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CYTOCHROME C553 AND BACTERIOCHLOROPHYLL INTERACTION AT 77°K IN CHROMATOPHORES AND A SUBCHROMATOPHORE PREPARATION FROM *CHROMATIUM* D

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SUMMARY

1. The liquid dye laser (868 nm) induced spectral changes at 77°K of *Chromatium* D chromatophores poised at different oxidation–reduction potentials, demonstrate that P600 is a spectral component of the reaction center bacteriochlorophyll which oxidizes cytochrome C553 ($t_{1/2}$ = 2–2.5 msec). At —60 mV when cytochrome C553 is chemically reduced and its photooxidation is evident, P600$^+$ (formed in less than 0.5 μsec) is reduced over a time range similar to that of cytochrome oxidation. The photooxidation of C553 is non-reversible; a second laser pulse induces no further oxidation of cytochrome C553 or P600. At +300 mV when cytochrome C553 is chemically oxidized, reduction of the light-generated P600$^+$ takes longer ($t_{1/2}$ = 20–25 msec). At —350 mV no laser-induced reactions are observed at 77°K since the primary electron acceptor of reaction center bacteriochlorophyll is chemically reduced at this potential.

2. The reactions displayed by the subchromatophore preparation are essentially the same as those of the chromatophore. The studies indicate that the membrane is not an obligatory part of the early chemical events of bacterial photosynthesis.

3. An Arrhenius plot of laser-induced cytochrome C553 oxidation suggests the existence of two modes of electron transfer from this cytochrome to bacteriochlorophyll$^+$.

4. There were no detectable differences in the reactions studied when induced by laser light of wavelength 694 or 868 nm.

INTRODUCTION

In the previous paper[1], cytochrome C553 in *Chromatium* D chromatophores appeared to be oxidized by a reaction center bacteriochlorophyll complex which included the spectral components P600, P435, P400 and, on the basis of similar oxidation–reduction midpoint potentials[2], P800 and P890 (P890 will be more precisely

Abbreviation: E'_0, the midpoint potential of an oxidation–reduction couple at pH 7.

termed P883; see ref. 3). Since the dual wavelength spectrophotometer employed in these studies[1] could not time-resolve the reactions, the interactions of cytochrome C553, reaction center bacteriochlorophyll and its primary acceptor in both chromatophores and a subchromatophore preparation of *Chromatium* D have been examined further using 20-nsec, 868-nm light pulses from a liquid dye laser.

The *Chromatium* D subchromatophore preparation (Fraction A) is one of three bacteriochlorophyll–protein complexes resolved in the fractionation studies of THORNBER[3]. Fraction A contains the known reaction center of the organism, P883, together with the majority of the chromatophore-bound cytochromes. This subchromatophore preparation is water soluble, has no membrane and little or no phospholipid material. Besides some carotenoids and light harvesting bacteriochlorophyll, it contains 2–3 heme moieties of cytochrome C555 and 6–7 heme moieties of cytochrome C553 per reaction center bacteriochlorophyll. The preparation has given an opportunity to assess the possible role of the membrane in the early chemistry of photosynthesis and to further establish the association with P883 of the light-induced absorbance changes at 400, 435 and 615 nm already observed in the chromatophores at 77°K.

MATERIALS AND METHODS

Cultural methods of *Chromatium* D, preparation of chromatophores, and experimental procedures are as described previously[1]. The *Chromatium* D subchromatophore preparation used in this work was Fraction A of THORNBER[3]. The laser excitation pulse was obtained from a liquid dye laser[4] pumped by a Q-switched ruby laser[5], or directly from the ruby laser (694 nm). The dye system used was 3,3′-diethylthiatricarbocyanine iodide (about $1 \cdot 10^{-4}$ M) in dimethylsulphoxide. This provided a 20-nsec, 6-mJ laser pulse at 868 ± 2 nm with a bandwidth (full width at half-maximum) of about 10 nm. The temperature of the sample was measured with a copper-constantan thermocouple attached to the reaction cuvette.

The oxidation–reduction potential mediators used were: potassium indigotetrasulphonate, E'_0 —46 mV; potassium indigotrisulphonate, E'_0 —80 mV; neutral red, E'_0 —325 mV; and potassium ferricyanide, E'_0 +430 mV. The potential was made more negative with freshly prepared sodium dithionite in 0.1 M Tris–HCl buffer, pH 7.8, and more positive with potassium ferricyanide.

RESULTS

Fig. 1A shows the time-course of the laser (868 nm) induced oxidation of cytochrome C553 (421 nm) and P600 (615 nm, the position at 77°K of the second bacteriochlorophyll absorption transition[7]) in chromatophores of *Chromatium* D, poised at —60 mV before freezing to 77°K; at this potential cytochrome C553 is over 90% reduced (*cf.* ref. 1). The cytochrome is photooxidized with a half-time of 2–2.5 msec which compares well with the value of 2.3 msec obtained by DEVAULT *et al.*[6] using intact cells. A second laser pulse 1 min later induces no further cytochrome oxidation, showing that one flash is sufficient to drive the reaction to completion, and that the oxidation is non-reversible; the non-reversibility of the reaction at 77°K agrees with earlier findings with continuous illumination[1,7]. At 615 nm the fast (less than 0.5 μsec) laser-induced photooxidation of P600 is observed, which is followed by

P600+ reduction at a rate which is similar to the oxidation rate of the cytochrome C553. (Previously[1,7], under the same conditions but using the dual wavelength spectrophotometer which had a slower response time than that of the oxidation of cytochrome C553, no P600 reactions had been observed.) These data provide more evidence that P600+ is the primary oxidant of cytochrome C553. Since cytochrome C553

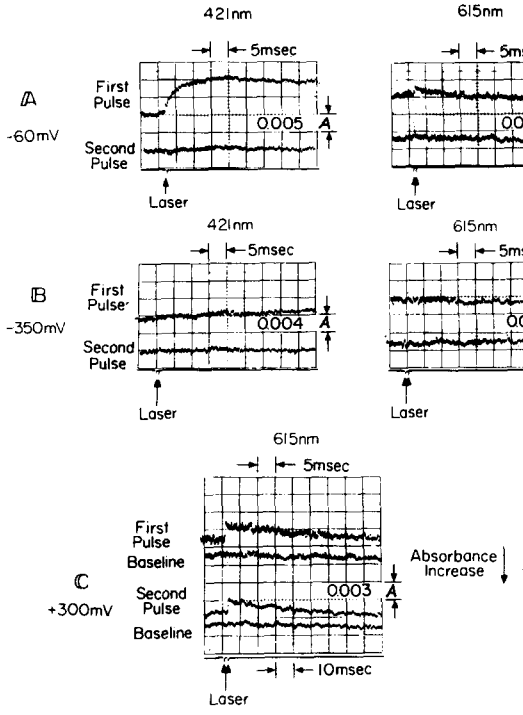

Fig. 1. Kinetics at 77 °K of cytochrome C553 oxidation and P600 oxidation–reduction in *Chromatium* D chromatophores poised at different oxidation–reduction potentials. The chromatophores, bacteriochlorophyll concn. 240 μM, in 100 mM Tris–HCl buffer, pH 7.4, containing potassium indigotetrasulphonate (100 μM), potassium indigotrisulphonate (100 μM) and neutral red (100 μM). Room temperature; argon atmosphere. Samples at the appropriate oxidation–reduction potential were transferred to the anaerobic cuvette and frozen to 77 °K (*cf.* ref. 1). Laser light 868 $\pm$ 2 nm; cuvette light path 1 mm. Time between first and second laser pulse, 1 min.

transfers an electron non-reversibly to P600+, the fact that a second laser flash causes no further P600 reactions implies that after the first flash the primary acceptor from bacteriochlorophyll remains reduced at 77 °K, thus preventing further bacteriochlorophyll photooxidation. At +300 mV (Fig. 1C) when cytochrome C553 is chemically oxidized before freezing, P600 undergoes a reversible laser-induced oxidation; the second laser pulse produces the same response as that of the first. The rate of dark reduction of the laser-generated P600+ is much slower than that observed when cytochrome C553 was the primary electron donor; the half-time is about 25 msec, and presumably the reductant is the primary electron acceptor of the reaction center bacteriochlorophyll. At these potentials at 77 °K, P435 measured at 435 nm shows similar laser-induced reaction kinetics as P600. At oxidation–reduction potentials lower than —300 mV (Fig. 1B) there are no detectable laser-activated cytochrome C553 or P600

reactions due to the chemically reduced state of the primary electron acceptor of bacteriochlorophyll, thus corroborating the earlier findings with continuous illumination[1].

Using the dual wavelength spectrophotometer and exciting with continuous illumination at 77°K, the subchromatophore preparation of *Chromatium* D displays similar light-induced absorbance changes to those observed in chromatophores[7]: at +300 mV the reversible light-induced absorbance changes measured at 400, 435 and 615 nm are evident, and at −70 mV these reversible reactions are replaced by the non-reversible oxidation of cytochrome C553. In both chromatophore and subchromatophore preparations the relative amplitudes of the laser-induced C553 and P600 absorbance changes agree well with each other and with those obtained under continuous illumination (compare findings at room temperature[12]), showing that at 77°K electron transport is confined between cytochrome C553 and bacteriochlorophyll, and bacteriochlorophyll and its primary acceptor.

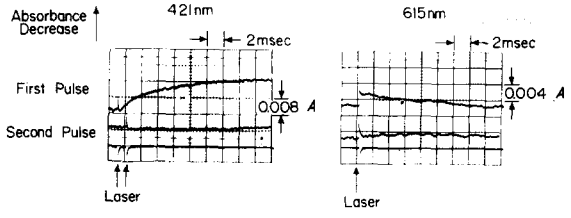

Fig. 2. Kinetics at 77°K of cytochrome C553 oxidation and P600 oxidation–reduction in a subchromatophore preparation of *Chromatium* D[3]. The subchromatophore preparation, bacteriochlorophyll concn. 110 μM, in 30 mM Tris–HCl buffer, pH 7.0, containing potassium indigotetrasulphonate (100 μM) and potassium indigotrisulphonate (100 μM) was maintained at room temperature at an oxidation–reduction potential of -70 ± 5 mV; argon atmosphere. Samples transferred to the low-temperature cuvette and frozen to 77°K. Laser light 868 $\pm$ 2 nm; cuvette light path 1 mm. Time between first and second laser pulse, 1 min.

Fig. 2 shows the laser-induced interactions of cytochrome C553 and P600 in the subchromatophore preparation. The traces are less noisy than those observed with the chromatophores because a lower concentration of bacteriochlorophyll could be used to observe the same absorbance changes, and in the absence of membrane material, the subchromatophore preparations were optically less dense. The cytochrome C553 photooxidizes with a half-time of 2.5 msec. Under the same conditions, the reduction of the light-generated $P600^+$ occurs over a similar time range (half-time about 3 msec), substantiating the view that P883 oxidizes cytochrome C553. At potentials of about +320 mV the P600 reaction was reversible, and the rate of $P600^+$ reduction had a half-time of about 20 msec. This is slightly faster than was observed in the chromatophores.

Excitation of chromatophore and subchromatophore preparations with the ruby laser (694 nm) produced the same effects as with 868 nm light. A feature of the decay kinetics of P600 and P435 after a laser pulse was that they were usually less than the expected first order. However, since the absorbance changes are small, the observed deviations could result from other slight optical changes.

Fig. 3 describes the temperature dependence (Arrhenius plot) of the rate of cytochrome C553 photooxidation in the subchromatophore preparation. From room temperature down to about 120°K cytochrome C553 oxidation requires an activation

energy of about 3.5 kcal/mole, but from 120°K to 77°K the reaction rate displayed very little temperature dependence. The amplitude of the absorbance change at room temperature (423 nm) and 77°K (421 nm) was approximately the same.

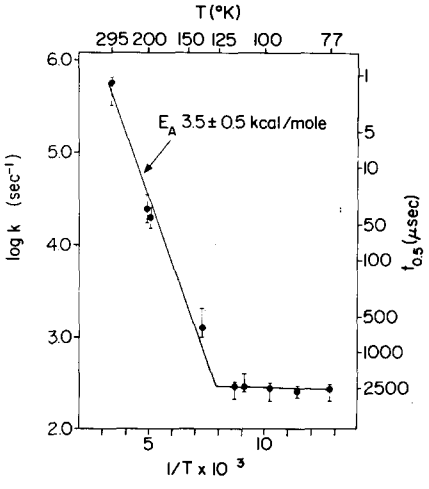

Fig. 3. Arrhenius plot of the temperature dependence of the rate of cytochrome C553 oxidation in a subchromatophore preparation from *Chromatium* D[3]. The subchromatophore preparation, bacteriochlorophyll concn. 103 μM, in 60 mM Tris–HCl buffer, pH 7.0, containing the same mediators as described in Fig. 2, was maintained at room temperature at an oxidation–reduction potential of -70 ± 8 mV; argon atmosphere. Samples were transferred to the anaerobic low temperature cuvette and at the appropriate temperature, the rate of absorbance change at 421 nm (423 nm at room temperature) was measured. Laser light, 694 nm.

DISCUSSION

PARSON[8] was the first to associate the time-course of cytochrome oxidation with the concomitant reduction of oxidized bacteriochlorophyll; in this way, P883 in *Chromatium* D has been assigned as oxidant of cytochrome C555. More recently P883 has been shown to be the oxidant of C553 in both chromatophores[9] and in the subchromatophore preparation[10]. In the previous paper[1], evidence was presented that P600 is, together with P883, a spectral component of the reaction center bacteriochlorophyll involved in the oxidation of cytochrome C553.

The light-induced reactions at 77°K in *Chromatium* D appear to be confined to non-reversible electron transfer from cytochrome C553 to reaction center bacteriochlorophyll, and reversibly between the reaction center bacteriochlorophyll and its primary electron acceptor; hence, study at 77°K provides a unique opportunity to elucidate the mode of interaction of these three electron carriers involved in the early phases of photosynthesis. The laser studies at 77°K presented in this paper confirm the conclusions[1] that the reaction center bacteriochlorophyll donates an electron to its primary electron acceptor (E_m —135 mV), and that the bacteriochlorophyll$^+$ thus formed is then reduced at 77°K by a dark process either non-reversibly at lower potentials by cytochrome C553 (half-time 2–2.5 msec), or reversibly at higher potentials by the primary electron acceptor (half-time 20–25 msec). At these higher potentials the rate of P600$^+$ reduction compares well with the decay rate of the light-induced

EPR signal at 77°K in *Chromatium* D (τ 30 msec) described by McElroy *et al.*[11]. The EPR signal is associated with the free radical formed during the photooxidation of reaction center bacteriochlorophyll, and the decay of the signal with its dark reduction.

The reactions and kinetics exhibited at 77°K by the membrane-free, water-soluble, subchromatophore complex prepared from *Chromatium* D are essentially the same as those observed in the chromatophores. This demonstrates that the chromatophore membrane is not an obligatory part of the organization of the electron carriers involved in the fast, early events of photosynthesis. The finding is given more substance by the Arrhenius plot of cytochrome C553 oxidation; besides the rate of its oxidation at room temperature (half-time 1.2 μsec) being of the same order as that described for the chromatophore or the intact cell[9,12], temperature dependence of the rate down to 77°K was within experimental error coincident with that described by DeVault *et al.*[6] for cytochrome oxidation in anaerobic, intact *Chromatium* D cells. This suggests that the parameters governing this electron transfer in the subchromatophore preparation are unimpaired by the fractionation procedure employed during its isolation. However, certain of the preparations when poised at −80 mV (cytochrome C553 over 95 % reduced) unexpectedly exhibited significant P600 and P435 reactions induced by the second and subsequent exposures to light. This suggested that in these subchromatophore preparations some photosynthetic units (30 % in one of the preparations) were devoid of cytochrome C553. However, since the overall ratio of cytochrome C553 to reaction center bacteriochlorophyll in the subchromatophore and chromatophore are similar (*cf.* refs. 3, 12), it seems more likely that a full complement of cytochrome C553 was present, but part of it was not capable of transferring an electron to the reaction center bacteriochlorophyll$^+$. There are 4–7 cytochrome C553 hemes[3,12] associated with each reaction center bacteriochlorophyll molecule, but previous data[1] have suggested that only two of them are light-oxidizable at 77°K. Since there are two hemes per cytochrome C553 molecule[13], the light-oxidizable hemes may be parts of the one cytochrome C553 molecule of those present in each unit, to be directly chemically bonded with the reaction center bacteriochlorophyll. Damage to this bonding in some photosynthetic units could explain the observation. Those cytochromes that are light-oxidizable, however, behave as they would in the intact cell system.

Chance *et al.*[14] considered the interesting biphasicity of the temperature dependence as due to either the existence of two modes of electron transfer in cytochrome C553, or to the combined effects of the different activation energies of cytochrome C553 and cytochrome C555. It has been shown using continuous illumination[2] and laser light[9,12] that at −70 mV, cytochrome C553 appears to be the only cytochrome photooxidized. This, therefore, suggests that cytochrome C553 has two distinct mechanisms of electron transfer.

It has been postulated by some investigators[2,15] that cytochrome C553 is associated with a different photosystem than the high potential cytochrome C555 in *Chromatium* D. Recent studies on this organism and its chromatophore fractions[1,3,9,10,12] have shown that the hypothesis for two photosystems is probably incorrect. The research discussed here substantiates the proposal that a single photosystem operates for the oxidation of two cytochromes in *Chromatium* D: Cytochrome C553 is oxidized at low potentials by a bacteriochlorophyll complex identified with

the spectral components P600 and P883. The latter component has, at higher potentials, been previously shown[8] to be the primary oxidant of cytochrome C555.

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