decay of the entire carotenoid shift by a factor of two and ten respectively under these conditions (Fig. 4). From data such as this it has been argued [7] that the target-site for the ionophore is the chromatophore vesicle rather than the electron transport chain (our current preparations of chromatophores possess about ten reaction centres per vesicle) [20] and that at least for the slow decay phase of the carotenoid shift, the response is to a delocalised field across the entire chromatophore membrane.

Fig. 4a shows the concentration dependence of valinomycin on the half-time of the carotenoid shift decay after pulsed electron transport through defined segments of the chain

(i) cytochrome $c_2 \rightarrow [BChl]_2 \rightarrow Q_I \overset{H^+}{\rightarrow} Q_{II}$ ($\rightarrow$ cytochrome b), (ii) $[BChl]_2 \rightarrow Q_I \rightarrow Q_{II} \rightarrow$ ($\rightarrow$ cytochrome b), (iii) $[BChl]_2 \rightarrow Q_I$.

The data shows no discontinuities and the profiles converge at high concentrations of valinomycin. Such profiles would be expected if a single pool of carotenoid pigment were responding to a delocalised membrane potential, $\Delta\psi$, which could be dissipated by both a back reaction (slow from Q_{II} or fast from Q_I) and by electrophoretic ion flux through the ionophore, viz. assuming first order dependencies on $\Delta\psi$ for the ionic flux (rate constants k_1 for basal membrane flux and k_2 for ionophore flux)

$$\frac{d(\Delta\psi)}{dt} = -k_1\Delta\psi - k_2\Delta\psi - k_3\, v_{br} \qquad (1)$$

where k_3 is an appropriate membrane capacitance and $v_{br}(t)$ is the rate of the back reaction at time t. The inset to Fig. 4 shows a graphical solution of this equation using $k_1 = -0.069\ s^{-1}$ and $k_2 = -0.07 \times$ (concentration of valinomycin in nM) s^{-1}. k_1 was measured from the slow decay phase at low redox potential in the absence of ionophore and k_2 was measured at low redox potential and at valinomycin concentrations between 100 and 200 nM, where it was assumed that back reaction contributions were negligible. The back reaction was taken to be approximately first order, with a 1 s half-time in the absence, an a 50 ms half-time in the presence of *o*-phenanthroline. At low redox potential, the back reaction was assumed to contribute to only 50% of the decay (i.e. 50% of the reaction centres lack cytochrome c_2). The model fits well with the experimental data. Deviations from the model are small but may arise from the assumed exponential nature of the decay processes and from a non-homogeneous distribution of ionophore among the vesicles at low concentrations. Such a good correlation would not be expected if the valinomycin was affecting localised electric fields when it was present at high concentrations.

A check on the internal consistency of the model is also shown in Fig. 4a. Chromatophores at low redox potential, capable of pulsed electron flow through cytochrome $c_2 \rightarrow [BChl]_2 \rightarrow Q_I \rightarrow Q_{II}$, were titrated with FCCP until the carotenoid shift decay rate was as fast as the back reaction from Q_I to $[BChl]_2$, i.e. another decay eterm is included in Eqn. 1 to match by ion transport the term '$-k_3 v_{br}(t)$'. In these circumstances the carotenoid shift decay kinetics approximately resemble those in chromatophores poised at high redox potential in the presence of *o*-phenanthroline and a subsequent titration with valinomycin gives a similar profile.

We think it is reasonable to conclude therefore that the field to which the carotenoids respond has a common origin for each of the three electron transport patterns. At high ambient redox potentials and with the H^+-binding reactions blocked, charge separation between $[BChl]_2$ and Q_I is believed to occur across only a part of the membrane. Under those conditions, $[BChl]_2 \rightarrow Q_I$ electron transfer is nevertheless capable of generating an electric field across the membrane, and this is the field to which the carotenoids respond.

A similar titration with FCCP (Fig. 4b) does not lead to such a simple interpretation. Again, however, the curves approach a limiting slope at high concentrations of FCCP, suggesting a similar mechanism of action for FCCP across the investigated concentration range. What is not expected from the simple model is the failure of the three curves to converge. It would appear that the FCCP behaves as a better ionophore in the presence of *o*-phenan-

throline. To explain this we propose that since the *o*-phenanthroline is positively charged, lipid soluble and is required in high concentrations to block the secondary electron transport reactions, it may partition into the chromatophore membrane and reduce the net negative surface charge. This might favour the binding of $FCCP^-$ and increase its ion-carrying activity.

Electrophoretic H^+ efflux driven by photochemical charge separation between $[BChl]_2$ and Q_{II}

We have sought other evidence that electron transport between $[BChl]_2$

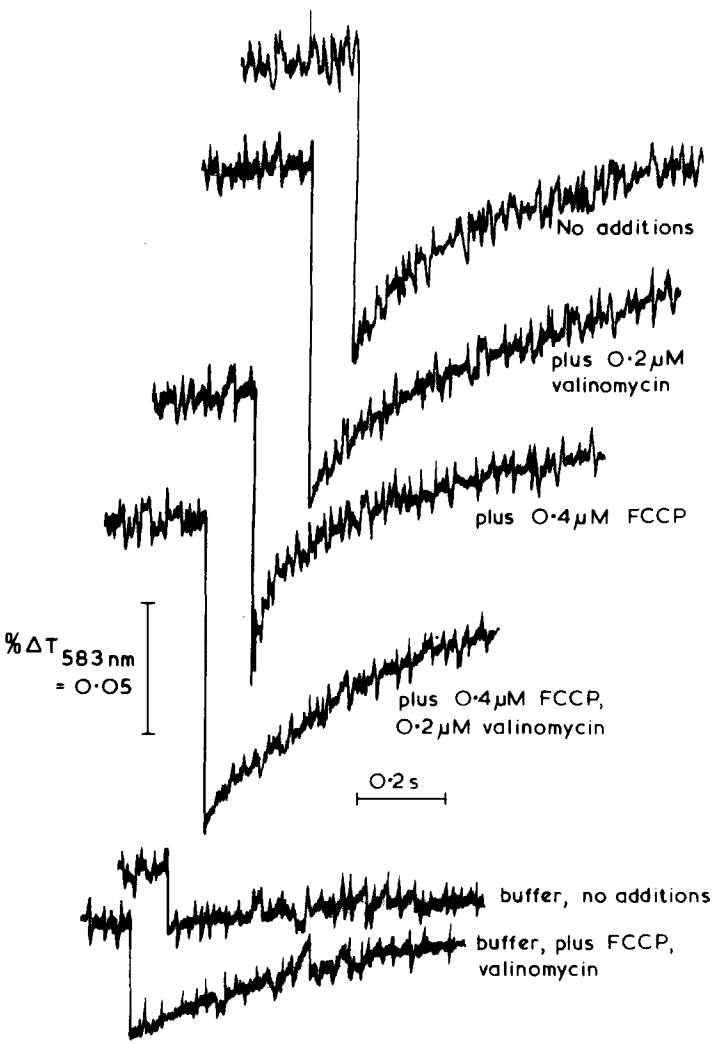

Fig. 5. Re-release of H^+ from chromatophores poised at high redox potential after short flash excitation. The top four traces were recorded in a medium containing 10% sucrose, 50 mM KCl, 8 mM $MgCl_2$, 40 μM cresol red, 0.24 mM potassium ferrocyanide, 0.56 mM ferricyanide, 5 μM antimycin A and chromatophores at a bacteriochlorophyll concentration of 21 μM (final pH 7.4, E_h = 450 mV) and other additions as shown. The experimental medium for the bottom two traces additionally contained 50 mM Tricine. In each case the traces are an average of 256 flashes, 50 s apart (the sample was changed every 32 flashes).

and Q_{II} gives rise to a difference in electric potential across the chromatophore membrane. In the experiment shown in Fig. 5c, the re-release of bound H^+ has been accelerated with FCCP. This simple observation in itself suggests that an electrochemical potential has been developed across the chromatophore membrane after the flash, but because FCCP may carry H^+ in response to either a membrane potential or a pH gradient, further interpretation becomes ambiguous. It may be significant, however, that only a portion of the decay of the cresol red signal is accelerated by the uncoupling agent.

More crucial is the observation that further addition of valinomycin decelerates the rate of H^+ re-release to that observed in the absence of FCCP (Fig. 5d). This suggests that H^+ efflux through the FCCP is driven by an electric potential that can be dissipated by valinomycin, i.e. H^+ efflux catalysed by FCCP under these circumstances is at least partly electrophoretic.

At low redox potentials, with cytochrome c_2 reduced prior to flash activation, valinomycin treatment gives rise to a deceleration of the cresol red decay even in chromatophores free of FCCP [10]. At high redox potentials this is not observed (Figs. 5a and b). This may be simply explained as follows: at low redox potential, a considerable fraction of the carotenoid shift and cresol red decay (viz. the slow phase) are due to electrophoretic H^+ transfer, probably a basal or leakage proton flux through the membrane; at high redox potential, however, rapidly bound H^+ is returned to the external aqueous phase in an electrically neutral manner, e.g.

$Q_{II}^- \xrightarrow{H^+} Q_I$ or $Q_{II}^- \xrightarrow{H^+} [BChl]_2^+$ (see above).

Discussion

Electron transport in each situation illustrated in Fig. 6 gives rise to a shift in the chromatophore carotenoid absorption spectrum. In II and III the carotenoid shift is dissipated mainly by an electronic back reaction (described by the dotted lines in the figure), and in case I by a basal leak of H^+. Both FCCP and valinomycin can accelerate the decay of the carotenoid shift in each case. The concentration dependences of the ionophores (Fig. 4) indicate that the electric potential to which the carotenoids respond is similar in each case. Since the titration of valinomycin, or FCCP on case I strongly argues for an effect on an electric field delocalised across the entire membrane, rather than upon a local field associated with discrete electron transport chains, we infer that in cases II and III the same applies. The fact that higher concentrations are required to produce a marked stimulation of the decay, particularly in case III, may be adequately explained by a simple competition between the ionophore and the back reaction in the decay process.

It is unnecessary to suppose that in the experiments of Fig. 4 the ionophores are acting in a manner other than as trans-membrane ion carriers. In the experiments of Fig. 5, it is difficult to think otherwise, since valinomycin (a K^+-carrying species) affects an FCCP-catalysed reaction (a H^+-translocating process). These experiments, and analogous ones performed at lower redox potentials where cytochrome c_2 is reduced prior to flash excitation, support the

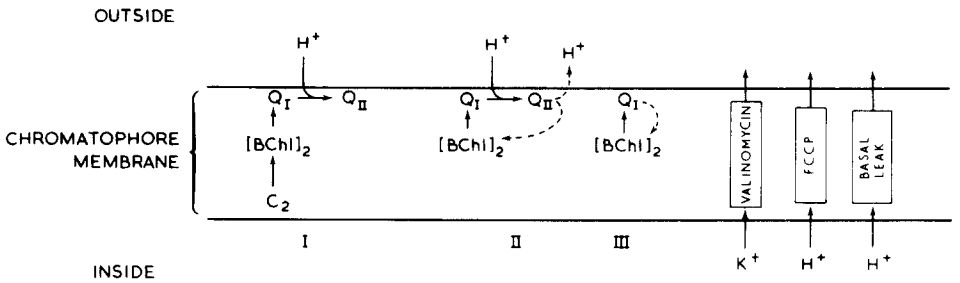

Fig. 6. Reactions responsible for the generation and decay of the carotenoid band shift in antimycin-treated chromatophores

idea that in cases I and II of Fig. 6, a transmembrane delocalised electric field is generated.

At low temperatures (between −35 and −50° C) the light-induced electron transport reactions of *Rps. sphaeroides* chromatophores are largely confined to the reaction centres. Under these conditions [27] the kinetics of the decay of the carotenoid band shift after a period of continuous illumination match those of $[BChl]_2$. The carotenoid shift (but not the reaction centre charge) is still sensitive to the ionophore Gramicidin D. These data can also most easily be explained if it is assumed that the shift is due to a delocalised field over the photosynthetic membrane caused by the $[BChl]_2 \rightarrow Q_I$ reaction [27].

The evidence that $[BChl]_2$ lies toward the centre of the membrane dielectric remains circumstantial [5,6]. However, recent work with reaction centres incorporated into planar phospholipid membranes has indicated that the reaction cytochrome $c \rightarrow [BChl]_2$ is indeed electrogenic (Packham, N.K., Dutton, P.L. and Mueller, P., unpublished observations). We therefore propose that the light generated $[BChl]_2^+Q^-$ dipole, a component of which is normal to the plane of the membrane, is able to raise the electric potential of the internal aqueous phase by a process of capacitative coupling. This explanation has also been proposed to interpret the dependence of the E_m of the *P*-870 of *Chromatium vinosum* on the ionic strength [21]. A capacitative coupling between the $[BChl]_2$ and the internal aqueous phase is anticipated to be fast and would be consistent with the rapid generation of phase 1 of the carotenoid shift.

The responsive pool of carotenoid pigment may therefore be expected to be quite remote from the reaction centre. In this context it is worth noting that the electrochemically-active carotenoids are associated with the light harvesting complex II of the membrane (Ref. 22, Webster, G.D. and Cogdell, R.J., personal communication and Holmes, N.K., personal communication) which, according to current models of energy transfer, is at least functionally isolated from the reaction centre complex [23].

When cytochrome *c* is available for oxidation, the incomplete, capacitative coupling is replaced by a complete trans-membrane electron transfer and membrane potential (phase II in the generation of the carotenoid band shift). The relative amounts of phases I and II will be dependent on the position of $[BChl]_2$ with respect to the membrane interfaces [5,6], the orientation of the $[BChl]_2^+Q_I^-$ dipole within the membrane and the effective dielectric constants

of the domains between $[BChl]_2$ and the inner and outer interfaces.

In the electron transfer sequence from cytochrome c_2 through the reaction centre to Q_{II} and cytochrome b, one H^+ is bound on the outside of the chromatophore membrane per electron per flash [4]. The stoichiometry of H^+ released into the chromatophore lumen is unknown. It would seem however that the electric field sensed by the carotenoids is not dependent on these protolytic reactions since: (i) at redox potentials where cytochrome c_2 is chemically oxidised before the flash, the rise of the carotenoid shift is complete before the H^+ is bound [5]; (ii) at these high redox potentials *o*-phenanthroline, which confines electron transport to $[BChl]_2 \rightarrow Q_I$ with no H^+ binding, does not attenuate the extent of the shift. The local field generated by individual electron transport chains must be communicated rapidly across the internal and external faces of the chromatophore membrane via the lateral diffusion of ions which may include H^+ [24]. Charge separation across the membrane may lead to changes in both the surface and bulk-phase electric potentials [25]. It seems likely that the carotenoids measure the field strength within the membrane [26]. Nevertheless, the electric potential gradient does extend between the membrane-aqueous phase interfaces, which can act as ion sources and sinks for ionophores and for the H^+-ATPase.

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