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ELECTRICAL POTENTIAL CHANGES, H^+ TRANSLOCATION AND PHOSPHORYLATION INDUCED BY SHORT FLASH EXCITATION IN *RHODOPSEUDOMONAS SPHAEROIDES* CHROMATOPHORES

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SUMMARY

1. The basal decay of the carotenoid shift of chromatophores from photosynthetic bacteria following short flash excitation is approximately biphasic. The decay indicates the dissipation of the transmembrane electrical potential.

2. The H^+ efflux following rapid H^+ binding after a flash, measured from the colour change of added cresol red, shows very similar kinetics to the carotenoid shift decay suggesting that the dissipation of the electric potential decay is a consequence of the H^+ efflux.

3. The electric potential decay is stimulated when the chromatophore suspension is supplemented with ADP and P_i (in either the presence or absence of antimycin A).

4. The stimulated electric potential decay by ADP and P_i has a similar pH dependence to that of phosphorylation in continuous light.

5. The stimulation of the electric potential decay by ADP and P_i is reversed, by aurovertin, an antibiotic which inhibits phosphorylation.

6. The stimulation of the electric potential decay by $ADP + P_i$ is also reversed by the inhibitors oligomycin and venturicidin. These inhibitors, but not aurovertin, also inhibit the fast phase of the decay under non-phosphorylating conditions.

7. Valinomycin accelerates the overall rate of decay of the electric potential, inhibits the ADP and P_i stimulated electric potential decay, and inhibits the flash-induced phosphorylation. The decay rate of the H^+ efflux however, is slower in the presence of this ionophore.

8. Nigericin-type ionophores accelerate the overall decay rate of the H^+ efflux and inhibit the ADP and P_i stimulated electric potential decay. The basal rate of the electric potential decay is unaffected by treatment with these ionophores.

9. When a coupling factor associated with the chromatophore ATPase is removed from the membrane, both the stimulation of the electric potential decay by ADP and P_i and ADP phosphorylation are inhibited. Both reactions are completely restored after reconstitution with the crude coupling factor extract. The basal electric potential decay rate is not affected by the removal of coupling factor.

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INTRODUCTION

The red shift of the carotenoid absorption bands observed when chromatophores from photosynthetic bacteria are illuminated is partially reversed in the presence of ADP, P_i and Mg^{2+} [1, 2]. Addition of ATP to a dark suspension of *R. rubrum* chromatophores also results in the absorption band shifts [3]. Evidence suggests that the band shifts [4, 5] and their counterparts in green plant photosynthesis [6–10] are an electrochromic response to the electric field generated across the membranes by electron transport reactions coupled to H^+ translocation (but see refs 3 and 11). In this case ATP synthesis may be driven by an electrochemical gradient of H^+ [12]. The reversal of the carotenoid band shift would represent a fall in the gradient resulting from H^+ current through a proton translocating ATPase.

The aim of the report is to ascertain whether or not the enzymic properties of the chromatophore ATPase and the carotenoid response are consistent with the hypothesis mentioned above. In particular, how do various chemical treatments of the ATPase affect the carotenoid response and how do energy dissipating reagents affect the phosphorylation in single turnover flashes? We have used saturating flashes of sufficiently short duration that the electron transport processes turn over only once after activation so that for example in the presence of the ionophore valinomycin the rate of electron transfer may be increased but the number of electrons transferred remains the same. The quality of the spectroscopic signals was improved by using repetitive flashes at low frequency so that electron transport is completed and the high energy state is largely dissipated during the dark period. Nishimura [13] and Geller [14] have used repetitive flash techniques to study photophosphorylation in chromatophores but using long low intensity flashes at high frequencies. That is under conditions where the rate of electron transport limited the net rate of ATP synthesis and the high energy state was built up to some indeterminate level.

METHODS

The bacteria were grown and the chromatophores were prepared as in ref. 15.

Resolution of chromatophore coupling factor

We have applied the techniques of Baccarini-Melandri et al. [16] for *Rps. capsulata* and Johansson [14] for *R. rubrum* to *Rps. sphaeroides*. Chromatophores were suspended in a medium containing 50 mM KCl, 50 mM tricine, 1 mM ethylene diamine tetra-acetic acid (EDTA) 10 % sucrose, pH 7.2 at a concentration of 0.2 mM bacteriochlorophyll, sonicated in the cold for 4 bursts of 30 s in an M.S.E. ultrasonic disintegrator and stirred for 1 h in the dark at room temperature. The suspension was centrifuged for 2 h at $140\,000 \times g$. The sediment of partially resolved chromatophores was washed once and resuspended in the above medium (with omission of the EDTA) and the supernatant was used as a source of coupling factor without further purification. The resolved chromatophores were reconstituted by incubation at room temperature for one hour with the crude coupling factor solution in the presence of 9 mM $MgCl_2$ [16, 17].

Spectrophotometric measurements

The single beam spectrophotometer and the signal averaging system is similar

to that described in ref. 15. The flash frequency was usually 1/15 Hz. Orthophosphate and ADP concentrations when added were 0.25 and 0.2 mM, respectively. The 10 ml reaction medium for carotenoid shift measurements routinely contained in 10 % sucrose, 50 mM KCl, 8 mM MgCl₂, 50 mM *N*-tris (hydroxymethyl)-methylglycine (tricine) or 50 mM glycylglycine pH 7.9, and bacteriochlorophyll in the concentration range of 10^{-5} M unless otherwise stated.

Rapid H⁺ uptake and release, following flash excitation was measured in unbuffered chromatophore suspensions, containing in 10 % sucrose, 50 mM KCl and 8 mM MgCl₂ pH 7.9. Cresol red [18] was used as a pH indicator in preference to bromocresol purple [19] and phenol red [20] because its higher pK_a permitted measurement at around pH 8.0, the optimum for photophosphorylation. Its properties were in accordance with the requirements for a reliable indicator of the pH of the chromatophore bathing fluid [19] and at the concentrations we have used ($2 \cdot 10^{-5}$ – $6 \cdot 10^{-5}$ M) it was without any significant poisonous effects on electron transport or on ATP synthesis. The measuring wavelength for colour changes of the cresol red was 575 nm. Where background changes occurred (i.e. when the solution was made 50 mM in tricine) they were subtracted electronically by the signal averager.

When simultaneous measurements of the carotenoid shift and cresol red absorbance changes were required, a double-beam instrument was used, with the two measuring beams at 90 °C [15].

Biochemical assays

The incorporation of ³²P_i into organic phosphate was measured according to the method of Avron [21]. The conditions were as follows: 50 mM KCl, 8 mM MgCl₂, 50 mM tricine (or glycylglycine), 0.66 mM ADP, 0.5 mM P_i, chromatophores containing approximately 50 nmol bacteriochlorophyll in 2.0 ml of 10 % sucrose at final pH 7.9, 25 °C. The reaction was terminated after 1 or 3 min of continuous illumination, with 0.2 ml of 40 % trichloroacetic acid.

Protein was estimated according to Lowry et al. [22] and bacteriochlorophyll according to the in vivo extinction coefficients given by Clayton [23].

Chemicals

ADP and ATP were purchased from Boehringer, Mannheim (Germany), valinomycin from Calbiochem, San Diego (USA), dianemycin from Lilly laboratories, Indianapolis, (USA), and oligomycin and nigericin from Serva, Heidelberg (Germany). Venturicidin and aurovertin were gifts from Prof. J. B. Chappell, Department of Biochemistry, University of Bristol.

RESULTS

The flash-induced carotenoid shift and cresol red absorbance change and their decays

Following a short actinic flash the carotenoid shift of *Rps. sphaeroides* chromatophores continues for about 100 ms due to electrogenic electron transfer between cytochromes *b* and *c* [24]. This phase of electric field formation is abolished by the electron transport inhibitor, antimycin A, to leave only the fast components generated by electron transport between cytochrome *c*, *P*-870 and X [24]. The decay of the field is more clearly apparent in the presence of antimycin, because the faster portion of the

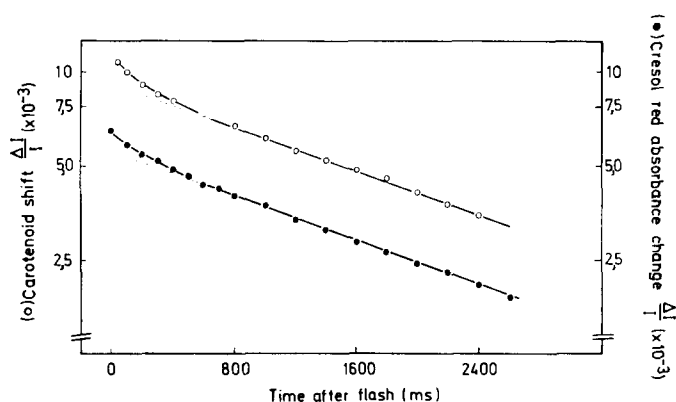


Fig. 1. The decay kinetics of the carotenoid shift and added cresol red absorbance change in chromatophores from *Rps.sphaeroides* (log plot). Chromatophores containing $4.6 \cdot 10^{-6}$ M bacteriochlorophyll were suspended in 10.0 ml medium. Simultaneous measurements with a double beam spectrophotometer. Average of 32 flashes at 1/18 Hz. The flash was fired at $t = 0$. Antimycin was present at a final concentration of $5 \cdot 10^{-7}$ M. For details, see Methods.

decay is masked by the rise of the shift in the absence of antimycin. The decay of the shift is at least biphasic (Fig. 1 and compare ref. 25), on average 15 % relaxing with $t_{\frac{1}{2}} \approx 150$ ms and 85 % with $t_{\frac{1}{2}} \approx 2500$ ms. Slight variations depend on the preparation.

The rapid proton uptake indicated by the colour change of added phenol red following single flash excitation has been described by Cogdell et al. [20]. It is of interest to compare the decay kinetics of the electric potential decay indicated by the carotenoid band shift with the kinetics of H^+ efflux indicated by the absorbance change of cresol red (see Methods). As shown in Fig. 1 the decay kinetics of the carotenoid shift and of the pH change following short flash activation are very similar, suggesting that electrophoretic proton transport is largely responsible for the dissipation of the membrane potential.

The effect of phosphorylating conditions on the decay of the flash-induced electrical potential

Fig. 2 shows that in both the presence and absence of antimycin, the decay of the faster component of the carotenoid shift is accelerated and its amplitude is increased at the expense of the slow phase when the suspension is supplemented with ADP and P_i . This effect is hereafter called "the carotenoid shift decay stimulated by ADP and P_i ". The half-time of the remaining slow decay phase is unaffected. The biphasic nature of the decay is more clearly apparent in Fig. 3 in the presence of antimycin (see above).

The stimulation of the decay by ADP and P_i may also be seen when the redox potential of the suspension is raised to ≈ 400 mV with ferricyanide i.e. under conditions where electric field formation is driven solely by the $P-870 \rightarrow X$ reaction [24]. Under anaerobic conditions in the presence of low concentrations of succinate, the initial amplitude of the carotenoid shift is increased, owing to enhanced electron flow through cytochrome *b* \rightarrow cytochrome *c* [24]. The carotenoid shift decay stimulated

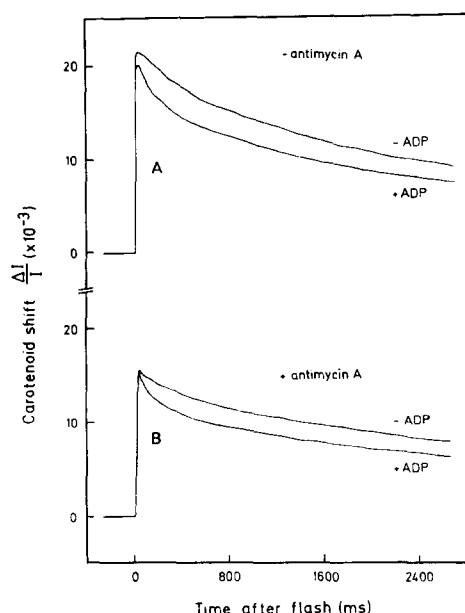


Fig. 2. The kinetics of the decay of the flash-induced carotenoid shift in phosphorylating and non-phosphorylating conditions. The traces are an averaged signal from 16 flashes at 1/18 Hz. Bacteriochlorophyll concentration was 10^{-5} M in 10 ml medium. Phosphate was present in all four samples and ADP was present where indicated. In (B) antimycin was present at a final concentration of $5 \cdot 10^{-7}$ M. For further details, see Methods.

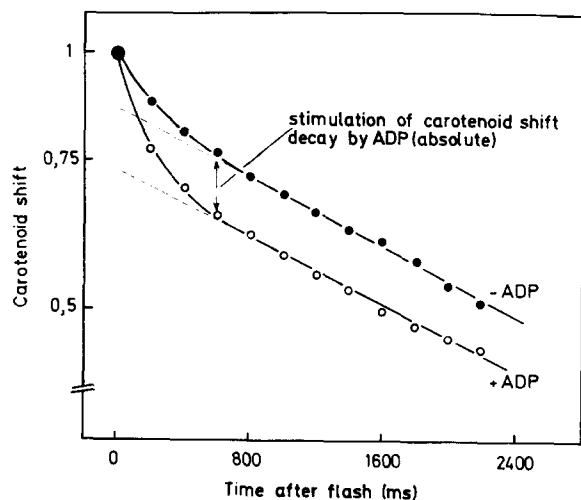


Fig. 3. The decay kinetics of the chromatophore carotenoid shift in phosphorylating and non-phosphorylating conditions (log plot). Antimycin was present at $5 \cdot 10^{-7}$ M. For other details, see Fig. 2 and Methods.

by ADP and P_i is increased under these conditions but only relative to the increased amplitude of the shift.

Similar results are observed at other wavelengths of the carotenoid bands shifts – both at the peaks (523 nm, 494 nm) and the troughs (510 nm) of the difference spectrum. We have prepared chromatophores using different isolation media, by grinding with alumina and at different pressures in the cell fractionator. In all cases the capacity of the chromatophores for photophosphorylation is proportional to the degree of stimulation of the carotenoid shift decay with ADP and P_i but stimulation is never more than 20 % of the total decay. Similar results were obtained with preparations of *R. rubrum* and *Rps. capsulata* chromatophores (unpublished observations). In chromatophores from all three species, showing normal rates of continuous light-induced photophosphorylation (1 μ mol ATP/ μ mole bacteriochlorophyll/min) the stimulation of the decay of the carotenoid shift by ADP and P_i after a flash, is of the order of 10 % of the total decay.

The pH profile of the accelerated decay of the carotenoid shift in the presence of ADP and P_i , Fig. 4, shows a pH optimum around pH 8.0 similar to that of the ATPase activity of the *Rps. sphaeroides* chromatophores [26] and photophosphorylation in continuous light in chromatophores from *R. rubrum* [27] and *Chromatium* [28].

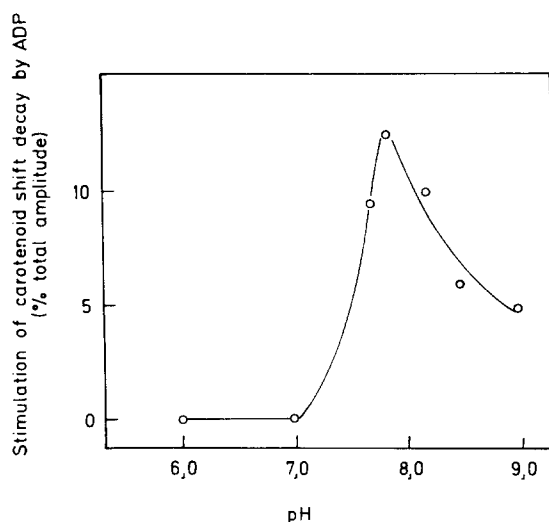


Fig. 4. pH dependence of the stimulation of the carotenoid shift decay by ADP. Taken from a series of experiments similar to that shown in Fig. 3 (in the presence of antimycin) except that the pH of the suspending medium, containing in 10 % sucrose, 50 mM KCl, 8 mM $MgCl_2$, 20 mM *N*-morpholinoethane sulphonic acid, 20 mM *N*-tris (hydroxymethyl)aminoethanesulphonic acid and 20 mM *N*-tris-(hydroxymethyl) methylglycine was varied as shown.

The effect of oligomycin, venturicidin and aurovertin

Even in well coupled chromatophore preparations there is always some faster decay phase of the electrical potential and pH change in the absence of ADP and P_i (see Figs 1 and 3). The faster decay, of the carotenoid shift, whether in the presence or absence of phosphate acceptor, is inhibited by treatment with either oligomycin or

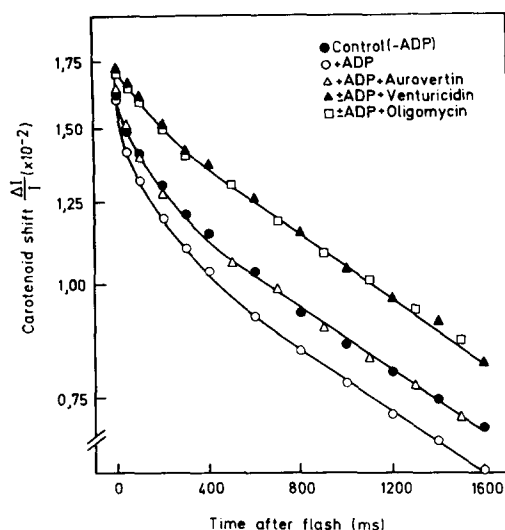


Fig. 5. The effect of energy transfer inhibitors on the decay of the carotenoid shift in phosphorylating and non-phosphorylating conditions (log plot). From a series of experiments similar to that shown in Fig. 3 all in the presence of antimycin and P_i ; 0, no ADP; ●, with ADP; △, with ADP and 4 μg aurovertin; ▲, with ADP and 1 μg venturicidin; □, with ADP and 42 μg Oligomycin. Bacteriochlorophyll concentration was 10^{-5} M.

venturicidin (Fig. 5), the slow decay phase remaining completely unaffected. Of the two antibiotics venturicidin is the more potent – in titration experiments it was found that one molecule per 300 bacteriochlorophyll (assuming the molecular weight of venturicidin = 793) [29] would completely abolish the ADP and P_i induced stimulation.

Aurovertin inhibited the stimulation of the decay produced by addition of ADP in the presence of phosphate (Fig. 5) but unlike oligomycin and venturicidin had no effect on the faster decay in the absence of ADP and P_i . This was true for concentrations of aurovertin up to five times higher than that shown in Fig. 5. This observation has a parallel in the effect of energy transfer inhibitors on submitochondrial particles – both oligomycin and aurovertin inhibit oxidative phosphorylation of ADP but only the former can be used to improve respiratory control [30].

Addition of ADP, even in the absence of added P_i , leads to a significant oligomycin sensitive acceleration of the carotenoid shift decay. This is apparently due to the presence of endogeneous or bound P_i . Addition of P_i produces a further stimulation, a maximal effect occurring at $\approx 10 \mu\text{M}$. The K_m for ADP is also very low (approximately $5 \mu\text{M}$) but the addition of P_i in the absence of added ADP has no effect.

ATP but not AMP gives rise to a P_i independent, oligomycin sensitive stimulated decay of the flash-induced change ($K_m \approx 5 \mu\text{M}$). In the presence of ATP and $^{32}P_i$ there was no detectable flash-induced radioactive incorporation into organic phosphate above the dark exchange level (compare $^{32}P_i$ incorporation in the presence of ADP, ref. 15).

We have seen above that the decay kinetics of the carotenoid shift and the cresol red absorbance change are similar (Fig. 1). The faster decay phase of the cresol red change is also inhibited by oligomycin or venturicidin as was shown in Fig. 5 for the carotenoid shift leaving the half-time of the slow decay phase largely unaffected by this type of antibiotic (not shown). The decay of the cresol red absorbance change is also stimulated in the presence of ADP and P_i but the stimulation is less than that observed with the carotenoid shift. This difference is very likely due to H^+ disappearance during the formation of ATP. We make use of this difference in an estimate of the proton translocation accompanying ATP synthesis [31].

The effect of nigericin and dianemycin

The class of nigericin-type ionophores including dianemycin has been shown to catalyse an electrically neutral exchange of K^+/H^+ across chromatophore membranes [32]. This principle is illustrated in Fig. 6, where nigericin is shown to accelerate the decay of the flash-induced cresol red change without affecting the decay of the carotenoid shift. Fig. 7A shows the concentration dependence of the same phenomena with dianemycin. Over the same concentration range which gave rise to an accelerated decay of the cresol red absorbance change (Fig. 7A) the stimulation of the carotenoid shift decay by ADP and P_i was inhibited (Fig. 7B). Similar results were observed with nigericin. The nigericin-catalysed decay of the cresol red absorbance change and the abolition of the stimulated decay of the carotenoid shift in the presence of ADP and

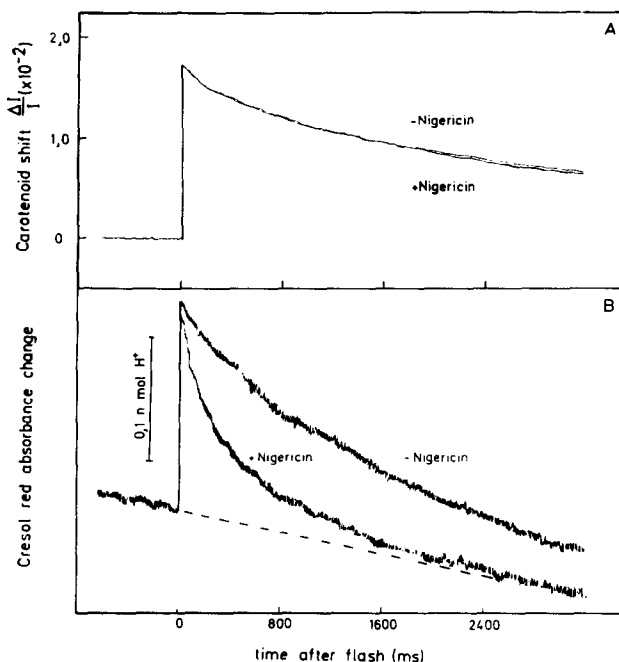


Fig. 6. The effect of nigericin on the decay of the carotenoid shift and cresol red absorbance change. Antimycin present at $5 \cdot 10^{-7}$ M. Nigericin was added to give a final concentration of $1 \mu\text{g/ml}$. 10^{-6} M bacteriochlorophyll.

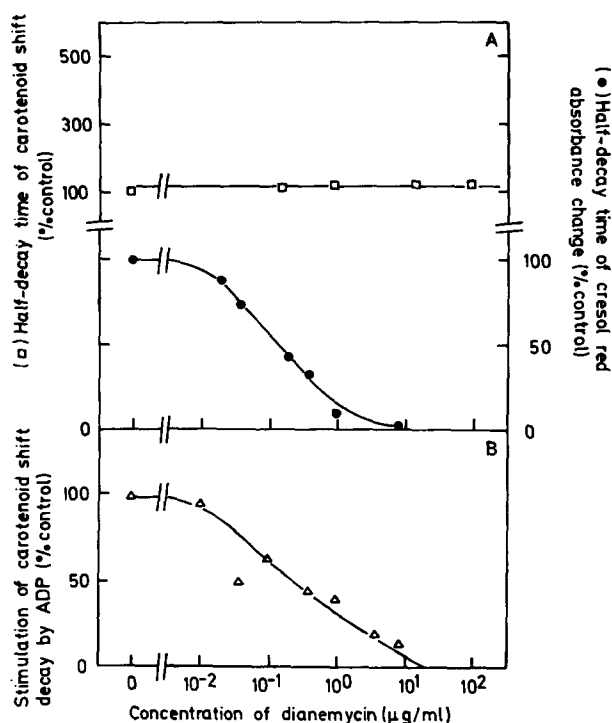


Fig. 7. The dependence of the stimulation of the carotenoid shift decay by ADP and P_i and the half-decay times of the carotenoid shift and cresol red absorbance change on dianemycin concentration. (A): \square , the overall half-decay time of the carotenoid shift in the absence of ADP; \bullet , the overall half-decay time of the cresol red absorbance change in the absence of ADP. (B): \triangle , the stimulation of the carotenoid shift decay by ADP, from a series of experiments similar to that shown in Fig. 3, except that the bacteriochlorophyll concentration was $1.2 \cdot 10^{-5}$ M. The stimulation of the carotenoid shift-decay by ADP in the control sample was 10 % of the total amplitude. Antimycin at $5 \cdot 10^{-7}$ M was present in all experiments.

P_i are entirely dependent on the presence of K^+ in the suspending medium. No effects were observed in a Na^+ medium but for dianemycin the K^+ specificity over Na^+ was not pronounced in keeping with the properties of this ionophore in other membrane systems [33].

The effect of valinomycin

If we assume that the stimulated decay of the carotenoid shift in the presence of ADP and P_i is a consequence of increased membrane potential dissipation during ATP synthesis then we should expect less stimulation when the electric potential is discharged by K^+ current through the valinomycin complex. This is found to be the case – see Fig. 8. This figure includes data taken from the accompanying manuscript [15] and shows that the carotenoid shift half-decay time, the synthesis of ATP and the stimulation of the decay of the carotenoid shift by ADP and P_i are all decreased over the same range of valinomycin concentration. The half-decay time of the cresol red change was increased by these ionophore concentrations (Fig. 8A).

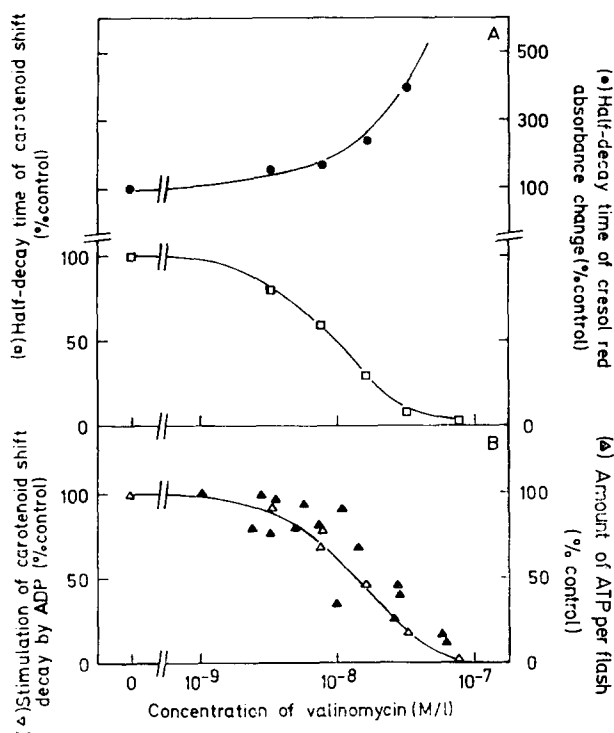


Fig. 8. The dependence of the stimulation of the carotenoid shift decay by ADP and P_i , the single flash yield of ATP synthesis and the half-decay times of the carotenoid shift and cresol red absorbance change on valinomycin concentration. (A); □, the overall half-decay time of the carotenoid shift in the absence of ADP, data taken from ref. 14; ●, the overall half-decay time of cresol red absorbance change in the absence of ADP. (B); ▲, ATP synthesis following a single turnover flash, data taken from ref. 14; △, stimulation of the carotenoid shift decay by ADP from experiments similar to that shown in Fig. 3, except that the bacteriochlorophyll concentration was $5 \cdot 10^{-6}$ M. The stimulation of the carotenoid shift decay by ADP of the control sample was 14 % of the total amplitude.

Resolution and reconstitution of the chromatophore coupling factor

The coupling factor removed from Mg^{2+} -depleted chromatophores of *Rps. capsulata* and *R. rubrum* after treatment with EDTA and ultrasonic irradiation appears to be a protein component of the ATPase complex [16, 17]. It was of interest to investigate the effect of coupling factor removal on the decay of the flash induced carotenoid shift in *Rps. sphaeroides* chromatophores. Resolution according to standard procedures [16, 17] led to a decline in the chromatophore ATPase and phosphorylating activities which were restored on subsequent reconstitution with crude coupling factor extract (Fig. 9B). The coupling factor itself showed a high oligomycin-insensitive ATPase rate dependent on either Mg^{2+} or Ca^{2+} and consequently there was no masking during reconstitution (Saphon, S., unpublished observations; compare ref. 34). When coupling factor was removed from *Rps. sphaeroides* chromatophores the basal decay of the carotenoid shift in the absence of ADP was unaffected (Fig. 10) but the resolved particles show no significant stimulated decay rate in the presence of ADP and P_i (Fig. 9A). The capacity for stimulation by ADP and P_i was

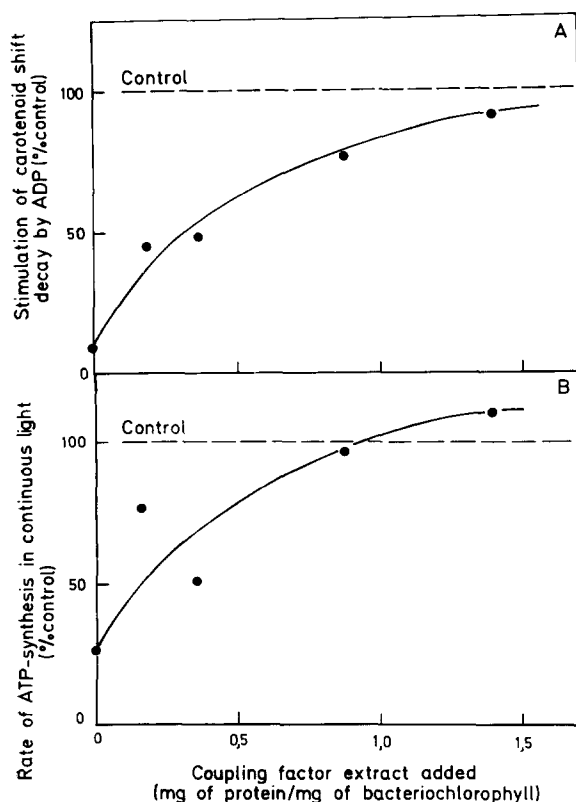


Fig. 9. The reconstitution of the stimulation of the carotenoid shift by ADP and P_i (A) and continuous light-induced phosphorylation (B) of resolved particles with crude coupling factor. Resolution and reconstitution techniques are described in Methods. The coupling factor extract was the supernatant from centrifugation of the sonicated, EDTA-treated chromatophore suspension. Photophosphorylation was measured as described in the Methods and stimulation of the carotenoid shift decay by ADP as in Fig. 3. The control samples, i.e. unresolved chromatophores, show phosphorylation rates of $0.5 \mu\text{mol ATP}/\mu\text{mol bacteriochlorophyll}/\text{minute}$ (absence of succinate) and the stimulation of the carotenoid shift decay by ADP was 11 % of the total amplitude.

restored after incubation with coupling factor in parallel with the recovery of the photophosphorylating activity (Figs 9 and 10).

DISCUSSION

The pH dependence (Fig. 4), sensitivity to the "energy-transfer" inhibitors oligomycin, venturicidin and aurovertin (Fig. 5), the parallelism to the inhibition of ATP formation by valinomycin (Fig. 8B) and the absolute requirement for the coupling factor proteins (Fig. 9 and 10) strongly suggest that the stimulated rapid decay of the chromatophore carotenoid shift following a short flash in the presence of ADP and P_i is associated with ATP synthesis. If we accept that the carotenoid shift is a reflection of a trans-membrane electric field, it follows that the synthesis of ATP is accompanied by the movement of positive charges outwards across the chromato-

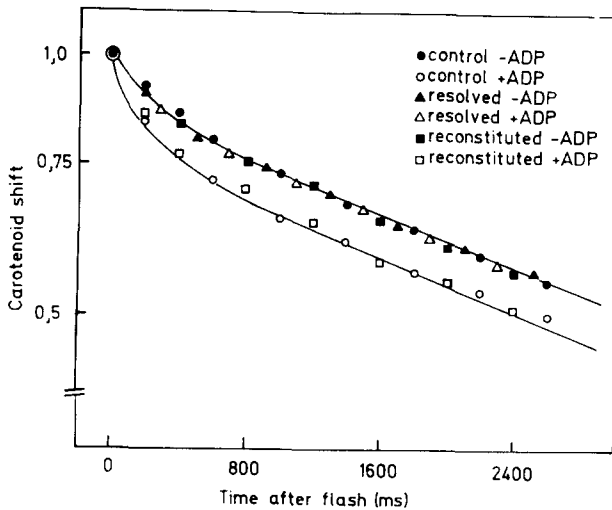


Fig. 10. The decay kinetics of native, resolved and reconstituted chromatophores in phosphorylating and non-phosphorylating conditions (log plot). From similar experiments to that shown in Fig. 3 all in the presence of antimycin and P_i using chromatophore particles as described in the Methods; ●, control (native chromatophores without ADP); ○, control chromatophores with ADP; △, resolved chromatophores with ADP; ■, reconstituted chromatophores without ADP; □, reconstituted chromatophores with ADP.

phore membrane and specifically through the ATPase enzyme complex. That these charges are protons is suggested by the similarity between the decay kinetics of the carotenoid shift and the cresol red change (Fig. 1). The phosphorylation dependent decay of the carotenoid shift is, however, only partial ($\approx 10\%$) even in carefully prepared chromatophores showing high rates of ATP synthesis in steady state light. Possible reasons for this observation are discussed more fully in the following communication [31].

When valinomycin provides a bi-pass for charge translocation, the stimulation of the carotenoid shift decay in the presence of ADP and P_i is decreased in parallel with the inhibition of ATP synthesis (Fig. 9). We deduce that the membrane potential, produced as a result of flash activation of the chromatophore suspension, is a major driving force for the expulsion of H^+ through the ATPase, which results in the synthesis of ATP. Note that in steady-state light, valinomycin in this concentration range does not inhibit photophosphorylation [32].

Inhibition of the stimulation of the carotenoid shift decay in the presence of ADP and P_i by nigericin and dianemycin (Fig. 7) argues for a significant energetic contribution from the chemical gradient of H^+ across the chromatophore membrane after a flash. In steady-state light, nigericin does not inhibit the synthesis of ATP [32, 35] and we have confirmed this using the same range of ionophore concentration as that shown in Figs 6 and 7 (not shown). We suggest two possible explanations for this different behaviour: (i) in continuous light the membrane potential (as indicated by the carotenoid shift) increases to partly compensate for the fall in ΔpH in the presence of nigericin [5, 36]. This does not happen under short flash conditions (Fig. 7A); (ii) the relative contribution of ΔpH to the proton motive force may be

greater after a short flash than during continuous illumination. Although we have calculated a membrane potential of approximately 100 mV following short flash excitation [8], comparative estimates of ΔpH under similar conditions are not yet available, but are currently under investigation.

It is known that the rise of the carotenoid shift of *Rps. sphaeroides* chromatophores is generated in three kinetically distinct steps [24]. A fast phase ($< 10^{-7}$ s) corresponding to electron transport between P and X is operative at ambient redox potentials between +450 mV and -20 mV; a low amplitude 100 μs phase II following cytochrome *c* \rightarrow P-870 is detectable only when cytochrome *c* is reduced before the flash and an antimycin sensitive phase III, a result of electron transfer between the *b* and *c* type cytochromes, is favoured by low redox potential (150– -20 mV). ADP and P_i produce an accelerated decay of the carotenoid shift whichever systems are operational – in the absence of antimycin (phases I, II and III), in the presence of antimycin (phases I and II) and in the presence of antimycin and ferri/ferrocyanide at $E_m = +400$ mV (phase I). The membrane ATPase is unable to distinguish between the electric fields produced at any of these electron transport sites with the possible exception of the low amplitude phase II.

In ATPase-resolved chloroplasts it has been shown that the H^+ permeability of the membrane is greatly increased and the decay of the 515 nm shift is accelerated by more than two orders of magnitude [37, 38]. The increased ionic permeability of resolved chloroplasts can be reversed by reconstitution with the coupling factor or by treatment with *N,N'*-dicyclohexylcarbodiimide. The permeability lowering after reconstitution has been explained in two ways: (i) the coupling factor merely blocks the open H^+ channels by non-specific binding or (ii) it recombines to give the native configuration. Since the coupling factors are probably not all resolved on extraction [37] simple blocking of open channels could also restore phosphorylation. In highly resolved submitochondrial particles the proton permeability decreases on reconstitution with either the so-called oligomycin sensitivity conferring protein (OSCP) or with the factor F_1 but the lowering of the permeability is most significant with a combination of F_1 and OSCP [39]. Low concentrations of oligomycin improve respiratory control [30] and it has been suggested that a H^+ leak through damaged ATPase enzymes devoid of their F_1 subunits is blocked by oligomycin binding to the naked F_0 sites [12, 40].

In chromatophores the steady-state, light induced H^+ uptake is increased in the presence of oligomycin [41] and the faster decay component of the flash-induced carotenoid shift in the absence of ADP and P_i is decreased by oligomycin or venturicidin treatment (Fig. 5). It seems that even in native (i.e. unresolved) chromatophores there is a H^+ leak through the ATPase enzyme, which is blocked by energy transfer inhibitors of the oligomycin type. It is possible that intact ATPase leaks H^+ in the absence of phosphorylation substrates and that some as yet undiscovered mechanism switches non-phosphorylating H^+ current to a phosphorylating H^+ current when ADP and P_i are present. Alternatively some of the chromatophore ATPase may be damaged (see Fig. 11D).

Removal of coupling factor, however, does not lead to enhanced membrane permeability: (i) in resolved *Rps. capsulata* chromatophores, light-induced H^+ uptake is not decreased in the steady state [41], (ii) the decay of the flash-induced carotenoid shift of *Rps. sphaeroides* chromatophores is not accelerated by coupling factor removal

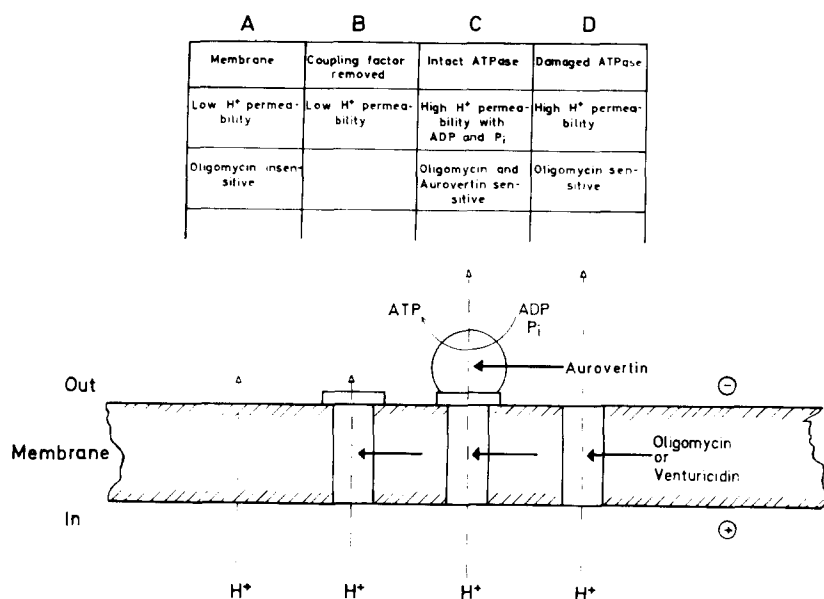


Fig. 11. Diagrammatic representation of the disaggregation states of the chromatophore ATPase and H^+ permeability. Details, see text.

(Fig. 10). M. Baltscheffsky (by personal communication) has obtained similar results with chromatophores from *R. rubrum*, (iii) the decay of the flash-induced cresol red absorbance change in *Rps. sphaeroides* is unaffected in resolved chromatophores (unpublished observations). These observations may be explained by assuming that the ATPase from which the coupling factor has been removed (see Fig. 11B) is different from a "damaged" ATPase (see Fig. 11D). In Fig. 11 we have shown the ATPase enzyme in three states of disaggregation (compare ref. 42). The intact enzyme (Fig. 11C) conducts protons only in the presence of ADP and P_i and this H^+ current is blocked by both oligomycin and aurovertin (Fig. 5). In ATPase devoid of coupling factor (Fig. 11B) the permeability to H^+ is low in the presence or absence of ADP and P_i (Fig. 10). In "damaged" ATPase (Fig. 11D) in both native and resolved chromatophores there is a high proton permeability which is sensitive to oligomycin but not to aurovertin (Fig. 5). The proposed sites of oligomycin and aurovertin action in this model are consistent with the suggestion that the former reacts with mitochondrial F_0 and the latter with F_1 [43, 44]. Since the basal decay of the carotenoid shift in chromatophores is not affected by removing the coupling factor and since the efficiency of recoupling is high [17, 45] in contrast to the corresponding experiments in chloroplasts [37, 38], it seems that the chromatophore coupling factor reconstitutes to give the native configuration.

The stimulation of the carotenoid shift decay by ATP (in the absence of P_i) was unexpected. However, the oligomycin sensitivity of the reaction and the inability of AMP to produce stimulation of the decay suggest the involvement of the membrane ATPase rather than non-enzymic, electrophoretic ATP^{4-} (or ADP^{3-}) translocation. A likely explanation is that added ATP is hydrolysed by the chromatophore ATPase during the dark time between the flashes and that the resultant ADP and P_i remain

bound to be rapidly combined after the flash. The net rate of ATP hydrolysis would therefore be limited by the dissociation of ADP and P_i from the enzyme complex. Consistent with this explanation is the lack of $^{32}P_i$ incorporation following flash activation in the presence of ATP.

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