

Properties of *Chromatium* Subchromatophore Particles Obtained by Treatment with Triton X-100*

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ABSTRACT: *Chromatium* chromatophores contain bacteriochlorophyll (BChl) in three forms which have absorption maxima around 800, 850, and 890 m μ . Treatment with 3% Triton X-100 produces two fragments which can be separated by sucrose density-gradient centrifugation. The lighter fraction contains both the 800- and 850-m μ forms of BChl as well as the usual complement of carotenoids, but is photochemically inactive. The 850-m μ BChl component is very sensitive to photooxidative destruction and is lost upon further treatment with the detergent. The heavier fraction contains the 890-m μ BChl component with small amounts of the 850- and 800-m μ forms. This heavy fraction contains cytochromes of the *c* type, catalyzes the aerobic photooxidation of tetramethyl-*p*-phenylenediamine and the anaerobic photooxidation of reduced phenazine methosulfate coupled to ubiquinone-6 reduction, exhibits the P890 reaction (photooxi-

dation of the special reaction center chlorophyll absorbing at 890 m μ), and thus contains the components involved in the primary photochemical reactions of photosynthesis in *Chromatium* chromatophores. The residual 850-m μ form (but not the small amount of the 800-m μ form) of BChl can be removed from the heavy particle by further treatment with Triton X-100. The detergent splits the chromatophore into two fractions. Electron microscopy shows that the heavy fraction consists of essentially separate subunits while the light fraction includes the membrane of the chromatophore and some attached material. The origin of the heavy particle is not known (it could arise from the interior of the chromatophore) but it is involved in the photochemical reactions of photosynthesis. The light fraction derives primarily from the chromatophore membrane and includes the bacteriochlorophyll forms involved in light harvesting and energy transfer.

The photosynthetic apparatus of *Chromatium* cells is contained in internal structures which are probably derived from the cell membrane (Fuller *et al.*, 1963). In this organized system the bacteriochlorophyll (BChl)¹ is associated with other compounds such as carotenoids, quinones, lipids, and various proteins, and all function together to carry out the energy conversion process (Vernon, 1964). Understanding the functions of these components will be made easier if one has available fragments of the complete apparatus which can be studied in terms of their composition and photochemical capabilities. Several investigators have tried to fragment and solubilize the bacterial pigment systems. The methods used have included sonic vibration (Newton and Newton, 1957; Frenkel, 1958), enzymatic proteolysis (Newton and Levine, 1959), enzymatic lipolysis (Cohen-Bazire and Kunisawa, 1960), chemical attacks on disulfide bonds (Bergeron, 1958; Newton, 1962), and treat-

ments with detergents (Bril, 1958; Clayton, 1962). Similar experiments have been performed with plant chloroplasts, and these studies are summarized in recent reviews by Kupke and French (1960) and Goedheer (1966).

The early experiments of Komen showed that sodium dodecyl sulfate causes a lowering of the *in vivo* absorption bands of BChl and produces a new band at 760 or 780 m μ with chromatophores of *Rhodospirillum rubrum* and *Chromatium* (Komen, 1956). The latter band would be due to the extraction and solubilization of some BChl by this detergent. Bril (1958) used Triton X-100 to convert *Rhodopseudomonas spheroides* chromatophores into two fractions, a heavy one with an absorption peak at 880 m μ and a lighter fraction with a maximum at 850 m μ . He later improved on this technique and isolated two fractions by means of sucrose gradient centrifugation, obtaining one fraction rich in the 890-m μ component (B890) and another rich in B850 and B800 components. Bril (1960, 1963) has also studied the effect of deoxycholate upon *Chromatium* chromatophores, observing a decreased absorption due to photobleaching, primarily of the B850. However, in this case the spatial arrangement was changed so that energy transfer between the different BChl types was inhibited. Clayton (1962) also studied the effect of deoxycholate upon *Chromatium* chromatophores, and separated by centrifugation a sediment which was enriched with the B890 component. Olson *et al.* (1963) and Olson and

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¹ The abbreviations used in this paper are: BChl, bacteriochlorophyll; B800, B850, and B890, the forms of bacteriochlorophyll which absorb at 800, 850, and 890 m μ , respectively; P890, the reaction center chlorophyll which shows a light-induced absorbancy change at 890 m μ ; PMSH₂, the reduced form of phenazine methosulfate; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; UQ6, the form of ubiquinone having six isoprene units in the side chain; esr, electron spin resonance; NAD, nicotinamide-adenine dinucleotide; DPIP, 2,6-dichlorophenolindophenol; H, heavy particles; L, light particles.

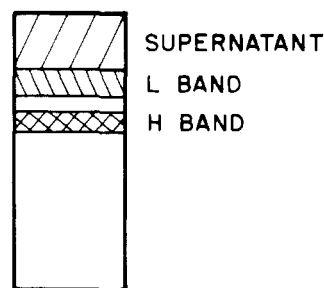


FIGURE 1: Fractions obtained by density gradient centrifugation of *Chromatium* chromatophores after treatment with Triton X-100.

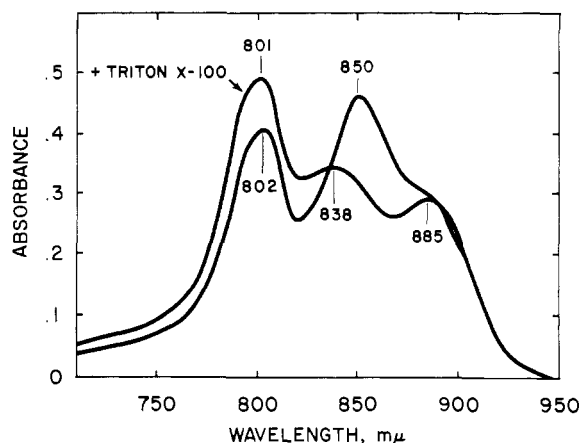


FIGURE 2: Absorption spectra of *Chromatium* chromatophores in the absence and presence of 3% Triton X-100. The chromatophores were suspended in 0.01 M buffer, pH 8.1. The absorbancies at 590 mμ for chromatophores in the absence and presence of Triton were 0.2 and 0.18.

Romano (1962) have succeeded in isolating a BChl-protein complex from the green photosynthetic bacteria. Izawa *et al.* (1963) have shown that sodium dodecylbenzenesulfonate effectively removes and solubilizes the BChl from *R. rubrum* and *Rps. spheroides*, producing a BChl compound with an absorption maximum at 778 mμ. This 778-mμ compound resembles BChl dissolved in organic solvents.

Triton X-100, an effective detergent for rupture of both chloroplasts and chromatophores, is more gentle than the ionic detergents since it allows some of the photochemical activities of the derived particles to be retained. We report below on the physical and photochemical properties of two BChl-containing fractions obtained from *Chromatium* by this detergent.

Methods

Cells of *Chromatium* strain D, grown according to the directions of Hendley (1955), were harvested

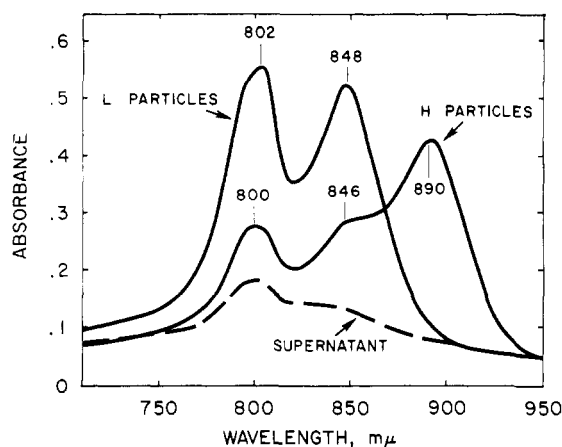


FIGURE 3: Absorption spectra of the dialyzed fragments obtained after density gradient centrifugation. Particles from the heavy (H) or light (L) bands were diluted with 0.01 M Tris buffer, pH 8.1, for the measurement. The absorbancies at 590 mμ were 0.14 (H) and 0.16 (L).

after 3 days of growth, washed once by centrifugation, and suspended in 0.01 M Tris buffer, pH 8.1, for a 3-min sonication in a 10 KC Raytheon sonic oscillator. The chromatophore fraction was obtained by centrifugation, collecting the material sedimenting between 20,000 and 110,000g during 1 hr. The chromatophores were suspended in 0.01 M Tris buffer, pH 8.1, centrifuged again, and resuspended in fresh buffer. Triton X-100 was added to the chromatophore preparation (2.6 mg of detergent/100 mg of protein) which was then allowed to stand in an ice bath, under argon, for 2 hr in the dark. The suspension was diluted to a concentration of 1.5 mg of protein/ml and applied to tubes containing a discontinuous sucrose gradient consisting of three different layers of sucrose (57, 24, and 14%) dissolved in 0.01 M Tris buffer, pH 8.1. The tubes were centrifuged for 15 hr at 110,000g in the 30 rotor of a Spinco Model L ultracentrifuge. Material was removed from the particle bands with a syringe and hypodermic needle. The separated particles were then dialyzed overnight in the cold room against 0.01 M Tris buffer, pH 8.1.

For protein determination a small aliquot was suspended in a mixture of acetone-methanol (7:2) and the suspension was centrifuged at low speed. The precipitate was washed twice with acetone-methanol and finally with acetone before drying under reduced pressure. The precipitated proteins were dissolved in 0.5 M NaOH and an aliquot was used to determine protein by the method of Lowry *et al.* (1961). It was not possible to determine the BChl present in each fraction since it was rapidly destroyed under dim light in the organic solvents used. However, the absorbance at 590 mμ is due to BChl, and was used as a measure of BChl concentration in the various preparations.

The aerobic photooxidation of TMPD was followed spectrophotometrically using a modified Bausch and Lomb 505 spectrophotometer as previously described

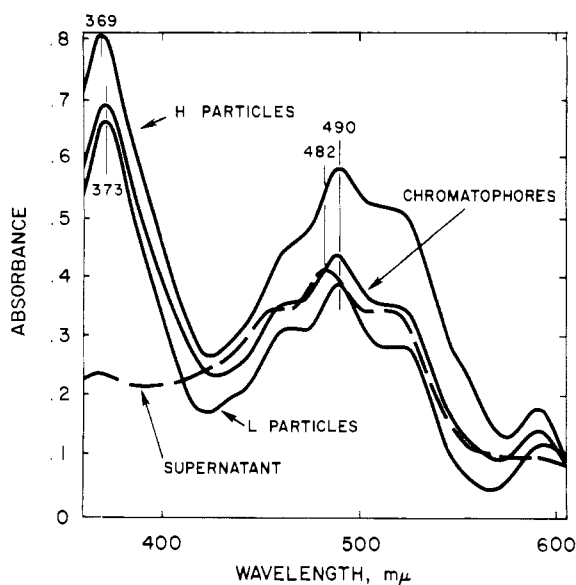


FIGURE 4: Absorption spectra of the various fractions for the visible region, showing the presence of carotenoids.

(Vernon, 1963). The reaction mixture contained 1.0 μ mole of TMPD and 400 μ moles of Tris buffer, pH 7.8, in addition to the chromatophore fractions in a volume of 6.0 ml. The PMSH₂ photooxidation system was the same as described by Zaugg *et al.* (1964). The PMSH₂ photoreactions were performed under anaerobic conditions using red actinic light (650-m μ sharp cut-off filter). Ubiquinone-6 (UQ6) was obtained from Mann Research Laboratories. The reduced UQ6 was prepared by the method of Green and Burkhard (1961). The absorption spectra of the fractions were taken with a Cary Model 14 spectrophotometer. The light-induced absorbancy changes at 890 m μ , a measure of the reaction center chlorophyll, were measured by Dr. R. K. Clayton, and the esr spectra were determined by Mr. R. W. Treharne.

Triton X-100, an alkylphenoxypolyethoxyethanol, is a nonionic detergent which was purchased from Rohm and Haas Co., Philadelphia, Pa. This detergent reportedly has 9-10 oxyethylene groups per molecule, making an average molecular weight of 625.

Results

Cells Grown at High Light. *Chromatium* chromatophores which had been treated with the detergent Triton X-100 fragmented into two major particles which could be separated by density gradient centrifugation. Figure 1 shows the separation pattern obtained after 15 hr of centrifugation at 110,000g. Some non-sedimenting material, which was yellow and appeared to be carotenoid, was located at the top of the tube. A brownish band, designated the light, or L band, was located in the upper portion of the tube and immediately below was another band, red, which is

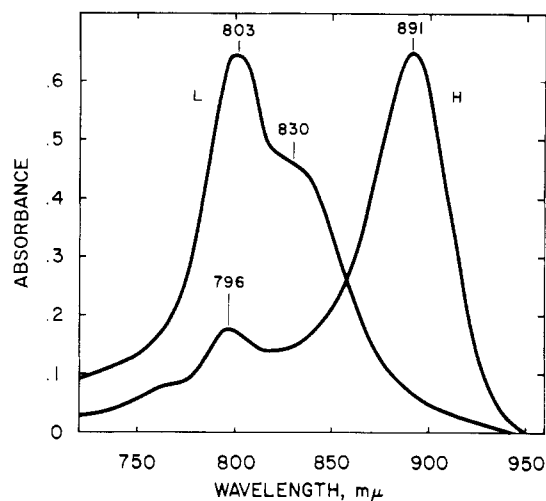


FIGURE 5: Absorption spectra of the heavy (H) and the light (L) fractions after an additional treatment with Triton X-100 and a second density gradient centrifugation. The absorbancies at 590 m μ were 0.10 (H) and 0.14 (L).

called the heavy, or H band. The drawing is made to scale and shows the relative widths of the separated bands. The densities corresponding to the various bands are not known. In some cases an additional band was observed in the lower part of the tube. All evidence indicates that this band consisted of incompletely ruptured chromatophores, since treatment of this material with more detergent and recentrifugation converted it into L and H fragments. Furthermore, the use of higher concentrations of Triton X-100 in the initial treatment prevented the appearance of this material.

The addition of Triton X-100 to *Chromatium* chromatophores caused some changes in the absorption spectrum, as shown in Figure 2. In discussing the spectra, we will call the three BChl components B800, B850, and B890, recognizing that in many cases the absorption maxima may occur at slightly different wavelengths. The most noticeable change caused by the detergent was a decrease of the 850-m μ band. A similar effect of this detergent was noted by Brill (1963), showing the sensitivity of this particular BChl component. The near-infrared absorption spectra of the isolated H, L, and supernatant fractions are shown in Figure 3. These data show clearly the different nature of the BChl pigments on the two particles. The heavier particle was greatly enriched in B890, yet still showed the presence of the other two BChl forms. The light fraction, L, was devoid of the B890 form, but contained both of the other BChl forms in almost equal concentration (assuming both forms have similar molar absorptivities). The material which remained at the top of the tube did have some B800, but as will be shown below, the light-absorbing material in this fraction was primarily carotenoid in nature.

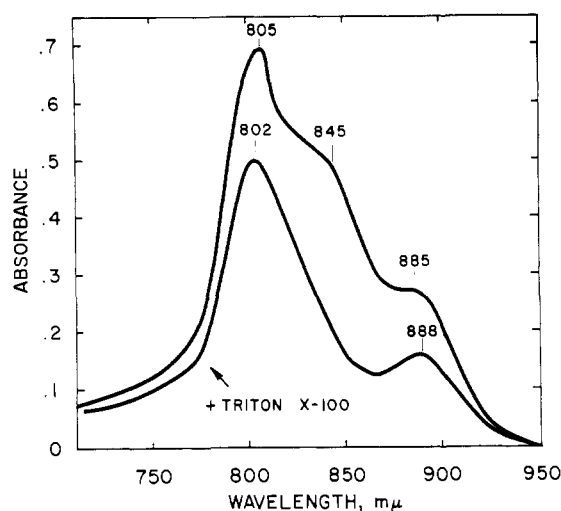


FIGURE 6: Absorption spectra of chromatophores obtained from *Chromatium* cells grown in low light. The spectra were taken with the chromatophores in the absence and presence of 3% Triton X-100 at pH 8.1 in 0.01 M Tris buffer. The absorbancy at 590 mμ was 0.35 in both cases. A tungsten lamp was used for growth of these cells, giving an intensity of 95 foot candles (compared to about 500 foot candles at the surface of the bottles for the normal cells).

The H and L fractions differ primarily in their absorption characteristics in the near-infrared region. Figure 4 shows the spectra obtained throughout the visible region for these fractions. It was not possible to make quantitative relationships between the pigments in these fractions, but it is apparent that they are all qualitatively alike, showing the typical carotenoid-dependent absorption in the 400- to 600-mμ region and the typical BChl peak below 400 mμ. There is some separation of the carotenoids by the procedures used. Figure 4 shows that the ratios of the bands at 520 and 460 mμ are different for the H and L fraction, with the 520 band being more prominent in the H particle. Another effect noted in this portion of the spectrum is the slightly different maxima for the 370- and 590-mμ bands in the different fractions. These reflect some slight modification in the environment of these BChl molecules.

Further treatment of either the H or L fractions with Triton X-100 showed that the three absorption bands for BChl in the near-infrared region for *Chromatium* chromatophores probably correspond to three separate BChl types. Reextraction caused a marked decrease in the 800 and 850 components of H, and yielded a particle containing primarily the 890 component (Figure 5). Similarly, reextraction of L partially removed or destroyed the 850 component, yielding a particle enriched in B800 (Figure 5). It has not been possible to separate the B800 and B850 components, which always appear together (although the 850 component sometimes appears as a shoulder of the B800

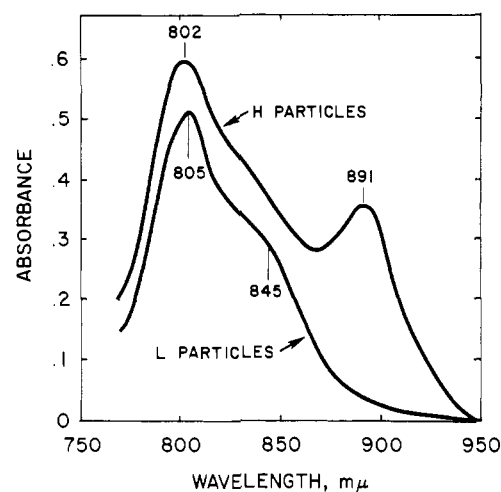


FIGURE 7: Absorption spectra of the fractions obtained by density gradient centrifugation of Triton-treated chromatophores obtained from *Chromatium* cells grown in low light intensity. The chromatophores were treated with 3% Triton X-100 before centrifugation. The absorbancies at 590 mμ were 0.22 (H) and 0.24 (L).

band). Although there is a close relationship between the B800 and B850, the relative absorptions at these wavelengths vary according to growth conditions and chromatophore treatment. For these reasons we feel that in this bacterium the three absorption bands correspond to three separate forms of BChl, and the B850 is particularly sensitive and is easily destroyed. This is different from *Rsp. spheroides* in which the 800 and 850 bands appear to be invariant in terms of their relative intensities, and it has been concluded that in this bacterium these bands represent a single BChl type (Sistrom, 1964). However, the experiments of Brill show that two distinct particles can be obtained from this bacterium, one enriched in B875 and the other particle containing the B800 and B850 (Brill, 1963).

Cells Grown at Low Light. Growth of *Chromatium* cells under low light intensity causes a change in the relative proportions of the three BChl forms, as shown in Figure 6. The most noticeable difference is the marked increase in B800 content. Subjecting these chromatophores to Triton X-100 results in a loss of the 850-mμ component, most likely due to destruction by bleaching in the dim light used during these experiments. Density gradient centrifugation of such detergent-treated chromatophores again yields two discrete particles (Figure 7). Except for the increased amount of the 800-mμ component in both fractions, the two fractions obtained resemble those obtained from cells grown under higher light intensity. The heavy fraction actually contained more B800 than B890. Again the light fraction was devoid of any 890-mμ pigment.

Photochemical Activities of the Derived Particles. The two major fractions H and L from *Chromatium* cells grown at high light intensity have been studied

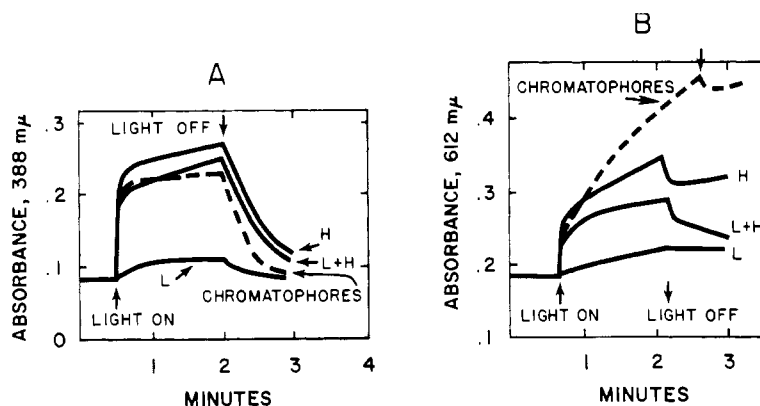


FIGURE 8: Photooxidation reactions catalyzed by the derived particles from *Chromatium* chromatophores. (A) Photooxidation of PMSH_2 coupled to the reduction of UQ6 under anaerobic conditions, using the assay procedure described by Zaugg *et al.* (1964). The BChl contents of the chromatophores and fragments were proportional to their absorbancies at 590 mμ, which were: L (4.4), H (0.3), and chromatophores (0.22). (B) Photooxidation of TMPD under aerobic conditions. The assay system is described under Methods. The absorbancies of the chromatophores and particles at 590 mμ were: L (4.4), H (0.4), and chromatophores (0.22).

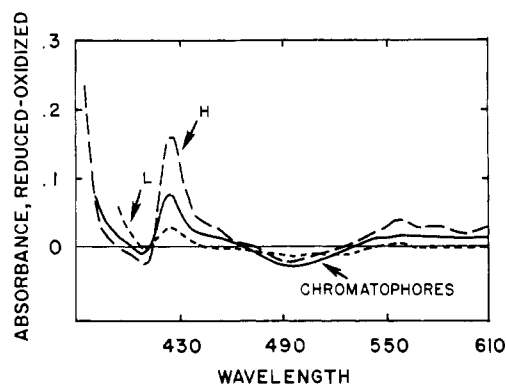


FIGURE 9: Cytochromes present in the derived particles. The spectra, obtained with a Bausch and Lomb spectrophotometer, are difference spectra between reduced (with hydrosulfite) and oxidized (ferricyanide) samples of the fractions. The fractions had first been extracted with acetone to remove the BChl. Original absorbancies (before extraction) at 590 mμ were: H (0.25), L (0.43), and chromatophores (0.43).

to determine their abilities to catalyze representative photochemical reactions. Treatment with even low concentrations of Triton X-100 destroys the photophosphorylation ability of intact chromatophores, and none of this activity remains in the derived particles. They are also inactive for NAD photoreduction with succinate or ascorbate-DPIP. However, even intact *Chromatium* chromatophores have low activity in this regard.

Both intact chromatophores and the derived particles are unable to photoreduce methyl red in the presence of ascorbate and DPIP, a reaction readily carried out by *R. rubrum* chromatophores (Ash *et al.*, 1961). The derived H and L particles are able to perform two

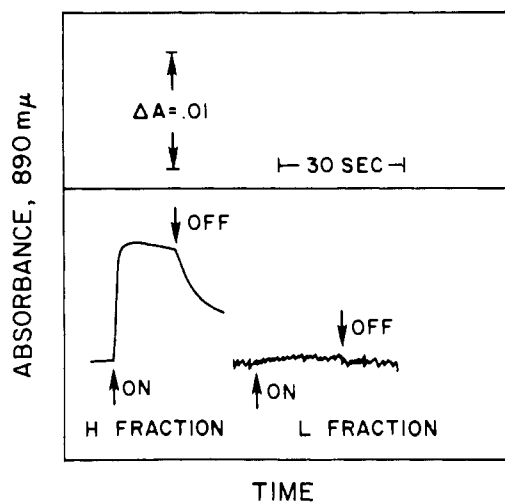


FIGURE 10: Localization of the P890 reaction in the heavy fraction. The light-induced absorbancy change was detected by Dr. R. K. Clayton with the apparatus previously described (Clayton, 1962). The absorbancies at 590 mμ were 0.05 (H) and 0.15 (L).

photochemical reactions: the aerobic photooxidation of TMPD and the anaerobic photooxidation of PMSH_2 coupled to reduction of UQ6. The details concerning these reactions with intact chromatophores have previously been published (Zaugg *et al.*, 1964), and Figure 8 shows the data obtained with the chromatophore fragments. Of the two fragments, H is clearly more active. Indeed, the small activity noted for the L particles could be due to contamination of this material with H particles.

It is generally held that physiologically significant photochemical reactions in bacterial systems are initi-

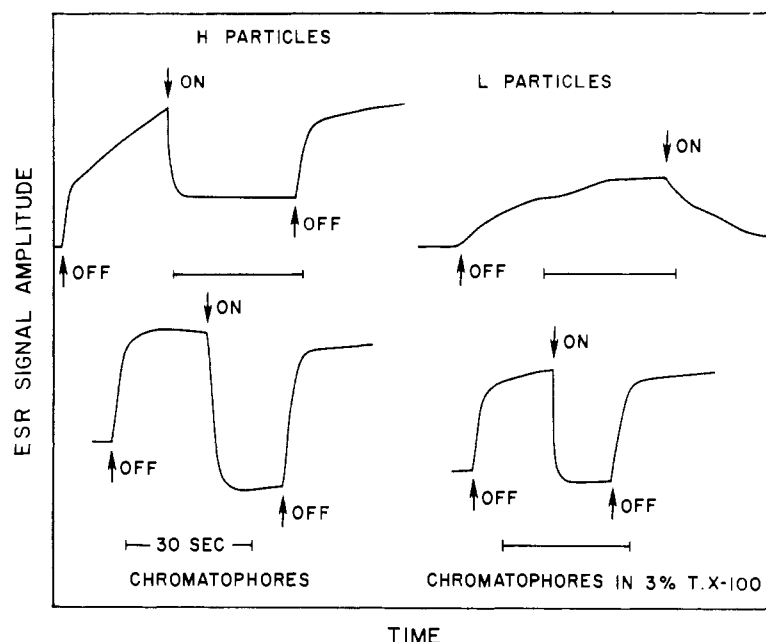


FIGURE 11: Kinetics of the light-induced esr signals for *Chromatium* chromatophores and particles derived by treatment with Triton X-100. These experiments were performed by Mr. R. W. Treharne on the apparatus described previously (Treharne *et al.*, 1963). The absorbancies at 590 $m\mu$ were 0.8 (H), 1.2 (L), and 0.8 (chromatophores).

ated at a "reaction center" which would contain a BChl which is special in the sense that it is complexed with (or at least adjacent to) the necessary electron transfer agents, such as cytochromes. Although it has not been isolated and characterized, the reaction center BChl is readily detected by means of its light-induced absorbancy change.

Photooxidation of TMPD and PMSH₂ by bacterial chromatophores requires an operative reaction center, as shown by Clayton *et al.* (1965) for *Rps. spheroides*. The fact that only the H particle efficiently catalyzes these photooxidation reactions indicates that the reaction center BChl is localized in this particle. If this be true, the cytochromes related to photosynthetic functions should also be localized in H, and Figure 9 shows this is the case. The position of the α band of the reduced cytochrome varies somewhat in different preparations, but is located around 553 $m\mu$. We are not able to identify which of the *c*-type *Chromatium* cytochromes is represented by the spectrum in Figure 9, or if it represents all of those previously reported (Vernon, 1964). However, the important datum is the concentration of these cytochromes in the H fraction.

Another criterion for the presence of reaction center chlorophyll is the absorbancy change at 890 $m\mu$ related to the reaction center P890 (Clayton, 1962). Again fraction H shows this response, and the L fraction is devoid of this activity, as shown in Figure 10. All these data, combined with the fact that the H fraction contains all of the detectable B890, indicates that the reaction center is indeed localized in this particle.

The concentrations of BChl and cytochrome relative to total protein in the chromatophores and the derived

TABLE I: Relative Concentrations of BChl and Cytochrome in *Chromatium* Chromatophores and the Derived Particles.^a

Fraction	Absorbance at 590 $m\mu$ per mg of Protein	Δ Absorbance at 423.5 $m\mu$ per mg of Protein
Chromatophores	0.86	0.16
H fraction	0.62	0.44
L fraction	1.2	0.075

^a The absorbance at 590 $m\mu$ is taken as a measure of BChl concentration, and the change of absorbance at 423.5 $m\mu$ is related to the cytochrome content.

particles are shown in Table I. The H fraction, as expected, is significantly enriched in cytochrome, while the L fraction has only trace amounts. Also the H fraction has relatively more protein (compared to BChl) than the L fraction. The absorbancies at 590 $m\mu$ for the two fractions, which are proportional to the BChl concentrations, show that the BChl from the chromatophores fractionates into the L and H fractions in the ratio of approximately 3:1 (L:H).

The H and L fractions have been examined for their electron-spin resonance properties using the apparatus described previously (Treharne *et al.*, 1963). Both particles gave light-induced esr signals which were located at $g = 2.002$. On the basis of BChl content,

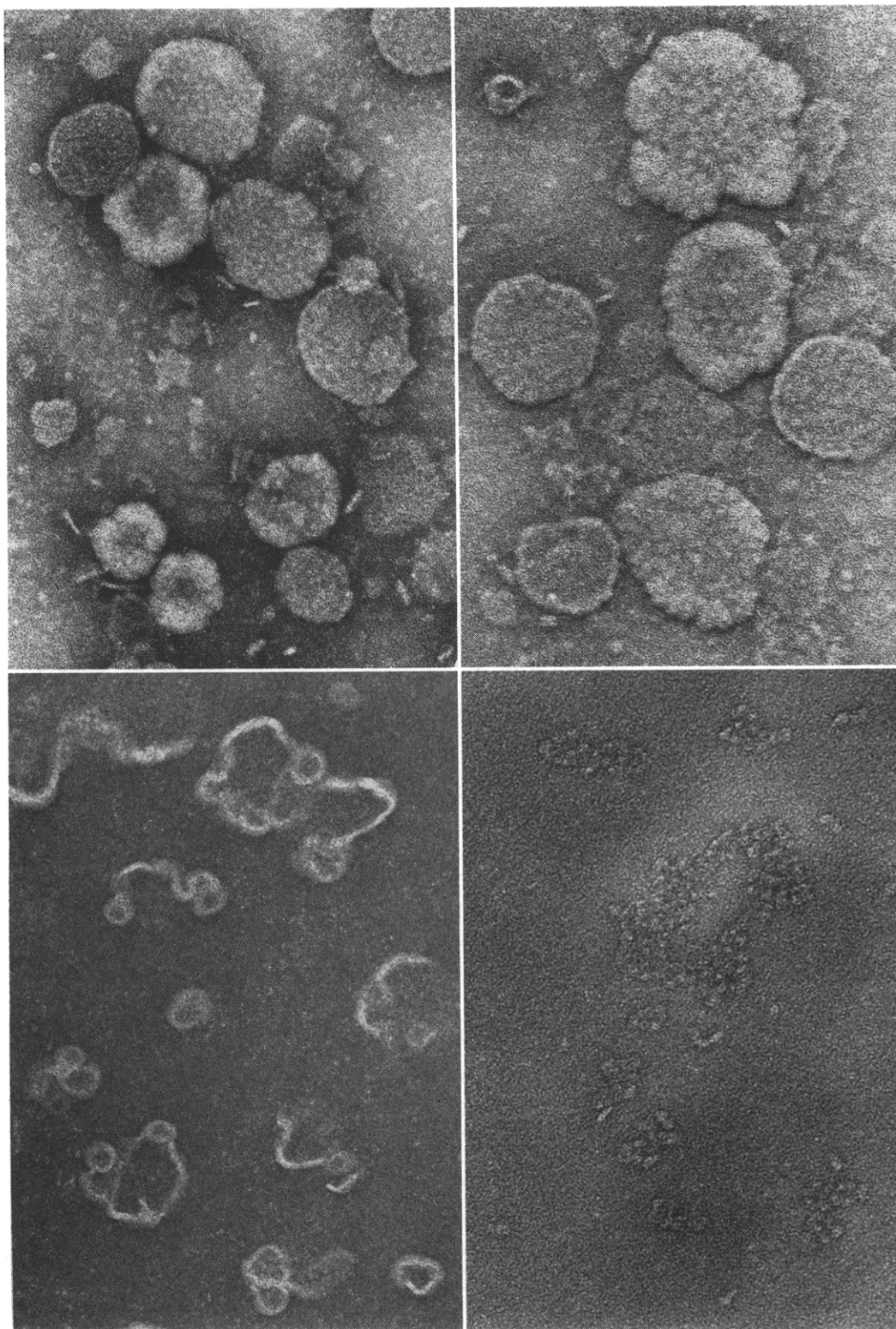


FIGURE 12 (upper left): Electron micrograph of *Chromatium* chromatophores, obtained by negative staining with phosphotungstic acid at pH 7.0; $\times 160,000$.

FIGURE 13 (upper right): *Chromatium* chromatophores prepared as in Figure 12 but printed by the masking technique described by Gonazles (1962); $\times 200,000$.

FIGURE 14 (lower left): Electron micrograph of the L fraction of *Chromatium* chromatophores; $\times 113,000$.

FIGURE 15 (lower right): Electron micrograph of the H fraction of *Chromatium* chromatophores; $\times 180,000$.

the H particle gave a larger signal. Examination of the kinetics of the light-induced signals gave the data shown in Figure 11. Whereas the signal associated with the L particles had a slow rise and decay, the corresponding signal for the H particle showed a rapid rise upon illumination. In the dark, however, the signal decayed in a biphasic manner, indicating the presence of two types of unpaired electrons in this particle. The response of the H particle more closely resembles that of intact chromatophores, which indicates that the esr signal seen in the intact chromatophores is probably related to the functional reaction center chlorophyll.

Structure. Electron micrographs of *Chromatium* chromatophores and the fragments produced by detergent action are shown in Figures 12–15. The chromatophores (Figures 12 and 13) appear as flattened disks. Frequently, they have surface depressions and sometimes appear cup shaped.

In some chromatophores substructure is clearly evident (Figure 13). Individual units of approximately 60–70 Å in diameter are arrayed in the chromatophore, sometimes in a regular, repeating fashion with rows of subunits separated by approximately 100–150 Å. The L particles (Figure 14), which are photochemically inactive, consist of the membrane of the chromatophore along with some attached material possibly deriving from the interior of the chromatophore. The H particles (Figure 15), which are photochemically competent, show none of the structure of the original chromatophore, and apparently consist of the released subunits from the chromatophore. Similar subunits are also seen in *R. rubrum* chromatophores (Garcia *et al.*, 1966), which show a similar response to Triton X-100.

Discussion

The ability of BChl to exist in three forms absorbing at different wavelengths enhances the bacterial cells' ability to utilize available light for its photosynthetic process, since studies on BChl fluorescence *in vivo* show that the three forms are structurally close enough to allow the transfer of excitation energy from the 800 and 850 forms to B890 (Duysens, 1952; Bril, 1964). The experiments of Bril (1964) show that low concentrations of Triton X-100 suffice to disrupt this association, and prevent the energy transfer to B890. The present study shows that higher concentrations of the detergent physically disrupt the chromatophore to yield two fragments and the BChl pigments segregate in the two particles. One particle contains all of the B890 and a little of the B850 and B800. Further extraction with the detergent removes this residual B850. This particle, which is the heavier one, contains the reaction center chlorophyll P890 and cytochrome, and shows limited photochemical activity. The lighter particle contains both B800 and B850, the latter in variable amounts. It is photochemically inactive and the B850 component is very labile.

The existence of three BChl forms *in vivo*, as evidenced by the near-infrared bands, has been interpreted in

terms of either BChl-protein interaction (Duysens, 1952), carotenoid-BChl interaction (Izawa *et al.*, 1963; Wassink *et al.*, 1939; Bergeron and Fuller, 1959; Clayton and Arnold, 1961), or different aggregated forms of BChl (Krasnovskii *et al.*, 1952). The present investigation does not give direct information on this important point. However, since there is no marked preferential separation of the carotenoids between the two particles, it seems less likely that the observed differences are caused by carotenoid-BChl interaction. This is also supported by the data of Sistrom *et al.* (1956) on *Rps. spheroides*, which show that growth of the bacterium under conditions which do not allow carotenoid synthesis does not significantly alter the absorption pattern of the near-infrared bands of the BChl. Pertinent to this question is the recent observation of R. K. Clayton (personal communication, 1965) that the BChl bands *in vivo* are intensified and narrowed by comparison to BChl in organic solvents. This would not be expected to occur for BChl going from a monomer in solution to an aggregated form *in vivo*. Thus, it appears likely that the band positions observed for BChl *in vivo* are due to specific BChl-protein complexes.

Triton X-100 at the concentrations employed splits the chromatophore into two fragments. One contains the reaction center chlorophyll and appears to be involved in the primary photochemical and perhaps secondary electron-transfer reactions of photosynthesis. The less-dense particle contains the B800 and variable amounts of the B850 components. Its function seems to be that of light harvesting and energy transfer. Since the B850 is distributed between both particles, and since after preparation of the particles the B850 is very labile and subject to photooxidation, it appears that Triton X-100 splits the photosynthetic apparatus in the area where the B850 is contained, perhaps by solubilizing the protein (lipoprotein) attached to the B850. After this action the B850 would be more exposed to external reagents and more labile than the other BChl components. This indicates a structural sequence in the chromatophore of B800-B850-B890, with B890 being situated adjacent to the electron-transfer components such as cytochromes, quinones, etc. This sequence was earlier proposed by Bril (1963).

Electron microscopic study shows that the H particle, the photochemically competent one, could derive from the interior of the chromatophore, while the L particle is associated with the chromatophore membrane. A similar situation obtains for *R. rubrum*, except that in the case of *R. rubrum* no segregation of the BChl forms between the two particles was observed (Garcia *et al.*, 1966). Since the L particle contains the BChl forms which may serve as light-gathering molecules, the arrangement of this particle on the surface of the chromatophore is logical. All light-harvesting BChl forms on the surface could transfer excitation energy to the photochemically active BChl situated somehow in the interior of the chromatophore. Under conditions of low incident light, the content of the B800 and B850 forms of BChl increases, thus allowing for a more efficient light-harvesting operation. About three-fourths

of the total BChl is present in the L fraction which contains the B800 and B850 forms. This agrees with the absorption spectrum of the intact chromatophore, which shows relatively more B800 and B850. If the chromatophore exists in the cell as a flattened disk, as is indicated in Figure 13, or if the H particles are intimately attached to the interior of the chromatophore membrane, the photoactive H particles could easily be in close enough contact with the chromatophore membrane system to allow for efficient energy transfer from the B800 and B850 forms. Of course the alternate possibility cannot be ruled out that the H particles arise from specialized enclaves within the chromatophore membrane, which is modified by the detergent treatment to yield the L fraction.

Acknowledgment

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