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THE KINETIC AND REDOX POTENTIOMETRIC RESOLUTION OF THE CAROTENOID SHIFTS IN *RHODOPSEUDOMONAS SPHEROIDES* CHROMATOPHORES: THEIR RELATIONSHIP TO ELECTRIC FIELD ALTERATIONS IN ELECTRON TRANSPORT AND ENERGY COUPLING

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SUMMARY

1. Three principal phases of the carotenoid band shift in *Rhodopseudomonas spheroides* chromatophores elicited by a single-turnover flash can be resolved both kinetically and potentiometrically. Phase I (complete in < 1 μs) is apparent over the redox potential limits of the light reaction, *i.e.* the potential range in which reaction centre bacteriochlorophyll is reduced and the primary electron acceptor oxidized before the flash; thus the band shift is consistent with its response to the formation of P^+X^-. Phase II (about 25% of the amplitude of Phase I) with an approximate 0.15-ms half time is observed if cytochrome *c* ($E_{m7.2} + 295$ mV) is chemically reduced before the flash and hence may be in response to the photooxidation of cytochrome *c* and re-reduction of P^+. A much slower phase (Phase III) can also be detected at positive potentials. It is enhanced both in extent and formation rate ($t_{1/2} > 5$ ms to about 1 ms) over the 150 mV potential range in which cytochrome b_{155} ($E_{m7.2}$ + 155 mV) becomes chemically reduced. Between 50 and 100 mV this phase is approx. 80–100% of the extent of Phase I. Phase III is abolished by antimycin A as are the oxidation of cytochrome b_{155} and the re-reduction of photooxidized cytochrome *c*. All phases are additive. Thus the formation of the carotenoid band shift is in response to pulsed electron transfer events. Further, using multiple, one-turnover flashes, the extent following each flash, and behaviour of the formation the carotenoid band shift can be clearly explained in terms of the electron flow patterns in the chromatophore.

2. The decay of the carotenoid band shift is stimulated by agents such as the uncoupler carbonyl cyanide *p*-trifluoromethylphenylhydrazone, and the potassium ionophore valinomycin, irrespective of the suspension redox potential. The decay half time, which after five flashes is in the region of 500 ms without addition, can be reduced to < 1 ms by addition of the membrane ion carriers. The kinetic behaviour of cytochrome *c* is used to demonstrate that the course of carotenoid band shift

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethylphenylhydrazone: MOPS, morpholinopropane sulphonate; PMS, phenazine methosulphate.

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decay has no obligate relationship to the redox state or electron transfer events of the electron carriers.

3. The carotenoid band shifts appear to be generated by electrostatic field alterations resulting from oxidation–reduction reactions between adjacent electron transport carriers. Once formed, the retention of the fields is a function of membrane ion permeability. The location of the field-forming reactions, with respect to the kinetically and thermodynamically defined spans of electron transport, and to their topology within the membrane, is discussed.

INTRODUCTION

We have recently characterized both thermodynamically and kinetically, the electron carriers of chromatophores from photosynthetically-grown *Rhodopseudomonas spheroides*[1]. We shall now describe the dependence of the flash-induced change in carotenoid spectrum, an absorption band shift to longer wavelengths, on the behaviour of the electron carriers and on factors which affect energy coupling.

The light-induced carotenoid absorption shift in *Rhodospirillum rubrum* and *Rps. spheroides* is modified by a variety of electron transport inhibitors, uncoupling agents and ionophorous antibiotics[2-5] and is decreased in amplitude under phosphorylating conditions[4,6]. The light-induced carotenoid band shift of *Rhodopseudomonas gelatinosa* at low temperatures has been shown to respond directly to the electron transfer events of cytochromes and the reaction centre bacteriochlorophyll system[7]. In addition to the effects of light, the carotenoid shift has been shown to be generated in the dark, upon ATP or pyrophosphate addition[8], or in response to electric fields generated by ion gradients across antimycin-treated chromatophore membranes[9].

It seems unlikely that the shift is a result of a chemical change of the carotenoid molecules, one reason being that the quantum efficiency of the reaction is high. Two mechanisms have been proposed, (i) the pigments respond to changes in their microenvironment as a result of a conformational change associated with the chromatophore energy conservation[2,8] and (ii) the shifts are an electrochromic response to a membrane electric potential[5,10] which is in equilibrium with the high-energy state[11]. These possibilities are discussed in ref. 5.

MATERIALS AND METHODS

Bacterial cultures were grown anaerobically in the light and chromatophores were prepared and stored as previously described[11].

The preparation and suspending medium for the chromatophores contained 50 mM KCl and 50 mM morpholinopropan sulphonate (pH 7.0). Both *Rps. spheroides* strain Ga (with modified carotenoid complement which does not interfere with the recording of cytochrome α-band absorbance changes), and wild-type organisms have been used in these experiments. The dependence of the flash activated carotenoid band shift on redox potential is similar in each strain. Bacteriochlorophyll concentrations were assayed using the *in vivo* extinction coefficient given by Clayton[12].

Chemical reagents were of analytical grade where available or otherwise of

the highest purity obtainable commercially. Antimycin and valinomycin were purchased from the Sigma Chemical Company.

Flash-induced absorption changes in chromatophores poised at fixed oxidation–reduction potentials were measured in the vessel of Dutton[7] as described by Dutton and Jackson[1]. The flash system was a saturating 5 μs (full width at half height) xenon-flash capable of being triggered every 8 ms. 5 μs is so short as to permit only one turnover of *Rps. spheroides* per flash since cytochrome *c* oxidation, the limiting factor for photooxidized reaction center bacteriochlorophyll reduction, has a half-time of 0.15 ms. We have observed the precautions discussed in the previous report[1] to ensure good equilibration between the chromatophores, mediator dyes and electrodes, whilst keeping the dye concentration low to avoid rapid chromatophore–dye interaction immediately after the flash. The system was judged to be close to equilibrium if a similar flash-induced response was observed for any redox poise when approached from either the low or high potential side. The kinetics of the carotenoid shift, appeared to be free from artifact due to any electron donating or

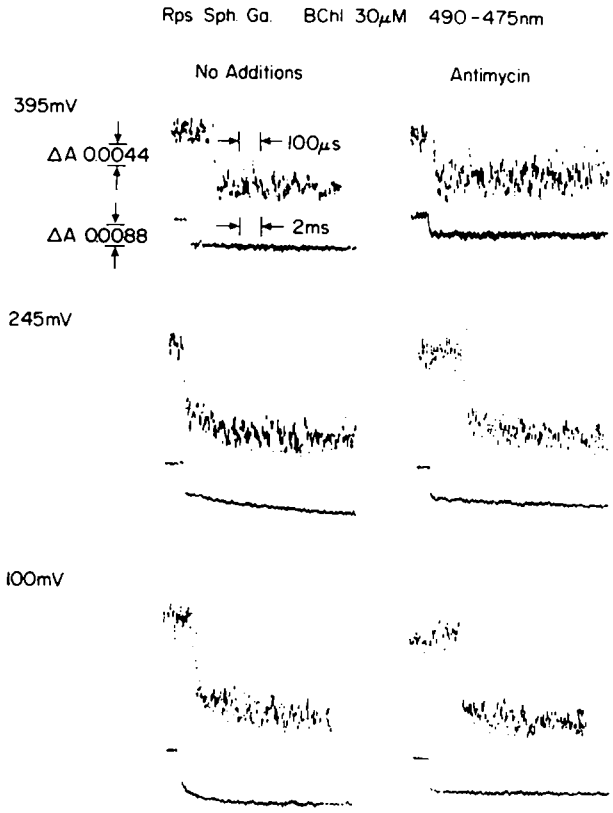

Fig. 1. The time course at various redox potentials of the carotenoid band shift in *Rps. spheroides* Ga. Chromatophores (bacteriochlorophyll, BChl, 30 μM) were suspended in 0.05 M KCl, 0.05 M morpholinopropane sulphonate buffer, pH 7.0, 0.5 μM diaminodurene and PMS were present as redox mediators; a fresh solution of sodium dithionite and potassium ferricyanide was also used to change the potentials to more electronegative and electropositive values. The concentration of antimycin added where indicated was 1 μM. The measuring wavelengths were 490–475 nm.

accepting properties of the dyes since changes of dye concentration were without significant effect on the absorption change. Also, the main features of the carotenoid shift *versus* redox poise (shown in Figs 1 and 2) could be duplicated in anaerobic suspensions in the absence of mediating dyes.

RESULTS

The kinetics of the flash-induced carotenoid absorption band shifts

Fig. 1 describes the time course of the flash-induced carotenoid band shift at various potentials chosen to maximize the resolution of three principal kinetic phases of the band shift. The effect on each phase of the electron transport inhibitor, antimycin A, is shown.

Phase I. At 395 mV where all electron carriers except reaction centre bacteriochlorophyll [its *in situ* oxidation–reduction midpoint potential at pH 7.2 ($E_{m7.2}$) is 440 mV] are chemically oxidized prior to the flash, the carotenoids respond rapidly (< 1 μs); the addition of antimycin A has no effect on this phase.

Phase II. At 245 mV, where in addition to the reaction centre bacteriochlorophyll, cytochrome *c* ($E_{m7.2}$ 295 mV[1]) is also mainly reduced and hence is available for photooxidation, an additional slower phase is detected. It has a half time of approx. 150 μs and is not affected by antimycin A.

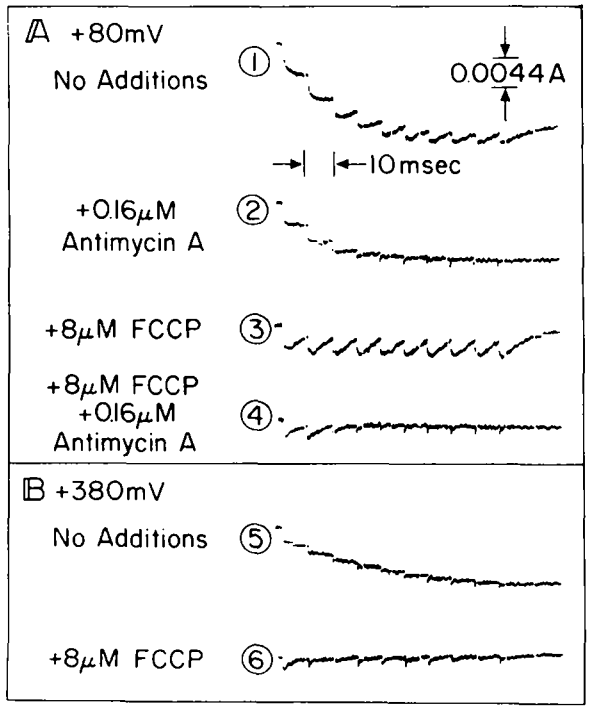

Fig. 2. The multipulse kinetics of the carotenoid band shift in *Rps. spheroides* Ga. The chromatophores (bacteriochlorophyll, 12.5 μM) were treated and assayed as in Fig. 1 except that in the 80-mV region 2 μM PMS was present and at 380 mV only ferri/ferrocyanide (approximately 200 μM) was present; other conditions are given in the figure. In all cases the chromatophores were activated by 12 flashes spaced at 8-ms intervals.

Phase III. At 245 mV there is also a much slower phase with a half time of several milliseconds. At 100 mV where cytochrome b_{155} is mainly chemically reduced (its $E_{m7.2}$ is 155 mV[1]) the millisecond phase is more rapid. Phase III, whether observed at 245 mV or 100 mV is completely abolished by antimycin A. The extents of Phases I, II and III are additive and are in the approximate ratio of 0.75:0.25:1.0, respectively.

Fig. 2 shows the response of the carotenoid band shift to a series of saturating flashes spaced 8 ms apart. As with the single flash response, a redox poise in the 100-mV region is optimal for a maximum generation of the carotenoid band shift (Trace 1). In Trace 1 it is clear that 5–6 flashes are required before a pulsed steady-state value is obtained and sustained by subsequent flashes. The inhibition of Phase III by addition of antimycin A (Trace 2) has a marked diminishing effect on the total extent of the change induced by the multiflashes. The maximum change is now achieved in 3–4 flashes. The stimulated decay rate of the band shift in the presence of high concentrations of the uncoupling agent carbonyl cyanide *p*-trifluoromethylphenylhydrazone (FCCP) (Trace 3) competes with the slow generation phase and leads to the observed limitation in the total extent of carotenoid band shift compared with that shown in Trace 1. Addition of antimycin A in the presence of uncoupler (Trace 4) results in band shifts following only the first 3 or 4 flashes, as seen in Trace 2. However, in contrast to Trace 2 there is full decay over the 8-ms dark period between each flash. At 380 mV (Part B) where only reaction centre bacteriochlorophyll is chemically reduced before flash activation, most of the rapid change occurs on the first flash, with little or no change on subsequent flashes. Phases II and III are barely detectable at this redox potential. Addition of uncoupler at 380 mV causes a rapid collapse of any flash-induced carotenoid band shift.

The addition of 1.5 μM valinomycin in the presence of K^+ induces the same kinetic patterns as described in Figs 2, 4 and 6 for uncoupler.

The dependence of the kinetic phases of the carotenoid band shift on the suspension redox potential

Fig. 3 shows the dependence of the flash-induced carotenoid band shift on the redox potential poise of the chromatophores. The dependence of the first 3 flashes is described. Two time divisions are shown; the 0–0.3-ms division to include Phase I and most of Phase II and the 0–8-ms division to include all three phases, the difference between the divisions, therefore, is mainly Phase III. The pertinent points are as follows. *First flash*: At 400 mV there is just the fast phase (Phase I) and, as we have shown previously[13], this phase attenuates as the potential is made more positive, the course of the attenuation following the chemical oxidation of reaction centre bacteriochlorophyll. As the potential is made more negative, a 25% enhancement due to Phase II appearance is seen in the 0–0.3-ms division over the 300-mV range. This enhancement is roughly commensurate with the course of chemical reduction of cytochrome *c*. Phases I and II are apparent down to below 0 mV where they are attenuated as the primary electron acceptor becomes chemically reduced ($E_{m7.2}$ −20 mV) which prevents photochemistry. Phase III becomes significantly apparent from 380 mV down and is enhanced in extent (and rate) in two steps: the first over the 300-mV range and the second over the 150-mV range, reaching a maximum at about +80 mV and then diminishing with the chemical reduction of the primary

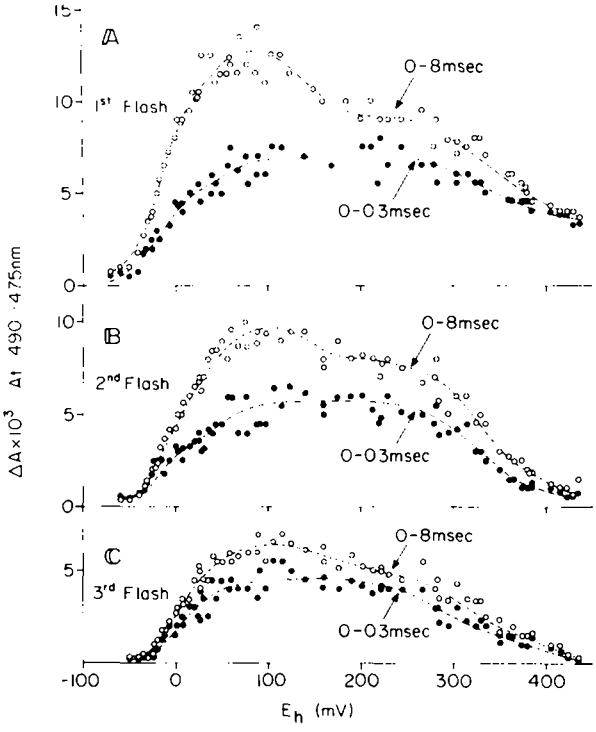

Fig. 3. The time-resolved flash-induced carotenoid band shift in *Rps. spheroides* Ga. The chromatophores (bacteriochlorophyll, 25 μM) are suspended and assayed as in Fig. 1. The 10 μM each of diaminodurene, PMS, phenazine ethosulphate and pyocyanine were used as mediators

electron acceptor. *Second flash*: At 400 mV there is very little band shift; both fast and slow phases of the band shift become evident over the 300-mV range. The Phase III enhancement over the 150-mV range is much less evident than that encountered on the first flash. *Third flash*: Again, little change at 400 mV; and as the potential is lowered, the course of appearance of the band shift over the 350–200 mV range is extended to somewhat lower potentials than is found with the second flash. The extent of Phase III is further decreased compared with the previous two flashes.

In the presence of antimycin A, the redox potential dependence of the amplitude of Phases I and II after the first, second and third flashes is essentially the same as that shown in Fig. 3 for untreated chromatophores.

Multipulse activated behaviour of coupled electron transfer

Although a fuller account will appear elsewhere on the behaviour of the electron transfer components following multipulse activation (Dutton, P. L., Morse, S. P. and Jackson, J. B., unpublished), it may be stated here that cytochrome *c*, being at the electropositive end of a site of energy conservation and also an immediate electron donor to the reaction centre, can serve to indicate the behaviour of electron flow patterns under various conditions activated on a multiple basis.

Fig. 4 shows the redox potential dependence of cytochrome *c* oxidation. Antimycin A is present to prevent any re-reduction of ferricytochrome *c* during the

multiflash sequence in order to simplify the experimental situation; the 0–8-ms change, then, represents the extent of cytochrome oxidation per light pulse. It takes three pulses to fully oxidize the cytochrome complement (see also Fig. 5), the change from the second and third flashes amounting to the same as that elicited by the first. The results imply the presence of two cytochrome *c* haems either of which may react with the flash-induced P^+. The solid lines drawn through the points are theoretical for this situation. Such an arrangement has also been shown in *Chromatium* D for

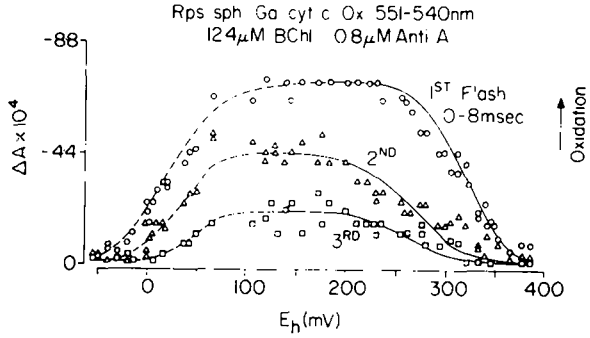

Fig. 4. Time-resolved flash-induced reactions of cytochrome *c* in *Rps. spheroides* Ga. The chromatophores (bacteriochlorophyll, 12.5 μM) were suspended in 0.05 M KCl, 0.05 M MOPS buffer, pH 7.0, with the same mediators as given in Fig. 3. The measuring wavelengths were 551–540 nm. Antimycin A (Anti A, 0.8 μM) was present to inhibit the re-reduction of flash-oxidized cytochrome *c* (cyt *c*); this was not significant 8 ms after the pulsed cytochrome *c* oxidation. Thus, the absorption changes 8 ms after each of 3 flashes are shown. The solid lines drawn through the points are theoretical for the existence of two cytochrome *c* haems (E_m +295 mV) being equivalent with respect to a single reaction centre bacteriochlorophyll (see text).

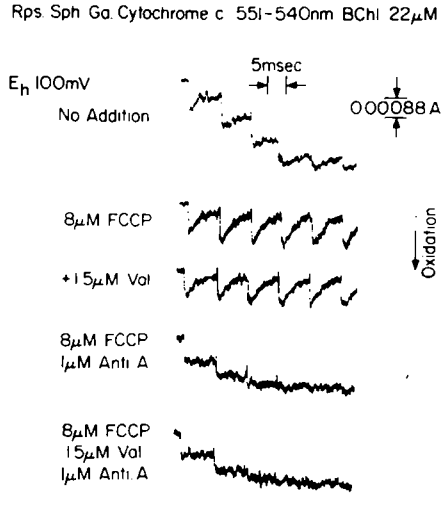

Fig. 5. The multiple flash-induced kinetics of cytochrome *c* reactions in *Rps. spheroides* Ga. The chromatophores (bacteriochlorophyll, 22 μM) were suspended in 0.05 M KCl, 0.05 M MOPS buffer, pH 7.0. The redox potential was 100 $\pm$ 5 mV, mediated by 10 μM each of PMS and diaminodurene. The measuring wavelengths were 551–540 nm. The conditions are given in the figure.

cytochromes c_{555} (ref. 15) and c_{553} (ref. 7). Thus, if x is the fraction of the cytochrome reduced at a redox potential, E_h, then the E_h dependence of the flash-induced extent of cytochrome c oxidation is given by the expression $2x-x^2$ for the first flash and x^2 for the second flash (see ref. 15). The x^2 curve is plotted through both the second and third flash induced changes because the 'second' cytochrome c haem is not fully oxidized on the second flash, the remainder being oxidized on the third flash. The lack of full oxidation of the second haem on the second flash is due to a kinetic limitation of electron transfer, perhaps in the carriers immediately after the primary electron acceptor; this will be dealt with elsewhere (Dutton, P. L., Morse, S. D. and Jackson, J. B., unpublished).

Fig. 5 shows the behaviour of the two cytochrome c haems in chromatophores poised at 100 mV under conditions comparable with those described for the carotenoid band shift in Fig. 2. In the coupled state it takes five flashes to achieve a pulsed steady-state in which the cytochrome c complement is maintained in a highly-oxidized state. Addition of uncoupler or valinomycin prevents the high level of oxidation by permitting a stimulated electron flow in the cycle which results in a complete re-reduction of flash-oxidized cytochrome c during each 8-ms interflash dark period. In contrast with the behaviour of the carotenoid band shift (see Fig. 2) the addition of antimycin A even in the presence of uncoupler or valinomycin, results in the typical inhibition of re-reduction of photooxidized cytochromes showing that the decay of the carotenoid band shift is not obligatorily governed by the redox state of the electron carriers.

DISCUSSION

The factors governing the pulsed formation of the carotenoid band shift

We are now in a position to relate each of the phases to electron transfer events following pulsed activation. The fastest phase, Phase I, is apparent over the redox potentiometric range in which the light induced formation of $P^+ X^-$ is possible (where P^+ is the reaction centre bacteriochlorophyll and X^- is its primary acceptor). Appropriately for it being a direct consequence of this event it has been shown to be complete in less than 50 ns (DeVault, D. and Kihara, T., unpublished). Working with green plant chloroplasts, Junge and Witt and their collaborators[14] have suggested that the analogous 518-nm shift, which is complete in 20 ns, is an electrochromic response to the light-induced charge separative act between the reaction centre chlorophyll and its primary acceptor. Similar conclusions have been derived from kinetic and low-temperature studies of the carotenoid band shift in chromatophores[3,5,7,9]; the new data are consistent with this hypothesis.

Phase II, apparent over the potential range in which cytochrome c is chemically reduced (and hence is available for photooxidation), we consider is a response originating in the oxidation of cytochrome c and reduction of P^+. In support of this is the finding that Phase II follows approximately the same time course as cytochrome c photooxidation ($t_{1/2} \simeq 150\ \mu s$). Our previous determination of cytochrome c oxidation ($t_{1/2} < 100\ \mu s$) was not accurate. The actual time being too close to the instrument risetime. A connection between cytochrome oxidation and P^+ reduction and the carotenoid band shift was clear in low temperature studies with *Rps. gelatinosa* chromatophores[7,16]. At these very low temperatures which confer very limiting

conditions on the system, the conclusion that the carotenoids are responding not only to electric field alterations operating between P^+ and X^-, but also between either of two clearly characterized *c*-type cytochromes and X^-, is not unreasonable; the same conclusion can be made from the room temperature experiments presented here with *Rps. spheroides.*

Phase III is more complex but kinetically related to antimycin A-sensitive electron transfer in the mid-potential span of the system. We are unable to assign this phase to precisely defined electron transfer events since the exact kinetic relationships between the *b*-type cytochromes and cytochrome *c* have not yet been established (see ref. 1). However, the enhancement which is observed over the 150-mV range (*i.e.* that with the 1–2-ms half time at 100 mV) is potentiometrically, kinetically and from its antimycin sensitivity, that expected from a carotenoid response to cytochrome b_{155} oxidation and ferricytochrome *c* reduction. It may be of relevance however, that with *Rps. capsulata*, studies (Evans, E. H. and Crofts, A. R., unpublished) have failed to reveal a *b*-cytochrome with a 150-mV midpoint although carotenoid enhancement does occur over the 150-mV potential region; this, therefore, raises the question of the functional generality of a *b*-cytochrome at this potential (although one has been shown to be present in *Rsp. rubrum*) or whether perhaps the carotenoids in *Rps. spheroides* respond to a hitherto undetected carrier.

On a multipulse basis the formation of carotenoid band shift depends on the number of components reduced prior to activation, namely P, the two cytochrome *c* haems and cytochrome b_{155}. As shown in Fig. 4, the first pulse extent is directly dependent on the state of reduction of P, cytochrome *c* (following the $2x-x^2$ 'first' cytochrome *c* haem oxidation E_h dependence curve) and cytochrome b_{155}. A carotenoid shift elicited by the second pulse requires that cytochrome *c* be reduced before activation to re-reduce P^+ in time for this second flash. Thus, as would be expected, the E_h dependence of the carotenoid band shift on the second flash also approximates to the $2x-x^2$ curve for the first cytochrome *c* oxidation. The third flash roughly follows that for the x^2 curve for the E_h dependence of the 'second' cytochrome *c* oxidation. After the first flash has oxidized cytochrome b_{155}, subsequent flashes do not produce an enhanced carotenoid shift over the 150-mV region: this agrees with the slow rate of re-reduction of this cytochrome[1]; *i.e.* once oxidized, the 8-ms dark period is not long enough for its re-reduction.

The decay of the carotenoid band shift

Uncouplers and the valinomycin group of ionophores increase the rate of decay of the shift after a flash[4,5,8]. The half time of the decay in the presence of valinomycin is not influenced by the redox potential. The course of the decay shows no obligate dependence on the state of reduction of the carriers. This is clearly demonstrated by comparing the cytochrome *c* kinetics in Fig. 5 with those of the carotenoid band shift in Fig. 2. The rate of decay of the carotenoid shift can be increased by three orders of magnitude by high concentrations of valinomycin or FCCP[5] whereas cytochrome *c* re-reduction is in comparison only marginally stimulated. Furthermore addition of antimycin A even in the presence of these agents inhibits cytochrome *c* re-reduction (and induces a stimulated cytochrome *b* reduction; see ref. 1). The important difference with the carotenoid band shift is demonstrated by its collapse under the influence of valinomycin or uncoupler regardless of the

presence of antimycin A. Thus, once formed, the retention of a field is dependent on membrane ion permeability.

Membrane-component organization

In view of the valinomycin and uncoupler stimulation of the decay of the rapid phases of the flash-induced chlorophyll and carotenoid shifts, the vectorial arrangement of the electron transfer components of the light reaction, P and X, in both plants[14] and bacteria[5] have been considered to span the membrane. In the case of the bacterial chromatophore, X has been placed on the outer side and P on the inner side of the membrane, consistent with the polarity of electric fields produced by ion gradients[9]. In view of the results presented in this communication, it now seems necessary to review the possibility that electric field alteration may also be generated by electron transfer between adjacent electron carriers which do not fully span the membrane. We shall consider two models: in the first (Fig. 6A), the carotenoid shift arises as a result of a trans-membrane electric field generated by electron flow at two sites. In the second (Fig. 6B), local fields between three adjacent oxidoreduction carriers perturb the carotenoid spectrum and the trans-membrane field is produced at one site.

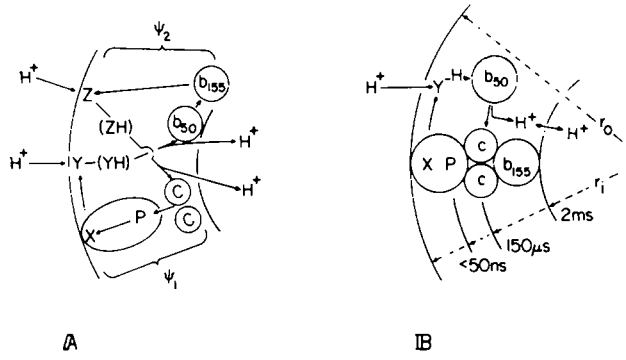

Fig. 6. Schemes showing possible membrane-component organization.

In Fig. 6A, the two-step generation of the chromatophore membrane potential is accompanied by H^+ translocation at two sites, a scheme based on Mitchell's concept of alternating trans-membrane electron and hydrogen carrying arms of the chemiosmotic loop[17]. The first electrogenic site, Ψ_1, is depicted between cytochrome *c* and X and hence Phases I and II of the carotenoid band shift are considered as response to this trans-membrane field. H^+ is picked up in a half time of about 300 μs (see refs 18 and 19) as a secondary acceptor, Y, is reduced by X. The second electrogenic site Ψ_2, is presumed to be between a *b*-cytochrome and a hypothetical hydrogen carrier Z (see ref. 19); the millisecond carotenoid band shift Phase III is considered to be in response to the transmembrane field generated at this site. In support of the expected second proton binding reaction is the identification of an approximate 2 ms half time hydrogen binding[18,19]. However, this is only observed after more than one turnover of the system or in the presence of valinomycin. Cytochrome b_{155} oxidation is a likely candidate for the electron carrier giving rise to Ψ_2

in Fig. 6A. In this case, and in view of the matching kinetics of cytochrome b_{155} oxidation and cytochrome c reduction[1], the reactions of Z, including H^+ translocation, must not be rate limiting.

Fig. 6B shows the second consideration based upon the assumption that the carotenoids respond to the formation of electric fields between adjacent carriers, prior to the ultimate formation of a trans-membrane field. In this arrangement we consider the possibility that both cytochrome b_{155} and b_{50} could serve as electron donors to ferricytochrome c (for a discussion see ref. 1). In this model, there is no requirement for a hydrogen carrying component between cytochrome b_{155} and cytochrome c. It is envisioned that the carotenoid band shift is caused by the movement of the oxidizing equivalents toward the centre of the chromatophore *via* P, cytochrome c and cytochrome b_{155}. The simplest reason for the three kinetic phases of the band shift in this model is that as the field operates over a wider distance there is an increase in the number of carotenoid molecules coming under the influence of the field. This possibility makes it worth looking at how the field will behave through a simplified, but useful first approximation which assumes the chromatophore membrane to be a capacitor. So far examination of fields in chromatophores have been based on parallel plate capacitors[5,20] but since the average chromatophore has an external radius of 250–300 Å and a probable internal radius of about 200 Å, it is more realistic to treat the system as a spherical dielectric. We appreciate that in any such treatment unless there is a full delocalization of the charges then the system is not a capacitor; a sufficient element of delocalization, however, may exist to be of significance. In such a system the potential difference, V, between the negative and positive charges (delocalized) is given by the expression:

$$V = \frac{Q}{\varepsilon 4\pi}\left(\frac{1}{r_i} - \frac{1}{r_0}\right)$$

where ε is the dielectric constant, Q is the charge, and r_0 and r_i are the respective distances from the centre of the chromatophore to the delocalized shells of the outer and inner charges. It can be seen that as r_i becomes smaller, as the positive charges move inward, the potential difference V, becomes larger assuming a constant ε and Q. The intensity of the electric field E at a point distance r from the centre of such a spherical system is given by $E = Q/\varepsilon 4\pi r^2$.

There is ample evidence that ionophores like valinomycin catalyse transmembrane transport of K^+ (see ref. 11). Moreover, approximately one valinomycin molecule per chromatophore[4] is sufficient to accelerate the decay of the carotenoid shift and the concentration dependence of ionophore[5] suggests that the chromatophore is the 'functional electric unit' (*cf.* experiments with gramicidin in chloroplasts, ref. 14). In order to explain the effect of valinomycin on the carotenoid shift in terms of the model shown in Fig. 6B we must assume that local intermolecular fields can also be affected by charge redistribution within the membrane by the valinomycin–K^+ complex.

The models we have outlined above, place constraints on the mechanism by which the oxidation–reduction energy may be used in the synthesis of ATP. Both models are consistent with the chemiosmotic hypothesis[17] (in that electron transfer generates trans-membrane potential H^+ gradients), there being either one (Fig. 6B)

or two (Fig. 6A) energy transducing 'loops'. The model shown in Fig. 6B allows for the conceptual difference that the trans-membrane gradients are in equilibrium with intramembrane energy conservation occurring over the cytochrome b_{50}-cytochrome *c* redox potential span.

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