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PHOTOOXIDATION OF CYTOCHROMES IN REACTION CENTER PREPARATIONS FROM *CHROMATIUM* AND *RHODOPSEUDOMONAS VIRIDIS*

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SUMMARY

In a reaction center preparation from *Rhodopseudomonas viridis*, which contains as its only bacteriochlorophyll a single reactive trimer, two bound cytochromes undergo photooxidation. Cytochrome C558 photooxidation occurs at redox potentials above +100 mV, with a half-time of approx. 1 μsec. Cytochrome C 553 photooxidation occurs at potentials below 0 mV, with a rate that is presently too fast to measure.

Similarly, a subchomatophore fraction from *Chromatium* exhibits photooxidation of bound cytochrome C555 with a half-time of 2.3 μsec at potentials above +100 mV, and cytochrome C552 with a half-time of 1.1 μsec at potentials below 0 mV. The changeover from one cytochrome to the other fits a one-electron titration curve with $E_m = +25$ mV, which is independent of the pH. At all potentials, the rate of $P883^+$ ($P870^+$) reduction is the same as that of cytochrome oxidation. Measurements of $P883^+$ reduction at both 882 and 785 nm allow the conclusion that this reactive bacteriochlorophyll species oxidizes both cytochromes.

The similarities between the light-induced reactions in *Chromatium* and *Rps. viridis* suggest that a general feature of bacterial photosynthesis may be the oxidation of both high- and low-potential cytochromes by a single photochemical system.

The cytochrome oxidation kinetics in the *Chromatium* fraction are essentially identical with those in *Chromatium* chromatophores; however, the reaction between the primary and secondary electron acceptors is slower by a factor of $1 \cdot 10^3$. The purified material appears to lack the secondary acceptor, Y. *N*-Methylphenazonium methosulfate (PMS) or methylene blue can replace Y in a reaction which, unlike the *in vivo* reaction, is insensitive to 1,10-phenanthroline.

INTRODUCTION

Recent studies[1,2] of cell suspensions and chomatophores from the purple sulfur bacterium *Chromatium* have presented several lines of evidence that a reactive bacteriochlorophyll trimer, called P870 or P883, can oxidize either of two different, membrane-bound cytochromes. Following a single actinic flash, oxidation of the

Abbreviation: PMS, *N*-methylphenazonium methosulfate; E_m, the midpoint of a symmetrical oxidation–reduction titration.

low-potential cytochrome C552 prevails over that of the high-potential cytochrome C555, if the ambient redox potential is sufficiently low. To observe C555 photooxidation, one must deplete the store of ferrous C552, by raising the ambient redox potential for example. In either case, light-induced absorbance changes at 882 nm, which we have attributed to $P883^+$ reduction, occur with kinetics matching the kinetics of the cytochrome oxidation[1].

THORNBER[3] has isolated three distinct bacteriochlorophyll–protein particles from *Chromatium*. One of these, Fraction A, contains P883, cytochromes C555 and C552, and some light-harvesting bacteriochlorophyll. Cytochrome C552 is in the oxidized state in isolated Fraction A; illumination causes reversible photooxidation of C555 and P883 (ref. 3). The present report will show that reversible photooxidation of C552 also occurs in Fraction A, if the redox potential is appropriate.

Prolonged illumination of Fraction A also causes an absorbance decrease at 890 nm, which has been attributed[3] to the oxidation of a second reactive bacteriochlorophyll species P'890. The discovery of P'890 calls into question our previous use of 882-nm absorbance changes to measure P883 oxidation and reduction. Thus a major aim of the present report is to reconsider whether P883 alone can oxidize both C555 and C552, or whether P'890 mediates the photooxidation of one of them.

THORNBER *et al.*[4] have also isolated a bacteriochlorophyll–protein complex from the non-sulfur purple bacterium *Rps. viridis*. Like *Chromatium* Fraction A, the *Rps. viridis* preparation contains a photochemically active bacteriochlorophyll trimer, called P960, and two *c*-type cytochromes, C558 and C553. Again the low-potential cytochrome, C553, occurs in an oxidized state in the isolated fraction, and illumination causes reversible photooxidation of C558 and P960. Unlike *Chromatium* Fraction A, the *Rps. viridis* preparation contains no component analogous to P'890, and no light-harvesting bacteriochlorophyll. It is interesting to ask, therefore, whether the single reactive bacteriochlorophyll present in the preparation (P960) is capable of oxidizing both C558 and C553.

In whole cells or chromatophores, the unidentified electron acceptor, X, which oxidizes P883 (or P960) in the primary photochemical reaction, subsequently transfers an electron to another membrane-bound carrier, Y (refs. 1, 5). One can determine the rate of X^- oxidation by measuring the ability of a second flash to cause cytochrome oxidation, as a function of time after the first flash. As long as X remains reduced, no photochemistry can occur on the second flash. In *Chromatium* chromatophores, the electron transfer reaction between X^- and Y has a half-time of 60 μsec at pH 7 (ref. 5). 1,10-Phenanthroline inhibits the reaction[1]. 100 μM PMS (oxidized form) has no effect on the rate of the reaction, but reduced PMS may be inhibitory (G. D. CASE AND W. W. PARSON, unpublished observations, also refs. 1, 5, 6). A final aim of the present report is to consider whether the normal reaction between X^- and Y occurs in *Chromatium* Fraction A, or whether an alternative mechanism of X^- oxidation develops in the purified preparation.

METHODS

Reaction center preparations from *Chromatium* Strain D and *Rps. viridis* were prepared according to published procedures[3,4]. The techniques for measuring cytochrome oxidation and P883 oxidation and reduction after Q-switched laser

flashes, and for controlling the redox potential have also been described[1,5]. Except as noted in Fig. 3, the redox buffers included 100 μM PMS, indigotetrasulfonate, and indigodisulfonate. In some cases, 100 μM methylene blue was also present. 0.1 M potassium phosphate or Tris–maleate buffer was used, and the pH was 7.0, except as noted in Fig. 2A.

Double-flash experiments with *Chromatium* Fraction A utilized procedures which have been described elsewhere[5,6], except that a Xe flash lamp with a Schott RG-9 filter replaced the second laser. The width of the Xe flash was 4 μsec at half-maximum amplitude. The redox potential was in the range +200 to +300 mV. The experimental cuvette had a 1-cm light path, unless otherwise indicated.

RESULTS

The upper part of Fig. 1 shows the α-band difference spectra of the cytochromes in the *Rps. viridis* reaction center preparation which respond to actinic flashes at redox potentials of +236 and —44 mV. From the spectra, one can conclude that a single flash results in the oxidation of cytochrome C558 at the higher redox potential, and of cytochrome C553 at the lower potential. This agrees with the report of FOWLER AND SYBESMA[7], who have studied cytochrome oxidation in *Rps. viridis* membrane fragments as a function of the redox potential. As there is no indication that any bacteriochlorophyll other than P960 undergoes reversible photooxidation in the reaction center preparation[4], it appears that $P960^+$ oxidizes both cytochromes.

Attempts to measure the rates of the *Rps. viridis* cytochrome oxidations were only partly successful. The quantum yields of these oxidations are lower than those of the *Chromatium* reactions[4,8–10], necessitating the use of stronger actinic flashes to saturate the primary photochemical reaction. Artifacts from the more intense flashes interfere with the measurements. The quantum yield is further lowered for ruby laser excitation (694 nm), because the reaction center preparation contains a pigment (probably oxidized bacteriochlorophyll *b*) with an absorption maximum at 685 nm (ref. 4). Energy absorbed by this pigment is not efficiently transferred to the reaction center.

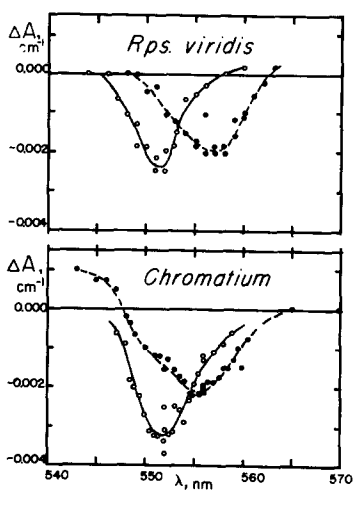
For the photooxidation of an unidentified cytochrome in intact cells of *Rps. viridis*, KIHARA AND CHANCE[11] give a half-time of < 2 μsec. We observed a half-time of approx. 1 μsec for C 558 oxidation in the reaction center preparation. The half-time of C553 photooxidation could not be measured, because the reaction appeared to be complete by the time the flash artifact had ended, 1 μsec after the flash. The rate of C553 oxidation may be sufficiently greater than that of C558 photooxidation to account for the prevalence of the former reaction at low redox potentials (*cf.* refs. 1, 2).

The lower part of Fig. 1 shows flash-induced difference spectra for *Chromatium* Fraction A. In agreement with observations[1,12] on *Chromatium* chromatophores, cytochrome C555 photooxidation occurs at a redox potential of +242 mV, and cytochrome C552 photooxidation at a potential of —25 mV.

Fig. 2A shows that the changeover from C555 to C552 photooxidation in *Chromatium* Fraction A fits a one-electron tritation curve, with an $E_m = +25$ mV. The E_m value is independent of the pH between pH 6.8 and 9.0. Similar titrations with *Chromatium* chromatophores gave a lower E_m value (+10 mV) which was also independent of the pH between pH 5.5 and 9.5 (G. D. CASE and W. W. PARSON, unpublished observations).

Fig. 2B and 2C allow a comparison, as a function of the redox potential, of the rate of cytochrome oxidation and the rate of $P883^+$ reduction. Within experimental error, the two rates are equal. Both rate constants increase from approx. $3 \cdot 10^5$ sec^{-1} (half-time = 2.3 μsec) to approx. $6 \cdot 10^5$ sec^{-1} (half-time = 1.1 μsec), as the secondary electron donor changes from C555 to C552. These rates are essentially the same as those which occur in *Chromatium* chromatophores[1] and intact cells[2].

The crosses in Fig. 2C were obtained from measurements made at 882 nm; the circles (open en filled) represent measurements made at 785 nm. Whereas the absorbance changes at 882 nm could be due to the reduction of either $P883^+$ or $P'890^+$, those at 785 nm can be due only to $P883^+$ (ref. 3). We conclude that $P883^+$ alone oxidizes both cytochromes.



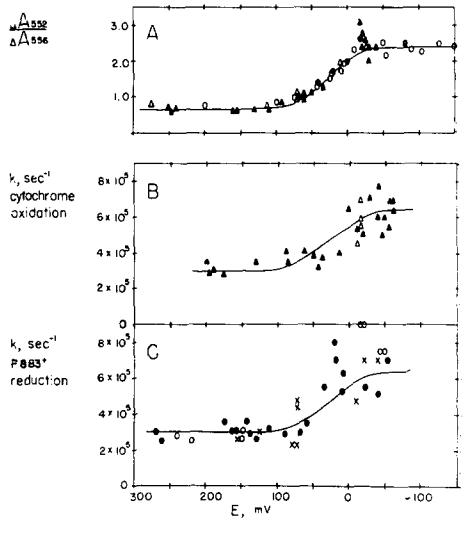


Fig. 1. Spectra of flash-induced absorbance changes in reaction center preparations from *Chromatium* and *Rps. viridis*, at different redox potentials. Upper figure: *Rps. viridis*; 2.0-nM monochromator bandpass; the redox potentials were $+236 \pm 2$ mV (●---●), and -44 ± 5 mV (○—○); the sample had an absorbance at 830 nm of 0.3 cm^{-1} (12-15-69). Lower figure: *Chromatium*; 3.6-nm bandpass; the redox potentials were $+242 \pm 8$ mV (●---●), and -25 ± 13 mV (○—○); the absorbance at 890 nm was 0.94 cm^{-1} (9-24-69). The redox potentials are the mean potential and the range during the recording of each spectrum. Small additions of $NA_2S_2O_4$ were made frequently to keep the potential within these ranges.

Fig. 2. Correlation of the rate of $P883^+$ reduction, the rate of cytochrome oxidation, and the changeover from cytochrome C555 to cytochrome C552, in *Chromatium* Fraction A, as a function of the redox potential. A. Changeover of cytochrome photooxidation from C555 to C552, measured by the ratio of the flash-induced absorbance changes at 552 nm to those at 556 nm. △, pH 6.8; ▲, pH 7.0; ○, pH 8.0; ●, pH 9.8. For the pH 7.0 experiment, the sample had an absorbance at 890 nm of 0.94 cm^{-1}, and the monochromator bandpass was 3.6 nm. For the other experiments, the 890-nm absorbance was 0.46 cm^{-1}; the bandpass, 2.0 nm. (9-24-69, 3-17, 24, 25-70). First-order rate constant of the cytochrome photooxidation. The rate of the flash-induced absorbance decrease at 422.5 nm was measured with a bandpass of 5 nm. △ and ▲, two different preparations of Fraction A; (9-24-69, 2-17-70). C. First-order rate constant of $P883^+$ reduction. The rate of the absorbance decrease at 785 nm (following a flash-induced absorbance increase) was measured with a bandpass of 10 nm, with two different preparations of Fraction A (○ and ●) (12-16-69, 2-19-70). The rate of the absorbance increase at 881.9 nm (Xe emission line) was measured, following a flash-induced absorbance decrease (×—×) (2-19-70). All data were corrected for artifacts due to bacteriochlorophyll fluorescence[8]. Absorbances at 890 nm ranged between 0.77 and 1.0 cm^{-1} in the experiments of B and C.

Fig. 3 shows experiments on the rate of reoxidation of the primary electron acceptor, X^-, in *Chromatium* Fraction A. The ordinate gives the amount of cytochrome C555 oxidation which occurs on a second actinic flash, as a function of time after the first flash. Each photosynthetic unit contains two (or possibly three) C555 hemes[3,6]. Separate measurements at 785 nm showed that a second flash given within 400 μsec of the first flash caused little or no P883 oxidation. This long refractory period for primary photochemistry indicates that the rate-limiting step must be that of X^- oxidation, rather than a step linking the second C555 heme to the P883 (*cf.* refs. 5, 6).

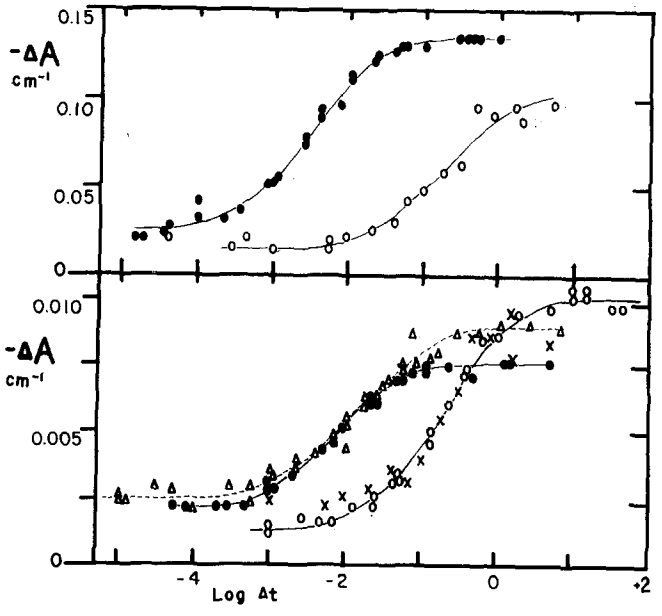


Fig. 3. Cytochrome oxidation due to a second actinic flash, in *Chromatium* Fraction A; absorbance changes measured at 422.5 nm, with 5-nm bandpass. The abscissa gives the $\log_{10}$ of the time (in sec) between the first and second flashes. Upper figure: Absorbance at 890 nm, 11.0 cm^{-1}; measurements made in a 0.1-cm cuvette. ●, 100 μM PMS. ○, no PMS (2-21, 27-70). Lower figure: Absorbance at 890 nm, 0.75 cm^{-1}; 1.0-cm cuvette. ●, 100 μM PMS. ○, 5 μM PMS. △, 100 μM methylene blue. ×, 100 μM 1,2-napthoquinone 4-sulfonate (2-21, 23, 25, 27-70).

The rate of X^- oxidation in Fraction A varied somewhat among different preparations, but it was consistently about three orders of magnitude below the rate which occurs in *Chromatium* chromatophores. In the absence of PMS (Fig. 3, upper) or in the presence of 5 μM PMS (Fig. 3, lower) the half-time of the reaction in Fraction A is approx. 200 msec. Separate experiments showed that, contrary to the situation in chromatophores[1], 1 mM 1, 10-phenanthroline does not inhibit the oxidation of X^- in Fraction A, either in the presence or absence of PMS. These observations suggest that the normal secondary electron acceptor Y is either missing or damaged in Fraction A. Unlike the reaction in chromatophores, the rate of X^- oxidation in Fraction A increases in the presence of high concentrations of PMS or methylene blue (Fig. 3, upper and lower) suggesting that these dyes act as inefficient substitutes for Y. Significantly, the fast H^+ binding observed in *Chromatium* chromatophores[13], which may be linked to the reduction of Y, does not occur in Fraction A (ref. 14).

NICOLSON AND CLAYTON[15] have found that, in a reaction center preparation from *Rps. spheroides*, dyes with midpoint redox potentials more positive than —50 mV will serve as oxidants for X^-, whereas those with potentials below — 50 mV will not. In *Chromatium* Fraction A, we found no simple correlation between the reactivities and the midpoint potentials of various dyes. Thus, PMS ($E_{m,7} = +80$ mV) and methylene blue ($E_{m,7} = -11$ mV) increased the rate of X^- oxidation (Fig. 3, upper and lower), but 1,2-napthoquinone 4- sulfonate ($E_{m,7} = +220$ mV) did not (Fig. 3, lower). Other dyes which failed to accelerate X^- oxidation (at a concentration of 100 μM) were 1,4-naphthoquinone 2-sulfonate ($E_{m,7} + = 113$ mV), indigotetrasulfonate ($E_{m,7} = -46$ mV), indigodisulfonate ($E_{m,7} = -116$ mV), NAD ($E_{m,7} = -320$ mV), neutral red ($E_{m,7} = -325$ mV), and methylviologen ($E_{m,7} = -420$ mV).

The kinetics of X^- oxidation in intact chromatophores[5] had suggested the possibility that interaction of separate reaction centers is necessary to give two electron equivalents for reduction of one molecule of Y. In the experiments of Fig. 3 and in other similar ones, we measured the X^- oxidation rate as a function of Fraction A concentration. Varying the concentration of Fraction A by a factor of 60 (corresponding to an 890-nm absorbance range from 0.18 to 11.0 cm^{-1}) had only slight effects on the half-time of X^- oxidation, either in the presence or absence of PMS. Thus we found no reliable indication of electron transfer between separate particles in the Fraction A preparation.

DISCUSSION

OLSON AND NADLER[16] were the first to draw an analogy between the two high-potential cytochromes, C558 of *Rps. viridis* and C 555 of *Chromatium*, and another between the low-potential cytochromes, C553 and C552. THORNBER[3] and THORNBER *et al.*[4] extended the analogy by finding that reaction center preparations from both species contain the reactive bacteriochlorophyll complexes (P960 and P883) and the high- and low-potential cytochromes in the molar ratio 1:(2–3):(5–7).

The similarities between the cytochrome photooxidation reactions in *Chromatium* and *Rps. viridis* are especially striking when one recalls that the two species of bacteria thrive under different culture conditions, exhibit different morphology and fine structure, and contain different types of bacteriochlorophyll[17–20]. With this consideration in mind, one is tempted to suggest that the existence of a single photochemical system which is capable of oxidizing both high- and low-potential cytochromes may be a basic feature of bacterial photosynthesis. Similar arrays of cytochromes occur in other species of photosynthetic bacteria[11,21,22]. Multiple photochemical systems have been postulated[7,12,22–24] to account for the photooxidation of different types of cytochromes, but only in the case of *Rhodospirillum rubrum* (*cf.* ref. 24) is there any support for this postulate (*cf.* ref. 1). The evidence in that case is, in our view, indecisive, whereas a single photochemical system evidently suffices in the cases of *Chromatium* and *Rps. viridis*.

The results presented here indicate that the primary photochemical events occur in an essentially unaltered form in the membrane-free bacteriochlorophyll–protein particles from *Chromatium* and *Rps. viridis*. In additional support of this assertion, OLSON AND OWENS[10] have found that the quantum yields of the cytochrome photooxidations in the purified complexes are similar to those in whole cells of the

two species. Further, P. L. DUTTON (personal communication) has found that at 77°K, C552 photooxidation occurs at identical rates in *Chromatium* Fraction A and in whole cells. The detergent sodium dodecyl sulfate, which is used for solubilization of the complexes, does not interfere with the primary photooxidation of P883 or P960, not with the oxidation of bound cytochromes. It does, hoewever, destroy the secondary reaction between X^- and Y, and this action may ultimately be advantageous in a search for the identity of Y.

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