THE PREPARATION AND PROPERTIES OF BACTERIAL CHROMATOPHORE FRACTIONS

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ABSTRACT

Chromatophore material from the bacterium *Rhodopseudomonas spheroides* was freed of ribosomes by centrifugation in 27 per cent RbCl and then separated into "heavy" and "light" fractions by centrifugation through a sucrose gradient. The fractions differed from one another in the following ways. (*a*) The isopycnic density of the heavy fraction was between 1.15 and 1.18 gm/ml and that of the light fraction was 1.14 gm/ml. (*b*) The heavy fraction was able to bind ribosomes; the light fraction was not. (*c*) The light fraction was homogeneous in the ultracentrifuge and had a sedimentation constant, extrapolated to infinite dilution, of 153 $s_{20,w}$. The heavy fraction was grossly heterogeneous. (*d*) Both the amount of bacteriochlorophyll relative to protein and the ratio of bacteriochlorophyll to carotenoids were greater in the light fraction. (*e*) The spectra of the two fractions in the near infra-red were different. Comparisons of the chromatophore fractions from cells with different amounts of bacteriochlorophyll showed that the specific bacteriochlorophyll contents of the two fractions did not change to the same extent as did that of the whole cells. The amount of heavy fraction from pigmented cells was roughly independent of the cellular pigment content and was about equal to that from pigment-free cells. The amount of light fraction depended on the pigment content of the cells; no light fraction was obtained from cells devoid of bacteriochlorophyll. The cytochrome complements of both fractions underwent quantitative as well as qualitative changes with varying growth conditions. The size of the photosynthetic unit in *R. spheroides* appeared to increase as the total cellular bacteriochlorophyll content increased; however, the number of units per light fraction particle remained constant.

INTRODUCTION

In 1952, Pardee, Schachman, and Stanier (1) discovered that the photopigments of the photosynthetic bacterium *Rhodospirillum rubrum* are associated with a particulate fraction in cell-free extracts. By repeated centrifugation they were able to obtain a pigmented preparation which was homogeneous in the ultracentrifuge and had a sedimentation constant of about 120 *s*. Under the electron microscope this preparation was seen to consist, at least partly, of discs approximately 80 mμ in diameter. It was assumed that the discs arose by the collapsing of spherical particles during drying. These particles were termed chromatophores by Schachman and coworkers. Unhappily, this term came to be applied to any sedimentable, pigmented material in cell-free extracts of photosynthetic bacteria regardless of its purity or method of preparation.

Soon after this work it was shown that the pigment contents of *R. rubrum* and *Rhodopseudomonas spheroides* are markedly influenced by the environment. When grown under highly aerobic conditions, cultures of these organisms are nearly devoid of photopigments. If such a "bleached" culture is placed under anaerobic conditions in the light (photosynthetic conditions) there is a lag in growth during which the pigment content of the culture increases. Under photosynthetic conditions the pigment content is inversely related to the light intensity (2).

Several workers have made use of these variations in pigment content to study cytological manifestations of pigment formation. When thin sections of pigmented cells of *R. rubrum* and *R. spheroides* were examined in the electron microscope, numerous round or elliptical profiles were seen in the cytoplasm. These apparently represented cross-sections of spherical, membrane-limited vesicles (3). None were seen in aerobically grown cells devoid of photopigments (4). When cells grown in bright light were compared with cells grown in dim light, it was found that the vesicles were more numerous in the latter case, which suggests that the increase in photopigment content of whole cells is due to an increase in the number of chromatophores or in the amount of chromatophore material (5, 6).

After the demonstrations of Frenkel (7) and Geller and Lipmann (8) that chromatophore fractions of *R. rubrum* can carry out photophosphorylation, several workers undertook chemical analyses of these fractions. In addition to containing bacteriochlorophyll and carotenoid pigments, the chromatophore fractions were shown to contain protein, phospholipids, and some carbohydrate as well as cytochromes and quinones (9–11).

The most careful study of the chromatophore fraction has been carried out by Cohen-Bazire and Kunisawa (12). These workers showed that two pigmented fractions could be obtained by centrifugation of the chromatophore fraction of *R. rubrum* in a sucrose gradient. The two fractions were called "heavy" and "light" chromatophore fractions according to their positions in the gradient. Both fractions were capable of carrying out photophosphorylation. The light fraction had a higher specific bacteriochlorophyll content (μg bacteriochlorophyll per 100 μg protein) than the heavy fraction. The specific pigment contents of the fractions were roughly proportional to the pigment contents of the cells from which they were obtained. This implies that the number of chromatophores is constant, in contradiction to the results of electron microscopy.

In spite of these studies, many questions about bacterial chromatophores have remained unanswered or in some cases unasked. For example, what is the relationship of the chromatophore fraction of cell extracts to the vesicles seen in electron micrographs? Do changes in the pigment content of whole cells reflect changes in the amount or in the specific pigment content of the chromatophore fraction? Are heavy and light chromatophore fractions artifacts, and if not, what is their relationship to the vesicles seen in electron micrographs?

In this paper we report the development of a convenient method for the isolation and purification of chromatophore fractions and describe some chemical and physical properties of these fractions. We conclude that the heavy and light fractions are probably derived from distinct structures within the cell; that increased cell pigment content is brought about both by an increase in the specific pigment content of chromatophore material and an increase in the amount of light fraction. The evidence suggests that the heavy fraction may be derived from the cell membrane itself, and the light fraction from invaginations of this membrane.

METHODS

Biological Material

Most of the present experiments were conducted with *Rhodopseudomonas spheroides*, strain Ga (13). The only carotenoid pigments in this strain are neurosporene and hydroxyneurosporene (chloroxanthin). This circumstance permits the carotenoid pigment content to be determined easily.

Medium A of Sistrom (14), with succinic acid as the carbon source and supplemented with 0.1 per cent casein hydrolysate, was used.

For photosynthetic growth the rectangular glass vessels previously described (2) were used. The cultures were incubated in glass aquaria and were bubbled continuously with 5 per cent (v/v) CO_2 in N_2. Illumination was provided by banks of reflector flood lamps. The light intensity was measured at the front of the aquaria with a Weston illumination meter calibrated in foot candles.

For highly aerobic growth, Fernbach flasks containing 250 ml of culture were incubated with mechanical shaking. For semi-aerobic growth 2 liter serum bottles containing 1.5 liters of culture were used; the cultures were aerated continuously with gas

mixtures containing 1 per cent or 5 per cent O_2 and 5 per cent CO_2 in N_2.

In all cases the temperature was 34°.

PREPARATION OF CHROMATOPHORE FRACTIONS: TSM buffer was used throughout; its composition is: tris(hydroxymethyl)aminomethane (Tris), 0.02 M; NaCl, 0.08 M; $MgSO_4$, 0.01 M; pH 7.8–7.9.

A cell-free extract was prepared by passing the cell suspension in TSM through a French pressure cell at 20,000 psi. The cell was chilled beforehand and the extract was collected in an ice bath; all subsequent operations were conducted in the cold. The extract was centrifuged at 20,000 *g* for 25 minutes and the pellet (P_1) was discarded; the supernatant (S_1) is referred to as "crude extract."

To the crude extract a volume of Tris buffer (0.1 M, pH 7.8) containing RbCl and $MgSO_4$ was added to give final concentrations of 27 per cent RbCl and 0.05 M $MgSO_4$. The high magnesium concentration was used to reduce the effect of high salt concentration on ribosomes (15). The suspension was centrifuged at 40,000 RPM for 1 or 2 hours. At the end of this time all the pigmented material had floated to the top of the tube and the ribosomes had sedimented. The pigmented band was removed, diluted with TSM and centrifuged at 40,000 RPM for 2 hours to sediment the chromatophore material.

The pellet was resuspended in TSM and layered on top of a linear sucrose density gradient. The gradient was prepared from 35 per cent and 47 per cent (w/v) sucrose in TSM. The gradient tubes were centrifuged in a Spinco SW 25.1 rotor at 23,000 RPM for 7 to 12 hours. The pigmented material had separated into two bands; the material in the upper band was designated the light fraction and that in the lower, the heavy fraction. The material in each band was collected through a hole in the bottom of the tube, diluted with TSM and centrifuged at 40,000 RPM for 1 hour.

Chemical Methods

PHOTOPIGMENTS: Bacteriochlorophyll and neurosporene were estimated from the optical densities at 775 mμ and 468 mμ respectively of methanolic extracts (2).

Protein was determined by the Folin-Lowry method after digestion of the sample in 1 M NaOH for 1 hour at 40° (16).

RIBONUCLEIC ACID: The sample was precipitated with cold, 0.25 *M* $HClO_4$, heated at 70° for 15 minutes and centrifuged; this extraction was repeated. The RNA content of the extract was determined from the optical density at 260 mμ or by the orcinol method (17).

DRY WEIGHT: The sample was precipitated with cold, 5 per cent trichloroacetic acid and the precipitate washed with cold trichloroacetic acid and distilled water. The precipitate was transferred to an aluminum foil weighing cup and dried to constant weight at 100–105°.

LIPID PHOSPHORUS: The sample was precipitated with cold, 5 per cent trichloroacetic acid; the precipitate was washed and dried and then extracted 3 times with a mixture of chloroform and methanol (2:1) and once with ethyl ether. The combined extracts were taken to dryness and the residue digested with sulfuric acid at 150–160° for 1 hour. Phosphorus was determined by the method of Dryer (18).

CYTOCHROMES: Total heme content was estimated from the reduced (dithionite) minus oxidised difference spectrum of the pyridine hemochromogen prepared by the method of Elliott and Keilin (19). The difference in optical density at 550 mμ is reported. In addition, dithionite reduced minus oxidized difference spectra of chromatophore preparations were recorded.

Physical Methods

IN VIVO BACTERIOCHLOROPHYLL SPECTRA: A Cary Model 14 spectrophotometer was used to record spectra of chromatophore preparations and cell free extracts.

In *R. spheroides* the near infra-red spectrum of bacteriochlorophyll has three absorption bands with maxima at about 800 mμ, 850 mμ, and 875 mμ. In order to estimate the magnitude of each of these the following corrections were made. These are based on the assumption that each of the bands is symmetrical (20). The observed optical densities at 800 mμ, 850 mμ, and 873 mμ were first corrected for light scattering by assuming that this increased linearly between 1000 mμ and 750 mμ. This was the only correction applied to the OD at 800 mμ. The OD at 850 mμ was corrected by subtracting the OD at 896 mμ, which represents the contribution of the 873 mμ band to the OD at 850 mμ. The OD at 873 mμ was corrected by subtracting the difference between the OD at 827 mμ and at 773 mμ, which is the contribution of the 850 mμ band to the OD at 873 mμ.

LIGHT-INDUCED ABSORBANCY CHANGES: A Zeiss PMQ-II spectrophotometer was modified to admit exciting light through a filter on the top of the sample compartment; a blocking filter was placed between the sample and the photocell. A microscope lamp was used as the light source; the filament was imaged on the cuvette. With the exciting light turned off, the optical density scale was set to an arbitrary value; the light was turned on and the optical density read after several seconds. One or 2 minutes were allowed between readings so that the optical density regained its initial value.

ULTRACENTRIFUGAL ANALYSES: A model E Spinco ultracentrifuge with Schlieren optics was used. Kodak metallographic plates were used for non-

pigmented preparations, and Kodak spectroscopic plates (Type 1-D) for pigmented preparations.

RESULTS

Development of the Method for Isolation of Chromatophore Fractions

The main difference between the method employed by Cohen-Bazire and Kunisawa and the one used here for the isolation of chromatophore fractions is the centrifugation in 27 per cent RbCl which separates the chromatophore material from ribosomes. The following considerations led to this procedure.

It was clear that contamination with ribosomes would be a major difficulty in the purification of chromatophore material. As can be seen in Table I, the material prepared by the method of Cohen-Bazire and Kunisawa contains RNA which can be removed only by repeated centrifugation. In their method, the crude extract, which contains both chromatophore material and ribosomes, is centrifuged at a high speed; the pellet will contain not only the chromatophore material but also the bulk of the ribosomes. In this pellet, ribosomes may become bound to chromatophore material.

TABLE I

Ribonucleic Acid Content of Cells and Chromatophore Fractions of R. Spheroides

Preparation	μg RNA per μg bacteriochlorophyll
1. Whole cells	5.8
2. Chromatophore material*	1.9
3. As (2) but resuspended in TSM and centrifuged 6 times	0.20
4. As (2) but treated with ribonuclease‡	0.20
5. Chromatophore material§	0.14

* Prepared by the method of Cohen-Bazire and Kunisawa; this material was the pellet from the first high speed (40,000 RPM) centrifugation.

‡ The chromatophore suspension was treated with excess EDTA and RNase (1 mg/ml) for 10 minutes and recentrifuged.

§ Prepared by the method described in the text; the material was the pigmented layer after centrifugation in 27 per cent RbCl which was diluted in TSM and recentrifuged.

If this is the case, then it should be possible to separate ribosomes and chromatophore material by centrifugation of crude extract in a medium of the proper density. Sucrose solutions are too viscous, and so we turned to RbCl. As can be seen in Table I, the chromatophore material prepared by the present method contains very little RNA. When the material which sediments in 27 per cent RbCl was suspended in TSM and examined in the ultracentrifuge, a sedimentation pattern typical of ribosomes was obtained.

Purity and Properties of Heavy and Light Chromatophore Fractions

There is little RNA in either the heavy or light fraction. The RNA contents shown in Table I are maximal since they were calculated from the optical density, at 260 mμ, of hot perchloric acid extracts, and spectra of these indicated the presence of other material absorbing at this wavelength.

Neither muramic acid nor amino sugars were detected by chromatography of hydrolysates of either fraction. These compounds were found in the material sedimented in the first low speed centrifugation (P_1) which presumably is composed largely of cell wall material. This pellet was pigmented and does contain chromatophore material; this point will be discussed in more detail later.

In the sucrose gradient the heavy fraction formed an almost membranous layer which was easily dispersed in TSM to give a turbid suspension. The position of the heavy fraction in the gradient varied somewhat with different growth conditions and corresponded to densities of between 1.15 and 1.18 gm/ml. The densities of heavy fractions prepared by the method of Cohen-Bazire and Kunisawa were between 1.18 and 1.20 gm/ml. The difference is most likely due to contamination by RNA in the latter case (see below). Analytical ultracentrifugation showed that the heavy fraction was grossly heterogeneous.

In the sucrose gradient the light fraction was at a position corresponding to a density of 1.14 gm/ml, which is the same as that reported by Cohen-Bazire and Kunisawa. Suspensions of light fraction in TSM were optically clear.

The light fraction was monodisperse in the ultracentrifuge (Fig. 1); it had a sedimentation constant, in water at infinite dilution, of 153 *s* (Fig. 2). This is considerably higher than the

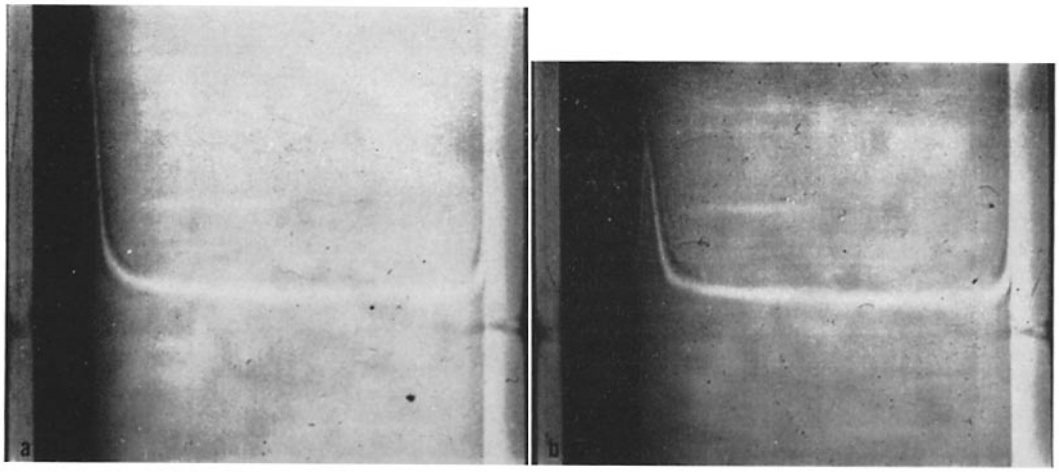

FIGURE 1 Appearance of the light chromatophore fraction in the analytical ultracentrifuge. The light fraction from *R. spheroides* grown photosynthetically in a light intensity of 600 ft-c was suspended in TSM to a bacteriochlorophyll concentration of 260 μg/ml. *a*, after centrifugation for 1 minute at 21,410 RPM; *b*, after 5 minutes. Note that the Schlieren peak corresponds with the light absorption boundary.

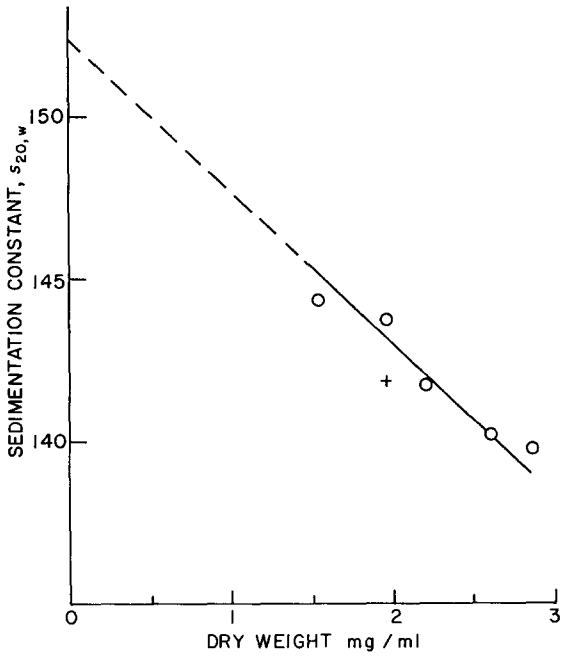

FIGURE 2 Dependence of sedimentation constant on concentration. The light fraction from *R. spheroides* grown photosynthetically in a light intensity of 600 ft-c was used. The sedimentation constant, $s_{20,w}$, has been plotted against the concentration of chromatophore material. The point (+) is for H(L) fraction (see Fig. 4).

values reported for chromatophore preparations from *R. rubrum* (1) or from *Chromatium* (9). These values were not, however, corrected for the effect of concentration. We have been able to obtain x-ray diffraction patterns from the light fraction (21). It is clear, therefore, that this fraction is remarkably homogeneous.

Differences between Heavy and Light Chromatophore Fractions

Chromatophore material prepared by the method of Cohen-Bazire and Kunisawa (12) contains RNA; when the heavy and light fractions are separated, the bulk of the RNA is in the heavy

fraction. This led us to look for an interaction of ribosomes with chromatophore fractions prepared by the present method which are almost free of RNA.

The pellet from the centrifugation in RbCl was resuspended in TSM to obtain a suspension of ribosomes. Mixtures of this with suspensions of heavy and light fraction were made containing 0.25 mg and 3.0 mg RNA per milligram chromatophore material (dry weight). The four mixtures were incubated for 1 hour at 4° and then layered on top of sucrose gradients (35 per cent to 61.5 per cent in TSM); these were centrifuged for 12 hours at 23,000 RPM. The samples of light fraction were unchanged in density, and there was no detectable increase in their RNA contents. The two samples of heavy fraction had increased in density; the increase was greater in the sample with the larger amount of RNA. The densities were within the range of those of heavy fraction isolated by the method of Cohen-Bazire and Kunisawa. Furthermore, the RNA contents of the heavy fraction samples had markedly increased.

In Table II are set out the results of an analysis of the bacteriochlorophyll and carotenoid pigment contents of heavy and light chromatophore fractions of cells grown under a number of conditions. It can be seen that the amount of bacteriochlorophyll relative to carotenoids is consistently higher in the light fraction.

This unexpected result led us to examine carefully the near infra-red spectra of the two fractions, since it has been suggested that the spectrum of bacteriochlorophyll is influenced by carotenoid pigments (2). Some typical spectra are shown in Fig. 3; in Table II are listed the corrected optical densities at the three absorption maxima for a wider range of growth conditions. It is clear that the spectra of the two fractions are different. In the heavy fraction, the relative size of the 873 mμ band is greater and those of the 850 and 800 mμ bands are smaller than in the light fraction. The relationship between the spectra and the ratios of carotenoid pigments to bacteriochlorophyll of the two fractions is the same, as was found for whole cells with different bacteriochlorophyll contents (2).

Recently, it has been shown that the relationship between carotenoid pigments and the spectrum of bacteriochlorophyll is not a simple or direct one but that the only such relationship is between the spectrum and the cellular content of bacteriochlorophyll (22). This is confirmed by the present results. It can be seen in Table II (column headed L/H) that the ratio of carotenoids to bacteriochlorophyll changes to the same extent in each fraction; however, the spectrum of the heavy fraction is modified to a greater extent than is that of the light fraction. This is seen clearly in Fig. 3 and also in the values for the 850 mμ absorption band in Table II.

TABLE II

The Spectra and Photopigment Contents of Chromatophore Fractions of R. spheroides Grown Under Various Conditions

	Corrected optical density per mg bacteriochlorophyll*						Molar ratio of bacteriochlorophyll to carotenoid pigments		
	Wavelength								
	800 mμ		850 mμ		873 mμ				
Growth condition	H‡	L‡	H	L	H	L	H	L	L/H
70 ft-c	49	65	71	87	44	37	2.6	3.3	1.3
600 ft-c	44	59	41	69	39	38	2.4	3.0	1.25
3,000 ft-c	43	59	37	56	50	46	2.1	2.7	1.3
8,500 ft-c	39	52	24	51	51	50	1.9	2.3	1.2
1 per cent O_2	48	61	44	61	39	39	2.6	3.1	1.2
5 per cent O_2	60	41	57	32	46	49	—	—	—

* See text for details of these corrections.

‡ H: heavy fraction; L: light fraction.

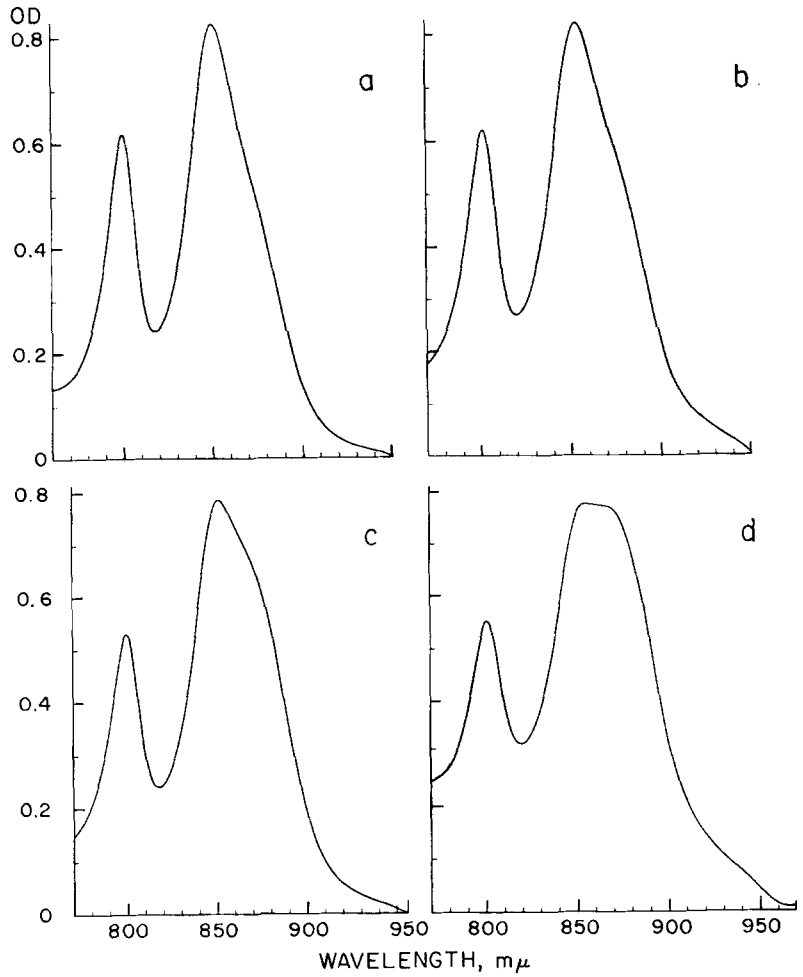

FIGURE 3 Absorption spectra of heavy and light chromatophore fractions from *R. spheroides*. *a*, light and *b*, heavy fraction from cells grown photosynthetically in a light intensity of 600 ft-c; *c*, light and *d*, heavy fraction from cells grown in a light intensity of 8,500 ft-c. In each case, the bacteriochlorophyll concentration was 16 μg/ml.

Formation of Material Similar to Light Fraction from Heavy Fraction

Cohen-Bazire and Kunisawa reported that when isolated heavy fraction from *R. rubrum* was treated with pancreatic lipase its rate of sedimentation in a sucrose gradient was the same as that of light fraction. We obtained entirely similar results with the heavy fraction from *R. spheroides*.

We have found that another, and probably less drastic, way of altering the density of isolated heavy fraction is simply to pass it through a French pressure cell. When this was done and the material centrifuged through a sucrose gradient, all the pigmented material was found at a position corresponding to a density of 1.14 gm/ml, which is the density of the light fraction. We refer to this material as the H(L) fraction. In the analytical ultracentrifuge it was monodisperse (Fig. 4) and had the same sedimentation constant as the light fraction (Fig. 2).

It can be seen from Table III that the ratio of bacteriochlorophyll to carotenoid pigments in H(L) was similar to that of the heavy fraction from which it was obtained. The spectrum of bacteriochlorophyll in the H(L) fraction was not identical with that of either the heavy or the light

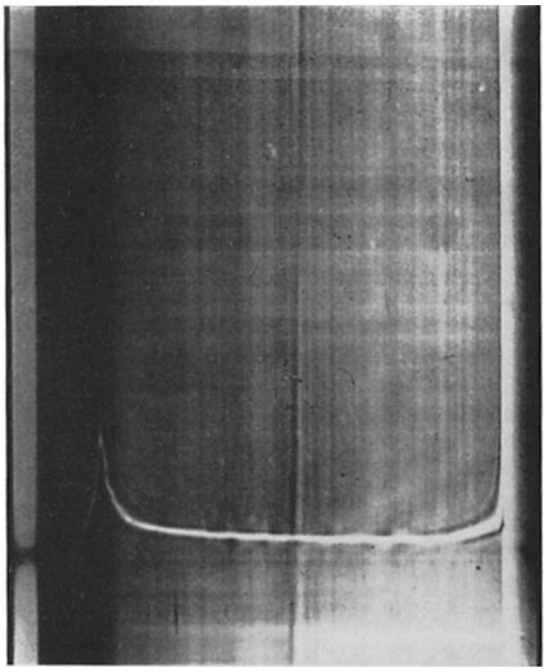

FIGURE 4 Appearance of H(L) fraction in the analytical ultracentrifuge. The fraction prepared from cells grown photosynthetically in a light intensity of 600 ft-c was used; the bacteriochlorophyll concentration was 280 μg/ml. The picture was taken after centrifugation at 21,410 RPM for 3 minutes. See Fig. 2 for the sedimentation constant.

fraction. Unlike the heavy fraction, H(L) did not bind ribosomes.

The conversion of heavy fraction to H(L) did not occur in crude extracts but only in suspensions of isolated heavy fraction. This was shown by the following experiment. A crude extract was divided into two parts and one part was passed through the French pressure cell a second time. The amounts of heavy and light fractions in each part were then estimated by centrifugation in RbCl and in a sucrose gradient. The results are shown in Table IV. Although there was a slight increase in the amount of light fraction in the sample which was treated a second time in the pressure cell, the increase is hardly sufficient to account for the amount of light fraction.

These results suggest that the isolated heavy fraction may be a complex of pigmented material having a density equal to that of the light fraction and non-pigmented, denser material which is capable of binding ribosomes. Further consideration of this point is given in the Discussion.

Influence of Growth Conditions on Chromatophore Fractions

CHEMICAL COMPOSITION: The experiments described in this section were undertaken to answer the question whether changes in the specific bacteriochlorophyll content of cells reflect changes in the amount or in the specific pigment content of chromatophore material. To this end we have determined the relative amounts and pigment contents of heavy and light fractions prepared from cells grown under different conditions.

TABLE III

Photopigments of Heavy and Light Fractions and of H(L) Fraction of Chromatophore Material of R. spheroides

Fraction	Specific bacteriochlorophyll content (μg/100 μg protein)	Weight ratio of bacteriochlorophyll to carotenoids
H(L)	14.3	5.3
Heavy	8.6	5.4
Light	14.8	6.6

Strain Ga was grown in light intensity of 600 ft-c; heavy and light fractions were prepared as usual and then a portion of the heavy fraction was treated a second time in the French pressure cell to produce the H(L) fraction.

For purposes of orientation, some typical analyses of whole cells grown under different conditions are shown in Table V.

In both chromatophore fractions, protein represented 55 to 65 per cent of the dry weight, and

lipid phosphorus about 0.65 per cent. These values did not depend on the conditions during growth of the cells.

In Table VI are shown values for bacteriochlorophyll contents relative to protein for heavy and light fractions. Comparison with Table V shows that the increase in specific bacteriochlorophyll content of the chromatophore material is not so great as that of the whole cells.

TABLE IV

Effect of Second Treatment of Crude Extract in French Pressure Cell on Relative Amounts of Heavy and Light Fractions of Chromatophore Material of R. spheroides

Treatment	Amount of bacteriochlorophyll in total	Weight ratio of bacteriochlorophyll to carotenoids
	per cent	
First passage through French pressure cell:		
1. Heavy fraction	45	5.4
2. Light fraction	55	6.6
Second passage through French pressure cell:		
1. Heavy fraction	42	5.1
2. Light fraction	58	6.8

Strain Ga was grown photosynthetically in light intensity of 600 ft-c, the cell suspension was treated in a French pressure cell and centrifuged at 20,000 *g* for 25 minutes; a part of the supernatant was again treated in the pressure cell and then the amount of bacteriochlorophyll and carotenoid pigments in the heavy and in the light fractions were determined after centrifugation through a sucrose density gradient.

CHANGES IN THE AMOUNTS OF THE CHROMATOPHORE FRACTIONS: The fact that the specific bacteriochlorophyll contents of the chromatophore fractions did not increase to the same extent as that of whole cells implies that the amount of the chromatophore material must increase. The following observations show that the amount of light fraction does increase but that the amount of heavy fraction remains constant.

Before giving the results, it is necessary to discuss the most important source of error: the loss of pigmented material in the low-speed centrifugation of the crude extracts. The material removed at this step (P_1) is certainly derived, at least in part, from the cell wall since it contains muramic acid and amino sugars. The amount of bacteriochlorophyll in P_1 represents 15 to 40 per cent of the pigment in the crude extract (Table VII). From counts of viable cells it appears that 80 to 90 per cent of the cells are disrupted by the treatment in the pressure cell; therefore, from one-third to one-half of the bacteriochlorophyll in P_1 is in unbroken cells. The bacteriochlorophyll to carotenoid pigment ratio of P_1 is slightly higher than that of the subsequently isolated heavy fraction. In Table VII this is seen as an increase in the bacteriochlorophyll to carotenoid ratio in the supernatant from the centrifugation. In calculating the amounts of heavy and light fraction, we have accordingly assumed that the material lost

TABLE V

Chemical Composition of Whole Cells of R. spheroides Grown Under Various Conditions

Growth condition	Dry weight					Molar ratio of BChl to carotenoid pigments	Total heme§
	RNA	DNA	Lipid P*	Protein	BChl‡		
	per cent						
70 ft-c	15	6.8	0.48	36	2.15 (9)	2.9	43
600 ft-c	24	8.8	0.33	37	0.94 (3.9)	2.5	35
3,000 ft-c	23	8.5	0.29	45	0.35 (1.5)	1.7	41
8,500 ft-c	26	8.1	0.22	43	0.24 (1)	1.6	33
1 per cent O_2	19	8.8	0.34	36	1.0 (4.2)	2.6	40
Aerobic	25	10.1	0.14	34	—	—	15

* Lipid phosphorus.

‡ Bacteriochlorophyll; figures in parentheses are values relative to cells grown in a light intensity of 8,500 ft-c.

§ Optical density at 550 mμ/10 mg protein.

TABLE VI

The Amounts and Specific Bacteriochlorophyll Contents of Heavy and Light Chromatophore Fractions From R. spheroides

Growth condition	Chromatophore protein (*per cent* total cell protein)		Specific bacteriochlorophyll content			
			μg/100 μg protein		Relative*	
	Heavy	Light	Heavy	Light	Heavy	Light
70 ft-c	26	17	19.7	22.3	5.05	3.05
600 ft-c	22	10	9.3	15.0	2.4	2.05
3,000 ft-c	—	—	5.5	10.9	1.4	1.5
8,500 ft-c	22	3	3.9	7.3	*1.0*	*1.0*
1 per cent O_2	—	—	9.5	16.4	2.45	2.35

* Relative to the values for cells grown in a light intensity of 8,500 ft-c.

in the first centrifugation is heavy fraction and have corrected the amount of heavy fraction isolated for this loss.

In Table VI are shown the amounts of heavy and light fractions isolated from cells grown at three different light intensities. It can be seen that the amount of light fraction increases with decreasing light intensity while the amount of heavy fraction remains constant. The amount of heavy fraction is about the same as the total amount of 'chromatophore' material in cells grown under highly aerobic conditions. In Fig. 5 the data of Table VI have been plotted to show the relative amount of light fraction as a function of the specific bacteriochlorophyll content of the cells. We have drawn the curve through the origin since we have been unable to detect any light fraction from cells grown under highly aerobic conditions. It is possible that a more extensive analysis would show that the curve is actually discontinuous at zero specific bacteriochlorophyll content.

Cytochromes and Reaction Center Chlorophyll in Chromatophore Fractions

Cytochromes are known to play an essential role in bacterial photosynthesis, and there is considerable evidence that a cytochrome *c* participates at a very early stage in energy conversion (23). It was of interest therefore to examine the cytochrome contents of heavy and light chromatophore fractions from cells grown under a variety of conditions.

In Table V the heme contents of whole cells are shown; it is evident that, relative to protein, the heme content of pigmented cells is more or less constant and that it is considerably smaller in cells grown under highly aerobic conditions.

TABLE VII

Loss of bacteriochlorophyll during low speed centrifugation of extracts of R. spheroides

Growth condition	Weight ratio of bacteriochlorophyll to carotenoid pigments		Bacteriochlorophyll in supernatant (*per cent* of broken cell value)
	Broken cells	Supernatant	
70 ft-c	4.8	4.9	85
600 ft-c	4.2	5.5	84
8,500 ft-c	2.8	3.4	63
1 per cent O_2	4.4	5.0	78

The cell suspensions were passed through a French pressure cell; bacteriochlorophyll and carotenoids were estimated in samples of the broken cell suspension; the remainder of these suspensions were centrifuged at 20,000 *g* for 25 minutes and the pigments in the supernatants measured.

Some representative difference spectra of heavy and light chromatophore fractions are shown in Fig. 6. The relative amounts of the cytochromes depend markedly on growth conditions. This can be seen by comparing the heights of the peaks at 553 mμ and at 560 mμ in the spectra in Figs. 6 *a* and 6 *c*. In Table VIII the values of the ratio of the optical density at 553 mμ to that at 560 mμ are given for the chromatophore fractions from cells grown under a variety of conditions. In each case, the ratios in the two fractions are similar. This similarity can also be seen by comparing the spectra of the fractions from cells grown in a light

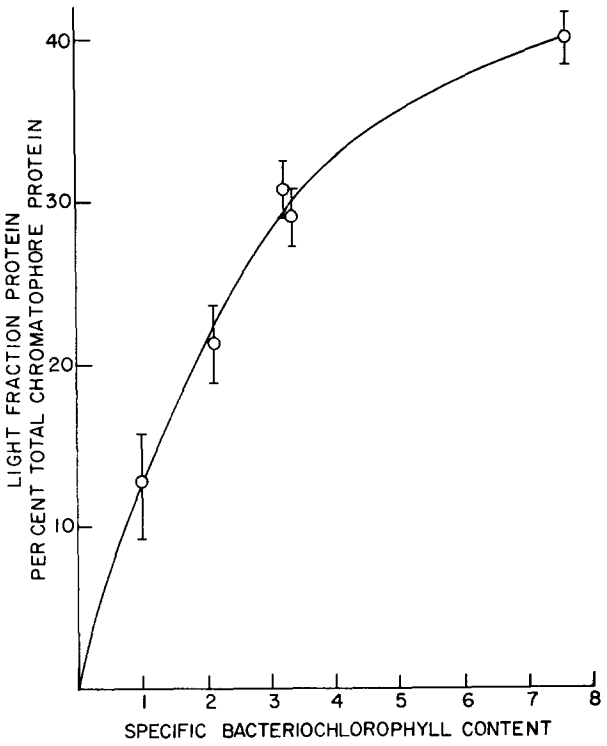

FIGURE 5 Light chromatophore fraction from cells with different specific bacteriochlorophyll contents. Cultures of *R. spheroides* were grown photosynthetically in different light intensities. The amounts of protein in the light and in the heavy chromatophore fractions were determined; the values for the heavy fractions were corrected for the loss at the low speed centrifugation, as described in the text. The sum of these values was the total chromatophore protein and the protein in the light fraction was expressed relative to this.

TABLE VIII

Cytochrome and Total Heme Contents of Chromatophore Fractions From R. spheroides Grown Under a Variety of Conditions

Growth condition	Fraction	Optical density at 560 mμ* (× 10²) per 100 μg protein	Optical density at 560 mμ* (× 10²) per 10 μmoles BChl¶	Relative‡ OD at 553 mμ	Pyridine hemochromogen value§ (× 10²) per 100 μg protein	Pyridine hemochromogen value§ (× 10²) per 10 μmoles BChl¶
70 ft-c	H	.49	.28	.51	.30	.18
	L	.39	.18	.50	.46	.21
600 ft-c	H	.31	.34	.60	.54	.58
	L	.35	.22	.59	.60	.37
3,000 ft-c	H	.32	.61	.74	.49	.83
	L	.31	.30	.69	.65	.56
8,500 ft-c	H	.32	.88	.67	.30	1.20
	L	.29	.39	.60	.31	.39
1 per cent O_2	H	.24	.21	.85	.37	.41
	L	.26	.16	.84	.45	.27
20 per cent O_2		.21	—	.96	.28	—

* From reduced minus oxidized difference spectra.

‡ Expressed as fraction of the OD at 560 mμ.

§ OD at 550 mμ (see Methods).

¶ Bacteriochlorophyll.

intensity of 70 ft-c shown in Fig. 6. In cells grown under photosynthetic conditions, the relative absorption at 553 mμ appears to depend on the specific bacteriochlorophyll content. In the chromatophore fractions from cells grown aerobically or semi-aerobically the optical densities at 553 mμ and at 560 mμ are almost equal. This is true regardless of the specific bacteriochlorophyll content, which is about the same in cells grown semi-aerobically (1 per cent O_2) or photosynthetically in a light intensity of 600 ft-c (see Table V).

It can be seen in Table VIII that neither the total heme nor the absorbancies at 560 mμ of the chromatophore fractions, when expressed relative

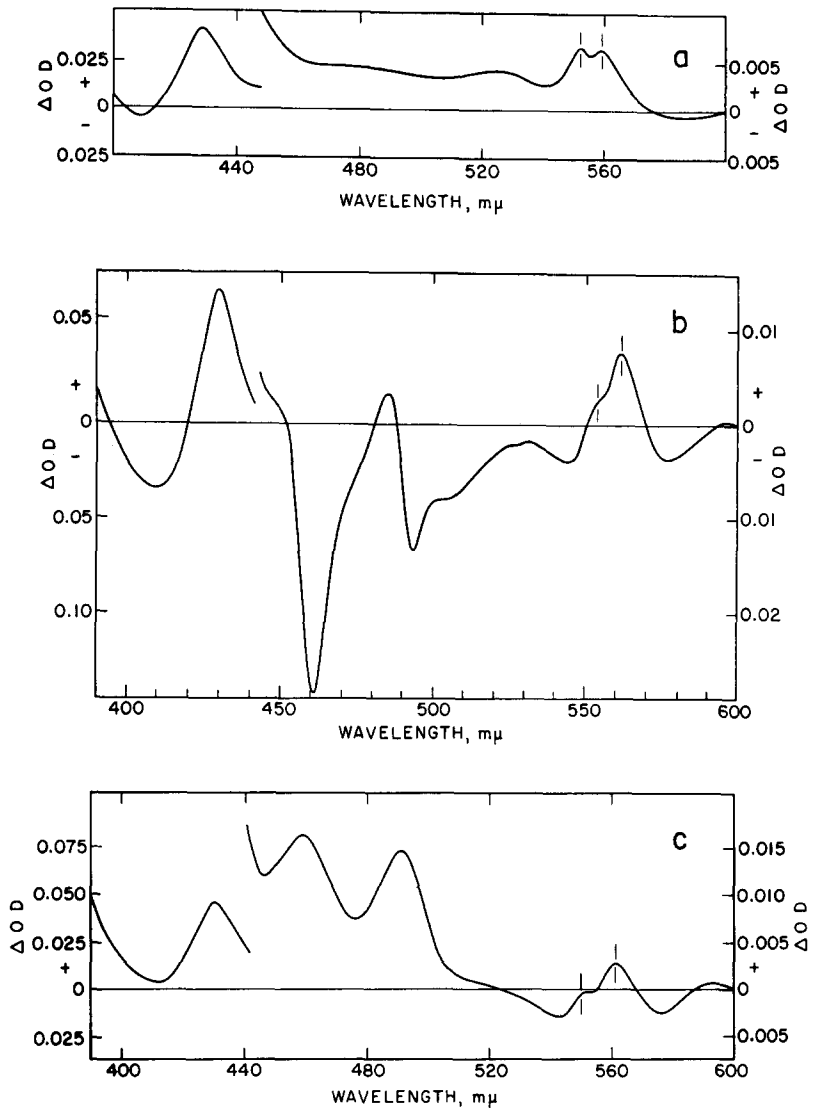

FIGURE 6 Difference spectra of chromatophore fractions from *R. spheroides*. The chromatophore fractions were suspended in TSM; a portion of the suspension was shaken in air and placed in the reference cell of the spectrophotometer; dithionite was added to the other portion to reduce the cytochromes. *a*, heavy fraction from cells grown under highly aerobic conditions (314 μg protein per ml); *b*, light fraction (189 μg protein per ml) and *c*, heavy fraction (50 μg protein per ml) from cells grown in a light intensity of 70 ft-c. The complexity of the spectra between 440 mμ and 500 mμ is due to changes in the spectrum of the carotenoid pigments.

to protein, were dependent on growth conditions. Similarly, the total heme contents of heavy and light chromatophore fractions in terms of protein were about the same; in terms of bacteriochlorophyll, however, the heme content of the light fraction was about half that of the heavy fraction.

Clayton (24) has observed light induced absorbancy changes in chromatophore material from a number of photosynthetic bacteria. He has ascribed these changes to the oxidation of a pigment which he termed P870 in the case of *R. spheroides*. Since the oxidation of P870 is probably closely associated with the primary photochemical reaction, it was of interest to investigate light induced absorbancy changes in heavy and light chromatophore fractions.

In Fig. 7 are shown difference spectra (illuminated minus dark) of the light fractions from cells grown in four different light intensities. It can be seen that all four are qualitatively similar but that they differ in the magnitudes of the changes relative to total bacteriochlorophyll. Thus, although in all four samples the amount of bacteriochlorophyll per milliliter was the same, the difference between the optical densities at 790 mμ and at 815 mμ was 0.059 for the fraction from cells grown in a light intensity of 8,500 ft-c but only 0.031 for the fraction from cells grown in a light intensity of 70 ft-c. There was a similar relationship between the bleaching at 875 mμ and the light intensity during growth.

In the heavy fraction, the bleaching at 875 mμ relative to total bacteriochlorophyll was consistently greater and that at 840 mμ less than in the light fraction.

It has been known for some time that illumination of chromatophore preparations may cause a red-shift in the spectra of the carotenoid pigments (23, 25). As can be seen from Fig. 8, the shift occurs in both chromatophore fractions, but to a much reduced extent in the heavy fraction in spite of its greater content of carotenoids.

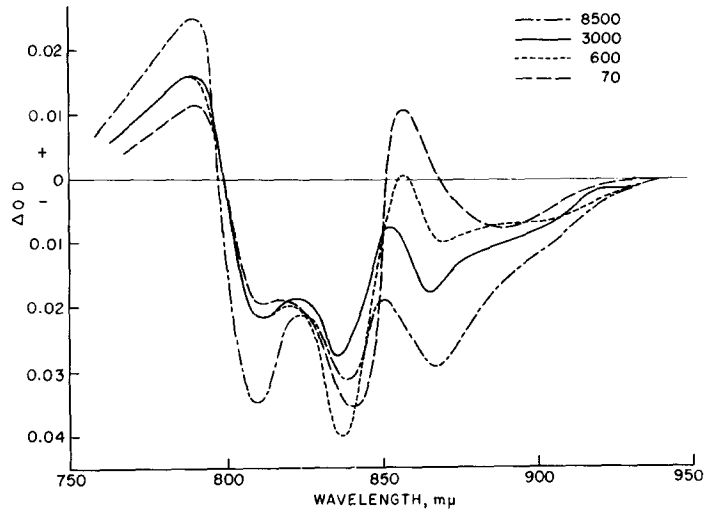

FIGURE 7 Light induced changes in the spectra of light chromatophore fractions in the region of bacteriochlorophyll absorption. Cultures of *R. spheroides* were grown photosynthetically in the light intensities shown in the figure. The light chromatophore fraction from each was suspended in TSM at a bacteriochlorophyll concentration of 30 μg/ml. Illuminated minus dark difference spectra were determined as described in the text; the exciting light was passed through a blue-green filter (Corning 4-97), and a Wratten 88A filter was placed between the sample and the photocell.

DISCUSSION

The heavy and light chromatophore fractions of *R. spheroides* described here are clearly analogous to those isolated by Cohen-Bazire and Kunisawa from *R. rubrum*. The principal difference is that in the latter case the heavy fraction was undoubtedly contaminated with ribosomes and therefore had a somewhat greater density than the heavy fraction we have isolated from *R. spheroides*. It is likely that the preparation obtained by Schachman and coworkers was the light fraction, since it was

purified by repeated cycles of low and high speed centrifugations. The chromatophore preparations studied by other workers were clearly mixtures of heavy and light fractions and in some cases were probably contaminated with ribosomes.

ORIGINS OF THE HEAVY AND LIGHT FRACTIONS: We have shown that there are several differences between heavy and light fractions in addition to those recognized by Cohen-Bazire and Kunisawa. The most clear cut are the differences in the *in vivo* bacteriochlorophyll spectra and in the ratios of bacteriochlorophyll to carotenoids; less certain are the differences in the cytochrome ratus of bacteria has two distinct forms within the cell.

However, the properties of the isolated fractions probably do not accurately reflect the state of the photosynthetic apparatus in the cell. This seems clear in the case of the heavy fraction, since we were unable to isolate this directly from a crude extract. In one experiment, a sample of crude extract was layered on top of a sucrose gradient (38 per cent to 61.5 per cent) and the gradient centrifuged for 23,000 RPM for 12 hours. From the density of the isolated heavy fraction it was expected that this fraction would have moved several

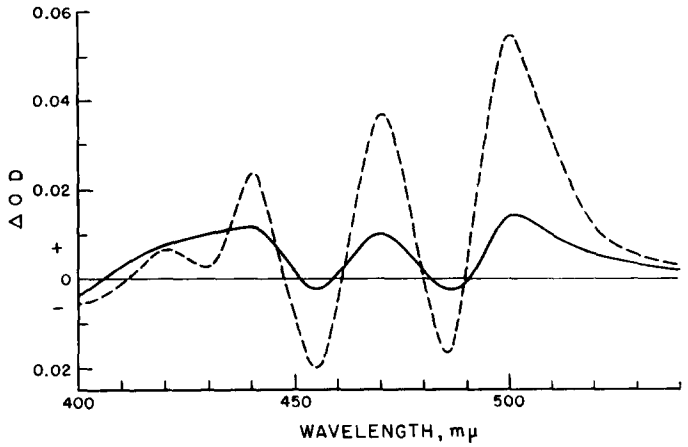

FIGURE 8 Light induced changes in the spectra of heavy and light chromatophore fractions in the region of neurosporene absorption. Light and heavy fractions from a culture of *R. spheroides* grown semi-aerobically in 1 per cent O_2 were suspended in TSM. Illuminated minus dark difference spectra were determined as described in the text; the exciting light was passed through a Wratten 88A filter, and a blue-green filter (Corning 4-97) was placed between the sample and the photocell. The suspension of heavy fraction (solid line) contained 12 μg neurosporene per ml; the suspension of light fraction (broken line) contained 15.5 μg/ml but the spectrum has been drawn on the basis of 12 μg neurosporene per ml.

to bacteriochlorophyll ratios and the magnitudes of the light induced changes in the spectra of the carotenoids. It is difficult to believe that these differences are artifacts, since they involve what might be called intrinsic properties; this is not the case with differences in densities or chemical composition which might arise from addition or removal of material during preparation of the fractions. Since it has been shown by Cohen-Bazire and Kunisawa that both the heavy and the light fraction from *R. rubrum* can carry out photophosphorylation and since we have shown that light induced changes of the spectrum of bacteriochlorophyll occur in both fractions from *R. spheroides*, we conclude that the photosynthetic apparatus of bacteria has two distinct forms within the cell.

centimeters in the gradient. In fact, all the pigmented material was still at the top of the tube. We concluded from this experiment that if the heavy fraction is a complex of pigmented and non-pigmented material, as is suggested by the formation of the H(L) fraction, the complex is formed in crude extracts only under certain conditions.

It should be pointed out that there is evidence that the two chromatophore fractions are not necessary for photosynthetic growth. We can detect little if any light fraction in extracts of strain uv-33 of *R. spheroides* which lacks colored carotenoids and on the other hand, strain MS-1, a histidine-requiring mutant of strain Ga, has little

heavy fraction. Both strains can grow photosynthetically.

We have seen that the amount of heavy fraction is more or less constant regardless of the specific bacteriochlorophyll content of the cells and that it is approximately equal to the total amount of particulate material from cells grown under highly aerobic conditions. It is difficult to estimate accurately the amount of heavy fraction, because of the loss, in the low speed centrifugation of the crude extracts, which can be corrected for only roughly. We have also seen that the amount of light fraction, relative to the amount of heavy fraction, increases with increasing cellular bacteriochlorophyll content (Fig. 5). It should be noted that the error due to the loss of heavy fraction would tend to decrease the relative amount of light fraction. This is so because, as is shown in Table VII, the amount of heavy fraction lost is smaller the higher the cellular bacteriochlorophyll content.

It has also been shown that changes in the specific bacteriochlorophyll content of the chromatophore fractions do not account for the changes in the pigment content of the cells from which they are derived. This is contrary to the results of Cohen-Bazire and Kunisawa with *R. rubrum* (12); we are unable to offer an explanation for this difference. Our finding implies that the amount of chromatophore material must change; this substantiates our conclusion that the amount of light fraction is not constant for cells with different bacteriochlorophyll contents.

The constancy in amount of heavy fraction suggests that it is derived from a component present in both pigmented and non-pigmented cells. This can not be the vesicles seen in the electron microscope, since these are not present in cells devoid of photopigments and since their number is not constant in cells with different pigment contents (6). We suggest that the heavy fraction may be derived from the cell membrane.

On the other hand, there are several points of correspondence between the light fraction and the vesicles seen in electron micrographs. Neither is found in cells grown under highly aerobic conditions and both increase in amount (or number) with increasing cellular bacteriochlorophyll (6). Furthermore, the size of the light fraction particles calculated from x-ray scattering (21) is about the same as the size of the vesicles seen in electron micrographs (6). Assuming a diameter of 60 mμ, we have calculated that there are about 4,000 light fraction particles per cell in a culture grown in a light intensity of 70 ft-c and about 700 particles per cell in one grown in a light intensity of 8,500 ft-c. Although no direct comparison is possible, these numbers are certainly reasonable in the light of the electron micrographs of *R. rubrum* published by Cohen-Bazire and Kunisawa (6). Thus it seems likely that the light fraction and the vesicles are derived from the same structure; this does not imply that the structure necessarily has the form of discrete spherical vesicles within the intact cell.

From preliminary observations it appears that the chromatophore material of *Rhodospirillum molichianum* and *Rhodomicrobium vannielii* can also be

TABLE IX

Size and Molecular Composition of Light Chromatophore Fraction Particles From R. spheroides

Diameter*	Wall thickness*	Volume‡	Weight§	Interfacial area‖	Protein molecules¶	Pigment molecules: Light intensity during growth	Pigment molecules: Bacteriochlorophyll	Pigment molecules: Carotenoids	Pigment molecules: P870**
A	*A*	A^3	μg	A^2					
600	60	5.5×10^7	6.3×10^{-11}	9.2×10^5	700	70	6,000	1,800	72
						8,500	2,000	900	67

* Calculated from x-ray scattering data (21).

‡ Volume of the shell with radii of 300 A and 240 A.

§ Weight of the shell, calculated from the volume and an assumed density of 1.13 mg/ml.

‖ Area assuming a radius of 270 A; *i.e.*, the midpoint of the wall.

¶ Assuming a molecular weight of 40,000.

** Calculated from illuminated minus dark difference spectra, assuming the extinction coefficient of P870 to be the same as that of bacteriochlorophyll.

separated into heavy and light fractions. Both organisms show lamellar, rather than vesicular, chromatophores when examined under the electron microscope (26, 27). In view of this similarity of the chromatophore material from organisms with very different cytologies, we should like to suggest that the structure of the photosynthetic apparatus is fundamentally the same in all purple bacteria and that it resembles the lamellae of *R. molichianum* more than the vesicles of *R. rubrum*. We suggest that the vesicles arise from the disruption and rounding up of intracellular membranes.

MOLECULAR COMPOSITION OF PARTICLES OF LIGHT CHROMATOPHORE FRACTION: In Table IX are shown some calculations of the molecular composition and geometry of light fraction particles. The dimensions are derived from x-ray diffraction data (21). We have assumed that the size of the particles is constant under all growth conditions. The volume and weight shown are for the wall of a spherical particle and not for the sphere itself, since the x-ray data are more consistent with a hollow sphere. There are several points of interest in these figures. In the first place, the futility of constructing models of chromatophores or of the photosynthetic apparatus with fixed amounts of the various components is obvious. Secondly, the interfacial area of the particle is too small to accommodate all the chlorophyll molecules in a monomolecular layer: they must somehow be stacked. Finally, it is of considerable interest that the number of reaction center chlorophyll (P870) molecules per particle seems to be a constant and a small number. This may represent the maximum number of photosynthetic units which can fit in the available space; if this is true then the maximum area occupied by a photosynthetic unit is about 10^4 A^2. It should be noted that according to these figures, the number of bacteriochlorophyll molecules associated with one molecule of P870 varies between 30 and 85.

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