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IN SITU CHARACTERISATION OF PHOTOSYNTHETIC ELECTRON TRANSPORT IN *RHODOPSEUDOMONAS CAPSULATA*

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SUMMARY

1. The effects of varying the ambient oxidation/reduction potential on the redox changes of cytochromes *c*, cytochromes *b* and P605 induced by a laser flash in chromatophores from *Rhodopseudomonas capsulata* Ala Pho$^+$ have been investigated.

2. The appearance and attenuation of the changes with varying ambient redox potential show that, of the cytochromes present, cytochromes *c* with $Em_7 = 340$ mV and 0 mV, and cytochrome *b*, $Em_7 = 60$ mV were concerned with photosynthetic electron flow.

3. The site of action of antimycin was shown to be between cytochrome b_{60} and a component, as yet unidentified, called Z.

4. The appearance or attenuation of laser-induced changes of cytochromes c_0 and b_{60} on redox titration was dependent on pH, but no effect of pH on the cytochrome c_{340} titration was observed.

5. The dependence on ambient redox potential of the laser-induced bleaching at 605 nm enabled identification of the mid-point potentials of the primary electron donor ($Em_7 = 440$ mV) and acceptor ($Em_7 = -25$ mV).

6. The interrelationship of these electron carriers is discussed with respect to the pathway of cyclic electron flow.

INTRODUCTION

In an earlier paper [1] we characterised the cytochromes of *Rhodopseudomonas capsulata* both spectrally and thermodynamically. Three *c*-type cytochromes, c_{340}, c_{120} and c_0 and two *b*-type cytochromes, b_{60} and b_{-25}, (where the suffix denotes the

Abbreviations: MES, 2-(*N*-morpholino)ethanesulphonic acid; TES, *N*-((trishydroxymethyl)-methyl)-2-aminoethanesulphonic acid; Tricine, *N*-((trishydroxymethyl)methyl)glycine; HOQNO, 2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide; PES, phenazine ethosulphate; PMS, phenazine methosulphate.

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mid point redox potential at pH 7.0) were identified. With the possible exception of cytochrome c_{340}, mid-point potentials of these cytochromes all exhibited some variation with pH in the range pH 6.0–8.6.

This paper describes an investigation of cytochromes involved in photosynthetic electron flow in *Rps. capsulata* chromatophores, and the light induced changes associated with the reaction centre.

Use has again been made of the carotenoid deficient mutant *Rps. capsulata* Ala Pho$^+$ [2] to characterise cytochromes *b* and *c* and the reaction centre.

METHODS

Preparation of chromatophores

The growth of *Rps. capsulata* Ala Pho$^+$ cells and preparation of chromatophores was as previously described [1].

The bacteriochlorophyll content was determined by acetone–methanol extraction as described by Clayton [3].

Measurement of light induced changes

Flash-induced changes at 605 nm in *Rps. capsulata* chromatophores were measured in a rapidly responding single-beam spectrophotometer with a variable time constant (RC network). Light from a Hilger D330 monochromator was passed through the sample and detected by an EM1 9592B photomultiplier covered by a Corning blue glass filter (No. 9782).

Flash-induced cytochrome changes were measured in a similar apparatus adapted as a rapidly responding dual-wavelength spectrophotometer by the addition of a second monochromator and photomultiplier at right angles to the first measuring beam. Illumination was from below, the laser beam entering the cuvette housing after deflection through a rightangle prism. The outputs of the two photomultipliers were fed through matched RC networks into the differential amplifier (Type 2A463, Tektronix) of the storage oscilloscope.

The RC values available were 0.01, 0.1, 1, 10 and 100 ms. In the experiments shown on RC value of 100 μs was used. Cytochromes *b* were measured at 561 nm with 570 nm as reference wavelength. Cytochromes *c* were measured at 551 nm with 540 nm as reference wavelength.

Illumination was provided by a Q-switched Ruby laser with a pulse width of 20 ns or by a xenon flash (200 μs duration at half height), as indicated.

Redox titrations of light-induced changes were performed using an anaerobic suspension of chromatophores, as previously described [4]. Chromatophores were suspended in 50 mM KCl, 50 mM buffer where the buffers used were 2-(*N*-morpholino)ethanesulphonic acid (MES) at pH 6.0, *N*-((trishydroxymethyl)methyl)-2-aminoethanesulphonic acid (TES) at pH 7.0 and *N*-((trishydroxymethyl)methyl)-glycine (Tricine) at pH 8.0.

Measurement of slower cytochrome changes

These were measured on a double-beam spectrophotometer as previously described [5]. Illumination was provided by a 100 W quartz–iodine bulb covered by a Wratten 88A filter. The photomultiplier was covered by a Corning blue glass filter (No. 9782).

MATERIALS

Simple organic or inorganic reagents were of Analar grade, or otherwise of the highest grade commercially available.

2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) and antimycin were obtained from Sigma (London) Chemical Co., and orthophenanthroline from British Drug Houses.

RESULTS AND DISCUSSION

Redox titration of the laser-induced change at 551–540 nm

Laser-induced cytochrome *c* changes in *Rps. capsulata* Ala Pho$^+$ chromatophores are shown in Fig. 1. Fig. 1a shows two typical kinetic traces observed at ambient redox potentials of 237 and 89 mV. and Fig. 1b shows the extent of different phases of the change over the range 450 to −100 mV. Fast and slow components of this reaction were apparent. The rapid phase (half-rise time $\tau_{\frac{1}{2}} < 100\ \mu s$) of cytochrome oxidation was elicited between potential values of 400 mV and −80 mV. The change titrated in with Em_7 is approx. 340 mV and disappeared with Em_7 is approx. −25 mV. Some diminution of the change was observed over the range 200–100 mV, possibly because of the rapid decay apparent over this range. The total extent of the change varied markedly with potential. Over the range 400–200 mV, a slower cytochrome oxidation ($\tau_{\frac{1}{2}}$ is approx. 1.5 ms), equivalent in extent to the fast phase was present, titrating in with Em_7 of 340 mV. Over the range 200–90 mV the slow oxidation was replaced by a decay ($\tau_{\frac{1}{2}}$ is approx. 3 ms at 90 mV), which led to a complete reversal of both phases of oxidation. This change occurred with Em_7 is approx. 130 mV. Over the range 90–10 mV, the decay titrated out with Em_7 is approx. 60 mV, so that at potentials below 10 mV, no additional slow oxidation or fast re-reduction were usually apparent. However, the rate of re-reduction varied somewhat from preparation to preparation.

We may interpret these results in the following way, on the basis of three *c*-type cytochromes characterised earlier [1]:

(a) Oxidation of the cytochrome Em_7 is approx. 340 mV (cytochrome c_{340}) occurred in a biphasic reaction. The characteristics of cytochrome c_{340} are similar to those of the previously identified cytochrome c_2 [5].

(b) Re-reduction of cytochrome c_{340} by a component Z_{130} of Em_7 is approx. 130 mV. This component is unlikely to be cytochrome c_{120} as no spectral change is seen corresponding to cytochrome c_{120} oxidation.

(c) The disappearance of the re-reduction at low potentials may be explained by the involvement of the cytochrome of $Em_7 = 0$ mV (cytochrome c_0). Either cytochrome c_{340} oxidation is replaced by c_0 which is reduced more slowly, or cytochrome c_0 is oxidised through cytochrome c_{340} in preference to the component Z_{130}, and its spectral change cancels that of cytochrome c_{340} reduction. The presence of two *c*-type cytochromes in the photosynthetic electron transport chain would be similar to those reported for *Chromatium* [6] and *Rps. viridis* [7]. An anomalous feature of the change below 0 mV is the absence of any phase of rereduction of cytochrome *c* which could correspond with the oxidation of cytochrome b_{60} observed over this potential range; our interpretation of these changes may therefore be oversimplified.

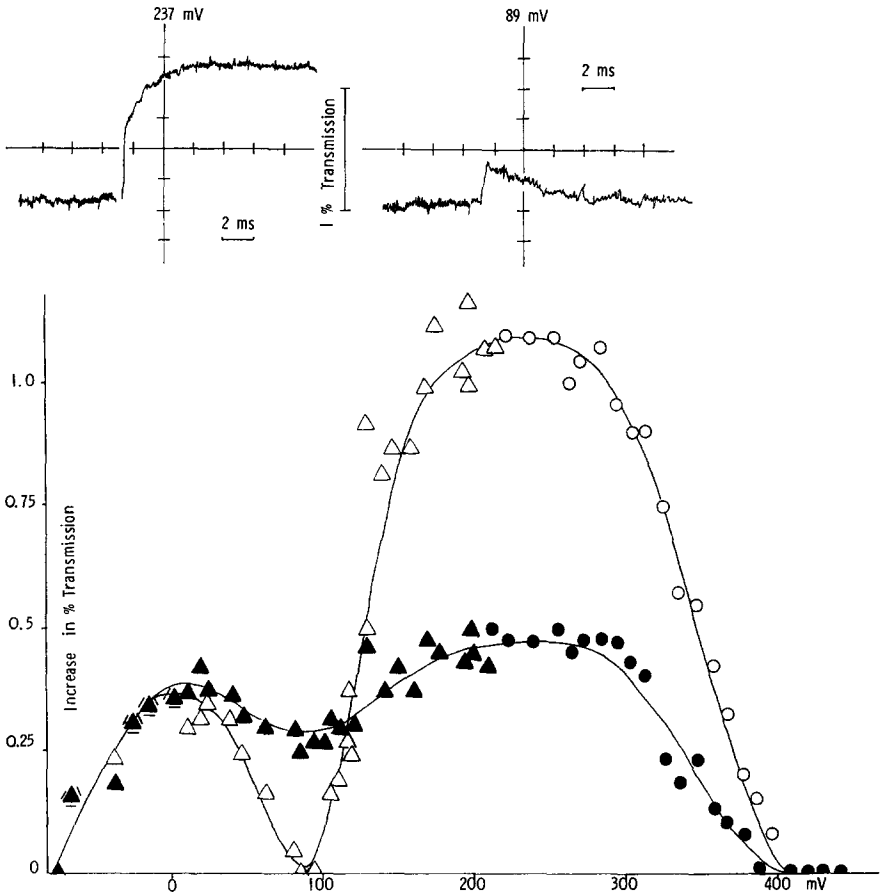

Fig. 1. (a) Laser-induced changes at 551–540 nm in *Rps. capsulata* Ala Pho$^+$ chromatophores. Chromatophores were suspended to 40 μg bacteriochlorophyll/ml in 50 mM TES, 50 mM KCl (pH 7.0). (i) Ambient redox potential: 237 mV; redox dyes present: 200 μM $K_3Fe(CN)_6$, 10 μM diaminodurene. (ii) Ambient redox potential: 89 mV; redox dyes present: 10 μM PES, PMS, diaminodurene. (b) Laser induced change at 551–540 nm as a function of ambient redox potential in *Rps. capsulata* Ala Pho$^+$ chromatophores. Conditions as for (a). ●, extent after 1 ms; redox dyes present: 10 μM diaminodurene, 200 μM $K_3Fe(CN)_6$. ○, extent after 14 ms; redox dyes as above. ▲, extent after 1 ms; redox dyes present: 10 μM diaminodurene, PMS, PES and 3.5 μM pyocyanine. △, extent after 14 ms; redox dyes as above.

From the spectral characteristics, it is unlikely that cytochrome c_0 is equivalent to cytochromes *cc'* of $Em_7 = 0$ mV reported by Kamen et al. [5]. However, low potential cytochromes c_3 have been isolated from other bacteria [8, 9], and have been associated with hydrogen lyase activity [8]. In view of the rapid growth of *Rps. capsulata* with H_2 as hydrogen donor, this may be the function of cytochrome c_0.

(d) The attenuation of the cytochrome *c* oxidation with a mid-point potential of −25 mV is ascribed to the reduction of the primary electron acceptor, X, Em_7 is approx. −25 mV.

Both cytochromes c_{340} and c_0 have shown some variation of mid-point

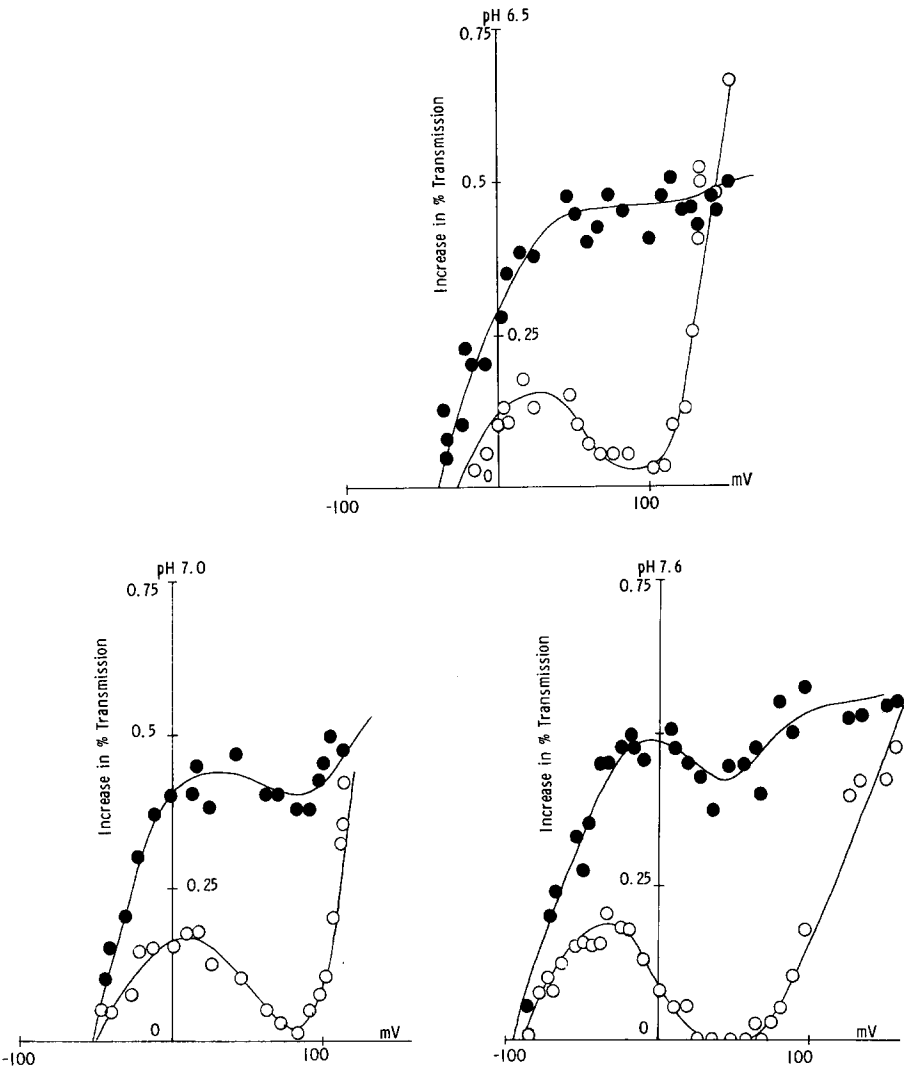

Fig. 2. The effect of pH on the variation of the laser-induced change at 551–540 nm with ambient redox potential in *Rps. capsulata* Ala Pho$^+$ chromatophores. Chromatophores suspended to 46 μg bacteriochlorophyll/ml in 50 mM MES, 50 mM KCl, pH 6.5; 50 mM TES, 50 mM KCl (pH 7.0); or 50 mM Tricine, 50 mM KCl (pH 7.6) as indicated. Redox dyes present were 10 μM diaminodurene, PMS, PES and 3.5 μM pyocyanine. ●, extent of change after 1 ms; ○, extent of change after 14 ms.

potential with pH [1]. Fig. 2 shows the effect of pH on the redox titration of cytochrome *c* below 100 mV. The disappearance of the re-reduction of cytochrome *c* between 80 and 0 mV ascribed to cytochrome c_0 oxidation has shifted to a lower potential at pH 8.0, and possibly to a higher pH at pH 6.0, since at this pH, complete re-reduction of cytochrome *c* is not seen over the time scale observed. These variations wih pH are consistent with the changes in the mid-point potential of cytochrome c_0 on dark

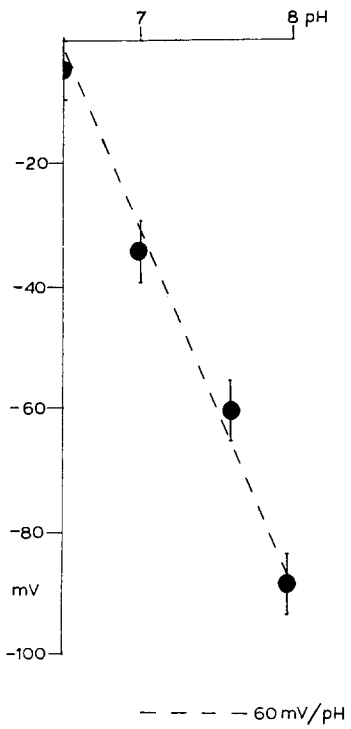

Fig. 3. The pH dependence of the primary acceptor. Each point an average of several experiments, calculated from the E_m of attenuation of cytochromes *b* and *c*, P605 and the carotenoid shift.

titration [1]. Fig. 2 also shows that the Em_7 of attenuation of cytochrome *c* oxidation varies with pH. This is associated with a pH dependence of the mid-point potential of the primary acceptor, which is shown in Fig. 3.

Titrations of the laser-induced cytochrome *c* oxidation at values of pH between 6 and 8 showed no variation with pH in the range over which cytochrome oxidation became apparent (half complete at Em_7 is approx. 340 mV), as the potential was lowered.

Neither antimycin nor HOQNO had any marked effect on the kinetics or the extent of the flash-induced cytochrome *c* changes at any potential. This is in contrast to the inhibitory effect of antimycin on the reduction of cytochrome *c* observed in *Rps. spheroides* [10].

Redox titration of laser induced change at 561–570 nm

Fig. 4a shows typical laser induced changes at 561 with 570 nm as reference, at two different ambient redox potentials in *Rps. capsulata* Ala Pho$^+$ chromatophores, while Fig. 4b shows a redox titration of the laser induced change. A slow phase of cytochrome *b* reduction ($\tau_{\frac{1}{2}}$ is approx. 2–4 ms) came in on titration below 420 mV (Em_7 is approx. 390 mV); between 140 and 20 mV the reduction changed to an oxidation of equivalent extent, the changeover having an Em_7 is approx. 60 mV. The oxidation disappeared with Em_7 is approx. −25 mV.

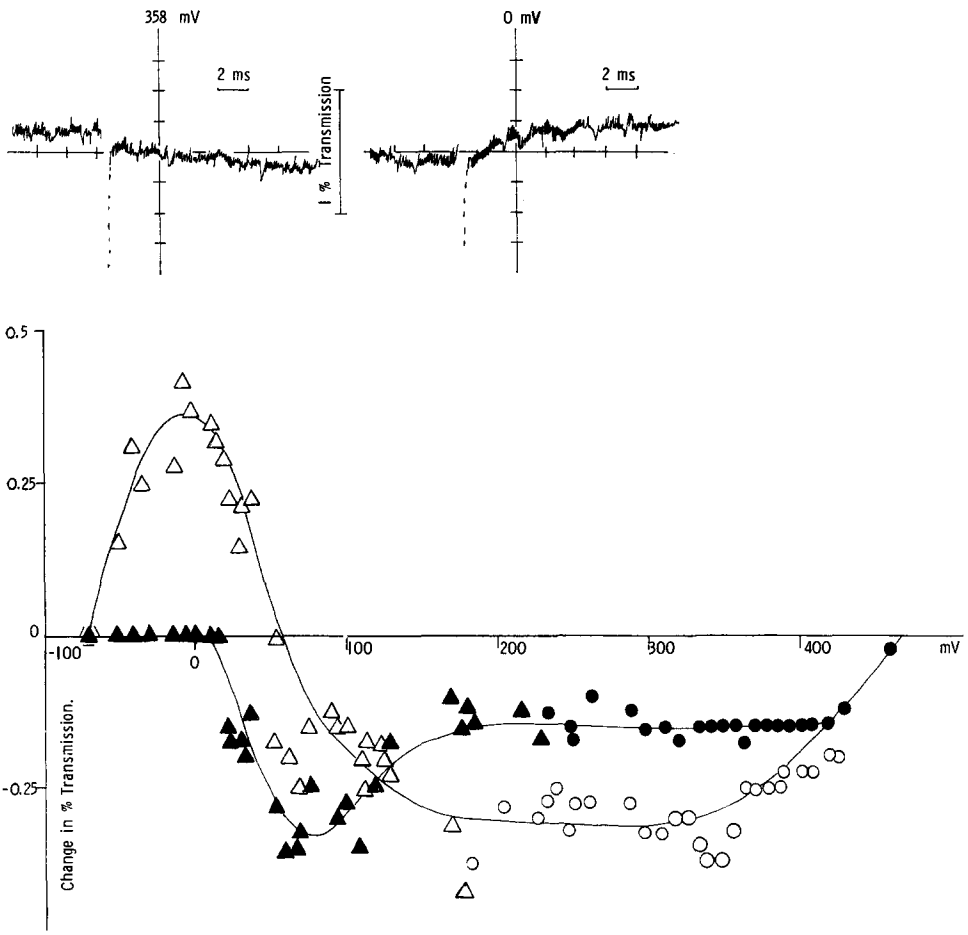

Fig. 4. (a) Laser-induced changes at 561 nm with 570 nm as reference in *Rps. capsulata* Ala Pho$^+$ chromatophores. Chromatophores suspended to 44 μg bacteriochlorophyll/ml in 50 mM TES, 50 mM KCl (pH 7.0). (i) At an ambient redox potential of 358 mV, 200 μM $K_3Fe(CN)_6$ present. (ii) At an ambient redox potential of 0 mV, 3.5 μM pyocyanine present. (b) Laser-induced change at 561–570 nm as a function of ambient redox potential in *Rps. capsulata* Ala Pho$^+$ chromatophores. Chromatophores as for Fig. 5a. Nomenclature of symbols as for Fig. 1a.

An initial change at the wavelength pair chosen, occurring during the flash artifact, remained constant over the range 400–100 mV, but a more extensive rapid phase was apparent from 120 to 80 mV, which could be interpreted as a rapid reduction followed by a slower reoxidation of the cytochrome *b*. Below 80 mV the extent of fast reduction decreased, with Em_7 is approx. 40 mV.

Fig. 5 shows the effect of antimycin on the redox titration of the cytochrome *b* changes. A constant extent of flash induced reduction was observed between 420 and 220 mV which was approximately the same as the maximal extent of absorbance change in the absence of antimycin. Between 210 and 110 mV the reduction doubled in extent, and titrated out completely by 30 mV with Em_7 is approx. 70 mV. No oxida-

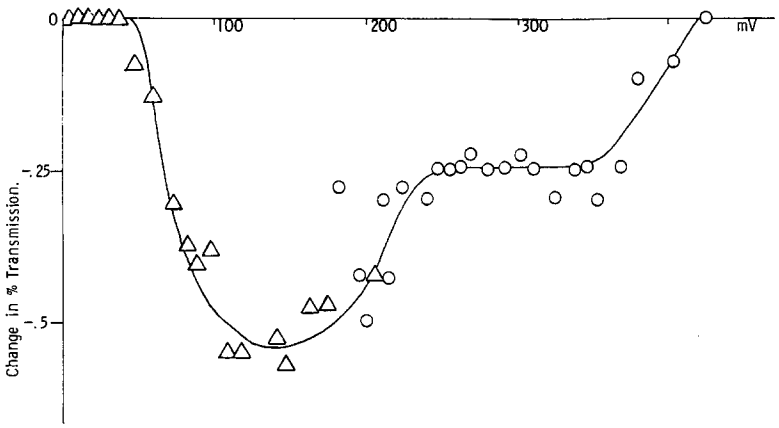

Fig. 5. Laser-induced change at 561–570 nm as a function of redox potential in the presence of antimycin. Conditions as for Fig. 5, but with 2 mM antimycin present.

tion of cytochrome *b* was seen. HOQNO had no marked effect on the cytochrome *b* changes.

We interpret these observations in the following way:

(a) It is likely that only cytochrome *b* with $Em_7 = 60$ mV (cytochrome b_{60}) is concerned in these changes. Participation of cytochrome b_{-25} is unlikely, as this could only be manifest in a reduction, and no reduction is seen below 30 mV. Cytochrome b_{60} is therefore photoreduced if chemically oxidised before a flash and photooxidised if chemically reduced before a flash. At intermediate potentials, the cytochrome first goes reduced, and then reoxidised.

(b) On reduction of the suspension over the potential range 130–80 mV a change occurs which is expressed as an increase in the rate of both the reduction of cytochrome *b* and its reoxidation. Over a similar range Z_{130} would have been reduced, and it is possible that the reduction of this component may have the general effect of speeding up the rate of electron flow in this region of the chain.

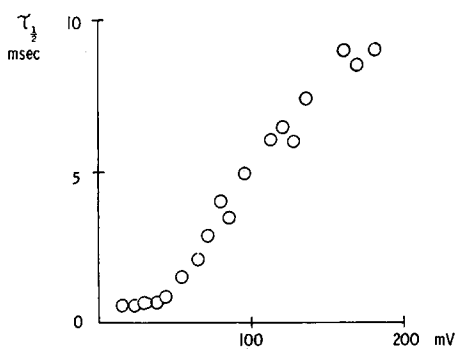

Fig. 6. The increase in the rate of cytochrome *b* reduction with decreasing ambient redox potential. The half-time of reduction of cytochrome *b*, in the presence of antimycin, was calculated from the results of Fig. 9.

(c) Antimycin A inhibited electron transport on the oxidising side of cytochrome b_{60}, thus preventing all photo-oxidation of the cytochrome. The doubling in extent of the photo-reduction of cytochrome b_{60} between 200 and 110 mV (Fig. 5) is not readily explained on the basis of a single turnover per flash. However, the

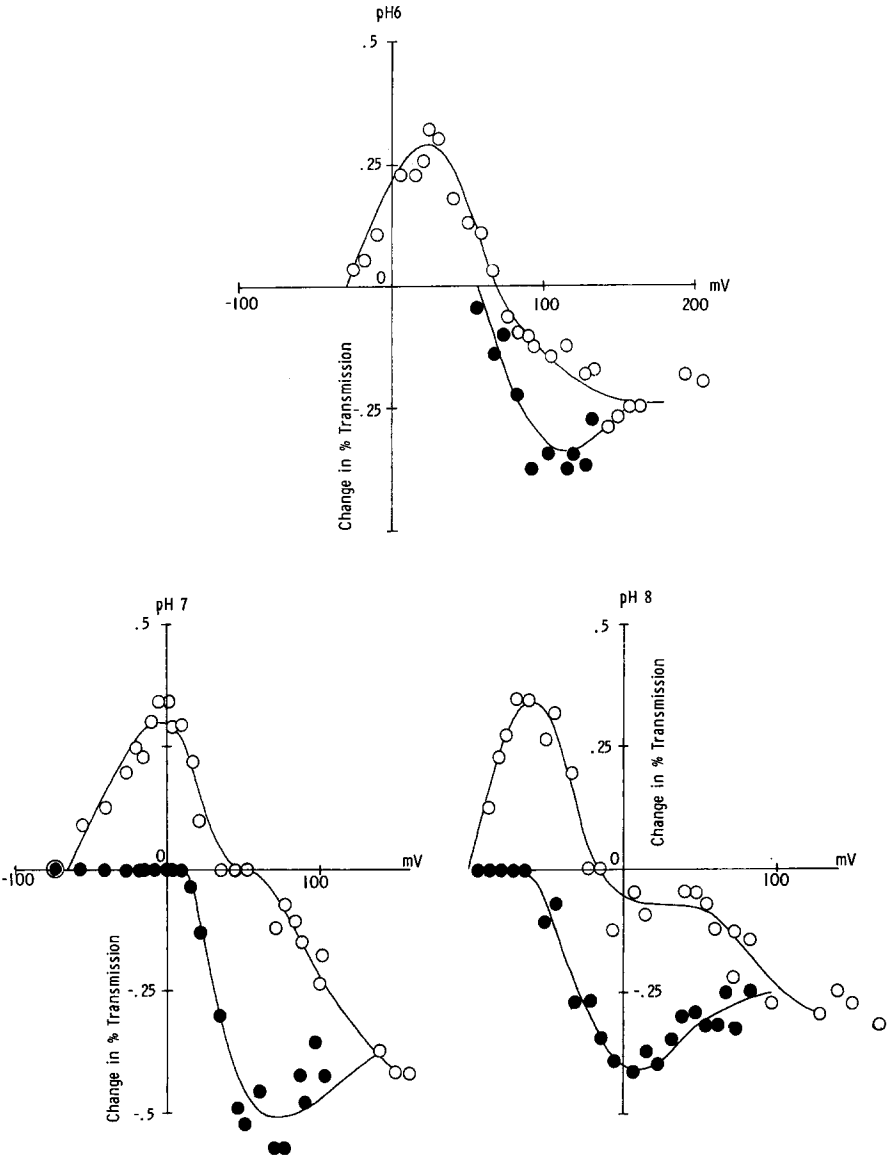

Fig. 7. Xenon-flash induced change at 561–570 nm as a function of ambient redox potential in *Rps. capsulata* Ala Pho$^+$ at different values of pH. Chromatophores suspended to 24 μg bacteriochlorophyll/ml in 50 mM MES, 50 KCl, (pH 6.0); 50 mM TES, 50 mM KCl, pH 7.0 or 50 mM Tricine, 50 mM KCl (pH 8.0) as indicated. Redox dyes present: 10 μM PES, PMS, 3.5 μM pyocyanine. ●, extent of change after 1 ms; ○, extent of change after 14 ms.

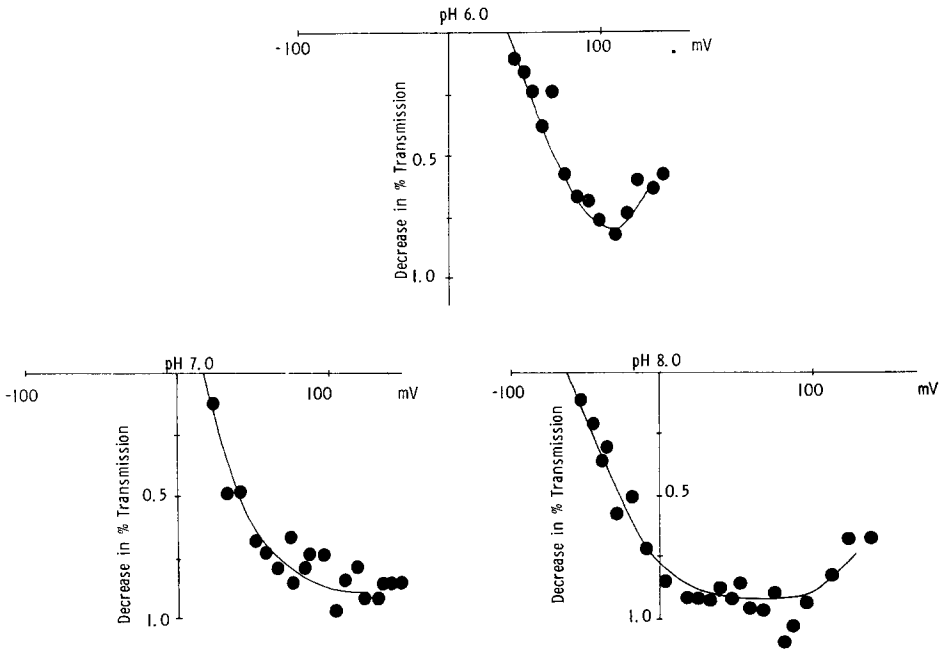

Fig. 8. Xenon-flash-induced change at 561–570 nm as a function of ambient redox potential, in *Rps. capsulata* Ala Pho$^+$ chromatophores. Conditions as for Fig. 8, except with 2 μM antimycin present. Nomenclature of symbols as for Fig. 8.

extent of laser induced change above 200 mV corresponded to reduction of about half the pool of cytochrome b_{60} while below 110 mV all the pool was reduced [1]. This may indicate that a component able to accept electrons from cytochrome b_{60} became chemically reduced over this potential range. The component is not equivalent to Z, since its reduction was not inhibited by antimycin, and may indicate the involvement of a further carrier as yet unidentified by spectrophotometric methods.

An increase in the rate of reduction of cytochrome *b* between 130–80 mV could also be seen in the presence of antimycin, and this is shown in Fig. 6, where the half-time of reduction of cytochrome *b* is plotted against potential.

The midpoint potential of cytochrome b_{60} has been shown to vary with pH [1]. The effect of pH on the flash-induced reduction of cytochrome b_{60}, in the presence and absence of antimycin A is shown in Figs 8 and 7, respectively. The attenuation of the fast reduction and the oxidation of cytochrome b_{60} both shifted to a higher potential at pH 6.0 and a lower potential at pH 8.0. In the presence of antimycin A, the attenuation of cytochrome *b* reduction shifted in a similar fashion at pH 6.0 and 8.0.

It is interesting to note that a speeding up of electron flow still occurred on poising the potential of the suspension between 130–80 mV. This effect must therefore be independent of pH over this range.

Redox titration of laser induced change at 605 nm

Fig. 9a shows typical laser-induced changes at 605 nm at two potentials, and

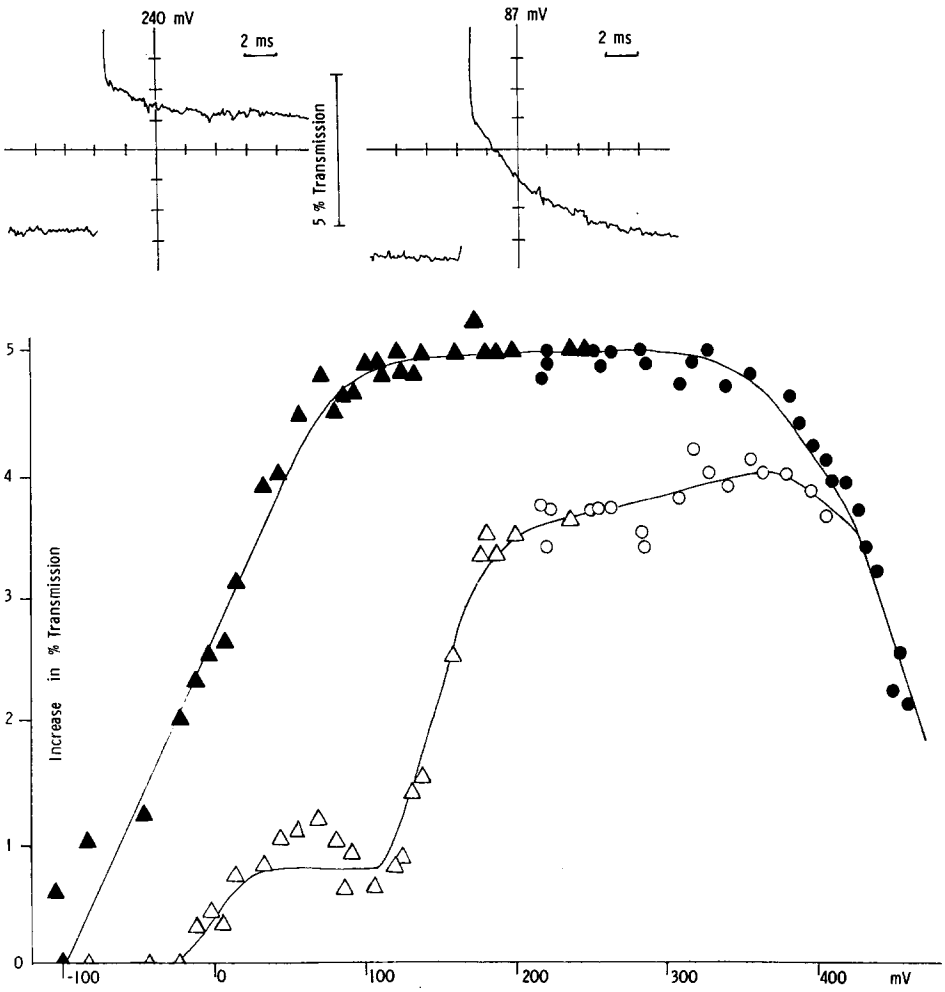

Fig. 9. (a) Traces of laser-induced changes at 605 nm in *Rps. capsulata* Ala Pho$^+$ chromatophores. Chromatophores were suspended to 42 μg bacteriochlorophyll/ml in 50 mM TES, 50 mM KCl (pH 7.0). 100 μs time constant. (i) Ambient redox potential 240 mV; 100 μM $K_3Fe(CN)_6$, 10 μM daiminodurene present. (ii) Ambient redox potential 87 mV; 10 μM PMS, PES and diaminodurene present. (b) Laser-induced change at 605 nm as a function of ambient redox potential in chromatophores from *Rps. capsulata* Ala Pho$^+$. Conditions as for Fig. 10a, nomenclature as for Fig. 1.

Fig. 9b the dependence of this change on ambient redox potential.

A rapid absorbance decrease was observed over the range 500 to -100 mV, which titrated in with Em_7 is approx. 400 mV and attenuated with Em_7 is approx. -25 mV. There was a reversal of the rapid phase, the rate of which varied with potential. Between 390 and 200 mV, 20 % of the change decayed with $\tau_{\frac{1}{2}}$ is approx. 2 ms. The rest decayed slowly. Between 200 and 100 mV, a much more extensive decay ($\tau_{\frac{1}{2}}$ is approx. 6 ms) appeared, titrating in with Em_7 is approx. 130 mV, and leading to a complete reversal of the rapid change.

The change was not affected markedly at any potential by antimycin or HOQNO.

Absorbance changes at 605 nm (P605) are thought to represent a bleaching of reaction centre bacteriochlorophyll, and to be due to the visible band of P870. In interpreting these results we are aware of a number of paradoxical observations, some of which have been discussed elsewhere [11], with respect to the similar change observed with *Rps. spheroides* chromatophores. These centre about apparent inconsistencies between the kinetics of cytochrome c_{340} oxidation and the reversal of the rapid P605 change. We have reported in an earlier paper [1] on the amount of P605 which underwent a change following a flash. The extinction coefficient was obtained by observing the re-reduction by mammalian cytochrome *c* of P605 in purified reaction centres prepared from *Rps. capsulata* Ala Pho$^+$ chromatophores essentially by the method of Clayton and Wang [12]. Since in this system the kinetics of re-reduction following a flash are identical to those of cytochrome *c* oxidation, an unambiguous value for the P605 extinction coefficient can be obtained by comparison with the absorbancy change and known extinction coefficient of cytochrome *c* (Prince, R. C., Cogdell, R. J. and Crowther, D., unpublished observations). From this value, it appears that in chromatophores, between twice and thrice as much P605 as cytochrome was oxidised by the flash, and this stoichiometric discrepancy may account for some of the kinetic anomalies noted above.

Thus it seems reasonable to suppose that the fast decay coming in below 400 mV reflected electron flow from cytochrome c_{340} as it became chemically reduced before a flash, and that the large decay appearing with Em_7 is approx. 130 mV was associated with the chemical reduction of the component Z already discussed above. The apparent discrepancy in kinetics of reduction by the Z pool ($\tau_{\frac{1}{2}}$ is approx. 6 ms for the P605 change and 2 ms for the cytochrome c_{340} reduction) may be due to the difference in the size of the pool of oxidant available. However, two aspects of the change are not accounted for: (i) the failure to observe a phase in the P605 kinetics corresponding to the rapid phase of cytochrome oxidation; (ii) following oxidation, cytochrome c_{340} became reduced before P605, despite the fact that electron flow from the cytochrome to the reaction centre is rapid. With these qualifications, the simplest assumption is that the P605 changes reflect the redox state of the primary donor. If this is the case, then the appearance and disappearance of the P605 change with Em_7 is approx. 440 mV and $Em_7 \approx -25$ mV reflect the reduction of the primary donor and primary acceptor before the flash. The discrepancies noted above may reflect different pools of cytochrome whose reactivities with the reaction centre and reductive pools differ.

CONCLUSIONS

From these results and the discussion, we would like to propose a scheme for electron transport in *Rps. capsulata* chromatophores.

This is shown in Fig. 10, and has much in common with that proposed by Klemme [13]. Six redox components can be identified with photosynthetic electron transport:

(1) Reaction centre, Em_7 is approx. 440 mV; (2) Primary acceptor, Em_7 is approx. −25 mV; (3) Cytochrome b_{60}, Em_7 is approx. 60 mV; (4) Cytochrome c_{340}, Em_7 is approx. 340 mV; (5) Cytochrome c_0, Em_7 is approx. 0 mV; (6) Z_{130} Em_7 is approx. 130 mV.

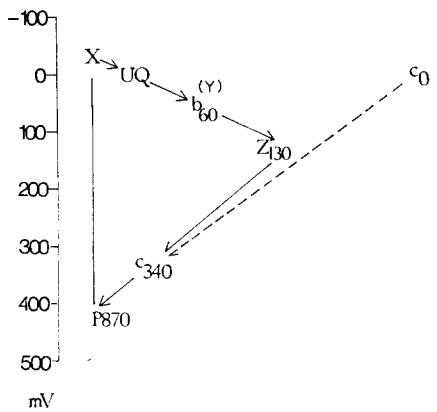

Fig. 10. Scheme for photosynthetic electron transport in *Rps. capsulata* chromatophores at pH 7.0.

The antimycin sensitive site is between cytochrome b_{60} and Z_{130}. In addition it has been noted that an additional redox component may be present close to cytochrome b_{60}.

The component Y, present in the scheme is that associated with H^+-uptake [14] (Evans, E. H., Cogdell, R. J. and Crofts, A. R., unpublished), many of whose characteristics are similar to those of ubiquinone. This will be discussed in a later paper (Evans, E. H., Cogdell, R. J. and Crofts, A. R., unpublished) when the relationship of the scheme of electron flow to energy conservation will be reported more fully.

The increased rate of electron transport over the redox potential range 150–90 mV is interesting in view of reports that an optimum redox potential is required for maximal rates of photo-phosphorylation [6].

ACKNOWLEDGEMENTS

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