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NATURE OF PHOTOCHEMICAL REACTIONS IN CHROMATOPHORES OF *CHROMATIUM* D

III. HETEROGENEITY OF THE PHOTOSYNTHETIC UNITS

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SUMMARY

The effect of isooctane extraction on photooxidation of *c*-type cytochromes was investigated in *Chromatium* chromatophores.

Photooxidation of cytochrome *c*-555 was not affected by isooctane-extraction except that the dark recovery was accelerated. Photooxidation of cytochrome *c*-552 was abolished by thorough extraction of ubiquinone-7, but the quantum yield of the cytochrome photooxidation remained unchanged until 90 % of the total ubiquinone was extracted. The photooxidation of cytochrome *c*-552 was recovered by the addition of ubiquinone-7 but not by menaquinone. A dark incubation of sufficient length was needed for maximal quantum yield of cytochrome *c*-555 photooxidation in the presence of 30 mM ascorbate.

It is proposed that there are two types of photosynthetic units (or associations of molecules involved in the primary redox reactions) in *Chromatium* chromatophores. The combinations of primary electron donor-reaction center chlorophyll-primary electron acceptor may be cytochrome *c*-552-P890-ubiquinone in one type and cytochrome *c*-555-P890-X in another.

INTRODUCTION

For the last ten years, the primary photochemical events in bacterial photosynthesis have been studied intensively. Cytochrome photooxidation at extremely low temperatures, first found in *Chromatium* [1, 2], was recognized as the general phenomenon observed in various species of photosynthetic bacteria [3]. The use of laser flashes made it possible to measure extremely fast photochemical reactions separated from subsequent slower processes. It was confirmed by Parson [4] that on illumination, charge separation between the reaction center chlorophyll (P) and the primary electron acceptor occurred first and the oxidized P subsequently oxidized cytochrome(s) as suggested earlier by Duysens [5]. Kinetic characteristics of the primary and secondary electron acceptors were also investigated by means of a "double flash technique" [6, 7].

Isolation and characterization of reaction centers from photosynthetic bacteria have been extensively carried out [8–12], and the nature of primary photochemical events has been investigated in isolated reaction center preparations [13–16]. All of the reaction center preparations isolated exhibited photobleaching of P870 or P890, indicating the presence of electron acceptor(s) to permit the oxidation of reaction center bacteriochlorophyll. The chemical entity of the primary electron acceptor has been studied by chemical and EPR analyses [12, 14–17]. At present, iron-sulfur protein and ubiquinone are the most likely candidates for the primary electron acceptor in bacterial photosynthesis [14–19]. However, Clayton and Straley indicated that the primary electron acceptor may not be a distinct molecule but rather a special configuration of protein molecules associated with the reaction center [20].

In a preceding paper [21], we suggested that in *Chromatium* chromatophores cytochrome *c*-555 and cytochrome *c*-552 were photooxidized in different photosynthetic units as opposed to the assumption that cytochrome *c*-555 and *c*-552 were photooxidized by one and the same reaction center [6, 7, 22–24].

In the present study, effects of isooctane extraction and readdition of ubiquinone and menaquinone on the quantum yield of photooxidation of both cytochromes in *Chromatium* chromatophores were investigated in order to elucidate the difference between the chemical constituents of the two types of photosynthetic units.

MATERIALS AND METHODS

Preparation of chromatophores

Chromatium D was grown photoautotrophically as previously described [21]. Chromatophores were isolated after the disruption of cells in a French pressure cell at 300 kg/cm^2 and by fractionation as described previously [21].

Isooctane extraction

An outline of the extraction procedure has been described elsewhere [18]. Fresh chromatophore suspension was recentrifuged at 80 000 $\times g$ for 1 h and the precipitated chromatophores were resuspended in a small volume of distilled water and lyophilized. Isooctane dried by anhydrous sodium sulfate was added immediately to the lyophilized chromatophores (100 ml isooctane/μmol bacteriochlorophyll). The extraction was carried out with magnetic stirring at 20 °C. Usually, after a 3 h-extraction, the mixture was centrifuged at low speed and the pellet was dispersed in the same volume of fresh isooctane and the extraction repeated overnight. The mixture was centrifuged and the pellet resuspended in the same volume of fresh isooctane and extracted further for a full day. When the extraction was almost complete, the final isooctane extract was colorless and the extracted chromatophores were greenish gray in color. Even when the isooctane extract was colorless, dark purple or purple pellet indicated that the extraction was not complete. The thoroughly extracted pellet was washed with a small volume of fresh isooctane and dried completely in vacuo. The dried pellet was resuspended in 60 mM phosphate buffer, pH 7.4, containing 0.25 M sucrose and 17 mM NaCl. The suspension was sonicated briefly and the chromatophores were homogeneously dispersed. The suspension was designated as the extracted chromatophore suspension. As a control, lyophilized chromatophores were resuspended in the same buffer (control chromatophores).

Determination of quinones

The isooctane extracts were combined and evaporated in vacuo in a rotary evaporator. The concentrated extract was spotted onto a thin layer of silica gel and developed with chloroform. Bands of ubiquinone-7 and menaquinone were scraped off and extracted with ethanol three times. Ubiquinone concentration was determined by the difference of absorbance at 275 nm before and after the addition of sodium borohydride. In order to detect any remaining ubiquinone in the extracted chromatophores, an aliquot of extracted chromatophores was further extracted by an acetone/methanol mixture (7 : 2, v/v) three times and the combined extracts were evaporated and spotted onto the thin layer of silica gel. Leuco-methylene blue was sprayed onto the developed chromatogram. The sensitivity of this detecting method was 1–0.1 nmol ubiquinone [25, 26]. Unless otherwise stated, less than 1 % of the total ubiquinone remained in the extracted chromatophores.

Reconstitution of chromatophores

Readdition of pure ubiquinone or menaquinone was carried out as follows: ubiquinone-7 or menaquinone in the amount comparable to that found in normal chromatophores was dissolved in the smallest volume of isooctane and added back to the isooctane-washed pellet (see above). The pellet was dispersed and dried in vacuo. Completely dried pellet was suspended in 60 mM phosphate buffer pH 7.4, containing 0.25 M sucrose and 17 mM NaCl. The ubiquinone- or menaquinone-readded chromatophores were homogeneously dispersed by a brief sonication.

Concentration of bacteriochlorophyll was determined as described previously [21].

Spectroscopic measurements

In spectroscopic measurements, an aliquot from each stock chromatophore suspension was diluted by 60 mM phosphate buffer pH 7.4, containing 0.25 M sucrose and 17 mM NaCl.

Absorption spectra of chromatophore suspensions and light-induced oxidation of cytochromes were measured with a Hitachi 356 two-wavelength, double-beam spectrophotometer as reported earlier [21], except that in most cases the light-induced absorption changes due to cytochrome photooxidation were measured with the "dual beam" mode with a reference wavelength at 440 nm.

RESULTS

Of the two cytochromes (*c*-555 and *c*-552) in *Chromatium* chromatophores, as only cytochrome *c*-555 is reduced in the dark in the presence of ferricyanide/ferrocyanide mixtures and both cytochromes are reduced in the dark in the presence of ascorbate, we can investigate the effect of isooctane extraction on photooxidation of each cytochrome. Fig. 1 shows the time courses of light-induced absorption decreases due to the photooxidation of cytochrome *c*-555 in lyophilized (control), isooctane-extracted, ubiquinone-readded (hereinafter referred to as "reconstituted") and menaquinone-readded chromatophores in the presence of 1 mM ferrocyanide and 10 μM ferricyanide. The dashed lines represent the initial slopes of the absorption decrease. Relative values of slopes are also indicated in parentheses. Small variations

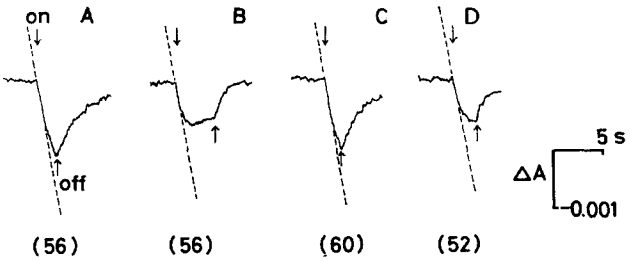

Fig. 1. Time courses of cytochrome photooxidation in *Chromatium* chromatophores. Absorption changes at 423 nm in the presence of 1 mM ferrocyanide and 10 μM ferricyanide. (A) control (lyophilized) chromatophores, absorption at 890 nm is 46 %; (B) extracted chromatophores, absorption at 890 nm is 41 %; (C), reconstituted chromatophores (extracted + ubiquinone), absorption at 890 nm is 38 %; (D) menaquinone-readded chromatophores, absorption at 890 nm is 39 %. Downward deflection corresponds to cytochrome oxidation. "On" and "off" indicate the time when the actinic light of 890 nm was turned on and off, respectively. Intensity of the incident actinic light of 890 nm was 5.5×10^{13} quanta/cm^2/s. The dashed lines indicate initial slopes of the "light on" traces (relative initial rates are shown in parentheses).

in the light absorption at 890 nm of these chromatophores were not taken into account to correct the relative rates of photooxidation. As the absorption (100 % – transmittance) of these chromatophores at 890 nm (1-cm light path) was in the range of 46 %–38 %, it is indicated that the initial rate or quantum yield of cytochrome *c*-555 photooxidation is not practically affected by the isooctane-extraction or the readdition of ubiquinone or menaquinone. (At this level of actinic light, the rate of cytochrome photooxidation is limited by the light intensity and is approximately linear with it [21].) Half-recovery time of photooxidized cytochrome *c*-555 was shortened by the isooctane extraction (2 s → 0.6 s). The half-recovery time was increased by readding ubiquinone (0.6 s → 1.5 s) but not by menaquinone (0.6 s → 0.7 s). The decrease of half-recovery time by extraction may explain the decrease of magnitude of light-induced absorption changes (steady state) in the extracted and the menaquinone-readded chromatophores (Fig. 1B and 1D).

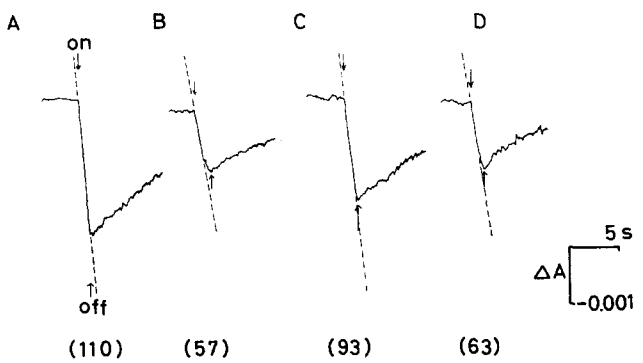

Fig. 2. Time courses of cytochrome photooxidation in *Chromatium* chromatophores. Absorption changes at 423 nm in the presence of 30 mM ascorbate. Experimental conditions are the same as in Fig. 1 except for the presence of 30 mM ascorbate instead of 1 mM ferrocyanide and 10 μM ferricyanide.

TABLE I

RELATIVE QUANTUM EFFICIENCY OF CYTOCHROME OXIDATION

Experiments were carried out on control, extracted, ubiquinone-readded and menaquinone-readded chromatophores. Values of quantum efficiency ($\Delta A_{423} \cdot s^{-1}$/number of absorbed quanta at 890 nm · $cm^{-2} \cdot s^{-1}$) were calculated from initial rates of light-induced absorption decrease. Relative values are given in parentheses.

Type of chromatophore	($\Delta A \cdot s^{-1}$/number of absorbed quanta at 890 nm · $cm^{-2} \cdot s^{-1}$) × 10^{17}	
	30 mM ascorbate	1 mM ferrocyanide + 10 μM ferricyanide
Control	9.9 (1.00)	5.1 (0.52)
Extracted	5.0 (0.50)	5.8 (0.59)
Ubiquinone-readded	8.5 (0.85)	6.3 (0.64)
Menaquinone-readded	5.9 (0.60)	6.1 (0.62)

In the presence of 30 mM ascorbate, as reported previously [21], the initial rate of absorption decrease in the control (lyophilized) chromatophores was about twice that in the presence of the ferrocyanide/ferricyanide couple (Fig. 2A). On the contrary, the initial rate of absorption change in the extracted chromatophores remained low in 30 mM ascorbate (Fig. 2B). The initial rate of absorption change in the extracted chromatophores in the presence of 30 mM ascorbate was restored by the readdition of ubiquinone (Fig. 2C), but not by the addition of menaquinone (Fig. 2D). Half-recovery times of the chromatophore preparations of four types were approximately in the same range in 30 mM ascorbate (7–10 s). In Table I, values of relative quantum efficiency of cytochrome oxidation ($\Delta A \cdot s^{-1}$/number of absorbed quanta at 890 nm · $cm^{-2} \cdot s^{-1}$) calculated from the initial slopes in Figs 1 and 2 (average of three measurements) are compared. In the presence of 1 mM ferrocyanide and 10 μM ferricyanide, extraction by isooctane and readdition of ubiquinone or menaquinone had little effect on the relative quantum efficiency. In the presence of 30 mM ascorbate, the high efficiency of the control (lyophilized) chromatophores was lowered by isooctane extraction to one-half (the level in the presence of 1 mM ferrocyanide and 10 μM ferricyanide). Readdition of ubiquinone considerably recovered the decreased initial rate but that of menaquinone did not. The results obtained in the measurements at lower redox potentials such as −49 mV and −52 mV were similar to those obtained in the presence of 30 mM ascorbate. As all the cytochromes are in the reduced form at these redox potentials, the decrease in the initial rate of cytochrome photooxidation by extraction should be due to the lower efficiency of the cytochrome photooxidation itself.

In order to determine if both cytochromes *c*-555 and *c*-552 are photooxidized in the extracted chromatophores in the presence of 30 mM ascorbate, we measured the light-minus-dark (steady state) difference spectra of the extracted chromatophores at the actinic light intensity similar to that used for measuring initial rates of cytochrome oxidation. A comparison of light-minus-dark difference spectra in the alpha region of cytochromes in the extracted and the reconstituted chromatophores is presented in Fig. 3. The difference spectrum of the extracted chromatophores (b in Fig. 3) had a minimum at 555–556 nm in 30 mM ascorbate. The difference spectrum

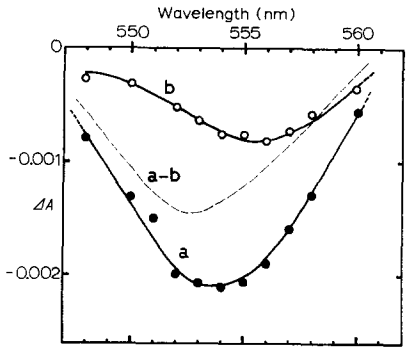

Fig. 3. Light-minus-dark difference spectra (steady state) of reconstituted and extracted chromatophores. ●—● (curve a), reconstituted chromatophores in the presence of 30 mM ascorbate. Concentration of bacteriochlorophyll, 16 μM. ○—○ (curve b), extracted chromatophores in the presence of 30 mM ascorbate. Concentration of bacteriochlorophyll, 16 μM. Extent of the first light-induced absorbance decrease was measured with different aliquots taken from the same suspension of reconstituted and extracted chromatophores after a dark preincubation for 5 min in the presence of 30 mM ascorbate (renewing sample after each measurement). Curve a—b represents the curve obtained by subtracting curve b from curve a.

of the reconstituted chromatophores (a in Fig. 3) in the presence of 30 mM ascorbate had a minimum at 553–555 nm and the band was noticeably broader than that of the extracted chromatophores. On the basis of concentration of bacteriochlorophyll, the extent of the light-induced absorbance change of the extracted chromatophores in the presence of 30 mM ascorbate was about one-third that of the reconstituted chromatophores in the presence of 30 mM ascorbate. Curve "a–b" in Fig. 3 represents the "difference spectrum" (a–b) between the two difference spectra. The curve had a minimum at 552–553 nm suggesting that in the extracted chromatophores, even in 30 mM ascorbate, only cytochrome *c*-555 was efficiently photooxidized. A similar result was obtained in experiments of the Soret region. The control chromatophores had a minimum at about 423 nm in 30 mM ascorbate, whereas in the extracted chromatophores the steady-state absorbance change was about one-third of that of the control and the minimum was located at 422–423 nm (not shown here). At the same time, it was noticed that in the presence of ascorbate, the fractions of reduced forms of cytochromes *c*-552 and *c*-555 were similar both in the reconstituted and the extracted chromatophores before illumination (not shown here), and dark recovery times of cytochrome photooxidation were in the same range (7–9 s) as shown in Fig. 2. These data are interpreted as the evidences that ubiquinone is required for an efficient photooxidation of cytochrome *c*-552 but not for cytochrome *c*-555 photooxidation. We investigated next the relationship between the quantum efficiency of cytochrome oxidation and the extent of ubiquinone extraction. In these experiments, an aliquot was removed from chromatophore suspension at an appropriate interval during isooctane extraction. The aliquot was centrifuged and the supernatant isooctane solution was concentrated in vacuo. Ubiquinone content in the isooctane solution was determined as described in Materials and Methods. The precipitated pellet was dried in vacuo and resuspended in the phosphate buffer. The total content of ubiquinone in chromatophores was determined on the bacteriochlorophyll basis after extraction of lyo-

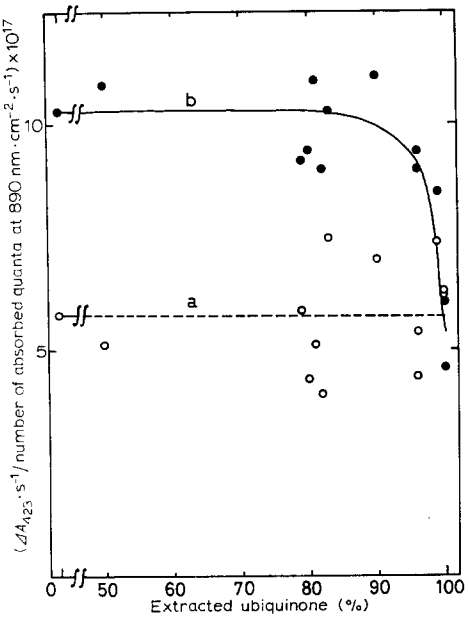

Fig. 4. Relationship between relative quantum efficiency of cytochrome oxidation and fraction of extracted ubiquinone. Ordinate indicates relative quantum efficiency ($\Delta A_{423} \cdot s^{-1}$/number of absorbed quanta $\cdot cm^{-2} \cdot s^{-1}$). Abscissa indicates percentage of extracted ubiquinone to total ubiquinone. ○- - -○ (curve a), in the presence of 1 mM ferrocyanide and 10 μM ferricyanide. ●—● (curve b), in the presence of 30 mM ascorbate. Each point indicates average of three determinations in two different series of experiments.

philized chromatophores by an acetone/methanol mixture (7 : 2, v/v). Chromatophores exhaustively extracted by isooctane were further extracted by the acetone/methanol mixture and the concentrated extract was chromatographed on a silica gel plate. If a blue spot of methylene blue did not appear when spraying the thin-layer chromatogram with leuco-methylene blue, we assumed that ubiquinone was completely extracted from chromatophores by isooctane extraction. In this case, the amount of isooctane-extracted ubiquinone was equal to the total amount of ubiquinone determined by the acetone/methanol method, within experimental error. The relative quantum efficiency of cytochrome oxidation in the presence of 1 mM ferrocyanide and 10 μM ferricyanide was independent of the fraction of extracted ubiquinone (Fig. 4, Curve a). Thus, we confirmed that the quantum yield of cytochrome *c*-555 photooxidation was not affected by ubiquinone. In 30 mM ascorbate, the quantum efficiency of the cytochrome oxidation did not decrease until about 90 % of the total ubiquinone was extracted (Fig. 4, Curve b). In the chromatophores from which ubiquinone was completely extracted, the quantum efficiency was low but still comparable to those in the presence of 1 mM ferrocyanide and 10 μM ferricyanide (see also Table I). Thus, ubiquinone seemed to be essential to the photooxidation of cytochrome *c*-552.

In the control and the reconstituted chromatophores in the presence of 30 mM ascorbate, as previously reported [21], light-induced cytochrome photooxidation was

reversible and a constant initial rate of light-induced absorption decrease was observed when the dark interval was about 2 min in repeated illumination-dark cycles. But in the cases of extracted and menaquinone-readded chromatophores, the initial rate of cytochrome oxidation on the second illumination was considerably lower than that on the first illumination even after the oxidized cytochrome was completely reduced in the dark before the second illumination. It should be noted that for this reason all the data with extracted and menaquinone-readded chromatophores in the presence of 30 mM ascorbate in Figs 2–4 and Table I were obtained from the initial rates during first illumination after a sufficiently long dark preincubation. The intensity of the measuring beam (423 nm) was kept sufficiently low to avoid the lowering of the apparent quantum yield for the cytochrome photooxidation.

DISCUSSION

In our previous paper [21] we proposed that cytochrome *c*-555 and cytochrome *c*-552 in *Chromatium* chromatophores were photooxidized by two different photosynthetic units. If the interpretation is correct, what would be the difference between two types of photosynthetic units besides cytochromes? From our previous and present data it is probable that the photosynthetic unit which can photooxidize cytochrome *c*-552 has ubiquinone as the primary electron acceptor and the other photosynthetic unit which can oxidize cytochrome *c*-555 has a substance other than ubiquinone as the primary electron acceptor. In other words, photosynthetic units in *Chromatium* chromatophores are not homogeneous concerning the chemical compositions of donor-reaction center chlorophyll-acceptor triplets. A few points about this proposition are discussed here.

First, 10 % of the total ubiquinone (the critical level for the oxidation of cytochrome *c*-552) is not a small amount compared with the content of P890 in *Chromatium* chromatophores, i.e. the molar ratio of ubiquinone to bacteriochlorophyll is 0.15 in *Chromatium* cells (ref. 27, see also ref. 28) and 0.2 in *Chromatium* chromatophores (unpublished data, see also ref. 28), whereas the molar ratio of P890 to bacteriochlorophyll in *Chromatium* chromatophores is 0.01–0.02 (unpublished data). Thus, 10 % of the total ubiquinone still has a large number of molecules to be a candidate for the primary electron acceptor in photooxidation of cytochrome *c*-552. Second, as for the role of ubiquinone as the secondary electron acceptor or in the pool of the electron transfer chain [13, 20, 29–31], a discussion may be necessary. If all of the ubiquinone serves as a secondary electron acceptor, the decrease in quantum yield of cytochrome *c*-552 photooxidation by the extraction of ubiquinone leads us to accept the possibility that an unknown primary electron acceptor present in a small amount is reduced by the reaction center chlorophyll and that it rapidly transfers electrons to ubiquinone. The upper limit of the amount of the unknown primary electron acceptor, if present, is calculated to be 1/20–1/50 of total P890, based on the number of absorbed quanta per cm^2 per s and on the sensitivity of the detecting system for the light-induced absorption change at 423 nm at low actinic light levels. However, from the data of Parson [4], Parson and Case [24] and Seibert and DeVault [22], it is indicated that the amount of the primary electron acceptor is approximately equal to or larger than that of P870, on a molar basis, in *Chromatium* chromatophores.

Another possibility remains that the rate of back reduction of oxidized P890

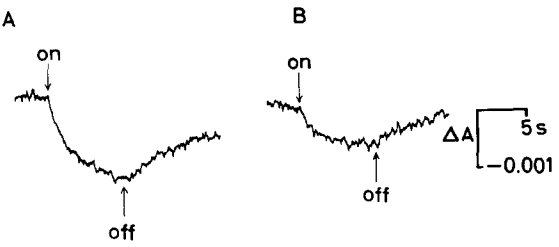

Fig. 5. Time courses of P890 photooxidation in the reconstituted and extracted chromatophores. Absorbance changes at 885 nm in the presence of 1 mM ferrocyanide and ferricyanide. (A) reconstituted chromatophores (extracted + ubiquinone) absorption at 590 mn was 26 %; (B) extracted chromatophores, absorption at 590 nm was 31 %. "On" and "off" indicate the time when the actinic light at 590 nm was turned on and off, respectively. Downward deflection corresponds to P890 oxidation. Intensity of incident light of 590 nm was 1.32×10^{14} quanta/cm^2/s.

by the reduced primary electron acceptor is accelerated by removing a secondary electron acceptor, ubiquinone, resulting in the partial decrease of photooxidation of cytochrome *c*-552. Although we cannot yet exclude this possibility definitely, Dutton et al. indicated that the rate of charge recombination of oxidized P890 and the reduced primary electron acceptor was much slower than that of electron donation from reduced cytochrome *c*-552 to oxidized P890 in *Chromatium* chromatophores [17]. One may still doubt whether the photooxidation of cytochrome *c*-552 was not detected with the response time of the instrument used because of the rapid dark reduction of photooxidized cytochrome *c*-552 in the extracted chromatophores. Therefore, we modified the Hitachi 356 spectrophotometer to give a single-beam, enabling us to measure rapid reactions with a time constant of about 1 ms. Cytochrome species oxidized and the relative quantum yield of cytochrome photooxidation observed by this procedure were not different from those obtained by the routine method.

If our present proposition is correct, the quantum yield of photooxidation of P890 in *Chromatium* chromatophores is expected to become lower by a complete extraction of ubiquinone from chromatophores and to recover by a readdition of ubiquinone. Our preliminary measurements showed that the initial rate of P890 photobleaching (or the quantum yield of P890 photooxidation) at higher potential (E_h of about +400 mV, at which potential all cytochromes are oxidized) decreased after an extraction of ubiquinone and it was partially recovered by a readdition of ubiquinone. The initial rate of absorbance change at 885 nm in the extracted chromatophores was about half of that in the reconstituted chromatophores (Fig. 5). In contrast to the results obtained in the measurements with laser flash [32], the rate of dark recovery of photooxidized P890 was not much diminished by ubiquinone extraction (in both the reconstituted and extracted chromatophores, the half-recovery times were in the range of several seconds). Thus, the dark recovery virtually did not affect the measurements of quantum yield of P890 photooxidation in the present study.

As there is no basis on which to assume that the unknown primary electron acceptor has the same normal redox potential as that of ubiquinone, it may be expected that light-induced absorption changes of cytochromes and reaction-center chlorophyll photooxidation versus redox potential (E_h) curves have two inflection points in the region of lower potential (below 0 mV). Though such experimental

evidence has not yet been fully documented in many species of photosynthetic bacteria [33–35], recently, Seibert et al. [36] suggested that in *Chromatium* chromatophores, a low-potential light reaction operates besides the widely recognized light reaction.

The suggestion that ubiquinone is the primary electron acceptor has appeared repeatedly [15, 16, 18, 19] since Clayton's first proposal [37]. Recently, a substance other than ubiquinone has been suggested as the primary electron acceptor [17, 38]. The existence of two types of photosynthetic units, in which different modes of molecular associations involving a primary electron acceptor and cytochrome are present, is highly probable in *Chromatium* chromatophores. Of the two types of molecular associations in the vicinity of reaction centers such as cytochrome *c*-552-P890-ubiquinone and cytochrome *c*-555-P890-X, it is possible that only one type of photosynthetic unit or reaction center preparation is easily purified during preparation of reaction centers.

Recently, Ke et al. described that exhaustively extracted *Chromatium* subchromatophore particles still showed light-induced absorption changes due to P890 and cytochrome(s) [39]. They suggested that ubiquinone was not the primary electron acceptor. From our point of view, however, it is possible that they observed the light reaction of the photosynthetic unit of a type which contains a primary electron acceptor other than ubiquinone.

More recently, Halsey and Parson [32] investigated the effect of ubiquinone extraction on the photooxidations of P890 and cytochrome *c*-555 by laser flash in *Chromatium* chromatophores. They observed that the magnitude of the oxidation of cytochrome *c*-555 by the first flash was not decreased by ubiquinone extraction, but the oxidation by the second flash at 15 ms after the first flash was abolished by the extraction. The abolished oxidation of cytochrome *c*-555 was recovered by readding ubiquinone. They suggested that ubiquinone was the secondary electron acceptor for cytochrome *c*-555 photooxidation. Their suggestion does not contradict our present result that cytochrome *c*-555 photooxidation is not affected by extracting ubiquinone.

Finally, we must comment on the differences between our data and the results from measurements with the laser pulse. One saturating laser pulse oxidized all P890 at higher redox potentials and in the succeeding dark period all oxidized P890 was reduced by cytochrome *c*-555 in *Chromatium* chromatophores [24]. As Halsey and Parson observed, extraction of ubiquinone did not decrease the extent of laser-flash photooxidation of P890 [32]. At present, these observations cannot be reasonably explained by our hypothesis. There are some other experimental disagreements with the results obtained by laser pulse. For example, as pointed out previously [21], at lower redox potentials (below 0 mV), the weak continuous illumination oxidized both cytochromes *c*-555 and *c*-552, whereas one saturating laser pulse oxidized only cytochrome *c*-552. These discrepancies may be attributed, at least in part, to the difference in experimental conditions, i.e., strong laser pulse and weak continuous illumination. Detailed experiments focused on these points are needed.

ACKNOWLEDGEMENTS

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