BBA 46074

SPECTRAL AND PHOTOCHEMICAL PROPERTIES OF SUBCHROMATOPHORE FRACTIONS DERIVED FROM CAROTENOID-DEFICIENT *CHROMATIUM* BY TRITON TREATMENT*

BACON KE AND THOMAS H. CHANEY
Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387 (U.S.A.)

(Received September 15th, 1970)

SUMMARY

Triton treatment of chromatophores of carotenoid-deficient *Chromatium* followed by density-gradient centrifugation led to a separation into three subchromatophore fractions. Unlike the case with chromatophores of regular *Chromatium*, Triton releases about 1/3 of the total bulk bacteriochlorophyll into one fraction (designated G, for green) whose major absorption-band maximum is at 780 nm. One fraction (H, for heavy) absorbs at 805 and 885 nm, with an absorbance ratio $A_{885\text{ nm}}/A_{805\text{ nm}}$ between 1.5 and 2; another fraction (L, for light) absorbs at 805 nm and has a shoulder at 825 nm. The absorption and fluorescence emission spectra of the three fractions at room temperature and 77°K indicate that the different bacteriochlorophyll forms are efficiently separated by Triton treatment.

The reaction center P890 is concentrated exclusively in the H-fraction, at a level of 5–7 % of the bulk bacteriochlorophyll. The solubilized bacteriochlorophyll absorbing at 780 nm can be totally and irreversibly bleached by 5 mM ferricyanide. The other bacteriochlorophyll forms in the H- and L-fractions are also irreversibly bleached by ferricyanide to variable extents. P890 is the only component that can be re-reduced by ascorbate after ferricyanide oxidation. The P890 content estimated by reversible chemical bleaching agrees well with that obtained by reversible light bleaching. The different bacteriochlorophyll forms, with the exception of the 780-nm absorbing form, are relatively stable toward light bleaching. Again, only P890 is reversibly bleached by light.

Cytochromes-555 and -553 are distributed in both the H-and L-fractions, but not in the solubilized-bacteriochlorophyll G-fraction. However, only cytochromes in the H-fraction which contains all of the P890 can undergo coupled oxidation. Excitation with 20-nsec ruby-laser pulses shows that cytochrome-555 can be oxidized in 2–3 μsec by photooxidized P890, indicating that necessary conformation for rapid electron transport is retained in the subchromatophore particles.

The data on fractionation and redox reactions obtained here, together with direct kinetic measurements recently reported in the literature lend further support to

---

Abbreviation: TMPD, *N,N,N′,N′*-tetramethyl *p*-phenylenediamine.

[*] Contribution No. 404 from the Charles F. Kettering Research Laboratory, Yellow Springs, Ohio.

the view that oxidation of these two cytochromes is mediated by the same reaction center, P890.

INTRODUCTION

Detergent treatment of the photosynthetic apparatus can lead to a fractionation into subparticles of differing spectral and photochemical properties. A successful fractionation of spinach chloroplasts with digitonin was first reported by BOARDMAN AND ANDERSON[1]. Subsequently, the nonionic detergent, Triton X-100, was successfully used for fractionating chloroplasts[2] as well as chromatophores from photosynthetic bacteria[3]. In the case of chloroplasts, detergent fractionation has led to the separation of particles representing the two photosystems of green-plant photosynthesis[4,5]. In the case of bacterial chromatophores, a fraction with enriched reaction-center bacteriochlorophyll and enhanced photochemical activity has been obtained by Triton[6] and more recently by sodium dodecyl sulfate[7] fractionation.

Recently, particles containing pure reaction-center bacteriochlorophyll have been obtained by detergent treatment of carotenoidless mutant strain R-26 of *Rhodopseudomonas spheroides*[8] and strain G-9 of *Rhodospirillum rubrum*[9]. Although pure reaction-center particles have not yet been obtained from green-plant chloroplasts, more recent attempts by VERNON and co-workers showed that subparticles obtained from spinach chloroplasts extracted with organic solvent to remove carotenoids prior prior to detergent treatment[10] are further enriched in P700 by a factor of several fold compared with the previously prepared subparticles[2,4]. Similar 'high-P700 particles' have also been obtained from a carotenoidless blue-green alga, Anabaena, grown in a medium containing diphenylamine and a carotenoidless mutant of Scenedesmus[11].

With this background, we felt it would be of interest in finding out how the spectral and photochemical properties of the subchromatophore particles might differ if *Chromatium* cells free from or deficient in carotenoids were subject to Triton treatment and fractionation. Present results show that, with carotenoid-deficient *Chromatium* cells, approximately one third of the bulk bacteriochlorophyll was solubilized. Consequently, the photochemically active fraction becomes further enriched in the reaction-center bacteriochlorophyll, P890. The photochemically active fraction retains activities in the photooxidation of P890 as well as dark oxidation by photooxidized P890 of either cytochrome-555 or -553 depending on the redox environment.

EXPERIMENTAL

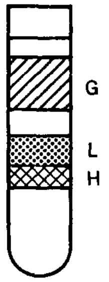
Cells of *Chromatium* strain D were grown in a medium described by HENDLEY[12] but containing additional diphenylamine at 12 mg/l[13]. Cells grown in this medium contained only 10 % of the normal level of carotenoid. The cells were harvested and chromatophores prepared by sonication and collected by centrifugation as previously described[3]. The chromatophores were resuspended in 0.02 M pH 8 Tris buffer to a bacteriochlorophyll concentration of approx. 1 mM. A 10–20 % Triton solution in the same Tris buffer was added to the chromatophore suspension to a Triton/bacteriochlorophyll mole ratio of 400. The mixture was then stirred near 4° in the dark for 2 h. The chromatophore-detergent mixture was then loaded onto a discontinuous sucrose density gradient (54, 24 and 14 % sucrose) and centrifuged overnight at 144000 × *g*. All chemicals were of reagent grade and were used without further purification.

The absorption spectra and chemical difference spectra were taken in a Cary model 14R spectrophotometer. The liquid-nitrogen-temperature spectra were taken in a cold-finger cuvette inside a Dewar adapted to the Cary scattered-transmission attachement and using a DuMont 6911 end-on photomultiplier. Bacteriochlorophyll concentration was determined using 75 as the millimolar extinction coefficient at 772 nm in a acetone–methanol (7:2, by vol.) mixture[14]. Room-temperature and liquid-nitrogen-temperature fluorescence spectra were taken in a spectrofluorometer similar to that described earlier[15]. Some steady-state light-*minus*-dark difference spectra were also taken in the Cary 14R spectrophotometer either by operating it in the IR-2 mode or by illuminating the sample with an external source from a direction perpendicular to the measuring beam. The flash kinetic spectrophotometer was used in conjunction with a Fabri-tek signal averager to improve the signal-to-noise ratio in examining the light-induced absorption changes[16,17].

RESULTS

Spectral properties of the subchromatophore fractions

Absorption spectra. Density-gradient centrifugation of the carotenoid-deficient *Chromatium* chromatophores treated with Triton yielded a separation pattern shown in Fig. 1. A heavy, dense band (H, for heavy), a diffused layer (L, for light) immediately above, and a dark, green band (G) near the top of the tube. This pattern of separation is quite different from that obtained when the same Triton treatment was applied to the regular *Chromatium* chromatophores[3,6]. The regular *Chromatium* chromatophores treated with Triton resulted in the separation into two major fractions with different near-infrared absorption spectra and a yellow supernatant containing



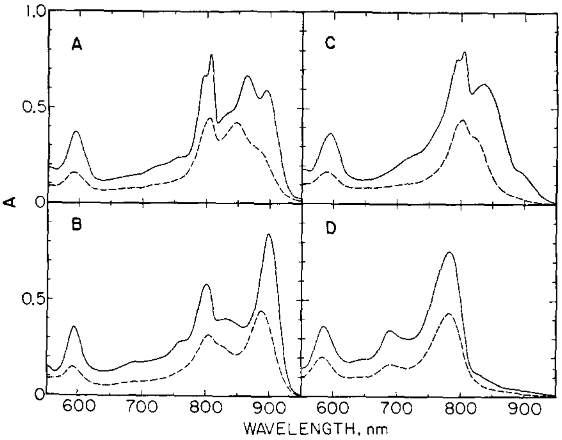


Fig. 1. Distribution in the centrifuge tube of the subchromatophore fractions obtained by Triton treatment of the carotenoid-deficient *Chromatium* chromatophores. H, heavy; L, light; and G, green supernatant.

Fig. 2. The near-infrared absorption spectra of *Chromatium* chromatophores (A); and subchromatophore particles: (B) the heavy fraction; (C) the light fraction; (D) the supernatant. – – –, at room temperature; ———, at 77°K.

mostly carotenoids *plus* some bacteriochlorophyll absorbing near 800 nm (ref. 3). In the case of carotenoid-deficient *Chromatium* chromatophores, an average of 1/3 of the bulk bacteriochlorophyll was solubilized and located in the upper green band. Analysis showed the range of bacteriochlorophyll distribution among the three bands was: H, 31–39 %; L, 37–39 %; and G, 27–32 %.

The absorption spectra at room temperature and 77°K of the unfractionated chromatophores and the three fractions in the near-infrared and red regions are presented in Fig. 2. The spectra show that a separation of the different bacteriochlorophyll forms by Triton treatment took place. The highest band in the H-fraction absorbs at 885 nm; and the 885/805-nm band ratio averages 1.5 (occasionally this ratio reached 2). A shoulder occurs at 825 nm. The L-fraction has a predominant band at 805 nm with a shoulder at 825 nm. There is very slight absorption near 890 nm, which is probably due to a contamination by materials from the H-fraction lying immediately below in the centrifuge tube. The G-fraction consists almost entirely of solubilized bacteriochlorophyll absorbing at 780 nm *plus* some oxidized bacteriochlorophyll absorbing near 680 nm.

In unfractionated *Chromatium* chromatophores, the major near-infrared bands are at 805 and 846 nm, with a shoulder at 880 nm. One prominent feature of the Triton treatment of carotenoid-deficient *Chromatium* chromatophores is the loss of the original 846-nm band (*cf.* Fig. 2). The sensitivity of the 850-nm band toward Triton was observed previously in *Chromatium* chromatophores grown in low light and after repeated Triton treatment[3]. This phenomenon was studied in more detail recently by SUZUKI *et al.*[18], who showed that the 850- and 810-nm bacteriochlorophyll forms can undergo reversible interconversions in the presence of a nonionic or cationic detergent.

The near-infrared absorption spectra of the unfractionated chromatophores and the three subfractions at 77°K are also presented in Fig. 2. In general, lowering the temperature sharpened the absorption bands and shifted the far-red bands toward longer wavelengths. In the unfractionated chromatophores, an additional band appeared at 794 nm next to the 806-nm band. The 846-nm band shifted to 862 nm and the 880-nm shoulder sharpened and shifted to 893 nm. In the H-fraction, the 805-nm band actually shifted toward a shorter wavelength at 800 nm. An intermediate band appeared at 842 nm, which was presumably from the 825-nm shoulder. The 885-nm band appeared at 897 nm. In the L-fraction, the 800-nm region has an appearance similar to the unfractionated chromatophores, with a doublet at 794 and 805 nm. The 825-nm shoulder was intensified into a band at 837 nm. A negligible shoulder appeared at 897 nm due to trace contamination by material from the H-fraction. In the G-fraction, the absorption band at 780 nm due to solubilized bacteriochlorophyll intensified with a red shift of only 2 nm. A faint shoulder developed near 380 nm. The 690-nm band also became more prominent at 77°K. For ease of comparison, the wavelength maxima of the fractions at room temperature and 77°K are listed in Table I.

Fluorescence emission spectra. The fluorescence spectra of the unfractionated carotenoid-deficient *Chromatium* chromatophores and the three subfractions excited with 590 nm (bandwidth 30 nm) light at room temperature and 77°K are presented in Fig. 3. At room temperature the unfractionated *Chromatium* chromatophores have a fluorescence emission spectrum with a maximum at 895 nm; at 77°K, the single emission band became greatly enhanced and the maximum was shifted to 925 nm. The single emission band indicates that all the 590 nm quanta were eventually transferred to the longest-wavelength bacteriochlorophyll form (880 nm at room temperature or 893 nm at 77°K). In the H-fraction, the emission maxima were at 910 nm at room temperature and 930 nm at 77°K, respectively. There is also a minor emission band at 870 nm. Apparently energy transfer occurs from the 800-nm band to either the 842-nm or 897-nm band, but does not occur between the latter two bands. In the L-fraction, the room-temperature emission band was very broad, spanning from 770 to 870 nm.

TABLE I

WAVELENGTHS (nm) OF THE ABSORPTION BANDS AND FLUORESCENCE EMISSION BANDS OF *Chromatium* CHROMATOPHORES (C) AND SUBCHROMATOPHORE FRACTIONS (H, L AND G) AT ROOM TEMPERATURE AND 77°K

Wavelengths in parenthesis indicate shoulder.

Fraction	*Absorption*		*Emission*	
	25°C	*77°K*	*25°C*	*77°K*
C		794		
	805	806		
		835		
	846	862		
	880	893	895	925
H	805	800		
	(825)	842		870
	885	897	910	930
L		794		788
	805	805	770	
	(825)	837	/	863
	890*	(894)	870	(920)
G	680	680		693
	780	782	800	800
		(830)		(850)

* Indicates trace amount.

The 77°K emission spectrum was resolved into two major bands at 788 and 863 nm, a minor band at 694 nm, and a faint shoulder at 920 nm. Thus, the major emission came from the 837-nm band (*cf.* ref. 18). The 920-nm shoulder may be attributed to the trace absorption at 894 nm. The 788- and 694-nm bands cannot be easily accounted for by any discernible absorption at 77°K in Fig. 2. In the G-fraction, the emission bands at 693 nm, 802 and 850 nm may be accounted for by the 680 (oxidized bacteriochlorophyll), 782 (solubilized bacteriochlorophyll), and the 830-nm shoulder, respectively. The G-particles presumably represent the different bacteriochlorophyll forms attached to a lipoprotein matrix with little interaction between the bacteriochlorophyll forms, since the shift of the far-red absorption maximum is slight. The wavelength maxima of the emission bands of the various chromatophore fractions are also summarized in Table I.

Chemical composition of the subchromatophore fractions

Reaction-center bacteriochlorophyll P890. The P890 content in the chromatophore and subchromatophore fractions was determined by chemical difference spectroscopy as well as light-*minus*-dark difference spectroscopy (to be discussed later). The results by chemical difference spectroscopy may be illustrated by the actual spectra obtained with the H-particles. Fig. 4A shows the absorption spectrum of the H-particle used in these measurements. After the absorption spectrum was taken, two identical samples were placed in the sample and reference compartments of the spectrophotometer and the zero baseline recorded. To the sample cuvette, ferricyanide was added to 5 mM and then the difference spectrum recorded, as shown in Fig. 4B. This differences spectrum represents the total bleachable bacteriochlorophyll forms at 890, 830, 805 and 780 nm. The difference spectrum in Fig. 4C was recorded with the ferricyanide-treated cuvette in the sample compartment, and the reference compartment consisted

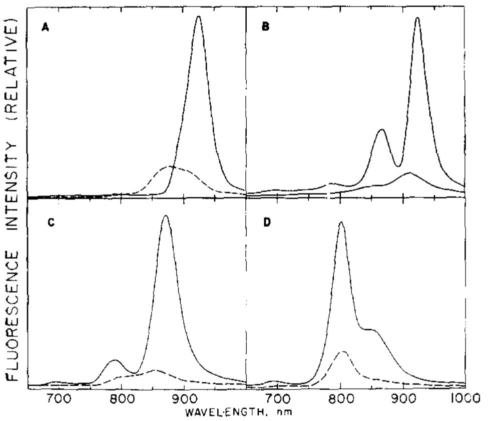


Fig. 3. Fluorescence emission spectra of *Chromatium* chromatophores (A); and subchromatophore particles: (B) the heavy fraction; (C) the light fraction; (D) the supernatant. — — —, at room temperature; ———, at 77°K.

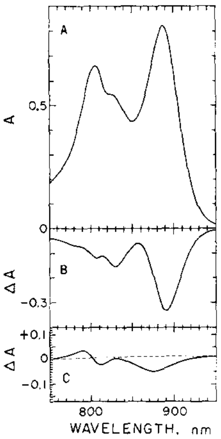


Fig. 4. (A) Absorption spectrum of the H-particles used for measuring the P890 content. (B) Difference spectrum: sample containing 5 mM ferricyanide *vs.* untreated sample. (C) Difference spectrum: ferricyanide-treated sample *vs.* ferricyanide-treated sample back titrated with ascorbate.

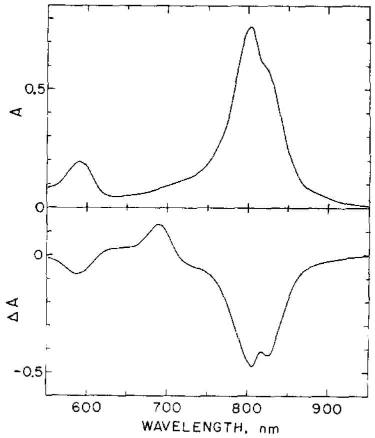


Fig. 5. Absorption spectrum of the L-particles (top) and absorption change due to ferricyanide oxidation (bottom).

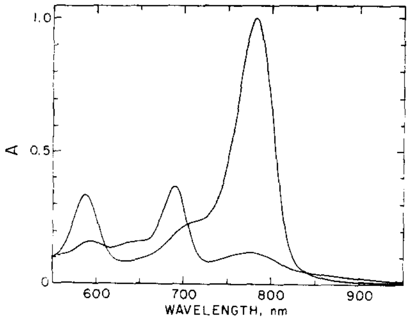


Fig. 6. Absorption spectra of the G-fraction before and after ferricyanide (5 mM) addition.

of the cuvette which was treated with 5 mM ferricyanide first and then back titrated with slight excess ascorbate. The ascorbate restores the reversibly bleached P890, leaving the irreversibly bleached bulk bacteriochlorophyll intact. Thus, the difference spectrum in Fig. 4C represents the amount of P890 present in the sample whose absorption spectrum is shown in Fig. 4A.

The irreversibly bleached bacteriochlorophyll represents 35 % of the total absorption of the 885 nm band. Assuming P890 (at 875 nm) and the bulk bacteriochlorophyll at 885 nm have identical absorbance, the reversibly bleachable P890 in this sample is 7 %. Analysis of P890 content in separately prepared H-fractions gave a range between 5 and 7%. It will be seen that this value agrees well with the P870 content determined by light-*minus*-dark difference spectroscopy to be discussed below

TABLE II

P890, CYTOCHROME AND UBIQUINONE COMPOSITIONS OF *Chromatium* SUBCHROMATOPHORE FRACTIONS (H, L AND G)

All components are expressed as number of molecules per 100 bulk bacteriochlorophyll molecules.

Fraction	*P890**	*Cytochrome-555****	*Cytochrome-553****	*Ubiquinone*
H	4.9**–6.7	9.5	4.3	24.0
L	Trace	7.8	3.3	8.6
G	0	0	0	0

* Estimated from the chemically reversibly bleachable portion of the 890-nm absorption band.

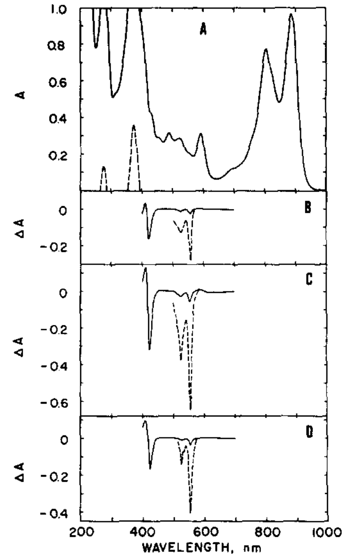
** Estimation by light bleaching on the same sample yielded 4.5 % (*cf.* Fig. 8).

*** 23 and 43 (ref. 19) were used as the millimolar extinction coefficients for cytochromes-555 and -553, respectively. It should be noted that cytochrome-553 has 2 hemes per mole.

Chemical difference spectroscopic measurements on the L- and G-fractions did not reveal any reversibly bleachable P870. In the L-fraction, the 805- and 825-nm forms are largely bleachable as shown in Fig. 5. Back titration by ascorbate did not cause any additional change in the magnitude of the absorbance change. In the G-fraction, 90 % of the 780-nm form was chemically bleached, but a small fraction was re-reduced by ascorbate. Typical results are shown in Fig. 6 and Table II.

Cytochromes. The two cytochromes in the fractions were estimated by chemical difference spectroscopy. Since both cytochromes-555 and -553 are present in the oxidized state, the former can be estimated from the ascorbate-*minus*-untreated difference and the latter from the dithionite-*minus*-ascorbate difference, respectively. A typical set of results for the H-particles is shown in Fig. 7. In this figure the dithionite-*minus*-untreated difference, which represents the sum of both cytochromes-555 and -553, is also shown. In all cases, when the untreated sample was substituted by a ferricyanide-treated sample, the net results were unaffected, indicating that both cytochromes were originally present in the oxidized state in these particles. Analytic data for the three fractions as well as unfractionated chromatophores are summarized in Table II. Compared with the unfractionated chromatophores, the cytochrome content is enriched by more than 2-fold in the H-fraction. It is also present in the L-fraction, even though this fraction contains only trace amount of P890. The G-fraction contains no cytochromes.

Ubiquinones. The ubiquinone content of the various fractions was determined by solvent extraction and spectrophotometric assay after $NaBH_4$ reduction and using 12 as the millimolar extinction coefficient[19]. The numerical values are listed in Table II.



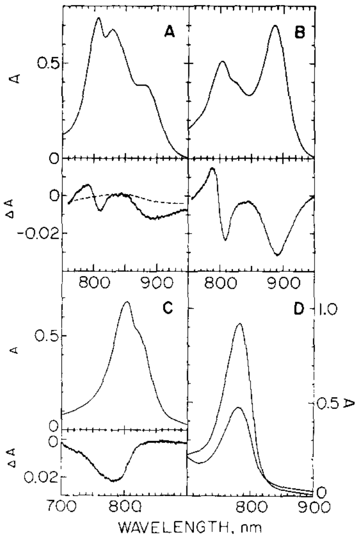


Fig. 7. (A) Actual absorption spectrum of the H-particle used for cytochrome estimation by chemical difference spectroscopy. (B) Ascorbate-*minus*-untreated difference. (C) Dithionite-*minus*-untreated difference. (D) Dithionite-*minus*-ascorbate difference. In A the dashed-curve portion has absorbance greater than 1. In B, C and D, the difference spectra in the α- and -β-band region of the cytochromes are also presented in a 10 × more sensitive scale by the dashed curves.

Fig. 8. Absorption spectra and steady-state light-induced absorption changes in the near-infrared region in the unfractionated chromatophores (A) and the H- (B) and L- (C) subchromatophore particles. (D) Absorption spectra of the G-fraction in the dark and under illumination.

Photochemical properties

Steady-state light-induced absorption changes in the near-infrared region. As seen in Figs. 8A and 8B, both the unfractionated *Chromatium* chromatophores and the H-particles show light-induced absorption decrease at 890 nm and a blue shift at 800 nm, typical of P890 photooxidation. The magnitude of the 890-nm absorption decrease representing P890 photooxidation is more than three times greater in the H-particles than in the unfractionated chromatophores. The H-particles were used without ascorbate present, so that the coupled cytochrome was not operating and maximum bleaching of P890 could result. The particular sample of H-particles used in this experiment yielded a P890 content of 4.5 %, in close agreement with the value obtained earlier by chemical difference spectroscopy (*cf.* Fig. 4 and Table II).

Although the near-infrared absorption bands in the L-particles are easily bleached by ferricyanide oxidation (*cf.* Fig. 6), they are quite stable toward light. Instead, as shown in Fig. 8C, steady-state illumination of the L-particles showed an irreversible bleaching of a band with a maximum at 780 nm, which is presumably the solubilized bacteriochlorophyll. The absorption band of the G-fraction also appears to be relatively more stable toward light than toward ferricyanide (*cf.* Fig. 7). Steady-state illumination, as shown in Fig. 8D, bleached about 50 % of the main band, and the change was irreversible.

Steady-state light-induced cytochrome oxidation in the H-particles. Cytochromes-555 and -553 are present in both the H- and L-fractions, albeit in the oxidized form. In the unfractionated chromatophores, cytochrome-555 is present in the reduced state whereas cytochrome-553 is autooxidizable. Illumination of unfractionated chromatophores causes the oxidation of cytochrome-555. Oxidation of cytochrome-553 in *Chromatium* chromatophores can be observed only under an environment of low redox potential[20–23].

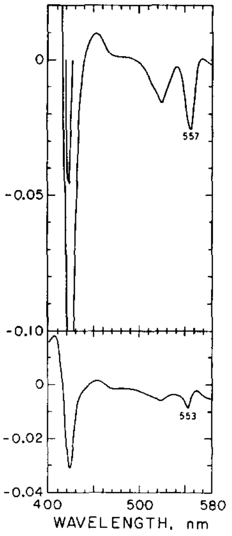
Illumination of the H-particles in the presence of ascorbate, where cytochrome-555 was brought into the reduced state, revealed the photooxidation of cytochrome-555, with the α-band maximum actually located at 557 nm, as shown in Fig. 9, top. Illumination of the H-particles in the presence of 2 mM dithiothreitol, on the other hand, gave a difference spectrum of cytochrome-553, with the maximum at 553 nm, as shown at the bottom of Fig. 9. The lesser magnitude of cytochrome-553 oxidation may have been caused by the decreased availability of components on the primary electron acceptor side under a more reducing environment.

Mention should be made here that a similar situation in the oxidation states of cytochromes also exists in the H-fraction obtained from the regular *Chromatium* chromatophores by Triton treatment[6]. Although cytochrome reactions were not reported for those particles at that time, subsequent experiments confirmed that, under appropriate redox conditions, the cytochromes in regular *Chromatium* H-particles can undergo similar oxidations as those reported here.

Laser-flash induced rapid absorption changes. Fig. 10 shows that the photochemically active H-fraction can undergo rapid absorption changes when excited with Q-switched 20-nsec laser flashes at 694 nm. The left column shows flash-induced changes in the untreated H-particles in which the cytochromes were present in the oxidized state. Under this condition, the 881 and 422 nm changes represent bleaching of the reaction-center bacteriochlorophyll and have very slow recovery kinetics. The right column shows absorption changes in the H-particles to which 10 μM *N,N,N′,N′*-tetramethyl *p*-phenylenediamine (TMPD) was added to bring cytochrome-555 into the reduced state. Under this condition, a rapid dark oxidation of cytochrome-555 (or -422) with a half-time of 2–3 μsec occurred, as shown by the absorption decrease at 422 nm. At 881 nm, P890 undergoes a rapid photooxidation in less than 1 μsec, followed by a partial decay also with a half-time of 2–3 μsec. Thus the oxidation of cytochrome-555 and the recovery of P890 are directly coupled. The cytochrome-oxidation rise-time and the direct coupling between cytochrome-555 and P890 are in agreement with the previous observation of PARSON[24], but the partial decay of P890 was not noted before. The latter phenomenon has also been observed by us at 810 and 785 nm, and has been observed in unfractionated chromatophores as well. The meaning of this partial decay is under further investigation.

The 280-nm change has been attributed to the reduction of ubiquinone[17]. Note that it is apparently unaffected in particles under the two redox conditions used. If the ultraviolet absorption changes were a part of the reaction-center bacteriochlorophyll change, as has often been suspected, it would have changed its profile like that at 881 nm when cytochrome oxidation was coupled to P890. As expected from results in

Fig. 9, top, the same rapid change at 881 and 422 nm can also be brought about when cytochrome-555 is reduced by ascorbate. However, in the presence of ascorbate the 280-nm change was abolished which we attributed to the chemical reduction of ubiquinone by ascorbate[17].



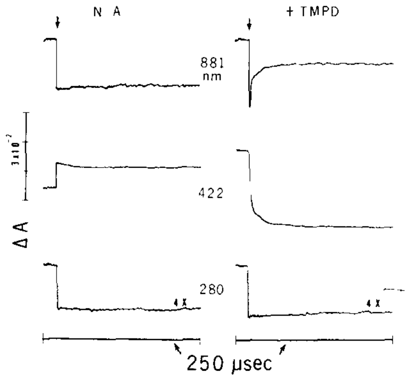


Fig. 9. Steady-state light-induced absorption changes due to cytochrome oxidation in the H-particles in the presence of ascorbate (top) or dithiothreitol (bottom).

Fig. 10. Rapid absorption changes at 881, 422 and 280 nm induced by Q-switched 20-nsec ruby-laser flashes in the photochemically active H-particles. Left column: sample with no addition (NA.) Right column: sample containing 10 μM *N,N,N',N'*-tetramethyl *p*-phenylenediamine (+ TMPD). Arrow indicates the moment the flash was applied. The signals were obtained by averaging with repetitive flashes spaced at intervals to allow signal recovery. The absorbance scale should be multiplied by 1/4 to be applicable to the 280-nm changes.

DISCUSSION

The absence of carotenoids causes no apparent alteration in the near-infrared absorption spectra of the chromatophores. In the unfractionated carotenoid-deficient *Chromatium* chromatophores, the room-temperature absorption spectrum agrees with that of the regular *Chromatium* grown under the same conditions. At 77°K, five distinct bands were observed in the near-infrared region, agreeing well with those reported by VREDENBERG AND AMESZ[25] for regular *Chromatium* strain D containing carotenoids.

From the absorption spectra at room temperature, and from those at 77°K in particular (see Fig. 2 and Table I), it is clear that Triton effectively separated the different bacteriochlorophyll forms into 3 fractions. As far as the H- and L-fractions are concerned, there is no substantial difference in their absorption spectra from the corresponding fractions obtained earlier from the regular *Chromatium* chromatophores [3,6]. In the present fractionation, however, the H-fraction has a consistently higher 890/800-nm band ratio, and that the L-fraction contains only trace amount of P890, if any.

The fluorescence spectrum of the unfractionated chromatophores from carotenoid-deficient *Chromatium* has a single emission band at 925 nm, indicating that the 590-nm excitation quanta were efficiently and successively transferred to the terminal 890-nm bacteriochlorophyll form. It also suggests that the absence of carotenoids has not altered the regular spacings between the different bacteriochlorophyll forms, as judged from the unaffected energy transfer between them. The H-fraction, however, has a 870-nm emission band in addition to the 930-nm band, suggesting that the relative spacings between the 890- and 842-nm forms were altered by Triton treatment, and so were the conditions for energy transfer between them. On the other hand, the fluorescence spectrum of the L-fraction appears to suggest that energy transfer between the 794/805-nm and the 837-nm forms was unaffected. In the G-fraction no energy transfer occurs between the solubilized bacteriochlorophyll and the trace amount of 830-nm form.

THORNBER[7] recently reported the fractionation of regular *Chromatium* chromatophores by treatment with the anionic detergent, sodium dodecyl sulfate. The sodium dodecyl sulfate treatment appears to cause no alteration in the various bacteriochlorophyll forms, since the original absorption spectrum of the unfractionated chromatophores can be reconstructed from those of the subfractions. The homogeneity in bacteriochlorophyll forms in the sodium dodecyl sulfate subfractions is also reflected in their fluorescence emission spectra[7].

The major difference between the regular and the carotenoid-deficient *Chromatium* chromatophores in their response toward Triton is that nearly 1/3 of the bulk bacteriochlorophyll became solubilized from the carotenoid-deficient chromatophores, whereas there was none from the regular chromatophores. It is worth noting that the original aim of this work was to isolate pure reaction-center particles free from bulk bacteriochlorophyll molecules from the carotenoid-deficient *Chromatium* chromatophores, as has been achieved with the carotenoidless mutant cells of *Rps. spheroides*[8] and *R. rubrum*[9]. The ease of solubilization of only part of the bulk bacteriochlorophyll molecules from the carotenoid-deficient cells suggests that the carotenoid molecules may play a somwehat different role in the binding of the bacteriochlorophyll molecules in the *Chromatium* cells. One may recall that a similar situation was also encountered in the preparation of P700-enriched particles from chloroplasts devoid of carotenoids either by solvent extraction or by growth in a medium containing diphenylamine, where a minimum number of bulk chlorophyll molecules was reached and could not be removed without altering the activity of P700[10,11].

As a result of the solubilization of part of the bulk bacteriochlorophyll molecules, the P890 content (5–7 %) in the photochemically active subchromatophore fraction is the highest obtained so far from *Chromatium* cells. The H-fraction from regular *Chromatium* chromatophores had one P890 per 45 bulk bacteriochlorophyll as determined by reversible chemical bleaching or by light bleaching (unpublished experiments). Light bleaching of the photochemically active subchromatophore fraction obtained by sodium dodecyl sulfate treatment also had one P890 per 45 bulk bacteriochlorophyll molecules[7].

Chemical bleaching by ferricyanide revealed different responses of the various bacteriochlorophyll forms toward this oxidizing agent. In the H-fraction, a rather large portion of the 890-nm and 825-nm bacteriochlorophyll forms *plus* some 810- and 780-nm forms were irreversibly bleached. The only reversibly chemically bleachable bacteriochlorophyll form in the H-fraction was P890, as revealed by back titration with ascorbate (see Fig. 4). The different bacteriochlrophyll forms in the L- and G-fractions were mostly irreversibly bleached with a concomitant appearance of a band at 680–690 nm due to oxidized bacteriochlorophyll[26].

Contrary to their response toward chemical bleaching, the various bacteriochlorophyll forms are generally quite stable toward light. P890 appears to be the only bacteriochlorophyll form in the unfractionated chromatophore and the H-fraction that is reversibly bleached by light. The only irreversibly bleached bacteriochlorophyll form is the solubilized 780-nm form in the G-fraction and trace amount of it in the L-fraction (see Fig. 8). Judging from the shape and magnitude of the 890 nm difference band in Figs. 8A and 8B, some so called 'P′890' form might have also been bleached. In the photochemically active subfraction obtained by sodium dodecyl sulfate treatment, the content of P′890 was much higher: P890:P′890:890-nm bulk bacteriochlorophyll was reported to be 1:4:45. The photochemical nature of P′890 is not exactly known; THORNBER[7] suggested that it might enter into a redox reaction with P890 under certain conditions.

The distribution of cytochromes in the Triton subfractions is also less than exclusive. Both cytochromes-555 and -553 are present in the H-fraction, which contains P890, as well as the L-fraction which contains only trace amount of contaminant P890. However, only the cytochromes in the H-fraction can be oxidized by P890 (see Figs. 9 and 10). In the sodium dodecyl sulfate subfractions, the two cytochromes are present only in the photochemically active subfraction, thus resulting in a much higher cytochrome/P890 ratio.

In connection with the cytochrome fractionation and the reaction of cytochromes-555 and -553 with one reaction-center P890 in the H-fraction under selective conditions, some comments may be made regarding several recent proposals on multiple reaction centers for the oxidation of the different cytochromes in photosynthetic bacteria (see ref. 27 for a review). OLSON AND CHANCE[28] were the first to postulate the existence of three separate electron-transport chains in *Chromatium* cells. In 1968, three separate groups almost simultaneously suggested the concept of multiple photochemical reaction centers associated with and responsible for the oxidation of different cytochromes. In a study of the light-intensity dependence of cytochrome oxidation in *Chromatium*, MORITA[29] found that the 810-nm light was more favorable for cytochrome-553 oxidation, the 850-nm light more effectively for cytochrome *cc′*, and the 890-nm light for cytochrome-555. From these results, three photochemical reaction systems were suggested. SYBESMA AND FOWLER[30] arrived at a similar conclusion for a different photosynthetic bacterium, *R. rubrum*. From the effects of variation in redox potential on the electron-transport reactions, CUSANOVICH *et al.*[31] proposed the existence of 2 functionally different electron-transport pathways: one involves the reaction-center P890 coupled to cytochrome-555, and the other consists of P905 coupled to cytochrome-553.

Although two of the fractions obtained here from carotenoid-deficient *Chromatium* by Triton contain both cytochromes-555 and -553, only those in the fraction containing the photoactive P890 can undergo oxidation. Both cytochromes are apparently reacting with the same bacteriochlorophyll reaction center under different redox conditions. In the sodium dodecyl sulfate fractionation of regular *Chromatium*, cytochromes-555 and -553 are found only in the photoactive sub-fraction containing

P890, and it was shown that both cytochromes were reacting with the same P890 under different redox conditions. These fractionation data together with the direct kinetic measurements of PARSON AND CASE[22] and SEIBERT AND DEVAULT[23] reported recently have now firmly established the thesis that oxidation of both cytochromes-555 and -553 are mediated by the same P890.

ACKNOWLEDGMENTS

The authors thank Mrs. Ferne Lubbers for supplying the carotenoid-deficient *Chromatium* cells used in this study.

This work was supported in part by Grant GB-8460 from the National Science Foundation.

REFERENCES

1 N. K. BOARDMAN AND J. M. ANDERSON, *Nature*, 203 (1964) 166.
2 L. P. VERNON, E. R. SHAW AND B. KE, *J. Biol. Chem.*, 241 (1966) 4101.
3 A. GARCIA, L. P. VERNON AND H. H. MOLLENHAUER, *Biochemistry*, 5 (1966) 2399.
4 L. P. VERNON, B. KE AND E. R. SHAW, *Biochemistry*, 6 (1967) 2201.
5 N. K. BOARDMAN, *Advan. Enzymol.*, 30, (1968) 1.
6 B. KE, L. P. VERNON, A. GARCIA AND E. NGO, *Biochemistry*, 7 (1968) 311.
7 J. P. THORNBER, *Biochemistry*, 9 (1970) 2688.
8 D. W. REED AND R. K. CLAYTON, *Biochem. Biophys. Res. Commun*, 30 (1968) 471.
9 G. GINGRAS AND G. JOLCHINE, in H. METZNER, *Progress in Photosynthesis Research*, Vol. 1, Tübingen, 1969, p. 209.
10 H. Y. YAMAMOTO AND L. P. VERNON, *Biochemistry*, 10 (1969) 4131.
11 T. OGAWA AND L. P. VERNON, *Biochim. Biophys. Acta*, 197 (1970) 282.
12 D. D. HENDLEY, *J. Bacteriol.*, 70 (1955) 625.
13 E. C. WASSINK AND G. H. M. KRONENBERG, *Nature*, 194 (1962) 553.
14 R. K. CLAYTON, in H. GEST, A. SAN PIETRO AND L. P. VERNON, *Bacterial Photosynthesis*, Antioch. Press, Yellow Springs, Ohio, 1963, p. 495.
15 B. KE AND L. P. VERNON, *Biochemistry*, 6 (1967) 2221.
16 B. KE, R. W. TREHARNE AND C. MCKIBBEN, *Rev. Sci. Instr.*, 35 (1964) 296.
17 B. KE, *Biochim. Biophys. Acta*, 172 (1969) 583.
18 Y. SUZUKI, S. MORITA AND A. TAKAMIYA, *Biochim. Biophys. Acta*, 180 (1969) 114.
19 F. L. CRANE AND R. A. DILLEY, *Methods Biochem. Anal.*, 11 (1963) 279.
20 M. A. CUSANOVICH, R. G. BARTSCH AND J. M. OLSON, in K. SHIBATA, A. TAKAMIYA, A. T. JAGENDORF AND R. C. FULLER, *Comparative Biochemistry and Biophysics of Photosynthesis*, University of Tokyo Press, Tokyo, 1968.
21 M. A. CUSANOVICH AND M. KAMEN, *Biochim. Biophys. Acta*, 153 (1968) 396.
22 W. W. PARSON AND G. D. CASE, *Biochim. Biophys. Acta*, 205 (1970) 232.
23 M. SEIBERT AND D. DEVAULT, *Biochim. Biophys, Acta*. 205 (1970) 220.
24 W. W. PARSON, *Biochim. Biophys. Acta*, 153 (1968) 248.
25 W. J. VREDENBERG AND J. AMESZ, *Biochim. Biophys. Acta*, 126 (1966) 244.
26 J. R. LINDSAY-SMITH AND M. CALVIN, *J. Am. Chem. Soc.*, 88 (1966) 4500.
27 G. HIND AND J. M. OLSON, *Ann. Rev. Plant Physiol.*, 19 (1968) 249.
28 J. M. OLSON AND B. CHANCE, *Arch. Biochem. Biophys.*, 88 (1960) 26.
29 S. MORITA, *Biochim. Biophys. Acta*, 153 (1968) 241.
30 C. SYBESMA AND C. F. FOWLER, *Proc. Natl. Acad. Sci. U.S.*, 49 (1968) 248.
31 M. CUSANOVICH, R. G. BARTSCH AND M. KAMEN, *Biochim. Biophys. Acta*, 153 (1968) 397.