Some Structural and Photochemical Properties of *Rhodopseudomonas* Species NHTC 133 Subchromatophore Particles Obtained by Treatment with Triton X-100*

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ABSTRACT: Treatment of *Rhodopseudomonas* species NHTC 133 with 0.6% Triton X-100 does not significantly influence the shape of the fluorescence spectrum. Treatment of chromatophores with 4% Triton X-100 produces two fragments which are separable by sucrose density gradient centrifugation into a heavy (H) and (L) fraction. Both fractions contain the 830-mμ form of bacteriochlorophyll (Bchl) b as well as the 1005-mμ form (this peak is at 1015 mμ in the chromatophores) in amounts similar to those in the original chromatophore. Light-induced photooxidation of the reaction center Bchl b (P985) is observed in both fragments, but only the L fragment shows a coupled photoreduction of an endogenous quinone. Examination of the quinone content shows the presence of two main quinones, one migrating in the region of ubiquinone during thin-layer chromatography, the other migrating in the vitamin K region. Light minus dark difference spectra show that both quinones are reduced in the L fragment under the influences of light. Structurally the L fraction consists of small spherical subunits which exist either as isolated particles or as aggregates. The particle has an outer diameter of 135 Å, and shows an inner core which is 60 Å in diameter. The aggregates form regular patterns of the subunit with 130-Å spacing and in some cases resemble growing crystals. Occasionally a series of small stacked disks are apparent in this fraction, each disc consisting of one or a few of the small subunits. The H fraction contains what appears to be partially degraded chromatophores along with some aggregates of subunits which occur in sheets, many of which have regular faces reminiscent of crystals.

The chromatophores themselves appear primarily as small disks which are formed by comminution of the original membrane system, but occasionally as isolated lamellar systems of thylakoids which reflect the stacked lamellar structure seen for the photosynthetic apparatus in the intact cell. The chromatophores themselves show the presence of the small 135-Å subunit, which makes a very prominent contribution to the over-all structure of the membrane.

The photosynthetic bacterium, *Rhodopseudomonas* species NHTC 133, was first isolated and described by Eimhjellen *et al.* (1963). A similar organism called *Rhodopseudomonas viridis* was independently isolated and studied by Drews and Giesbrecht (1965). This bacterium is unique in that it contains a bacteriochlorophyll (Bchl)[1] which differs from that found as the major Bchl in all purple photosynthetic bacteria. Jensen *et al.* (1964) suggested the name of Bchl b for the new Bchl, and proposed Bchl a for that found in the purple photosynthetic bacteria such as *Chromatium*, *Rhodospirillum rubrum*, etc. Recent studies have shown (Eimhjellen *et al.*, 1963; Jensen *et al.*, 1964) that light energy absorbed by or transferred to Bchl b sensitizes Bchl b fluorescence as well as a bleaching of a pigment absorbing at 985 mμ (Holt and Clayton, 1965). Other photoreactions include a photooxidation of a cytochrome and a shift of the 830-mμ Bchl b band to shorter wavelengths (Holt and Clayton, 1965; Olson and Nadler, 1965). The bleaching of the Bchl b at 985 mμ is significant since this indicates the reaction center Bchl absorbs at this wavelength, which is shorter than the major Bchl b band located at 1015 mμ. A less intense absorption band is at 830 mμ. The presence of two main Bchl bands in this organism places it in the category of *R. rubrum*, which also has two Bchl bands, one at 880 mμ and a smaller one at 800 mμ. The data presented below indicate that in other ways also *Rhodopseudomonas* NHTC resembles *R. rubrum*. In one important aspect, however, the former is unique. It has a lamellar structure for the membrane system which constitutes the photosynthetic apparatus of the bacterium (Giesbrecht and Drews, 1966). For this reason it was thought that an investigation of the effect of Triton X-100 upon this organism and a study of the fragments so produced would be of value to our general study of the structure and function of the photosynthetic apparatus of bacteria. Three previous investigations of a similar nature have been performed with *Chromatium* (Garcia *et al.*, 1966a), *R. rubrum* (Garcia *et al.*, 1966b), and *R. palustris* (Garcia *et al.*,

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[1] Abbreviation used: Bchl, bacteriochlorophyll.

1968). The publications covering the work done with *Chromatium* and *R. palustris* also contain a more detailed account of the history of detergent treatments of bacterial chromatophores, and explain the rationale for the experiments described below.

Methods

Rhodopseudomonas NHTC 133 cells were grown photosynthetically using the medium described by Eimhjellen *et al.* (1963). Chromatophores were released from the washed cells by sonication for 3 min and were separated from other cellular material by centrifugation (Garcia *et al.*, 1966a). The experimental details for detergent treatment, density gradient centrifugation, fluorescence measurements, protein and quinone determination on the separated fragments, and light-induced absorbance changes are described in the accompanying paper (Garcia *et al.*, 1968). Bacteriochlorophyll concentration was determined after extraction of the pigments with acetone–methanol (7:2, v/v) by means of a millimolar extinction coefficient of 122 at 790 mμ. The value for the extinction coefficient was calculated from the *in vivo* value of 144 mM^{-1} cm^{-1} at 1015 mμ as reported by Clayton (1966).

Results

Fluorescence Measurements. Both Triton X-100 and deoxycholate at a final concentration of 0.2% are without significant effect on the emission spectrum of the *Rhodopseudomonas* NHTC chromatophore fraction. The emission spectrum for the untreated chromatophore shows a single symmetrical emission band with a peak at 1045 mμ. The addition of either Triton X-100 or deoxycholate does not change the position or the shape of the emission spectrum. Furthermore, upon addition of either detergent no new emission bands between 830 and 900 mμ were detected, indicating that the efficient energy transfer from B830 and B1015 was not disrupted by the presence of the detergent. These results are shown in Figure 1. As discussed previously (Garcia *et al.*, 1968) concerning the fluorescence properties of photosynthetic bacteria, we may predict that since the detergents did not alter the fluorescence spectrum of *Rhodopseudomonas* NHTC chromatophores, no actual physical separation between B830 and B1015 would be produced by treatment with higher concentrations of the detergent. This was the case, as shown below. Figure 2 shows the different fractions obtained after a sucrose density centrifugation of the treated chromatophores. Two main Bchl-containing bands are detected, which have been named according to their position in the gradient light (L) and heavy (H).

Chromatophore Fragments. Figures 3 and 4 show the absorption spectra in the near-infrared and visible regions for *Rhodopseudomonas* NHTC chromatophores and L and H particles derived by treatment with Triton X-100. As expected because of the constancy of the fluorescence emission in the presence of detergents, the two fragments produced by the higher concentration of Triton do not show any significant difference in their absorption properties. The absorption peak of the main Bchl b band at 1015 mμ is shifted to 1005 mμ in both of the derived fragments, but the general shape of the band is the same. This represents a shift of a few millimicrons due to the presence of the

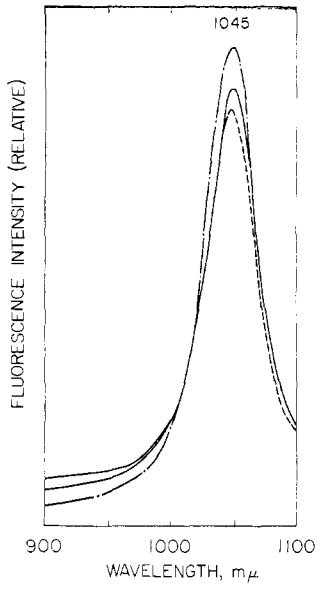

FIGURE 1: Fluorescence spectra of *Rhodopseudomonas* NHTC chromatophores suspended either in 0.05 M Tris-HCl buffer (pH 7.5) (solid curve) or after 30-min incubation in the same buffer with 0.6% Triton X-100 (dashed and dot curve) or 0.2% deoxycholate (dashed curve) present. The fluorescence was excited by light between 360 and 600 mμ obtained with the aid of a sharp cut-off filter (Corning 9782). The Bchl concentration was 1.7 μmoles in both cases.

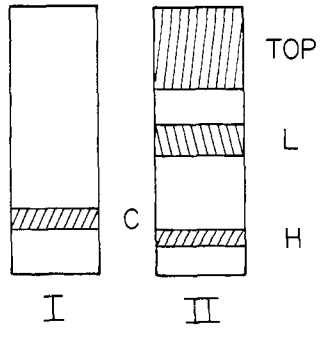

FIGURE 2: Sketch showing the distribution of untreated chromatophores (I) and the fragments produced through the action of 4% Triton X-100 (II) after sedimentation through a discontinuous gradient of sucrose (Garcia *et al.*, 1968). For the incubation with Triton, the ratio of Bchl b to Triton was approximately 50 mg of Triton X-100/1 μmole of Bchl b.

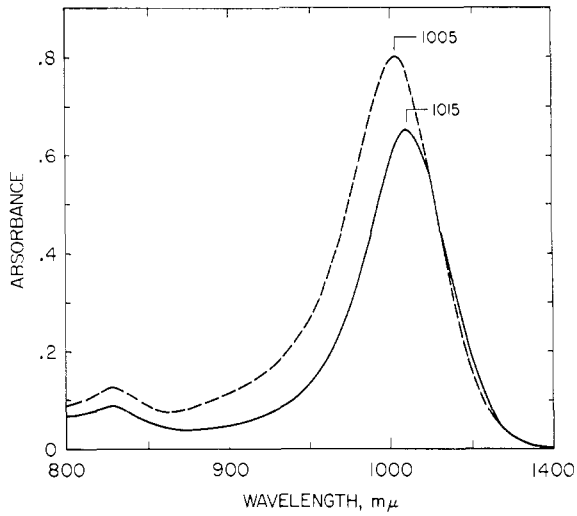

FIGURE 3: Absorption spectra of *Rhodopseudomonas* NHTC chromatophores (solid curve) and derived H and L fragments (dashed curve) in the near-infrared region. The H and L fragments were diluted with 0.05 M Tris buffer (pH 7.5) prior to measuring the absorption spectrum. The Bchl concentrations were in 3 ml: chromatophores, 0.012 μmole; H and L fragments, 0.016 μmole. The optical path length was 1 cm.

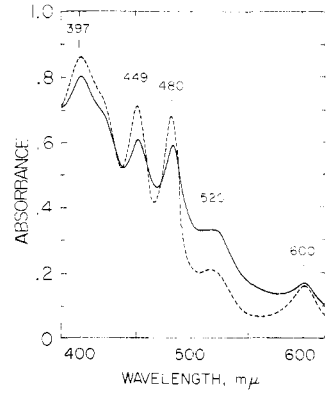

FIGURE 4: Absorption spectra of *Rhodopseudomonas* NHTC chromatophores (solid curve) and derived H and L fragments (dashed curve) in the visible region. The Bchl concentrations were in 3 ml: chromatophores, 0.012 μmole; H and L fragments, 0.016 μmole. The optical path length was 1 cm.

detergent and does not represent a major separation of Bchl types as is found in *Chromatium* and *R. palustris*. The spectra in the visible region (Figure 4) also show the similarities between the chromatophores and the derived fragments. The Bchl absorption bands at 600 and 397 mμ are apparent, as are the bands due to carotenoid absorption between 400 and 500 mμ. It appears, therefore, that the Triton X-100 separates the chromatophore membrane into two fragments which are separable on the sucrose density gradient centrifugation, but the environment of the Bchl b is apparently the same, or nearly the same, on each of the derived fragments, and from the fluorescence data it appears that the relative relationship of the B830 and B1015 has not been significantly changed by the treatment.

Light-Induced Absorbance Changes. Since the H and L fragments of *Rhodopseudomonas* NHTC both contain the long-wavelength Bchl b, B1005, it is of interest to know if both of these fractions contain the reaction center Bchl also. Figure 5 shows the light-induced absorbance changes measured at wavelengths appropriate to follow the photooxidation of P985 (the reaction center Bchl) and ubiquinone changes. Because of lack of a suitable interference filter at 980 mμ, the absorbance changes of the reaction center Bchl were measured at 940 mμ instead. The quinone changes were measured at two wavelengths, 275 and 255 mμ. As shown in Figure 5, both the H and L fractions, as well as the original chromatophore showed the light-induced photooxidation of the reaction center Bchl. On a Bchl basis, the L fraction showed a greater reaction. In contrast to these results, the absorbance changes in the ultraviolet region showed that only the L fragment catalyzed a photoreduction of ubiquinone. The H fragment was almost totally inactive in this reaction. In the photosynthetic bacteria examined to date this is the only case where a fragment of the chromatophore catalyzes a photooxidation of the reaction center Bchl and does not show a coupled photoreduction of a quinone. This will be discussed in more detail below.

The data of Figure 5 show that no absorbance change was observed at 255 mμ for any of the material examined. This is unexpected if one assumes that the ultraviolet changes are related to the photoreduction of only ubiquinone, which would show a negative change at 255 mμ (Ke *et al.*, 1968). To examine this matter further, the light-induced difference spectrum was determined through the region where ubiquinone would be expected to change in absorbance. Figure 6 shows such spectra for both the L fraction and chromatophores. The chromatophore spectrum shows a rather broad minimum, centering at 284 mμ. This differs from the expected value of 275 for ubiquinone. Even greater differences are seen in the spectrum obtained for the L fraction, which shows two minima located in the regions of 260 and 285 mμ. This spectrum indicates there are more than one compound reacting in this fraction. Consequently, the quinone compositions of the L fraction, as well as chromatophores, were examined by thin-layer chromatography (Garcia *et al.*, 1968). Whereas a major spot was observed in the region corresponding to ubiquinone, another significant spot was observed which migrated further when chloroform was used as the developing solvent. The limited amount of this material did not allow for definitive spectroscopic work to be done, but preliminary evidence points to a compound with an absorption maximum in the region of 267 mμ which is decreased

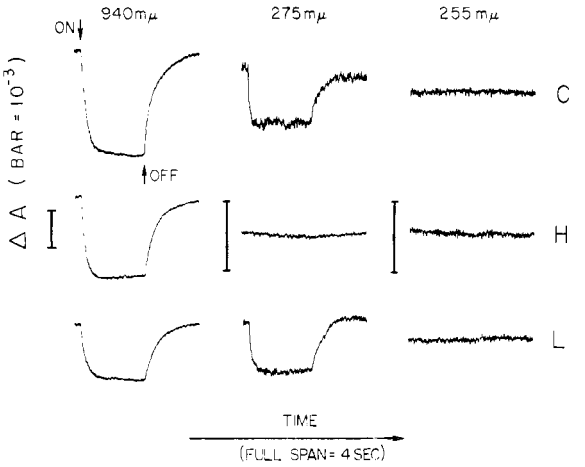

FIGURE 5: Light-induced absorbance changes at 940 mμ (oxidation of P985, the reaction center Bchl b) and the ultraviolet region (275 and 255 mμ) for quinone reduction. Actinic light at 830 mμ was used for all measurements, with an intensity of $\sim 5 \times 10^4$ ergs/cm^2 sec. The Bchl b concentrations (in 3.0 ml) were 0.014 (H), 0.010 (L), and 0.023 μmole (chromatophores).

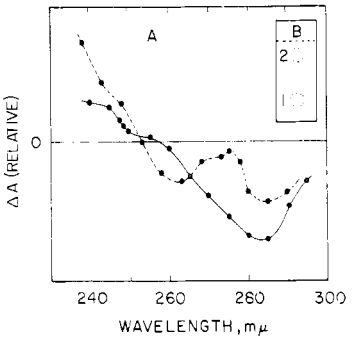

FIGURE 6: Spectral studies. (A) Light minus dark difference spectrum in the ultraviolet region, showing the photoreduction of quinones by the chromatophore (solid curve) and the L fraction (dashed curve). The conditions employed were those given in Figure 5, except that the concentrations of Bchl b were (in 3.0 ml) 0.007 (L) and 0.020 μmole (chromatophore). (B) Thin-layer chromatographic pattern of quinones from the chromatophores; (1) ubiquinone; (2) vitamin K like compound.

when borohydride is added. All these data indicate that this bacterium contains two quinone compounds, one of which is ubiquinone (however, see Figure 6) and another which resembles vitamin K. The light minus dark spectrum of the L fragment, shown in Figure 6, indicates that both of these quinones can be photoreduced in this fragment, with no evidence now available indicating which is more primary. The vitamin K like compound is less reactive in the chromatophore, however. The reason for this is not apparent, and the reason for the minimum in the difference spectrum at 285 mμ (as opposed to 275 mμ for ubiquinone) is not known. Further study is needed on the quinones in this bacterium.

The distribution of Bchl b and quinone between the different fractions is shown in Table I. The salient feature of this study is the constancy of the Bchl:protein ratios and the ratios of Bchl:quinone. The highest values for these ratios are found in the H fraction, but the differences are not very great. This is in contrast to the data for *R. palustris* (Garcia *et al.*, 1968), where there is a concentration of Bchl (on a protein basis) in the H particle.

TABLE I: Distribution of Bchl and Ubiquinone between the Chromatophore Fragments.[a]

Fraction	μmoles of Bchl b/mg of Protein	μmoles of Bchl b/ μmole of Ubiquinone
Chromatophores	0.024	1.33
L fraction	0.033	1.42
H fraction	0.040	1.62

[a] Bchl b was determined as reported in the Methods section and ubiquinone was determined as reported in the accompanying paper (Garcia *et al.*, 1968).

Structure. Electron microscopy of *R. viridis* cells, which are similar to *Rhodopseudomonas* NHTC 133 has shown that the membrance system involved in photosynthesis is in the form of stacked lamellae, very similar to the system of stacked thylakoids found in plant chloroplasts (Giesbrecht and Drews, 1966). Figures 7 and 8 show electron micrographs obtained for the chromatophores and fragments obtained from *Rhodopseudomonas* NHTC 133 through the use of Triton X-100. In Figure 7 (7) is shown the chromatophore fraction, which contains several of the smaller, rather typical cup-shaped disks which make up the usual chromatophore fraction of bacteria. Also present is an aggregated system of membranes, which is one of the original photosynthetic apparatus of the bacterium which has remained more or less intact during the sonication process used to break up the bacterial cell. Apparently this sonication procedure does comminute the majority of the membrane structures found in the cell, forming the smaller, typical disk-shaped structures. The presence of small subunits is very obvious on the larger lamellar structure shown in Figure 7. The whole surface of the thylakoids is composed of these subunits, which are ordered to give 130-Å spacing. These subunits have been seen by Giesbrecht and Drews (1966). Also seen on the figure (arrow) are some of the hexagonal arrangements of these particles reported by those authors.

Figure 7 (8) shows an electron micrograph of the H fraction. There are present some structures which

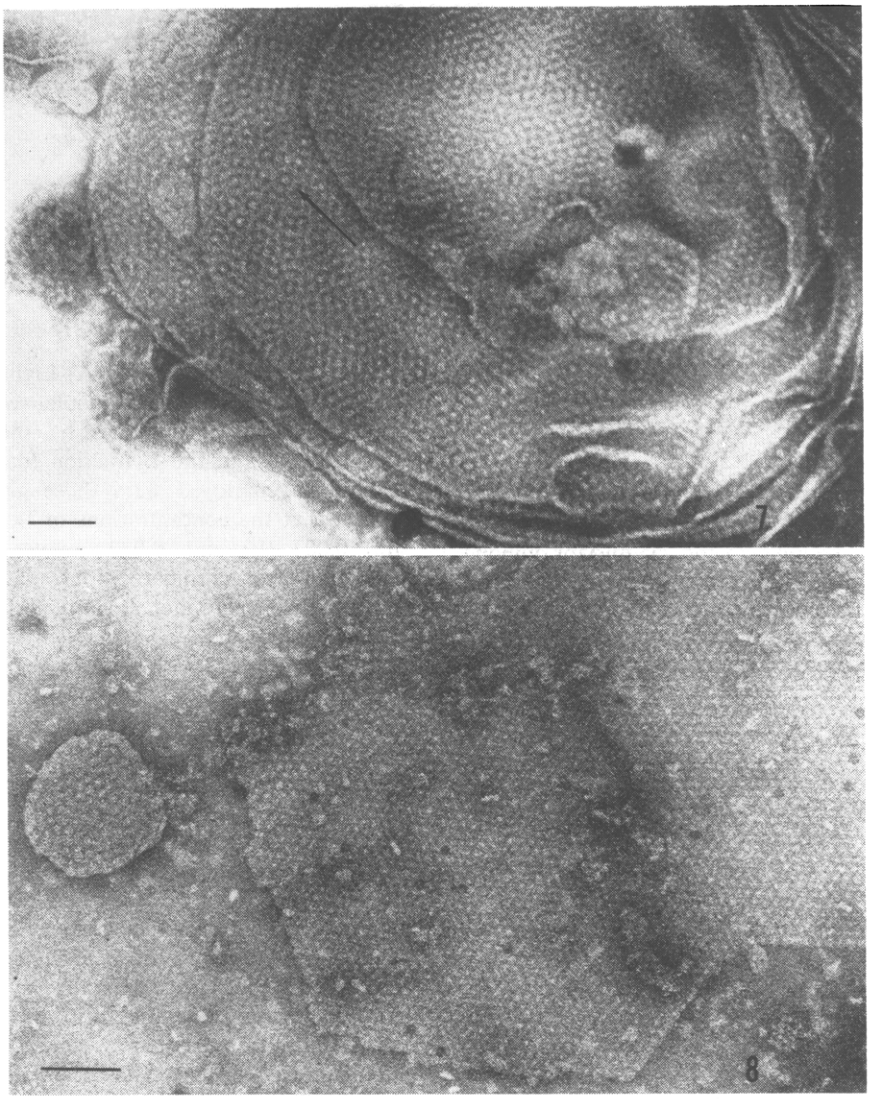

FIGURE 7: Electron micrographs. (7) Electron micrograph of a chromatophore preparation of *Rps.* NHTC. Negative staining with phosphotungstic acid at pH 5.9 in the presence of 5×10^{-3} M $MgCl_2$. The large, stacked-lamellar structure is representative of the chromatophore structure in the cell. The arrow indicates one of the hexagonal arrangements frequently seen in such preparations. The bar represents 1000 Å. (8) Electron micrographs of the H fraction of *Rps.* NHTC chromatophores prepared by the action of Triton X-100. The electron micrographs were obtained by the technique of negative staining as in 7. Note the clearly defined faces of the aggregated sheets which resemble crystal faces. The spacing between the rows of particles is 130 Å. The bar represents 1000 Å.

look like the original membrane structure. Also present are organized aggregates of the small subunits of the thylakoid membrane. This material appears as a sheet of such subunits, and a crystalline arrangement is suggested by the regular pattern of the subunits, particularly the faces which are formed. These planar aggregates could be formed either as intermediate breakdown products of the original chromatophore thylakoids, or they could be formed by reaggregation of these small particles. Because of the occurrence in the micrographs of what appear to be more-or-less typical chromatophores, it appears that these planar sheets of subparticles are incompletely fragmented thylakoid membranes.

Figure 8 (9) shows an electron micrograph of the L fraction obtained through the action of Triton X-100, showing the individual particles which have been separated from the thylakoid membrane by the detergent. Also apparent in the figure are some linear aggregates of these small particles. When these are formed, the individual particle in the linear aggregate changes shape and size. The individual spherical particle is of the order of 135-Å o.d., with an inner core of 60 Å.

In some cases the L fraction shows a rather unique

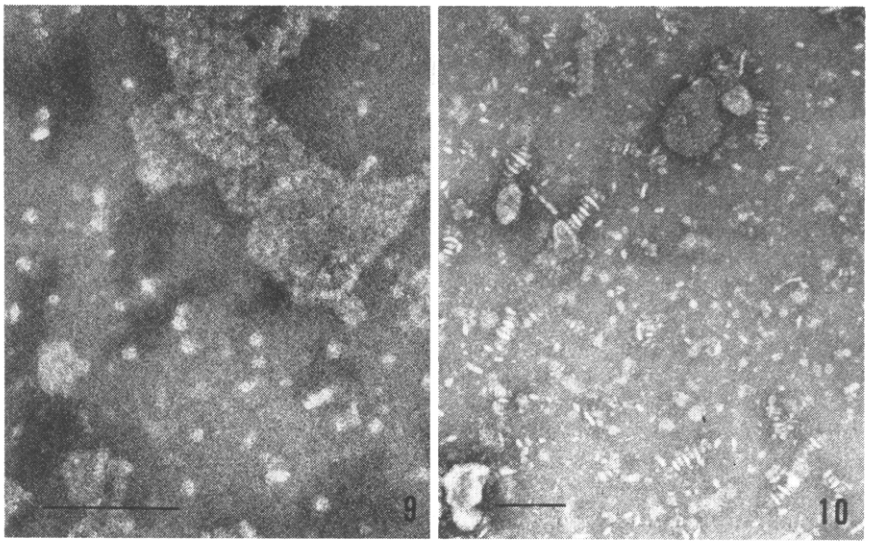

FIGURE 8: Electron micrographs. Electron micrograph of the L fraction, obtained by negative staining. Both the usual spherical particle and linear aggregates of the small particle are seen. Both types of particles appear to derive from the subunits seen on the original chromatophore membrane. The individual particle is 135 Å with an inner core of 60 Å. The bar represents 1000 Å. (10) Another preparation of the L fraction, showing in this case the aggregation of the smaller particles into small stacked aggregates. This could reflect the physicochemical property of these particles which leads to the formation of stacked lamellae in the chromatophore as it occurs in the intact cell. The bar represents 1000 Å.

structure. Figure 8 (10) shows such a preparation, revealing the presence of some small aggregates which appear as stacks of small flattened disks. Also present in this preparation are the usual spherical subparticle which occurs in aggregates. The nature of the stacked disks is not known, but they could be formed from the same subunit which is liberated through the action of the detergent and which ordinarily appear as the spherical 135-Å subunit. Some property of these subunits causes them to aggregate in this form, which is reminiscent of the condensation of the individual thylakoids into the lamellar system found in the intact cell. It could be that the physicochemical property which impels these subunits to recombine in this way is the same force which causes the individual thylakoid membranes in the cell to form the closely appressed membrane system so typical of this bacterium.

Discussion

The photosynthetic bacteria investigated to date in our laboratory fall into one of two groups. The first group, including *Chromatium* and *R. palustris*, have in the chromatophore structure three forms of Bchl whose absorption maxima fall generally in the regions of 800, 850, and 880 mμ. The addition of 0.6% Triton X-100 disrupts the flow of excitation energy between the 850- and 880-mμ Bchl forms, and allows fluorescence from the B850 form. Treatment with higher concentrations of Triton X-100 separates the chromatophore into two fragments, whose Bchl composition correspond to the fluorescent systems in the chromatophores treated with 0.6% Triton; *viz.*, one fragment contains the B800 and B850 and gives fluorescence from the B850 form, while the other fragment contains the B880 form. The fragment containing the B880 is a small particle (the heavy (H) fragment obtained by sucrose density gradient centrifugation) which derives from the membrane and is released through the action of the detergent. This particle contains the reaction center Bchl, as evidenced by a light-induced bleaching of a small portion of the long-wavelength form Bchl and by a coupled photoreduction of endogenous ubiquinone. The fragment containing the B800 and B850 forms is the light fraction (L) on sucrose density gradient centrifugation, appears to be membranous upon examination with the electron microscope, and is devoid of any photochemical activity. Therefore, in these two bacteria, the membrane system making up the photosynthetic apparatus consists of a continuum of lipoprotein material on which is deposited the B800 and B850 forms of Bchl. These serve as light-harvesting molecules, since no photochemical activity is observed in the fraction containing only these two Bchl forms. Deposited on this membrane continuum are the photosynthetic units, or particles, which contain the long-wavelength form of Bchl and a functional reaction center. Light induces a bleaching (oxidation) of the reaction center Bchl and a coupled photoreduction of endogenous ubiquinone.

R. rubrum and *Rhodopseudomonas* NHTC comprise the other class of bacteria. The addition of 0.6% Triton does not significantly alter the shape of the fluorescent spectra of these chromatophores. Treatment with 4%

Triton produces two fragments, one particulate and one membranous, which both exhibit similar Bchl composition. There is no separation of the B800 and B880 forms (*R. rubrum*) or the B830 and B1015 forms (*Rhodopseudomonas* NHTC). The derived fragments both show a light-induced bleaching of the long-wavelength form of Bchl, which is indicative of an oxidation of the reaction center Bchl a (*R. rubrum*) or Bchl b (*Rhodopseudomonas* NHTC). In the case of *R. rubrum*, both fragments carry out a coupled photoreduction of endogenous ubiquinone, while with *Rhodopseudomonas* NHTC a coupled reduction of quinone is observed only in the particulate fraction, which is the L fraction in the sucrose density gradient centrifugation. Whereas *R. rubrum* contains only ubiquinone as a major quinone, *Rhodopseudomonas* NHTC also contains a quinone which migrates during thin-layer chromatography to the region expected of vitamin K and it also has an absorption spectrum with a maximum at 267 mμ.

There are some unique features of the *Rhodopseudomonas* NHTC fragments. One is the occurrence of a reaction center in the H (membranous) fraction which does not show a coupled photoreduction of quinones. Another unusual feature is the apparent photoreduction of the new quinone (vitamin K?) in the L fraction. Although the bacterium contains Bchl b, it is distributed in the chromatophore membrane in a manner similar to the Bchl a in *R. rubrum*, with a small amount of a shorter wavelength form and a major band at a longer wavelength.

Electron microscopy of *Rhodopseudomonas* NHTC shows the presence of very discrete subunits on the surface of the stacked thylakoid membranes (Giesbrecht and Drews, 1966). Treatment with Triton X-100 breaks down the membrane structure and frees these subunits which are then subsequently found in the L fraction which exhibits both reaction center Bchl b and a coupled reduction of quinone. This, then, is the usual photochemical small particle which is liberated from all of the photosynthetic bacteria by the action of this detergent.

The photosynthetic membrane system of photosynthetic bacteria is composed of a basic membrane matrix containing Bchl which may be of the accessory light-harvesting type (B800 and B850 in *Chromatium* and *R. palustris*) or may also include some of the photochemically competent type (*R. rubrum* and *Rhodopseudomonas* NHTC). Situated on this basic membrane structure are the smaller particles which are removed by the detergent. In all cases these particles contain the reaction center Bchl and show a coupled photoreduction of endogenous quinone. In this regard the bacterial system resembles that found in spinach chloroplasts. In the latter case, Triton X-100 also liberates a small particle which appears to contain the reaction center Chl (P700) which is competent to carry out nicotinamide-adenine dinucleotide phosphate photoreduction. The other chloroplast fraction is a membranous fraction containing photosystem 2 activity (Vernon *et al.*, 1966a,b, 1968; Ke, 1967). In the sense that photosystem 2 of plants can be considered as an accessory system to the photosystem 1 reaction center (the latter is the one directly involved in nicotinamide-adenine dinucleotide phosphate photoreduction) the analogy between the plants and bacteria is very good. In both cases the small particle which is distributed along the membrane contains the reaction center Chl or Bchl associated with the long-wavelength form of the Chl or Bchl, and the primary reaction caused by light is a photooxidation of this reaction center Chl or Bchl in a reaction coupled to the reduction of some closely associated acceptor molecule (X for plant chloroplasts and ubiquinone for bacteria).

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