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

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ABSTRACT: Treatment of *Rhodospirillum rubrum* chromatophores with Triton X-100 yields three fragments which are separable by sucrose density gradient centrifugation. Two contain bacteriochlorophyll (BChl) and have absorption spectra which resemble that of the original chromatophores, except for a slight displacement of the main absorption band in the near infrared. Both particles show the light-induced absorption change related to P890, the reaction center BChl. The heavy fraction contains the major portion of the BChl, and the light fraction contains relatively more protein. Both particles catalyze cytochrome *c* and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) photooxidation coupled to oxygen. They also support the photooxidation of reduced phenazine methosulfate coupled to ubiquinone-6. Only the light fraction has activity for nicotinamide–adenine dinucleotide (NAD) and methyl red photoreduction coupled to ascorbate–2,6-dichlorophenolindophenol (DPIP). Succinic dehydrogenase activity is found in the light particle but not in the heavy one. In addition to the two BChl-containing particles, a bacteriopheophytin-containing band is obtained. This fraction is most active in catalyzing the simple photoreactions such as cytochrome *c* and TMPD photooxidation and methyl red photoreduction. This band, which sediments only slightly, also contains cytochrome *c* and has succinic dehydrogenase activity. The cytochrome and bacteriopheophytin appear to be on the same particle. The two BChl-containing particles arise from different environs in the chromatophore; the light particle most likely arises from specific subunits within the chromatophore while the heavy particle consists of chromatophore membranes with some attached subunits. These different particles could relate to different photoreactions in the chromatophore.

The accompanying paper (Garcia *et al.*, 1966), which is concerned with *Chromatium*, presents the historical background for our present experiments on the fractionation of the photosynthetic apparatus of bacteria with the detergent Triton X-100. This detergent fragments the photosynthetic apparatus of both *Chromatium* and *Rhodospirillum rubrum* into two major BChl[1]-containing fragments which are separable by density gradient centrifugation. Some of the physical and photochemical properties of the fragments obtained from *R. rubrum* are reported in this communication.

Methods

The *R. rubrum* cells, strain S-1, used for this study were grown on malate medium according to the directions of Newton (1961). Chromatophores were released from the cell by sonication and separated from other cellular material by centrifugation (Garcia *et al.*, 1966). The experimental details concerning apparatus, the procedure used for detergent treatment, density gradient centrifugation, dialysis of the separated particles, and protein determination are those described in the previous paper (Garcia *et al.*, 1966). In the present case the ratio of Triton X-100/BChl could be determined, and was routinely 70 mg of detergent/mg of BChl (which was also equal to 2.5 mg of Triton X-100/mg of protein). The detergent was in contact with the chromatophores for 3 hr before centrifugation. Acetone–methanol was used to extract BChl from the particles, and the concentration of BChl was determined from the absorption at 772 mμ using a value of 75 as the millimolar absorptivity as described by Clayton (1963).

For methyl red and tetrazolium blue photoreduction, the system described by Ash *et al.* (1961) was used, which employed ascorbate–DPIP as the electron donor system. DTNB photoreduction was assayed according to the procedure of Newton (1962). The procedures used for $PMSH_2$ photooxidation were the same as reported by Zaugg *et al.* (1964). The reactions were performed under anaerobic or aerobic conditions, using red light. In order to preserve activity in NAD photoreduction, all preparation steps involving the chromatophores were carried out under argon. The light-induced absorption changes were measured by Dr. R. K. Clayton, to whom the authors express their gratitude. The esr spectra were obtained with the kind help of Mr. R. W. Treharne using the Varian

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[1] The abbreviations used in this paper are: DPIP, 2,6-dichlorophenolindophenol; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; BChl, bacteriochlorophyll; PMS, phenazine methosulfate (phenazonium methosulfate); UQ6, ubiquinone with six isoprene units in the side chain; H, heavy particles; L, light particles; Pheo, bacteriopheophytin-containing particle; NAD, nicotinamide-adenine dinucleotide; esr, electron-spin resonance, DTNB, 5,5'dithiobis(2-nitrobenzoic acid), disulfide form.

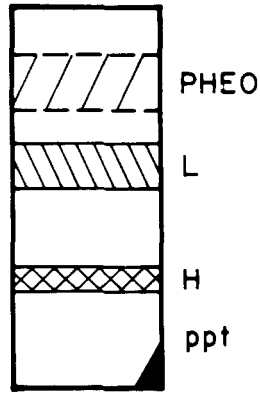

FIGURE 1: The separation obtained by sucrose density gradient centrifugation of *R. rubrum* chromatophores treated for 3 hr with Triton X-100. See the section on Methods for details.

V4502 spectrometer previously described (Heise and Vernon, 1963) without an interference filter. The electron micrographs were obtained with a Philips 200 electron microscope.

Results

Equilibrium centrifugation of *R. rubrum* chromatophores following treatment with Triton X-100 produced five different zones in a sucrose gradient. Figure 1 shows a typical separation obtained after 15 hr of centrifugation at 110,000*g*. Practically all the pigmented material was localized in two sharp, red-purple bands, designated the H (heavy) and L (light) bands. A diffuse, brown band was designated the Pheo (pheophytin-containing) band. The zones in between the bands contained varying small amounts of BChl, and therefore have not been directly investigated. Most of the BChl was contained in the H band, which was dark red in color because of the associated carotenoids.

Composition. The absorption spectra of the three pigment-containing bands are shown in Figure 2. Both the H and L particles yield spectra which do not differ significantly from that of the intact chromatophore. There is a displacement of the 880-mμ band to 882.5 mμ for the H particle. There is no separation of the 880- and 800-mμ components between the two fractions, however. The Pheo fraction shows an absorption spectrum which is similar to that of bacteriopheophytin. [See Vernon and Seely (1966) for absorption spectra of bacteriochlorophyll *in situ* and isolated bacteriopheophytin.] The Pheo fraction represented in Figure 2 was obtained after dialysis of the original Pheo material and recentrifugation at 144,000*g* for 2 hr. Thus the pigment contained in this fraction is "soluble" to the extent that it cannot be sedimented by centrifugation under these conditions.

The fractions obtained in the density gradient centrifugation were analyzed for both their BChl and protein content. Table I shows that BChl was concentrated in the H fraction, while protein was relatively more abundant in the L fraction. This has significance also in the potentiality of these two particles to catalyze

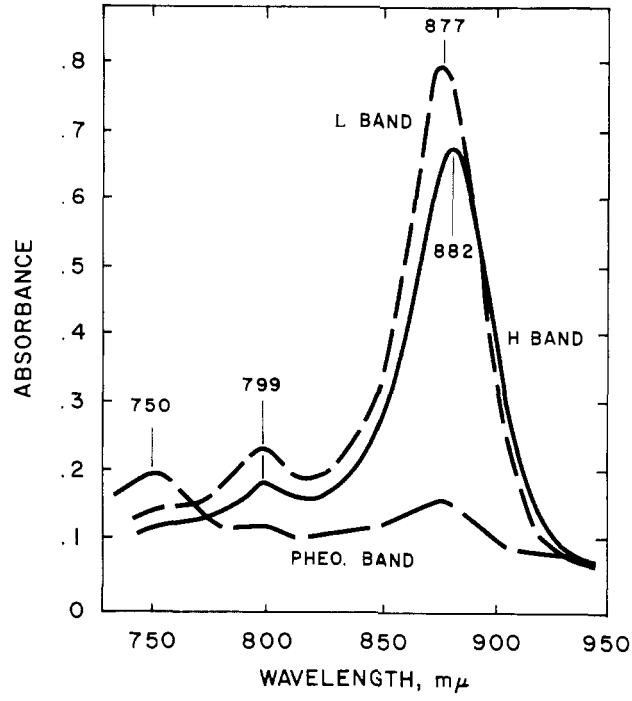

FIGURE 2: Absorption spectra of the heavy (H), light (L), and pheophytin-containing (Pheo) fractions separated by density gradient centrifugation. The BChl concentrations were 4.2 (H) and 5.0 μg/ml (L).

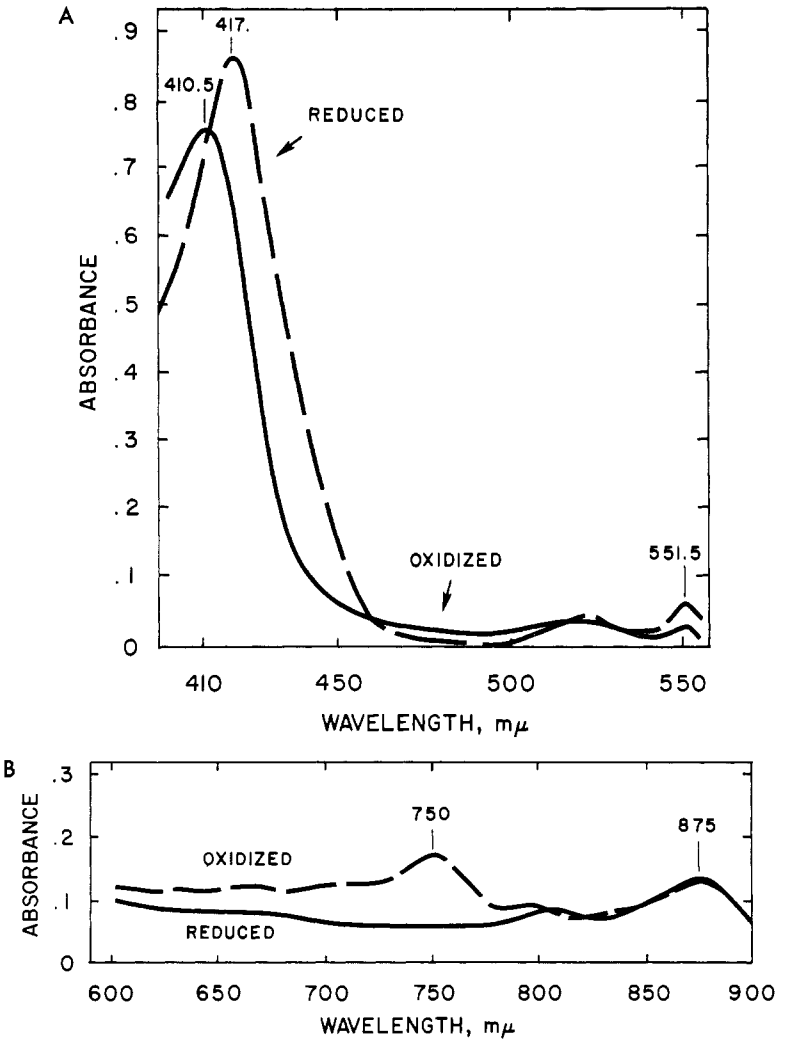

FIGURE 3: Spectra in the visible (A) and near-infrared (B) regions of the reduced and oxidized Pheo fraction described in Figure 2. The reduced fraction was treated with dithionite and the oxidized fraction was produced with ferricyanide. The preparation contained 0.8 mg of protein/ml.

photoreactions, since those reactions requiring the participation of enzymes were restricted to the L particle, the one which had relatively more protein.

Cytochromes were observed in the Pheo band, as shown by the reduced and the oxidized spectra of this fraction. The position of the 410-mμ peak observed in direct absorption spectra of these fractions varied among preparations. This may be caused by different oxidation states of the cytochrome. Figure 3A shows the spectra obtained for the Pheo band in the visible part of the spectrum. The cytochrome contained in the Pheo material is of the *c* type, showing a peak at 410 mμ in the oxidized form and at 419 and 551.5 mμ in the reduced form. When hydrosulfite was added to reduce the cytochrome, the 756 band of the bacteriopheophytin disappeared, as shown in Figure 3B. It is not presently known if the pheophytin and cytochrome are complexed to a protein or small particle in some way, nor is it known whether the pheophytin exists as such *in vivo* or if it is formed from BChl upon exposure to the detergent. In either case, a small amount of the total BChl appears in the pheophytin form and accompanies the cytochrome during centrifugation. Dialysis does not separate the cytochrome from the pheophytin.

Retreatment of the H fraction with Triton X-100, employing the original conditions, does not yield significant amounts of the Pheo band. Only one major band, the H band, is observed when the retreated material is subjected to density gradient centrifugation. Also observed are lighter, red-pigmented bands which may represent different aggregates of the L particles.

Photochemical Activities. The two BChl-containing particles and the Pheo fraction were tested for their ability to catalyze some of the electron-transfer reactions characteristic of *R. rubrum* chromatophores. Figure 4 shows that all three chromatophore-derived fragments catalyzed both TMPD and cytochrome *c*

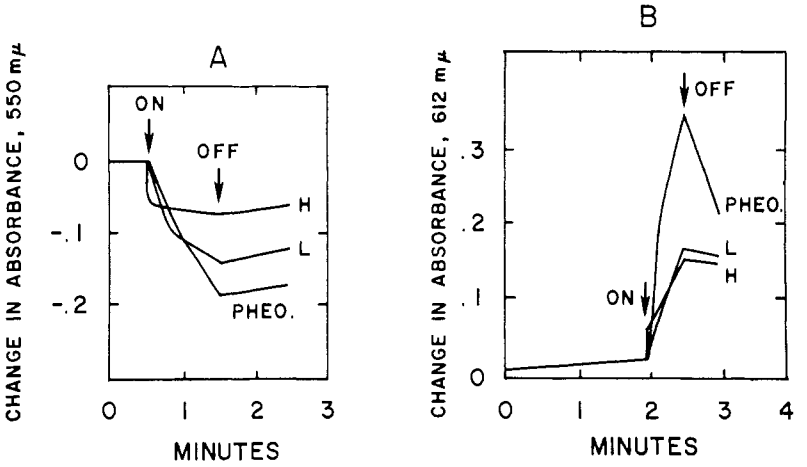

FIGURE 4: Photooxidation of cytochrome *c* (A) and TMPD (B) by the particles obtained from chromatophores with Triton X-100. The reaction mixtures contained in micromoles: Tris buffer, pH 7.8, 400; (A) reduced mammalian cytochrome *c*, 1.8; (B) TMPD, 1.0. Also present were: H fraction, 0.12 mg of BChl; L fraction, 0.06 mg of BChl, or Pheo fraction, 0.48 mg of protein; final volume 6.0 ml.

TABLE I: Bacteriochlorophyll and Protein Content of the Different Fractions Obtained from *R. rubrum* Chromatophores by Sucrose Density Gradient Centrifugation.

Fraction	Protein %[a]	Bacteriochlorophyll	μg of BChl/mg Protein
Pheo	10	0	...
L	6–8	2	7.1
H	18	80–85	125
Precipitate	45–50	8–10	7.1
Chromatophores	100	100	35.0

[a] The different percentages are related to the original chromatophores as 100%.

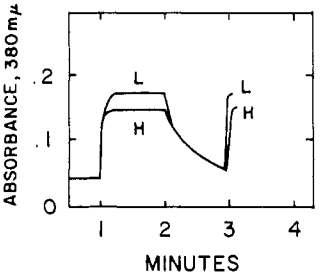

FIGURE 5: $PMSH_2$ photooxidation coupled to UQ6 reduction under anaerobic conditions. The reaction mixtures contained in micromoles: PMS, 0.2; phosphate buffer, pH 7.5, 250; UQ_8H_2, 0.01. Also present were: H fraction, 0.07 mg of BChl, or L fraction, 0.05 mg of BChl; final volume, 3.0 ml.

photooxidation, but in both cases the reaction catalyzed by the Pheo fraction proceeded further. Of the two BChl-containing particles, the H fraction gave a faster initial reaction, but in terms of total reaction both were quite similar.

The photooxidation of $PMSH_2$ coupled to UQ2 reduction is catalyzed by bacterial chromatophores and isolated BChl at high rates (Zaugg *et al.*, 1964). *Rps. spheroides* chromatophores lacking in reaction center BChl (the special BChl component showing light-induced bleaching at 890 mμ) but containing a normal amount of bulk BChl (that Bchl which is not bleached by light) are inactive in this regard (Clayton *et al.*, 1965). This reaction, then, is apparently limited only by the accessibility of the reagents to the BChl. As expected, both BChl-containing fractions were active in this reaction (Figure 5). As will be shown below, both fractions also show the P890 reaction, and therefore contain reaction center BChl. The Pheo fraction was not active when tested in this reaction, but this reaction is complicated by the fact that the detergent in this fraction made evacuation (to obtain anaerobic conditions) impossible.

Some distinction between the pigment-containing fractions is apparent in their ability to catalyze methyl red photoreduction. This reaction, also catalyzed by intact chromatophores (Ash *et al.*, 1961), is supported by ascorbate–DPIP as the source of electrons (Figure 6). Again the Pheo band was most active, followed by the L fraction. The H particles were quite inactive in this reaction. Again, this type of reaction does not require intact chromatophores, but is catalyzed by isolated Chl *a*, either in ethanol or in aqueous media with detergents present (Vernon *et al.*, 1966). Therefore, it appears that this reaction also requires primarily contact between the BChl and the reactants.

The reactions listed above are nonphysiological in

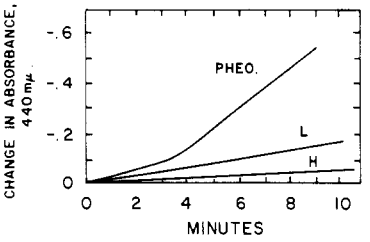

FIGURE 6: Methyl red photoreduction coupled to ascorbate–DPIP under anaerobic conditions. The reaction mixtures contained, in micromoles: methyl red, 0.8; Tris buffer, pH 7.8, 200; DPIP, 0.5; ascorbate, 20. Also present were: H fraction, 0.12 mg of BChl; L fraction, 0.06 mg of BChl, or Pheo fraction, 0.48 mg of protein; final volume, 6.0 ml.

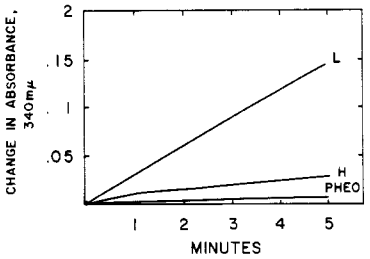

FIGURE 7: NAD photoreduction coupled to ascorbate–DPIP under anaerobic conditions. The reaction mixtures contained, in micromoles: NAD, 3.0; Tris buffer, pH 8.1, 200; DPIP, 0.5; and ascorbate, 20. Also present were: H fraction, 0.1 mg of BChl; L fraction, 0.06 mg of BChl; final volume, 6.0 ml.

the sense that the donors and acceptors used are not involved in the photosynthetic process as it takes place within the cell. A more physiological partial reaction is the photoreduction of NAD, supported by either succinate or the ascorbate–DPIP couple as the electron donor system (Vernon, 1963). Of the three pigmented fractions, only the L fraction has significant activity in this reaction, as shown by Figure 7. The rate of NAD reduction was low, however, when compared with rates observed for chromatophores (5 for L fraction *vs.* 20–40 μmoles/hr/mg of BChl for chromatophores). When the fractions were assayed for succinic dehydrogenase activity, only the light fraction and the Pheo fraction were found to contain this activity. Thus the activities which require the participation of both enzymes and BChl are restricted to the L particle. This is in agreement with the information given in Table I, which shows that the L fraction has relatively more protein per BChl molecule.

Two other photoreactions were studied in a preliminary fashion. All three pigmented fractions showed activity in tetrazolium blue photoreduction (Ash *et al.*, 1961) and in DTNB photoreduction (Newton, 1962). The latter reaction, studied in detail by Newton, involves the preliminary photoreduction of methyl viologen which is a cofactor in the reaction. Newton also observed that DTNB photoreduction was markedly stimulated by the addition of ammonium sulfate. In our experiments also, the presence of 0.3 M ammonium sulfate caused a stimulation of DTNB photoreduction with all three fractions. Even more marked stimulation by ammonium sulfate was observed for methyl red photoreduction (Figure 8). All the other photoreactions we have investigated were not responsive to added ammonium sulfate. The reason for the simulation in the two cases mentioned is not known, but could be due to some structural modification in the presence of the high salt concentration which favors interaction of the reactants.

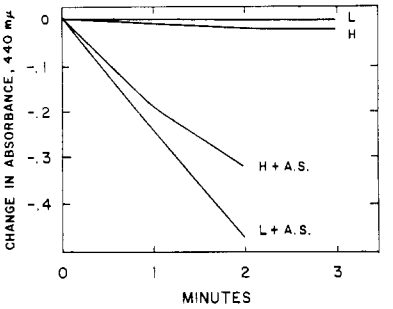

FIGURE 8: Stimulation of methyl red photoreduction by ammonium sulfate. Ascorbate–DPIP was used as the electron-donor system. The reaction mixture was the same as for Figure 6 and contained in addition 0.3 M ammonium sulfate.

R. rubrum chromatophores show a light-induced absorption change at 890 mμ which is related to a small, specialized fraction of the total BChl which is designated as the reaction center BChl or P890 (Clayton, 1962). Both the H and L fraction show this light-induced absorption change to about the same degree. A preparation of H particles (880-mμ absorption = 1.84) gave an absorption change of 0.043 at 890 mμ upon illumination. A preparation of the L particles (880-mμ absorption = 1.88) gave an absorption change of 0.034. It would appear that P890 is somewhat concentrated in the H particles, but it is questionable if the difference observed in this experiment was significant. It is apparent that, in contrast to the data observed for *Chromatium*, the P890 is partitioned between the two particles. As shown above, there was also no gross separation of the two BChl types (B800 and B880) by detergent treatment either.

The H and L fractions both show light-induced esr signals which have a *g* value of 2.002 and show somewhat slower kinetics than do chromatophores. For comparison purposes, chromatophores treated with Triton X-100 were examined along with the two derived particles. Figure 9 shows the kinetics of the generation and decay of the esr signals in these preparations. On a BChl basis, less signal was generated by the H particle. Although the kinetics of the L particle differ

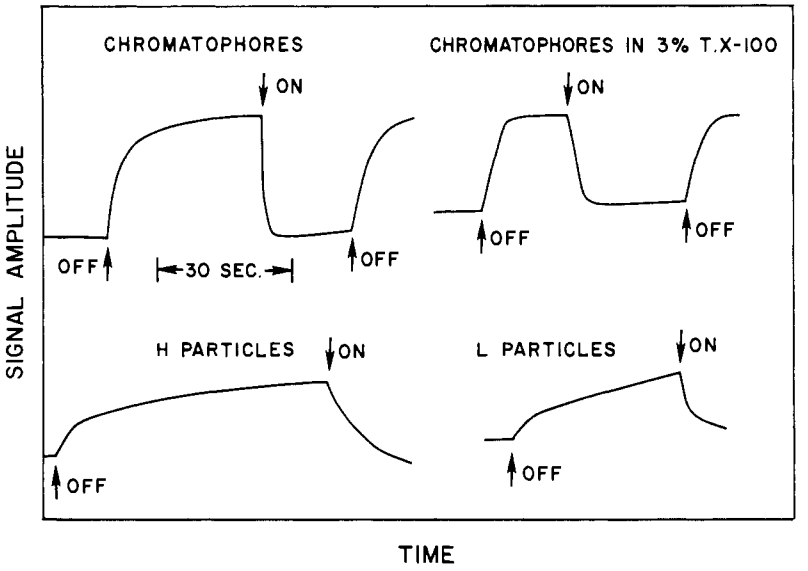

FIGURE 9: Kinetics of the light-induced esr signals of *R. rubrum* chromatophores and the particles derived by the action of Triton X-100. Chromatophores: 0.3 mg of BChl/ml; Tris, pH 8.1, 100 mM. Chromatophores and Triton X-100: 0.3 mg of BChl/ml; Tris, pH 8.1, 100 mM; Triton X-100, 3% final concentration. H particles: 0.55 mg of BChl/ml; Tris buffer, pH 8.1, 100 mM. L particles: 0.02 mg of BChl/ml; Tris, pH 8.1, 100 mM. The apparatus employed was described by Heise and Vernon (1963). The curve represents the change of signal amplitude at the field strength giving the maximum of the light-induced signal. The lower arm of the esr derivative curve was followed.

somewhat from those of chromatophores, this particle does exhibit a fast onset of the signal upon illumination. The H fraction produced a slowly developing signal which also decayed slowly in the dark. In view of the suggestion that the fast esr signal of chloroplasts is related to the P700 (reaction center chlorophyll) of chloroplasts (Beinert and Kok, 1963), one would expect that in the present case there would be a positive correlation between the P890 and esr responses of the derived particles. However, the P890 is about equally distributed between the particles while the esr response is greater in the L fraction. The reason for this is not apparent.

Structure. Electron microscopy has been used to determine the structure of the fractions obtained through the action of Triton X-100. The samples were negatively stained using phosphotungstic acid neutralized to pH 7.0 with NaOH. The chromatophore fraction (Figures 10 and 11) consisted of nearly circular appearing structures of varying sizes. Some substructure, consisting of particles about 50 A in diameter, is apparent in some of the chromatophores (see arrows in Figure 11). These subunits possibly correspond to those previously described by Holt and Marr (1965).

The H fraction consisted primarily of cup-shaped structures (Figure 12) which probably correspond to the membranous portions of the chromatophores. Many of these structures appear disrupted and none of them show the substructural, or particulate, detail of the chromatophore. The L fraction (Figure 13) consists of granular material derived from the chromatophores.

Discussion

Treatment with Triton X-100 fragments *R. rubrum* chromatophores into two main BChl-containing fractions which are readily separable by density gradient centrifugation. The salient features of these two particles are the following: (1) There is no significant difference in the BChl composition. Both the H (heavy) and L (light) fractions contain the 800- and 880-mμ forms of BChl in the same proportion found in the original chromatophores. (2) Both fractions contain reaction center BChl, as shown by light-induced absorbancy changes at 890 mμ. (3) The L fraction contains relatively more protein than the H fraction, and the H fraction contains the major portion of the BChl. (4) Both fractions catalyze cytochrome *c* and TMPD photooxidation coupled to oxygen as well as $PMSH_2$ photooxidation coupled to UQ2. (5) The L fraction is the only one containing significant activity for NAD photoreduction, methyl red photoreduction, and succinic dehydrogenase activity. In addition to the BChl-containing fractions, a bacteriopheophytin-containing band is obtained. This fraction is most active in catalyzing the simple photoreactions such as TMPD and cytochrome *c* photooxidation and methyl red photoreduction. The bacteriopheophytin-containing particle does not pass through a dialysis membrane.

Although there is no apparent fractionation of the BChl types (B800 and B880) between the two BChl-containing fractions, there are sufficient differences to show that they derive from different environs within

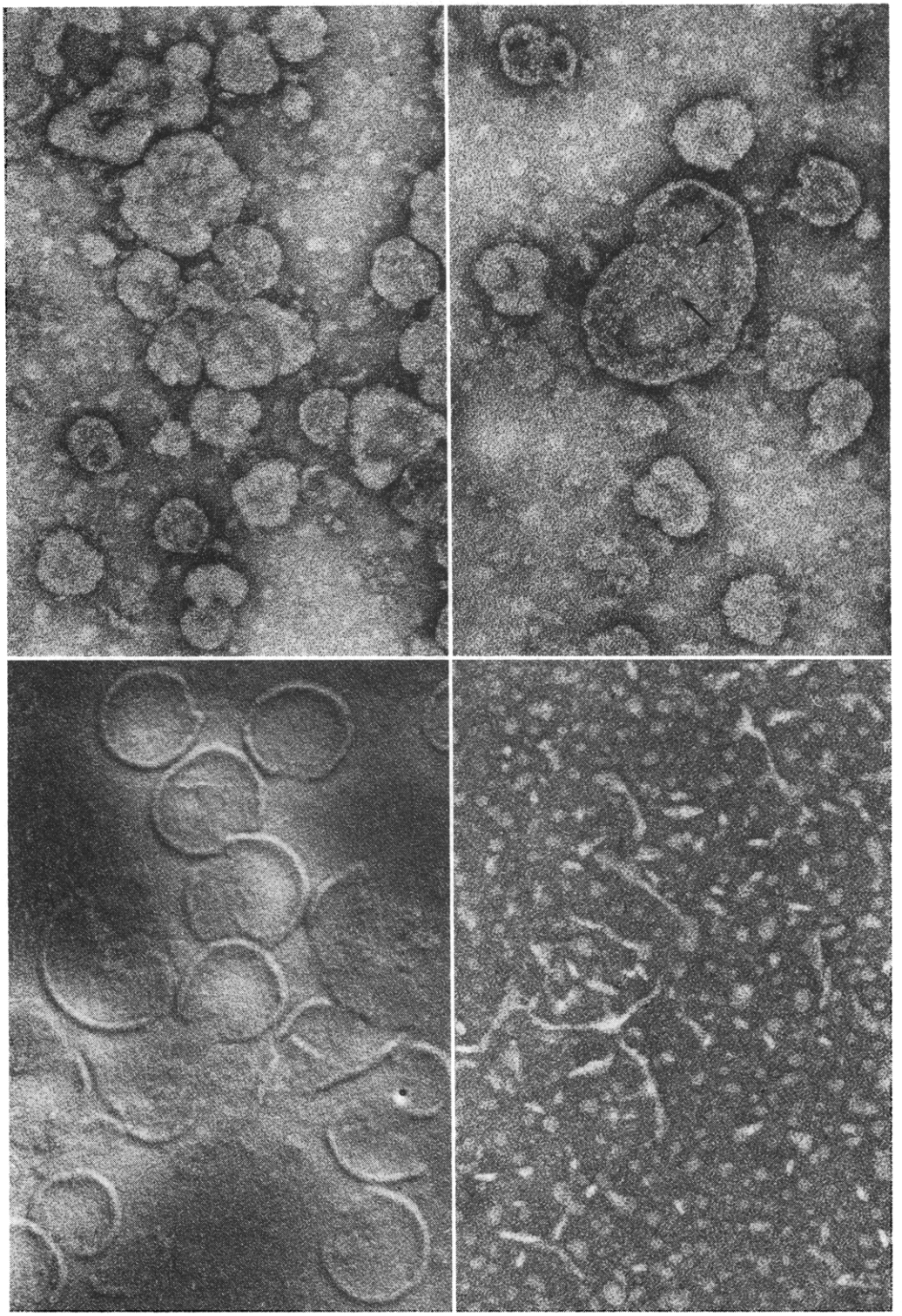

FIGURE 10 (upper left): Electron micrograph of *R. rubrum* chromatophores prior to detergent treatment. The sample was negatively stained utilizing phosphotungstic acid at pH 7.0; ×150,000.

FIGURE 11 (upper right): *R. rubrum* chromatophores prepared as in Figure 10 but showing some particulate substructure (see arrows); ×180,000.

FIGURE 12 (lower left): Electron micrographs of the H fraction illustrating the cup shape and smooth appearance of the structures. The micrograph was printed by the masking technique described by Gonzales (1962); ×190,000.

FIGURE 13 (lower right): Electron micrograph of the L fraction; ×160,000.

the chromatophore. The L particles, which are photochemically more active, probably derive from the interior of the chromatophore, and have a lower BChl/protein ratio. The higher relative protein concentration relates to the occurrence within this particle of succinic dehydrogenase activity and NAD photoreduction activity, indicating that enzyme activities related to the photochemistry of the chromatophore concentrate in this particle.

The H fraction contains some unruptured chromatophores, but consists primarily of the chromatophore membrane and some residual attached material. Reextraction of this fraction with Triton X-100 does not produce additional pheophytin-containing material, but does produce a small additional amount of pigmented particles which are lighter than the original H fraction. This further indicates that the L particle is the internal structural unit of the chromatophore, and it is removed through the action of the detergent. Some of the properties of the H fraction could well be due to residual L subunits which remain attached to the ruptured particle. This could be the case for P890, the reaction center BChl, which occurs in both fractions. However, there is a definite separation of NAD photoreduction activity between the L and H particles, which indicates a fairly complete separation of the two entities.

The bacteriopheophytin-containing particle is routinely obtained by the treatment with Triton X-100. The bacteriopheophytin is not dialyzable, and does not appear to be separable from the accompanying cytochrome *c* by chromatography on DEAE-cellulose. It appears that the cytochrome *c* is associated with the pheophytin on a small particle. No detectable cytochrome *c* is observed on the H or L particles. Further treatment of the H fraction with Triton X-100 does not produce more of the Pheo fraction, showing that the initial Pheo particle either exists as such in the chromatophore, or is derived from a particular type of BChl-containing particle. We are presently unable to decide between these two alternatives. Kihara and Frenkel (1963) have observed a pheophytin-containing particle from young *R. rubrum* cells. These investigators do not believe the pheophytin arises by alteration of the BChl.

There is good evidence that the chromatophores, as obtained by the procedures employed in this investigation, are derived from an interior membrane system of the bacterial cell, the membrane being continuous with and derived from the cell membrane (Holt and Marr, 1965; Cohen-Bazire and Sistrom, 1966). In the process of sonication this membrane system is broken at susceptible places (constrictions between adjoining enlarged areas) and the stable chromatophores are formed. The electron micrographs of Holt and Marr (1965) show the presence of subunits within the isolated chromatophores, which indicates that these interior units are functional and distinct within the chromatophore prior to cell rupture. This leads us to believe that the L fraction which we have isolated and partially characterized is this functional interior subunit of the chromatophore. This subunit probably corresponds to the one shown in the elegant electron micrographs of Frenkel and Hickman (1959). They did not state how this particle was prepared, but it was also found to be photochemically active.

The chromatophores of *R. rubrum* and *Chromatium* respond in a similar manner to treatment with Triton X-100. In both cases a small particle is released from the chromatophore, and this particle contains more protein and is photochemically more active than the remaining chromatophore membrane and residual attached particles. The characteristics of the *Chromatium* system are given in the accompanying paper (Garcia *et al.*, 1966). For *Chromatium* there is a fractionation of the B800 and B890 BChl types between the two particles, and a destruction of the B850 during the detergent treatment. This accords with the more complex pattern of BChl forms in *Chromatium*. For *R. rubrum* there is no separation of the B800 from the major B880 BChl form in the two particles, indicating a tight coupling between these two BChl forms.

In a recent review Vernon and Ke (1966) suggest that *R. rubrum* has two light reactions, one being involved with quinone photoreduction and the cyclic electron-transfer system involved in the photophosphorylation process of this bacterium, and the other reaction being involved in the terminal reactions leading to NAD photoreduction. The main reason for proposing the two reactions was the apparent independence of these two systems, *i.e.*, the rate of cyclic electron flow related to the photophosphorylating system was unaffected by terminal photochemical reactions of the type examined in this report (W. S. Zaugg and L. P. Vernon, manuscript in preparation). Although this is not the only explanation for two separate BChl systems, the data reported in this investigation do support the concept of two separate photoreactions in the *R. rubrum* chromatophore. The L particle would be the one involved in the terminal photoreduction of NAD, since it alone has this activity and succinic dehydrogenase is restricted to this particle. The H particle would be the one involving quinone photoreduction and cyclic electron flow leading to photophosphorylation. The association of the H particle with the chromatophore membrane and its ability to catalyze the photoreactions other than NAD photoreduction would be consistent with this idea.

Acknowledgments

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Interaction of Antimycin A with Biological Systems*

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ABSTRACT: A soluble protein (CAAF) obtained from washed chicken liver mitochondria mitigates the inhibition of respiration caused by antimycin A in isolated, intact mitochondria. The protein is active in differentiating chick muscle cultures and in explanted chick embryos treated with antimycin A. Bovine serum albumin (BSA) is one of several proteins which are inactive. A system of CAAF, antimycin A, and BSA has been used to investigate the mode of action of antimycin A. Antimycin A fluoresces at 421 mμ when excited at 348 mμ. The fluorescence of antimycin A at pH 7.5 is enhanced fivefold by an equimolar concentration of BSA. Addition of CAAF to the BSA-bound antimycin quenches this fluorescence. The BSA-bound antimycin also exhibits a characteristic fluorescence on excitation of the protein at 278 mμ, due probably to dipole–dipole coupling. This fluorescence is also quenched by CAAF. These data are interpreted to mean that antimycin A is capable of forming a ternary complex. The mechanism of complex formation is the likely cause of the effects of antimycin A in intact mitochondrial systems.

Antimycin A causes a pronounced specific effect(s) on tissues undergoing differentiation in addition to its action at the subcellular level. When explanted chick embryos are exposed to antimycin A heart formation is prevented. In muscle culture monolayers antimycin prevents maintenance and further formation of muscle fibrils. Single cells do not appear to be affected morphologically at concentrations of antimycin which destroy myofibrils. The effects of antimycin on these systems can be prevented by a purified protein (CAAF)[1] prepared from liver mitochondria of adult chickens (Reporter and Ebert, 1965). The present report describes a restorative effect of CAAF on isolated mitochondria treated with antimycin A and the interactions among BSA, antimycin A, and CAAF.

Experimental Section

Materials. Antimycin A_3 was obtained from Wisconsin Alumni Research Laboratories (lot order no. 12,243)

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[1] Abbreviations used: CAAF, the protein factor from chicken liver mitochondria which alleviates effects of antimycin A on chicken embryo explants and mitochondria; BSA, bovine serum albumin; ADP, adenosine diphosphate.