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NATURE OF PHOTOCHEMICAL REACTIONS IN CHROMATOPHORES OF *CHROMATIUM* D

II QUANTUM YIELD OF PHOTOOXIDATION OF CYTOCHROMES IN *CHROMATIUM* CHROMATOPHORES

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SUMMARY

Initial rates of the light-induced absorption decrease in *Chromatium* chromatophores due to the oxidation of cytochromes were measured under various experimental conditions The initial rate in the presence of 10 mM potassium ferrocyanide and 50 μM potassium ferricyanide was about one-half to two-thirds of that in the presence of 30 mM ascorbate or in a medium with a redox potential (E_h) of -78 mV

Light-minus-dark difference spectrum indicated that, in the presence of 10 mM ferrocyanide and 50 μM ferricyanide, only cytochrome *c*-555 was photooxidized In the presence of 30 mM ascorbate or at E_h values lower than about 0 mV, both cytochrome *c*-555 and cytochrome *c*-552 were photooxidized The quantum yield of cytochrome *c*-555 photooxidation was calculated to be about 0 4

The results obtained in the present study are compared with other investigators' and the possibility of the presence of two types of associations between the cytochromes and reaction-center bacteriochlorophyll is discussed

INTRODUCTION

It is widely believed that oxidation–reduction reaction in photosynthesis is initiated by charge separation between the primary electron donor (pigment "P") and the primary electron acceptor [1–4] In bacterial photosynthesis, the oxidized "P" rapidly oxidizes cytochromes in the dark [3–6] The photooxidation of cytochromes in bacterial photosynthesis has been investigated in greatest detail in intact cells, chromatophores and subchromatophore particles of *Chromatium* [5–20, 22] The cytochrome oxidation occurs at extremely low temperatures in many bacterial species including *Chromatium* [9, 14, 19–21, 23] The quantum yield of cytochrome photooxidation in *Chromatium* is high [6, 8, 11, 16] This indicates that cytochrome(s) in *Chromatium* is functionally in close contact with the reaction center bacteriochlorophyll

Two cytochromes are photooxidized in chromatophores or subchromatophore

particles of *Chromatium* [15–17, 19, 20, 24–26] Parson and other investigators suggested that only high potential cytochrome (cytochrome *c*-555) is photooxidized at higher redox potentials (E_h above +200 mV) and only low potential cytochrome (cytochrome *c*-552) is photooxidized at lower redox potentials (E_h below 0 mV) by a single laser flash [16, 19, 20, 24] and by continuous light [15]
The changeover of cytochrome *c*-555 photooxidation to that of cytochrome *c*-552 at a redox potential of around 10 mV by an unknown mechanism, has been suggested [16, 25, 26]

In the present paper, dependency of the quantum yield of cytochrome photooxidation on oxidation-reduction potential in chromatophores of *Chromatium* is investigated, and the results obtained are compared with those previously reported A possibility of the presence of different modes of association and interaction of cytochrome molecules and P890 molecules is discussed

MATERIALS AND METHODS

Preparation of chromatophores

Chromatium strain D was grown photoautotrophically in an inorganic medium described by Bose [27] at 30 °C After 5–7 days the bacterial culture was centrifuged at 2000 *g* for 30 min The cells harvested were resuspended in 60 mM phosphate buffer, pH 7 4, containing 0 25 M sucrose and 0 1 % (17 mM) NaCl The suspended cells were disrupted with a French pressure cell at 300 kg/cm^2 The cell debris was removed by centrifugation at 2000 × *g* for 30 min and the supernatant was centrifuged at 10 000 × *g* for 30 min Chromatophores were obtained by centrifuging the supernatant fluid further at 80 000 × *g* for 1 h The chromatophores were resuspended in the same buffer and centrifuged at 80 000 × *g* for 1h The precipitated chromatophores were resuspended in the same buffer The chromatophore suspension was evenly dispersed by a brief sonic treatment (15 s, 20 kHz) Preparations obtained by this procedure (washed chromatophores) were used throughout this work Preparation procedures were carried out at 0–4 °C

Determination of bacteriochlorophyll

5 ml of acetone–methanol mixture (7 2, v/v) were added to 0 05–0 1 ml of concentrated chromatophore suspension After an extraction for 15 min, a supernatant solution was obtained by centrifugation at 2000 × *g* for 15 min The concentration of bacteriochlorophyll in the solution was calculated from the absorbance at 770–772 nm A value of 75 000 was used as the molar absorption coefficient of bacteriochlorophyll in acetone–methanol mixture [28]

Spectroscopic measurements

Near-infrared absorption (or transmission) spectrum of chromatophore suspension was measured with a Hitachi 356 two-wavelength double-beam spectrophotometer with a S-1 type photomultiplier (HTV R473)

Light-induced absorption changes of cytochromes were measured with the spectrophotometer with the split-beam mode using a multialkali type photomultiplier, HTV R375 The front of the photomultiplier was guarded with a Corning 9782 glass filter The actinic light of 890 nm was provided by a Shimadzu-Bausch &

Lomb high intensity monochromator in combination with a Wratten 88A filter and appropriate neutral density filters The half-band width of the monochromator corresponded to 9 6 nm The actinic beam was at right angle to the measuring beam Energy of the actinic beam was measured with a calibrated thermopile (Kipp & Zonen)

The basic reaction mixture was composed of 60 mM phosphate buffer, pH 7 4, 0 25 M sucrose, 17 mM NaCl and chromatophores corresponding to 2 7–9 μM bacteriochlorophyll

All measurements were carried out at room temperature (20–25 °C) For redox poising, an appropriate combination of oxidant and/or reductant was added as required A ferricyanide–ferrocyanide system was used in measurements at high redox potentials Since the chromatophore suspension may have a large internal redox-buffering capacity [32], concentrations of chromatophores were kept as low as possible (2–5 μM bacteriochlorophyll) and concentrations of ferricyanide and ferrocyanide were higher than 30 μM in most cases Measurements at low redox potential values (E_h below 0 mV) were carried out anaerobically in a four-sided cuvette of the Thunberg type, containing chromatophores and 5 mM sodium oxalate and an appropriate ratio of ferric and ferrous ammonium sulfate ($E'_0 = +2$ mV) [29] In some cases, the redox potential of the above mentioned redox buffer was monitored by measuring the absorbance of added indigotetrasulfonate at 590 nm (33 μM, $E'_0 = -46$ mV) [29] The contribution of absorbance of bacteriochlorophyll in vivo at 590 nm was corrected In other cases, low redox potential was obtained anaerobically by adding a few crystals of sodium dithionite to a mixture in the Thunberg-type cuvette The redox potential of the chromatophore suspension was also calculated from the absorbance of 33 μM indigotetrasulfonate at 590 nm

RESULTS AND DISCUSSION

Fig 1 shows the time courses of cytochrome photooxidation at 423 nm under three different redox conditions, i e , a) under aerobic conditions in the presence of 10 mM potassium ferrocyanide and 50 μM potassium ferricyanide, b) aerobic conditions in the presence of 30 mM ascorbate, c) anaerobic conditions at E_h of -78 mV The dashed lines represent initial slopes of the absorption decrease Relative values of slopes are also indicated in parentheses These absorption changes were reversible and the initial rate of light-induced absorption decrease was constant when the dark interval was sufficiently long, for the maximal rates, dark periods of less than 5 min were needed under the conditions used for Fig 1a and 1b, but a longer period ($\approx$ 10 min) was required for Fig 1c

The initial rate of the light-induced absorption change was proportional to the number of absorbed light quanta in the intensity range used in the present study (Fig 2) In most cases, the number of absorbed light quanta at 890 nm was in the range of 1 0–1 5 10^{13} per cm^2 per s (1 6–2 5 10^{-11} Einsteins per cm^2 per s)

In bacterial photosynthesis, the initial rate of cytochrome photooxidation is a function of the fraction of the reduced form of the cytochrome before illumination [22, 26, 30] In chromatophores of *Chromatium* poised at a redox potential between +200 mV and +400 mV, only cytochrome *c*-555 is in the reduced state in the dark [15, 16, 22, 24] Fig 3 shows the redox potential dependency of the initial rate

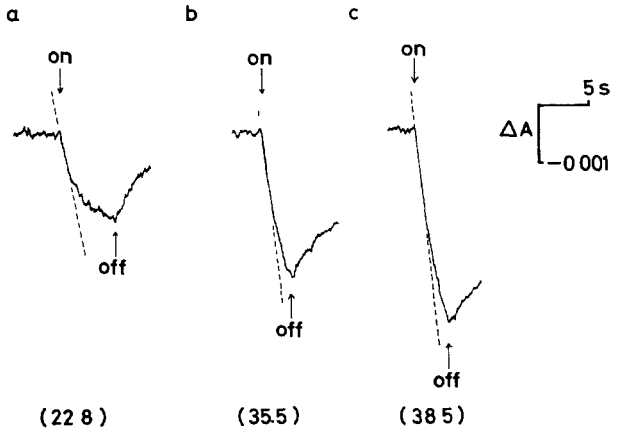

Fig 1 Time courses of cytochrome photooxidation in *Chromatium* chromatophores Absorption changes at 423 nm under three redox conditions a, in the presence of 10 mM ferrocyanide and 50 μM ferricyanide under aerobic condition, b in the presence of 30 mM ascorbate under aerobic condition c at E_h of −78 mV under anaerobic condition in the presence of 6 mM sodium oxalate, 1 mM ferrous ammonium sulfate, 33 μM ferric ammonium sulfate and 33 μM indigotetrasulfonate as a redox indicator Downward deflection corresponds to cytochrome photooxidation - - -, indicate initial slopes of the "light on" traces (relative initial rates are shown in parentheses) The number of absorbed actinic light quanta at 890 nm was 1 05 10^{13} per cm^2 per s Concentration of bacteriochlorophyll, 2 7 μM

of cytochrome photooxidation (cytochrome *c*-555) in chromatophores in ferricyanide/ferrocyanide redox buffers The initial rate practically reached a maximum at the ferrocyanide/ferricyanide ratio of 100–200 As P890 is fully reduced at these redox levels, the initial rate or quantum yield of photooxidation of cytochrome *c*-555 is expected to be maximal at the ratio of 200, as in Fig 1a As the potential lowers the cytochrome *c*-552 becomes reduced in the dark [15, 16, 24] In the presence of 30 mM ascorbate as in Fig 1b, all cytochrome *c*-555 is fully reduced and

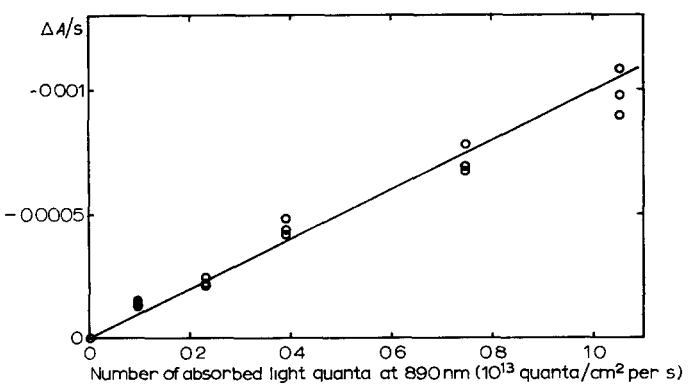

Fig 2 Relationship between initial rate of light-induced absorption change at 423 nm and number of absorbed quanta with actinic light at 890 nm Concentration of bacteriochlorophyll 2 7 μM, ascorbate 3 mM

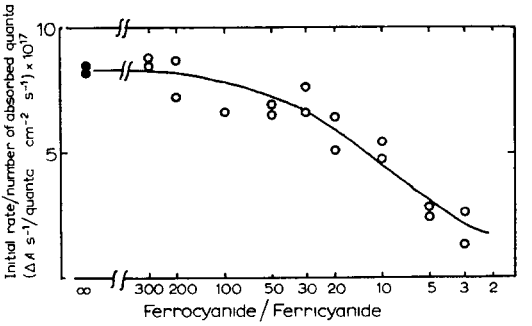

Fig 3 Relationship between initial rate of cytochrome *c*-555 photooxidation and redox potential Ordinate indicates initial rate of absorption change at 423 nm per number of absorbed quanta (wavelength of light, 890 nm) Abscissa indicates ratio of ferrocyanide to ferricyanide on a logarithmic scale Final concentrations of ferricyanide were varied between 20 μM and 100 μM Closed circles represent values obtained in the presence of 10 mM ferrocyanide only Concentration of bacteriochlorophyll, 2 7 μM

cytochrome *c*-552 is partially reduced in the dark As the potential further lowers, as in Fig 1c, cytochrome *c*-552 is also fully reduced in the dark

In order to ascertain which of the cytochrome(s) is photooxidized, light-minus-dark difference spectra were measured in the α- and γ-regions of cytochromes under continuous illumination In the presence of 1 mM ferrocyanide and 33 μM ferricyanide, the light-induced absorption decrease was maximal at 556 nm and 422 5 nm, suggesting that at the potential corresponding to the ferrocyanide/ferricyanide ratio of 30, only cytochrome *c*-555 was photooxidized (Fig 4) In the presence of 30 mM ascorbate, the α-trough was located at 554 nm and the γ-trough at 423 nm (Fig 5B) Based on the noticeably broader α- and γ-bands and the increased absor-

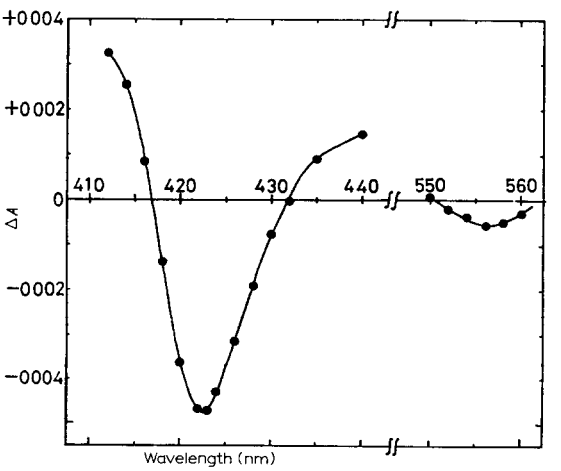

Fig 4 Light-minus-dark difference spectrum (steady state) of chromatophores in the presence of 1 mM ferrocyanide and 33 μM ferricyanide under aerobic condition Intensity of incident light (890 nm), 4 06 10^{14} quanta per cm^2 per s Concentration of bacteriochlorophyll, 9 μM

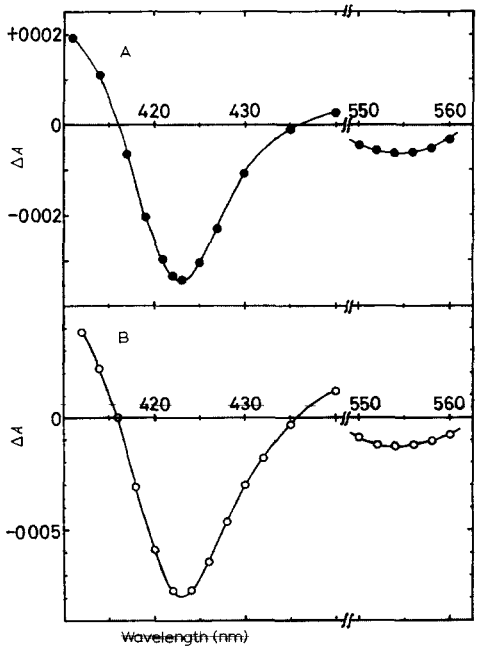

Fig 5 Light-minus-dark difference spectra (steady state) of chromatophores A in the presence of 6 mM sodium oxalate, 300 μM ferrous ammonium sulfate, 30 μM ferric ammonium sulfate and 33 μM indigotetrasulfonate Anaerobic condition, E_h, −30 mV Concentration of bacteriochlorophyll, 4 5 μM B, in the presence of 30 mM ascorbate under aerobic condition concentration of bacteriochlorophyll, 9 μM Intensity of incident light at 890 nm was the same as in Fig 4

bance changes, it was suggested that both cytochrome *c*-555 and *c*-552 were photooxidized A similar result was obtained at E_h of −57 mV (Fig 5A) probably indicating that both cytochrome *c*-555 and *c*-552 were photooxidized at −57 mV under continuous illumination The results at lower redox potentials (below 0 mV) were in disagreement with other investigators' who reported that, below 0 mV, cytochrome *c*-552 but not *c*-555 was photooxidized under continuous illumination [15] We obtained light-minus-dark difference spectra similar to that in Fig 5A at several low redox potentials (e g ,+2 mV, −51 mV, −57 mV, −85 mV)

As both cytochromes *c*-552 and *c*-555 were fully reduced in the dark at potentials lower than about −50 mV, the initial rate of light-induced absorption change in Fig 1c was maximal Initial rates of light-induced absorption changes at 423 nm under these redox conditions are compared in Table I. These are typical values in a series of experiments and are expressed in ratios of initial rate to the number of absorbed quanta (initial rate of absorption change at 423 nm (1 cm light path) divided by the number of absorbed light quanta per cm^2 per s) The ΔA/quantum value at low potential (−78 mV) was about twice that at higher potentials (in the ferrocyanide-ferricyanide system) The value in the presence of 30 mM ascorbate was slightly smaller than that at −78 mV

The quantum yield of cytochrome *c*-555 photooxidation can be easily calculated from the ΔA/quantum value if the reduced-minus-oxidized difference molar

TABLE I

INITIAL RATE OF ABSORPTION CHANGE AT 423 nm PER ABSORBED QUANTA AT 890 nm AND QUANTUM YIELD OF CYTOCHROME PHOTOOXIDATION

Bacteriochlorophyll concentration, 5 6 μM Intensity of absorbed light at 890 nm, 1 05 10^{13} quanta per cm^2 per s

Condition	$-\Delta A$ s^{-1}/No of absorbed quanta cm^{-2} s^{-1} (10^{17})	Quantum yield
Ferrocyanide/ferricyanide = 200	8 0	0 38
10 mM Ferrocyanide	8 5	0 41
30 mM Ascorbate	12 9	(0 92)[a]
Ferric-ferrous oxalate E_h = −78 mV	14 8	(1 06)[a]

[a] Values calculated assuming that cytochrome *c*-552 is solely photooxidized [15 24-26]

absorption coefficient at 423 nm is known for cytochrome *c*-555, which is the only photooxidizable component at higher redox potentials But at lower potentials at which both cytochromes are reduced in darkness and oxidized by light, the situation is more complex If cytochrome *c*-552 only is photooxidized at lower potentials (below 0 mV) as in the case of illumination by a single laser flash [16, 19, 24–26], the quantum yield of cytochrome *c*-552 photooxidation can be calculated from the difference molar absorption coefficient On the contrary, if both cytochromes are photooxidized at lower potentials (below 0 mV), the quantum yield of photooxidation of each cytochrome cannot be calculated easily For the calculation, at least, we must know the relative contribution of two cytochromes to the absorption change

If each P890 oxidizes both cytochromes *c*-555 and *c*-552 as Parson and Case suggested [24], the initial rate of absorption change due to cytochromes should depend on the difference molar absorption coefficients of the cytochromes involved, as well as on the fractions of reduced forms before illumination The fact that the initial rates of decrease in absorbance at higher redox potentials are smaller than those at lower redox potentials (Table I), suggests to us that difference molar absorption coefficient for cytochrome *c*-552 is larger than that for cytochrome *c*-555 at 423 nm, if the assumption that each P890 can oxidize both cytochromes is correct

The values of difference molar absorption coefficients for both cytochromes bound to chromatophores have not yet been obtained Among the cytochrome preparations from *Chromatium* [31–33], a cholate-solubilized cytochrome complex isolated by Kennel and Kamen seems to be closely similar to the membrane-bound cytochromes [33] In the cholate-solubilized cytochrome complex, $\Delta\varepsilon_{red-ox}$ (556 nm) for cytochrome *c*-555 is 15 1 mM^{-1} (on heme basis, also see the following discussion) and $\Delta\varepsilon_{red-ox}$ (552 5 nm) for cytochrome *c*-552 is 13 1 mM^{-1} [33] $\Delta\varepsilon_{red-ox}$ (γ band) for both cytochromes in the cytochrome complex has also been calculated [33] The ratio (6 8) of $\Delta\varepsilon$ (422 nm) to $\Delta\varepsilon$ (556 nm) of cytochrome *c*-555 is slightly smaller than the ratio (8 3) calculated from the α- and γ-bands of the light-minus-dark difference spectrum in Fig 4 This value of 8 3 is in good agreement with that (8 2–8 3)calculated from the light-minus-dark difference spectrum by Kennel et al [22] Therefore, the difference absorption coefficient for cytochrome *c*-555 at 423 nm can be

estimated to be 125 mM^{-1}(15 / 8 3) Although in the present study we could not obtain the light-minus-dark difference spectrum for cytochrome *c*-552 in chromatophores, in later experiments we were able to obtain the light-minus-dark difference spectrum of cytochrome *c*-552 in isooctane-extracted chromatophores (unpublished data) The ratio calculated from the data was 6 4 and was slightly smaller than that for cytochrome *c*-552 in the cholate-solubilized cytochrome complex (6 8) [33] Thus the reduced-minus-oxidized difference molar absorption coefficient for bound cytochrome *c*-552 can be calculated to be 84 mM^{-1}

In our calculation, as well as in the data shown by Kennel and Kamen [33], the difference molar absorption coefficient for cytochrome *c*-555 at 423 nm is larger than that for cytochrome *c*-552 at 423 nm This contradicts the expectation described above that the difference molar absorption coefficient for cytochrome *c*-552 is larger than that for cytochrome *c*-555 This contradiction suggests that we should reexamine the assumption that the same P890 molecule can oxidize both cytochromes

The quantum yield for *c*-555 photooxidation calculated from the initial rate of absorption change and the difference molar absorption coefficient at 423 nm is also shown in Table 1 The value of 0 4 is considerably lower than those reported earlier [6, 11, 16] If we assume that only cytochrome *c*-552 is photooxidized at low potentials as Parson and other investigators have suggested [15, 16, 19, 24], the quantum yield for *c*-552 photooxidation can be calculated to be 1 06 from our data The reason why the quantum yield for *c*-555 photooxidation is lower than that for *c*-552 cannot be explained if one assumes that a single P890 can oxidize both cytochromes

Although the possibility that the quantum yield for *c*-555 photooxidation is low for other unknown reasons(s) cannot be excluded (e g , slow reduction of P890 by an electron donor(s) other than cytochrome *c*-555 under continuous illumination at high redox potentials in competition with cytochrome *c*-555, thereby decreasing the quantum yield), an alternative mechanism in which only cytochrome *c*-555 is oxidized by a fraction of P890 and cytochrome *c*-552 is photooxidized by the remaining fraction of P890 may reasonably explain the experimental results In this model, each cytochrome belongs to a different photosynthetic unit This does not necessarily mean that all the chemical constituents other than cytochromes are different (e g , only primary electron acceptor may be different in two types of photosynthetic unit as will be discussed in a forthcoming paper) In a wider sense, this mechanism is included in the multi-light reaction theories in bacterial photosynthesis described earlier [15, 34, 35]

It is proposed that only cytochrome *c*-555 is oxidized by the P890 molecule which belongs to the photosynthetic unit containing cytochrome *c*-555, at higher redox potential The overall quantum yield for photooxidation of cytochrome *c*-555 is low Light quanta absorbed by or transferred to a photosynthetic unit which does not contain cytochrome *c*-555 cannot be utilized for photooxidation of cytochrome *c*-555 Consequently, the observed quantum yield (0 4) for cytochrome *c*-555 may imply that the fraction of the photosynthetic unit containing cytochrome *c*-555 is at least 0 4

Although in the present paper the experimental foundation for the above-mentioned proposition is not firm enough, this hypothesis can be supported by additional data which will be published in the near future

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