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LIGHT-INDUCED ABSORPTION CHANGES IN *CHROMATIUM* SUBCHROMATOPHORE PARTICLES EXHAUSTIVELY EXTRACTED WITH NON-POLAR SOLVENTS*

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SUMMARY

Chromatium subchromatophore particles completely freed of ubiquinone were prepared by Triton treatment preceeded by an exhaustive extraction with non-polar solvents at different stages of their preparation. Reconstituted particles were prepared by recondensing appropriate amounts of the quinone extract onto the extracted particles. The following observations are made and conclusions drawn from the light-induced absorption changes.

1. In the extracted particles, where cytochrome-422 is present in the oxidized state, light causes charge separation, which is followed by charge recombination in the dark with a half time of 20 ms; the light-*minus*-dark difference spectrum between 240 and 950 nm is essentially the same as that of the unextracted subchromatophore particles.

2. In the reconstituted particles, presumably because of the presence of ubiquinone as the secondary electron acceptor, charge recombination is prevented. After addition of 1 mM *o*-phenanthroline, which blocks electron transfer from the primary reductant to the secondary acceptor, charge recombination is re-established, as indicated by the return of the decay kinetics to the 20-ms half time.

3. The room-temperature light-*minus*-dark difference spectrum of the extracted particles indicates that the majority of the absorption decrease at 280 nm may be attributed to P890 photooxidation and not ubiquinone reduction. This is further confirmed by the fact that the light-*minus*-dark difference spectrum at 77 °K is essentially the same for extracted and unextracted particles.

4. If the oxidized cytochrome-422 in the extracted or reconstituted particles is reduced chemically, the reduced cytochrome can couple to the photooxidized $P890^+$, leading to its own oxidation and the reduction of $P890^+$ in the dark.

5. With the reconstituted particles, a steady, slow absorption decrease associated with the reduction of ubiquinone can be observed in the ultraviolet region when an electron donor such as *N,N,N',N'*-tetramethylphenylenediamine is present. No such reaction occurs in the extracted particles.

Abbreviation: TMPD, *N,N,N',N'*-tetramethylphenylenediamine.

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6. Studies with the extracted particles have provided evidence that endogenous ubiquinone does not play the role of a primary electron acceptor in bacterial photosynthesis. Recent trends in the studies of the primary electron acceptor and possible approaches to the problem are briefly commented upon.

INTRODUCTION

Light-induced absorption changes in the ultraviolet region have been observed in photosynthetic bacterial chromatophores since 1962[1,2]. Because of their resemblance to the reduced-*minus*-oxidized difference spectrum of ubiquinone in an organic solvent, the light-induced absorption decrease in the 270–280-nm region has often been assigned to the redox reactions of ubiquinone, a component known to be present in photosynthetic bacteria.

In 1968, Ke *et al.*[3] examined the absorption changes in a photochemically active subchromatophore particle derived from *Chromatium* by Triton treatment over a spectral region from the near infrared to the ultraviolet. The light-*minus*-dark difference spectrum in the near-infrared and visible regions was typical that for the photooxidation of the reaction-center bacteriochlorophyll, P890, and that in the ultraviolet region again resembled that of ubiquinone reduction. The near-infrared and ultraviolet changes were affected almost equally by pH of the medium and by heat treatment, and both occurred at 77 °K. These observations together with the fact that the absorption changes in the near-infrared and ultraviolet regions had practically identical kinetics prompted the suggestion that the changes represent, respectively, the photooxidation of P890, and the photoreduction of ubiquinone, presumably in a primary photochemical reaction. However, one inconsistency persisted: the quantum yield for ubiquinone photoreduction was twice that for P890 photooxidation when the normal extinction coefficient of ubiquinone in an organic solvent was assumed (*cf.* also ref. 1). A similar observation on such a quantum yield anomaly was reported independently about the same time by Beugeling[4] for ferricyanide-treated chromatophores of *Rhodospirillum rubrum*.

A role for ubiquinone as the primary electron acceptor, however, was soon questioned on some other experimental grounds. Ke[5] found that the ultraviolet absorption change disappeared when ubiquinone was chemically reduced by ascorbate, without affecting the P890-photooxidation absorption change. Reed *et al.*[6] found that when the medium containing *Rhodopseudomonas spheroides* reaction centers was poised at a sufficiently negative redox potential, the ultraviolet absorption change similarly disappeared, but the fluorescence-yield transient, which is an indicator of the primary photochemical reaction, still remained.

To gain more insight into this problem, *Chromatium* subchromatophore particles completely freed of ubiquinone were subsequently prepared by Triton treatment preceeded by an exhaustive extraction of the chromatophores with nonpolar solvents at different stages of their preparation. These ubiquinone-free subchromatophore particles were photochemically active and exhibited light-induced absorption changes with a difference spectrum very similar to that of the unextracted subchromatophore particles containing ubiquinone. Since the extracted particles were known to be completely free of ubiquinone, it was speculated[7] at that time that one

possible candidate producing the prominent ultraviolet absorption changes might be the pteridines, as some pteridines are known to show similar absorption changes upon reduction. However, no additional experimental support was available for the latter assignment.

Takamiya and Takamiya[8] independently reported similar observations from *Chromatium* chromotophores which had been extracted with isooctane. They observed that light-induced absorption changes in the near-infrared as well as the ultraviolet region were not affected by extraction of less than 80% ubiquinone; and even after extraction of 99% of the quinones from chromatophores, nearly 50% of these absorption changes remained.

Model studies of reversible one-electron oxidation of bacteriochlorophyll by Fuhrop and Mauzerall[9,10] have provided support for the assignment of the photo-induced absorption changes in the near-infrared and visible regions to the *in vivo* reaction-center bacteriochlorophyll, P890, in chromatophores. Subsequently, Loach *et al.*[11] extended the bacteriochlorophyll model studies into the ultraviolet region. Their results clearly showed that except for the major red shift of the near-infrared band in chromatophores, there was a striking resemblance between the redox difference spectrum of bacteriochlorophyll in methanol and the light-*minus*-dark difference spectrum of chromatophores of *Chromatium* and *Rsp. rubrum*; the majority of the light-induced ultraviolet absorption changes in the chromatophore preparations can be accounted for by the photooxidation of bacteriochlorophyll.

In view of these findings, we thought it would be of interest to re-examine the ultraviolet absorption changes in *Chromatium* subchromatophore particles which had been exhaustively extracted with non-polar solvents to completely remove the quinones. Presently available evidence indicates that the major portion of the ultraviolet absorption changes observed previously[3] originated from the bacteriochlorophyll pigment, and that ubiquinone absorption changes may be observed in a secondary reaction with much slower kinetics.

EXPERIMENTAL

Chromatium strain D was grown according to the directions of Hendley[12] under weak light. Washed *Chromatium* cells were lyophilized and subsequently extracted 15 times with a benzene–light petroleum mixture (1:1, v/v), then 10 times with pure benzene, followed by 10 times extraction with pure petroleum ether. The extracted cells were resuspended in Tris buffer, sonicated and centrifuged to yield chromatophores. The chromatophores were treated with 4% Triton X-100 (Rohm and Haas) and fractionated in a sucrose-density gradient to obtain a heavy brown band as described previously[3]. This brown fraction was oxidized with K_2IrCl_6 and the reaction stopped by the addition of excess $K_4Fe(CN)_6$. The mixture was dialyzed against 0.001 M Tris at pH 7.6 and then lyophilized. The lyophilized material was again treated with 4% Triton and refractionated on a sucrose-density gradient to remove additional solubilized bulk bacteriochlorophyll. This purified brown fraction was again dialyzed against water and extracted 10 times with pure *n*-hexane, 10 times with a *n*-hexane–benzene mixture (1:1, v/v) 5 times with trimethyl pentane, 10 times with benzene, and finally 10 times with light petroleum. The resulting subchromatophore fraction was designated as H_{ex} (H for the heavy fraction on the sucrose gradient).

To reconstitute the subchromatophore particles, the organic solvent extracts containing the quinones were combined, evaporated to dryness under a stream of high-purity argon, and dissolved in a known volume of petroleum ether. An appropriate amount of the redissolved quinone extract was recondensed onto the H_{ex} preparation described above to yield the reconstituted fraction (designated as $H_{ex}+Q$) with a quinone content comparable to that in the unextracted cells.

The technique of kinetic spectrophotometry has been described previously[3,5,13]. Q-switched ruby-laser flashes have been used as a routine excitation source. For steady illumination, near-infrared light isolated by a broad-band interference filter (875 ± 30 nm, Baird Atomic) and a shutter was used. A Bausch and Lomb monochromator was placed in front of the photomultiplier for measurements in the near-infrared region. For ultraviolet measurements, the WHS-200 deuterium lamp (Kern and Sprenger, Göttingen) or the ST-75 high-pressure mercury lamp (Quarzlampen, Hanau) was used in conjunction with a monochromator to produce the measuring light, and the Schott reflection-type interference filters were placed in front of the photomultiplier detector as a shield[3].

All chemicals were reagent grade and were used without further purifications

RESULTS AND DISCUSSION

Light-induced absorption changes in extracted (H_{ex}) and reconstituted ($H_{ex}+Q$) subchromatophore particles of Chromatium

Exhaustive extraction of *Chromatium* cells and chromatophores by organic solvents during detergent fractionation yielded the H_{ex} particles which contained no ubiquinone or naphthoquinone, as shown by spectrophotometric assay of the ethanol extract before and after addition of $NaBH_4$. Illumination of H_{ex} particles caused absorption changes, having a difference spectrum which is essentially identical to the detergent-fractionated but unextracted subchromatophore particles. Fig. 1 shows the absorption spectrum (top) for the H_{ex} fraction and its light-*minus*-dark difference spectrum (bottom) from 240 to 950 nm obtained by 2-s steady illumination at room temperature. The near-infrared region shows two absorption bands typical of bacteriochlorophyll in *Chromatium*. The ultraviolet band at 275 nm is mostly due to Triton, which largely masked the absorption by other components. Residual Triton, which apparently had replaced the lipids freed by extraction, could not be completely removed by prolonged dialysis.

The difference spectrum in the near-ultraviolet, visible and near-infrared regions is typical that of P890 photooxidation. In the far-ultraviolet region the absorption decreased at 278 and 325 nm and increased at 302 nm. The far-ultraviolet difference spectrum resembles that of a reduced-*minus*-oxidized difference spectrum of ubiquinone in an organic solvent, and this similarity had previously led to the assignment of absorbance changes in this spectral region to ubiquinone reduction[3]. Since ubiquinone and naphthoquinone were completely removed from these extracted subchromatophore particles, the assignment of these changes to ubiquinone is no longer tenable. As shown by evidence presented below, most of the ultraviolet absorption changes in the extracted particles as well as in the detergent-fractionated particles can be attributed to P890 photooxidation.

The H_{ex} fraction recombined with the lipid extracts (resulting in the $H_{ex}+Q$

fraction) also gave light-induced absorption changes, as shown in Fig. 2 by the dashed curve together with that of the H_{ex} fraction (solid curve) for comparison. Over most of the spectral regions, the difference spectra of the two samples are very similar; the slight difference in the magnitudes of the two spectra is most likely due to different bacteriochlorophyll contents of the two samples. The small differences between the far-ultraviolet difference bands of the two samples could be due to ubiquinone reduction in the $H_{ex}+Q$ particles when the spectrum was obtained by steady illumination (see below).

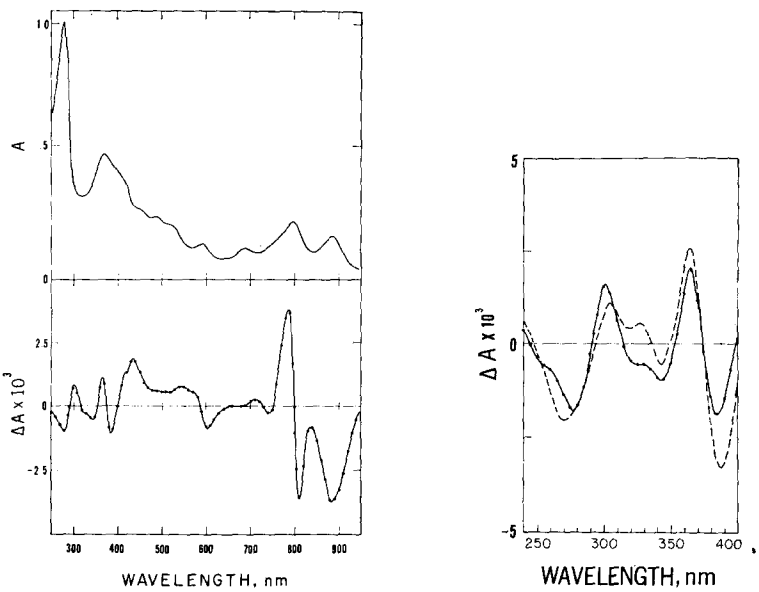


Fig. 1. Absorption spectrum (top) and the light-*minus*-dark difference spectrum (bottom) of the organic solvent-extracted *Chromatium* subchromatophore particles (H_{cx}).

Fig. 2. Light-*minus*-dark difference spectra of the organic solvent-extracted (H_{cx}) (solid curve) and the reconstituted ($H_{cx}+Q$) (dashed curve) *Chromatium* subchromatophore particles. Bacteriochlorophyll content: approximately 2 μg/ml.

Dark decay kinetic of the extracted and reconstituted subchromatophore particles and the effect of o-phenanthroline

In order to gain some more insights into the nature of these absorption changes, the kinetics of this reaction were examined. Fig. 3 shows the absorption-change transients at 870 and 280 nm induced by laser-pulse excitation. The 870-nm absorption decrease in the H_{ex} fraction occurred rapidly, and the rate was separately measured to be faster than 1 μs. The decay at 870 nm was monophasic with a $t_{1/2}$ of 20 ms and was completed in less than 100 ms. In the absorption-change transient for the same H_{ex} sample at 280 nm, about 80% of the change had kinetics identical to the 870-nm transient. The remaining portion of the absorption decrease recovered slowly.

The decay kinetics for the reconstituted $H_{ex}+Q$ preparation at 870 and 280 nm (Fig. 3, second row) are quite different. In the reconstituted particles, the decay at 870 nm was slower; at 280 nm, the slow decay (shown by the initial absorption

increase in the dark) appeared to be superposed by a slow absorption decrease running in the opposite direction. The slow absorption decrease presumably represents a secondary reaction of ubiquinone reduction (*cf.* Fig. 2). After addition of 1 mM *o*-phenanthroline to the reconstituted particles containing ubiquinone, decay at 870 nm became identical to that of the H_{ex} particles again, with a $t_{1/2}$ of 20 ms (Fig. 3, bottom). No effect on the decay kinetics of the absorption change of the H_{ex} fraction by *o*-phenanthroline was observed. Because of the high absorption by *o*-phenanthroline ($A > 10$), a reliable measurement of its effect on the 280-nm change in the reconstituted particles could not be made.

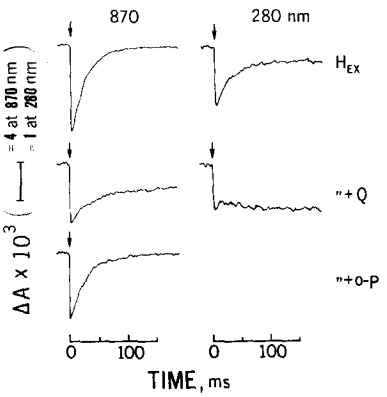


Fig. 3. Laser pulse-induced absorption-change transients of the extracted and reconstituted subchromatophore particles at 870 and 280 nm. Bacteriochlorophyll concentration same as in Fig. 2. *o*-Phenanthroline (*o*-P) concentration at 1 mM when present. See text for details.

The results presented thus far may be interpreted as follows: illumination of the H_{ex} particles induced the primary photochemical charge separation producing $P890^+$ and X^-, and this reaction accounts for the rapid absorption decreases at both 870 and 280 nm (at least 80% of the 280-nm decrease). When cytochrome-422 was present in the oxidized state, as was the case in the H_{ex} particles, and when the native secondary electron acceptor, presumably ubiquinone, was absent, the separated charges ($P890^+$ and X^-) recombined with a $t_{1/2}$ of 20 ms. These events are represented by the absorption-change transients in the top row of Fig. 3.

When ubiquinone is recondensed into the subchromatophore particles, and while cytochrome-422 is still in the oxidized state, the photoinduced charge species would have a different fate; the charge recombination would be truncated by electron discharge from X^- to the ubiquinone, accounting for the complex decay kinetics at 280 nm in the middle row of Fig. 3, and the partner $P890^+$ would have to seek some endogenous electron donor other than the cytochrome for its recovery (represented by the slower-decaying 870-nm transient in the middle row of Fig. 3).

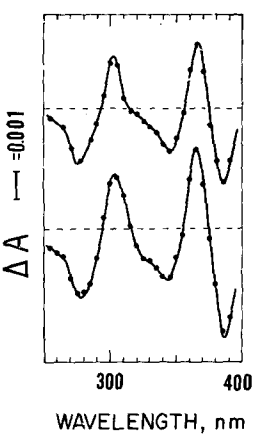
o-Phenanthroline has been shown to block electron discharge from the primary acceptor (X^-) to the secondary acceptor (presumably ubiquinone)[14,15]. The return of the kinetics of $H_{ex}+Q$ particles to exactly the original 20 ms upon addition of *o*-phenanthroline is consistent with the contention that electron discharge from X^- to ubiquinone was blocked, and since cytochrome-422 was in the oxidized state, charge recombination remained the only route for recovery.

Clayton *et al.*[15] recently reported a similar observation with the *Rps spheroides* reaction-center preparation, where 1 mM *o*-phenanthroline evidently blocked the electron transfer from the primary electron acceptor to the added ubiquinone, and the charge recombination was restored with a half time of 70 ms (compared to the original recombination time of 110 ms). They further found that *o*-phenanthroline had another distinctly different effect of preventing photochemistry. The slightly smaller magnitude of the transients in Fig. 3 measured in the presence of *o*-phenanthroline might be caused by such an effect.

Light-minus-dark difference spectra of extracted and unextracted subchromatophore particles of Chromatium at 77 °K

Taking the above interpretation, the absorption changes (Fig. 1, bottom) in the near-infrared and visible regions and a major portion of the absorption decrease in the 280-nm region, would represent a difference spectrum of P890 photooxidation. Further evidence supporting this contention has been obtained from the 77 °K light-*minus*-dark difference spectra for both extracted and unextracted subchromatophore particles derived from *Chromatium*, as shown in Fig. 4.

At 77 °K, all secondary electron-transport reactions are eliminated, and illumination causes a charge separation of the primary photochemical reaction, which is followed by charge recombination in the dark. Since the H_{ex} particles contain no ubiquinone, and since the difference spectra for the extracted and unextracted subchromatophore particles are essentially identical, the results in Fig. 4 lend additional and more decisive support to the interpretation that the 280-nm negative band in the difference spectrum is not caused by an ubiquinone reaction. Although the difference spectra in Fig. 4 were obtained by 2-s steady illumination, separate measurements showed the recombination times at 77 °K was comparable to that at room



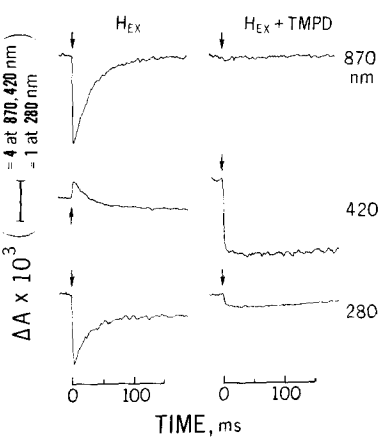


Fig. 4. Light-*minus*-dark difference spectra of unextracted (top) and organic solvent-extracted *Chromatium* subchromatophore particles (bottom) at 77 °K. Experimental procedures described in ref. 26; 2-s illumination (through a shutter) was used.

Fig. 5. Laser pulse-induced absorption-change transients of the extracted subchromatophore particles in the absence and presence of *N,N,N',N'*-tetramethylphenylenediamine ($2 \cdot 10^{-5}$ M). See text for details.

temperature. Also, although not presented in Fig. 4, both samples show absorption changes typical of P890 photooxidation in the other spectral regions at 77 °K.

Cytochrome-422 oxidation coupled to P890$^+$ reduction in the dark

In the extracted particles prepared by this procedure, cytochrome-422 was present in the oxidized state. If the cytochrome is chemically reduced, say, by *N,N,N',N'*-tetramethylphenylenediamine (TMPD), the resulting reduced cytochrome may now couple with the photooxidized P890$^+$, leading to its own oxidation and the re-reduction of P890$^+$ in the dark. These results are shown in Fig. 5 for the extracted H_{ex} particles and similar cytochrome coupling occurs in the $H_{ex}+Q$ particles.

The transients in the left column of Fig. 5 are the same as those shown in Fig. 3 with the additional transient at 420 nm and show that in the absence of cytochrome oxidation, an absorption increase occurred at 420 nm with kinetics which are also ascribable to P890 photooxidation. When $2\cdot10^{-5}$ M TMPD was added to the H_{ex} sample, cytochrome-422 became chemically reduced, and consequently coupled dark oxidation was observed, as shown by the 420-nm transient in the right column of Fig. 5. No light-induced absorption change was observed at 870 nm, but this is an instrumental artifact. Actually a rapid absorption decrease occurred with a very rapid recovery with $t_{1/2}$ of about 2 μs and a complete recovery in much less than 1 ms, and thus the signal was lost since the instrument time response was only 1 ms. The oxidation of cytochrome-422 occurred with a half time of 2 μs[5,17] corresponding to the decay of the absorption decrease at 870 nm, *i.e.* the re-reduction of P890$^+$.

Since a major portion of the absorption decrease at 280 nm was accounted for by P890 photooxidation, that portion should also be lost when the instrument time response of 1 ms was used. Therefore, the 280-nm transient remaining (Fig. 5, right column, bottom) under these conditions could be the portion of this same absorption change which exhibits the slow recovery, the nature of which is yet unknown. Since the kinetics of the 280-nm transient are similar to those of the 420-nm signal, part of the 280-nm change may also be the ultraviolet absorption change associated with cytochrome-422 oxidation. Although the exact oxidized-*minus*-reduced difference spectrum in the 280-nm region is not known for cytochrome-422 of *Chromatium*, the oxidized-*minus*-reduced difference spectrum of mammalian cytochrome *c* does show an absorption decrease at this wavelength[16].

Ubiquinone reduction as a secondary reaction; effect of ascorbate

As reported earlier[3], in studies with detergent-fractionated subchromatophore particles from *Chromatium*, when reduced forms of TMPD or 2,6-dichlorophenolindophenol or phenazine methosulfate was present, a steady, slow absorption decrease was also observed in addition to the rapid one. This slow absorption decrease was previously attributed to a bacteriochlorophyll-catalyzed electron transfer from the artificial donor to ubiquinone. With the extracted and reconstituted subchromatophore particles, it can now be confirmed that this steady, slow absorption decrease is associated with the reduction of endogenous ubiquinone.

In Fig. 6 are shown the light-induced absorption changes at 280 nm in the H_{ex} and $H_{ex}+Q$ particles in the absence and in the presence of $2\cdot10^{-5}$ M TMPD. The case for H_{ex} particles has been discussed earlier: in H_{ex} particles as prepared, charge recombination occurred; and in the presence of TMPD, the 280-nm absorption

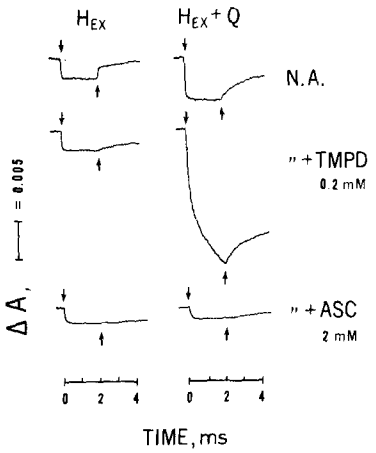


Fig. 6. Flash(2-s)-induced absorption-change transients in the extracted and reconstituted subchromatophore particles at 280 nm in the absence and presence of TMPD ($2 \cdot 10^{-5}$ M) or TMPD *plus* ascorbate ($2 \cdot 10^{-3}$ M). N.A. = no addition.

change possibly represents cytochrome oxidation and/or the original slow-decaying absorption decrease (*cf.* Fig. 3). The slower absorption-decrease phase occurred only in the reconstituted $H_{ex}+Q$ particles and only when reduced TMPD was present to furnish a continuous supply of electrons. Furthermore, changes with this type of kinetics occurred only in the spectral region corresponding to that of ubiquinone. Since cytochrome oxidation occurred in both H_{ex} and $H_{ex}+Q$ particles as long as TMPD was present to keep the cytochrome reduced, this steady, slow absorption-decrease phase can only be ascribed to ubiquinone reduction. The wavelength of 280 nm is near an isosbestic point on the spectra of both the oxidized and reduced TMPD[18]; thus the slow absorption decrease observed here would not be significantly affected by changes due to TMPD oxidation.

The assignment of this steady, slow absorption decrease at 280 nm to ubiquinone reduction is further supported by the fact that upon addition of sufficient ascorbate to chemically reduce ubiquinone, the steady, slow absorption decrease phase diminished and finally disappeared until only a small absorption decrease having a slow decay remained (see Fig. 6, right column, bottom). On the other hand, the addition of ascorbate to the H_{ex} particles which contain no ubiquinone had little effect. Also, the transient profile of H_{ex} and $H_{ex}+Q$ particles in the presence of ascorbate are practically the same. This may be interpreted as due to the fact that the reactivities in the absence of ubiquinone and in the presence of reduced ubiquinone are equivalent.

The transient observed here in the presence of ascorbate appears to be at variance with an earlier observation that the 280-nm absorption change completely disappeared[5,6,11]. This may be caused by some experimental anomaly. The explanation forwarded by Loach *et al.*[11] for their observation was an optical anomaly caused by high absorbance of the sample. We have also found that even though a single 2-s excitation with a dark-adapted sample such as that used in Fig. 5 yielded the transient shown, on successive excitations (the case in the previous experiment reported in ref. 5), the 280-nm absorption change also disappeared. Perhaps under

these conditions, the photoreduced form of the primary electron acceptor is accumulated and insufficient time was allowed for recovery by oxidation, and consequently all photochemistry ceased.

CONCLUDING REMARKS

Experiments with *Chromatium* subchromatophore particles which had been exhaustively extracted with non-polar solvents and completely depleted of their quinones have provided the basis for deciding that endogenous ubiquinones do not play the role of a primary electron acceptor in bacterial photosynthesis. If a quinone were the primary acceptor, light-induced absorption changes due to P890 photo-oxidation would not have occurred without the reaction partner in these extracted subchromatophore particles.

The extracted and reconstituted *Chromatium* subchromatophore particles have provided useful experimental materials for studying the nature of the light-induced absorption changes in the ultraviolet region, and for confirming the secondary nature of ubiquinone reduction in *Chromatium* chromatophores. It has been demonstrated, on the basis of kinetic correspondence, that a major portion of the 280-nm absorption change may be attributed to P890 photo-oxidation, in agreement with the bacteriochlorophyll model studies of Loach *et al.*[11]. It is shown in this work, that the ultraviolet absorption changes may also accompany ubiquinone reduction or cytochrome oxidation, depending on the particular experimental conditions, and the only means for differentiating these reactions has to be based on their kinetics.

A possible optical absorption change associated with the primary electron acceptor in bacterial photosynthesis has been a subject of wide interest, but no definitive evidence has yet been available. Although the absorption change of the primary electron acceptor is beyond the scope of this report, some recent trends in this area may be commented upon. If absorption changes do exist for the redox reactions of the primary electron acceptor, they would be a part of the difference spectrum shown in Fig. 1 obtained for the recombination reaction, where no secondary electron-transport reactions were involved. However, the changes due to the primary acceptor cannot be kinetically differentiated from that of P890. Loach *et al.*[11] made computational analysis of the light-*minus*-dark chromatophore spectrum and the oxidized-*minus*-reduced bacteriochlorophyll spectrum in an attempt to extract the primary-acceptor signal, but obtained no conclusive evidence for any discernible absorption changes which could be attributed to the primary acceptor.

Several other optical spectral approaches remain at least hypothetically feasible for the study of the identity of the primary acceptor. One approach used by Clayton and Straley[19] to isolate the absorption change of the primary acceptor was to illuminate a reaction-center preparation in the presence of an electron donor which suppresses the appearance of $P890^+$. They observed a relatively small absorption change (extinction coefficient about $2 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$) having a positive band at 450 nm which they attributed to the conversion of the primary acceptor to its reduced state.

The primary electron acceptor of greenplant Photosystem I has recently been identified[20,21]. The evidence was based mainly on the correlation of the reaction kinetics of the recovery of the primary photoproducts with the kinetics of the onset of the secondary electron carriers. With Photosystem I, it has been possible to use

appropriate artificial donors and acceptors to create favorable reaction kinetics which allow the reactions of the primary donor and acceptor to be differentiated and recognized. A similar kinetic approach in the bacterial system remains to be seen.

Several reports (refs 22–24, also ref. 25) on the identification of ESR signals due to the primary electron acceptor of bacterial systems have appeared recently.

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