

Structure and Photochemical Activity of Chlorophyll-Containing Particles from *Rhodospirillum rubrum**

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ABSTRACT

Comparative studies on isolated chromatophores and on sectioned cells of the photosynthetic bacterium *Rhodospirillum rubrum* confirm the assumption expressed in earlier investigations that the photochemically active chromatophores isolated from disrupted cells represent structural chlorophyll-bearing components of the protoplast.

Actively growing cells from light-grown cultures about 12 hours old do not release chromatophores when disrupted in dilute buffers, but do release smaller, chlorophyll-containing structures about 25 $m\mu$ in diameter. Sections of such cells do not reveal chromatophores, but contain in the ground cytoplasm numerous particles somewhat smaller in size than the 25 $m\mu$ chlorophyll-containing particles released from disrupted cells. Similar particles are obtained by the sonication of isolated chromatophores obtained from cells of 1-day-old cultures.

The small, subchromatophore particles described here appear to be functionally complete units which are photochemically active in photo-oxidation, photoreduction, and photophosphorylation, and it is postulated that they represent the basic biochemical and structural components of the chromatophore.

INTRODUCTION

The first observations on photochemical activities of cell-free preparations from photosynthetic bacteria were reported by French (4, 5) who noticed that such preparations catalyzed a light-sensitized oxidation of ascorbic acid by molecular oxygen. Vernon and Kamen (20, 21) extended these observations and coined the term "photo-oxidase" for this system which was capable of photo-oxidizing a variety of reduced substrates in the presence of molecular oxygen.

In 1952, Pardee, Schachman, and Stanier (14, 15) published electron microscope observations on extracts from *R. rubrum* and reported the presence of chlorophyll-containing particles, measuring about 110 $m\mu$ in diameter in air-dried preparations, which they termed "chromatophores." Schachman *et al.* (15) were able to isolate these chromatophores in relatively pure form by differential centrifugation. They concluded that the chromatophores contain all

the photosynthetic pigments of the bacterial cell, thereby suggesting strongly that these particles represent the sites of photosynthetic activity. Particles of similar size were obtained from *R. rubrum* cells by Thomas (17), who called them "grana."

More recently, the observation was made that cell-free preparations of *R. rubrum*, when illuminated under suitable conditions, will carry out a light-induced phosphorylation with the formation of ATP¹ from ADP¹ and orthophosphate (6, 7). At that time the sedimentation characteristics of the active, chlorophyll-containing fraction suggested that the bacterial chromatophores were responsible for this activity. Other investigators in the field of bacterial photo-phosphorylation who noted the particulate character of the phosphorylating material usually assumed that they were working with isolated chromatophores (1, 12, 13).

The present investigation was undertaken to study simultaneously the morphological char-

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¹ Abbreviations used: ADP, ATP, 5' isomers of adenosine diphosphate and adenosine triphosphate; DPN, DPNH for oxidized and reduced diphosphopyridine nucleotide; TPN, TPNH for oxidized and reduced triphosphopyridine nucleotide.

acteristics and the photochemical activities of cell-free, chlorophyll-containing structures isolated from disrupted cells of *R. rubrum*, to establish whether the chromatophores actually are the particles catalyzing the photochemical reactions, and, if so, whether they are the only type of structure capable of such activity. An effort also has been made to relate the appearance of the isolated structures to those observed in sections of *R. rubrum* cells by electron microscopy.

Materials and Methods

Methods for the culture of *R. rubrum* cells and the description of procedures and equipment employed in electron microscopy are given in the preceding paper (11).

Cell-free preparations were obtained according to the procedure described earlier (7) through the use of a Raytheon 10 KC magnetostrictive oscillator. The cell suspensions were exposed to high frequency sound for 1½ to 2½ minutes at an initial temperature of 0 to 1°C. which rose to 3°C. in the course of the treatment. The preparations thus obtained were fractionated according to the procedure outlined in Table I.

The fractionation procedure presented here was based in part on earlier work (7-9) on the isolation of photochemically active particles from this organism. In this procedure, intact cells and cell fragments were removed first (step 1); then the chromatophores were sedimented, resuspended, and sedimented again (steps 2 to 4). The greatest degree of purification was achieved in step 2, where a large amount of soluble protein remained in the supernatant, which also contained many enzymes not directly associated with the photochemical system of the isolated chromatophores. In earlier work it was apparent that isolation of the chromatophores in glycyglycine buffer yielded preparations with the highest photochemical activity. Fractionation steps 1 through 4 were carried out in an angle head rotor in which it was impossible to obtain clear fractionations of materials having similar sedimentation behaviors. Through the use of sucrose gradient columns in free swinging centrifuge tubes (Spinco rotor SW 39 L) it was possible to remove more of the colorless proteinaceous material from the chromatophore fraction. Steps 5 and 6 were not carried out routinely in studies on the photochemical activities of chromatophores as they yield relatively small amounts of material. Nevertheless, the chromatophores obtained from the sucrose gradient columns were fully as active, on a chlorophyll basis, as chromatophores which had not undergone this fractionation, yielded material which showed a slightly higher chlorophyll-to-protein ratio (as evidenced by a higher ratio of absorption—880/280 $m\mu$), and were relatively free of extraneous material when examined in the electron microscope (Fig. 6).

The photochemical activities of the chlorophyll-containing particles were tested by the light-induced synthesis of ATP from ADP and orthophosphate, either by determining the rate of disappearance of orthophosphate (6, 7) or by following the rate of formation of TPNH¹ spectrophotometrically in an illuminated cuvette containing a suspension of bacterial particles to which TPN, glucose, hexokinase, and glucose-6-phosphate dehydrogenase had been added in addition to ADP and orthophosphate (8). Photo-reducing activity was measured in anaerobic cuvettes by observing the formation of DPNH in the light in the presence of succinate (8, 9). Photo-oxidase activity could be followed by the light-accelerated oxidation of added ferrocytochrome *c* (20, 21); however, a quantitative study of this reaction was not carried out.

RESULTS

Purified, photochemically active chromatophores can be obtained by fractionation (Table I) of sonically disrupted cells from 24-hour-old light-grown cultures. When such chromatophores are fixed with osmium tetroxide and sectioned, structures are observed (Figs. 3, 4) which are similar in appearance to the vesicles observed in sections of intact cells (Figs. 1, 2) (11, 18, 19). In general, the peripheral areas of the sectioned chromatophores are of greater density than the central areas, but these chromatophores do not appear to be hollow structures as was postulated for the chromatophores of the purple sulfur bacterium *Chromatium* (2). Some of the chromatophores in Figs. 3 and 4 contain distinct localized areas of greater density which also have been observed in sectioned cells from older cultures (11, Fig. 18; 19). At present we have no evidence as to the actual localization of the photosynthetic pigments within the chromatophores.

In every case studied in sufficient detail by the authors, chromatophores can be isolated only from those cells which reveal chromatophores when sectioned. The preparation shown in Fig. 5 was obtained without chemical fixation by air drying a droplet of material, which had sedimented in a low gravitational field, revealing in the electron microscope cell fragments and chromatophores still surrounded by protoplasm released from ruptured cells. These observations are presented as additional evidence that the chromatophores must be real structures within the protoplast of the bacterial cell.

In an effort to minimize distortions caused by high surface tension produced in the drying of large droplets, chromatophore preparations were

TABLE I

Fractionation steps															
	Sonic preparation														
1	30 min. at 25,000 g														
2	<table border="0" style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;">Sediment (intact cells and cell fragments)</td> <td style="width: 50%; vertical-align: top;">Supernatant</td> </tr> <tr> <td></td> <td style="text-align: right;">50 min. at 54,000 g</td> </tr> </table>	Sediment (intact cells and cell fragments)	Supernatant		50 min. at 54,000 g										
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6	<table border="0" style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;">0.59 M sucrose layer (pink) 0.88 M " " (colorless) (both layers discarded)</td> <td style="width: 50%; vertical-align: top;">0.29 M sucrose layer (red) layered on top of sucrose gradient columns made up as follows: top: 1 ml. 0.29 M red sucrose layer 1 " 0.59 M aqueous sucrose solution 1 " 0.88 M " " " bottom: 2 " 1.17 M " " " 60 min. at 32,000 g in Spinco bucket rotor</td> </tr> <tr> <td style="vertical-align: top;">0.88 M and 1.17 M sucrose layers colorless (discarded)</td> <td style="vertical-align: top;">0.59 M sucrose layer (red) diluted 1:10 with glycylglycine buffer. Chromatophores then sedimented at 50,000 g for 30 min.</td> </tr> </table>	0.59 M sucrose layer (pink) 0.88 M " " (colorless) (both layers discarded)	0.29 M sucrose layer (red) layered on top of sucrose gradient columns made up as follows: top: 1 ml. 0.29 M red sucrose layer 1 " 0.59 M aqueous sucrose solution 1 " 0.88 M " " " bottom: 2 " 1.17 M " " " 60 min. at 32,000 g in Spinco bucket rotor	0.88 M and 1.17 M sucrose layers colorless (discarded)	0.59 M sucrose layer (red) diluted 1:10 with glycylglycine buffer. Chromatophores then sedimented at 50,000 g for 30 min.										
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sprayed in a fine mist onto electron microscope grids without previous chemical fixation. These preparations when shadowed (Fig. 6) do not reveal the outer ridge and central depression observed in Fig. 5, which must have resulted from a partial collapse of the chromatophores on the electron microscope grid when air-dried from large droplets. Isolated and purified chromatophores when air-dried on these grids have a mean diameter of 90 μ (with a standard deviation of 14.5 μ based on random measurements in 8 photomicrographs of 238 chromatophores which ranged in diameter from 50 to 160 μ).

In addition to chromatophores, smaller chlorophyll-containing particles with a mean diameter of about 25 μ were obtained from *R. rubrum* cells by three different procedures: (a) By sonic disruption of cells from cultures transferred every 12 hours, which will release the 25 μ chlorophyll-containing particles shown in Fig. 8. Sections of such cells reveal small particles 10 μ to 20 μ in diameter in the cytoplasm, but do not contain chromatophores (11, Fig. 3). (b) By the sonic disruption of chromatophore-containing cells in a viscous medium, such as a 0.2 M sucrose solution, which yields abundant small particles with diame-

TABLE II
Rates of Photophosphorylation and of DPN
Photoreduction of Chlorophyll-Containing
Particles from *Rhodospirillum rubrum*

Preparation (obtained from light-grown cultures)	Initial rates of photophosphorylation $\mu\text{M ATP formed per hr. per } \mu\text{M bacteriochlorophyll}$	Initial rates of DPN photoreduction $\mu\text{M DPNH formed per hour per } \mu\text{M bacteriochlorophyll}$
Purified chromatophores from 24-hour-old cultures	125-175	26.0
Particles from 12-hour-old cultures, with a diameter of 20 to 30 μm .	110	22.6
Particles from 24-hour-old cultures sonicated in 0.2 M sucrose. Diameter of particles 20 to 40 μm .	149	(not measured)

ter extremes of 20 μm to 40 μm (Fig. 7). (c) By the sonic disruption of isolated chromatophore preparations obtained from light-grown cells from 24-hour-old cultures. The particles described under (a) and (b) exhibit high photochemical activity (Table II), but the particles obtained in procedure (c) by the sonication of isolated chromatophores, show greatly reduced activity, probably resulting from excessive exposure to high frequency sound in two consecutive treatments.

Thus, at present two different types of photochemically active particles from *R. rubrum* have been isolated and their photochemical activity examined: the chromatophores about 90 μm in diameter (when air-dried on electron microscope grids), and what might be called the subchromatophore particles, about one-fourth the diameter of the chromatophores. Qualitative determinations indicate that both types of particles have photooxidase activity, and, in addition, can catalyze the formation of ATP from ADP and orthophosphate in the light (Table II). Furthermore, purified chromatophores and the small particles isolated from cells of 12-hour-old cultures carry out photoreduction of DPN in the presence of succinate; the small particles obtained by sonic disruption of chromatophore-containing cells in

viscous media remain to be tested for their ability to catalyze this reaction.

Cells of *R. rubrum* grown aerobically in the dark after repeated transfers lose their photosynthetic pigments (3). Sectioned cells from cultures which were transferred every 2 days do not contain chromatophores (15, 19), but smaller particles are apparent in the ground cytoplasm (Fig. 9), though these are less distinct than the 10 to 20 μm particles in the light-grown cells transferred every 12 hours. Other differences also are apparent in the dark-grown cells. The chromatin areas are smaller than in the sections of any of the light-grown cells, and electron-dense bodies assumed to be polyphosphate were found to be infrequent in the dark-grown cells in contrast to light-grown cells (11, Fig. 6). Future investigations may reveal what changes take place in such colorless, dark-grown cells as they are transformed into photochemically active organisms under suitable environmental conditions.

DISCUSSION

It has been demonstrated by Schachman, Pardee, and Stanier (15) that the chromatophores of *Rhodospirillum rubrum* contain all the photosynthetic pigments of the bacterial cell. They suggested that these particles represent "the site of the primary photochemical reaction of bacterial photosynthesis." This assumption appears to have been well founded, and in the meanwhile it has been recognized that the chromatophores also contain numerous enzyme systems concerned with the elaboration of the primary photochemical products (1, 6-10, 13, 16, 20, 21). From the information presented in this paper it would appear that, at least in *Rhodospirillum*, the chromatophore is not the smallest complete photochemical structure which can catalyze these reactions. Structures can be isolated which occupy only about $\frac{1}{50}$ the volume of the chromatophore and which on a chlorophyll basis are as active as the larger chromatophores. Thus, it is quite possible that these smaller particles represent building blocks from which the larger chromatophores can be formed, suggesting that these 25 μm particles are part of a repeating structure within the chromatophore (Fig. 2). The chemical and physical makeup of these particles is of considerable interest and will receive more attention in the future.

Comparative studies on isolated chromatophores and on sections of whole cells and studies on the appearance of partially ruptured cells reported here support the assumption that the vesicles observed in sections of whole cells (18, 19) represent sections through the chromatophores, and that the chromatophores must exist as real structures within the bacterial protoplast.

No attempt has been made thus far to test the photochemical activity of cell-free fractions from older cells showing the lamellae described in the preceding paper (11). It will be of interest to learn whether these lamellae, when isolated, contain photosynthetic pigments and, if so, can carry out photochemical reactions.

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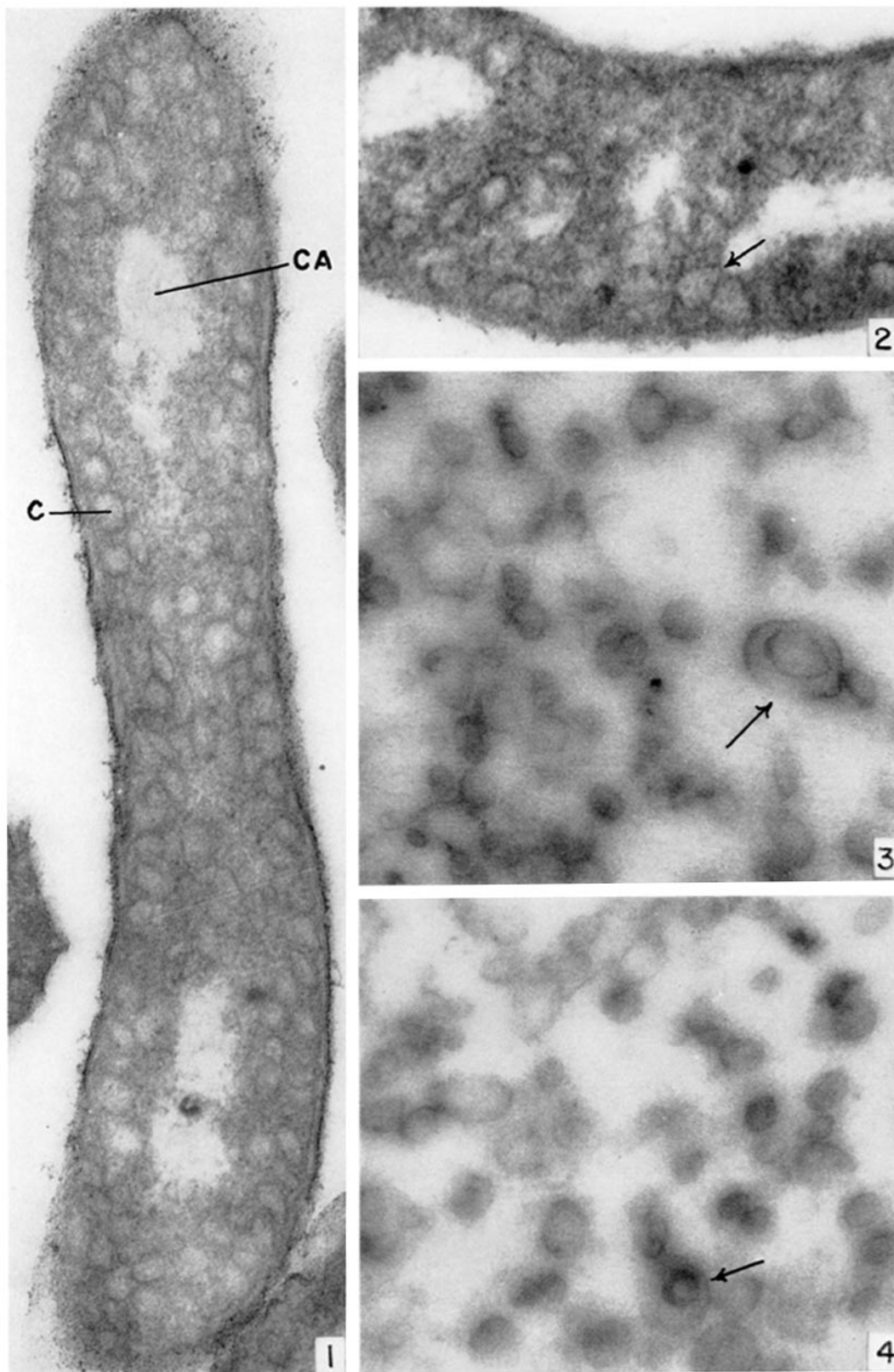
EXPLANATION OF PLATES

PLATE 152

FIG. 1. Section of a *R. rubrum* cell from a 1-day-old light-grown culture, showing the presence of chromatophores (C), and low-density chromatin areas (CA). The multiple layers of the peripheral envelope are visible at several points along the edge of the cell. $\times 75,000$.

FIG. 2. Section of a *R. rubrum* cell from a 22-hour-old culture. Several small particles are visible within the chromatophore indicated by the arrow. $\times 110,000$.

FIGS. 3 and 4. Sections of isolated, purified *R. rubrum* chromatophores. The concentric structures, indicated by arrows, occur relatively infrequently in such preparations. $\times 75,000$.



(Frenkel and Hickman: Photochemical activity of *Rhodospirillum rubrum*)

PLATE 153

FIG. 5. Sediment obtained from clarification centrifugation following sonic disruption of *R. rubrum* cells. Preparation air-dried without chemical fixation, and shadowed. Isolated chromatophores and scattered cellular remnants are visible. $\times 40,000$.

FIG. 6. *R. rubrum* chromatophores purified by sucrose density-gradient centrifugation and sprayed onto supporting film for electron microscopy. $\times 75,000$.

FIG. 7. Chlorophyll-containing, photochemically active particles obtained by sonic disruption of *R. rubrum* cells in 0.2 M sucrose. Particles purified by differential centrifugation in glycylglycine buffer. $\times 75,000$.

FIG. 8. Chlorophyll-containing, photochemically active particles obtained from cells from cultures 12 hours old by sonic disruption in glycylglycine buffer. Particles partially purified by differential centrifugation. $\times 75,000$.

FIG. 9. Section of a *R. rubrum* cell from cultures maintained aerobically in the dark. Such cells do not contain chromatophores, have smaller chromatin areas and fewer high-density polyphosphate particles than do cells from anaerobic light-grown cultures. $\times 80,000$.

