

Isolation and Fractionation of the Photosynthetic Membranous Organelles from *Rhodopseudomonas spheroides*¹

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Molecular sieve chromatography and sucrose gradient centrifugation were used to prepare large quantities of purified chromatophores from *Rhodopseudomonas spheroides*. Electron micrographs of these chromatophores revealed that the final preparations were very homogeneous and free of non-chromatophore particulate material. As an additional check on purity, ¹⁴C-L-phenylalanine-labeled aerobic cells, devoid of chromatophores, were mixed with unlabeled photosynthetic cells. The resulting preparation contained less than 1% of the radioactivity, originally located in non-chromatophore protein. The purified chromatophores were solubilized in 2-chloroethanol and separated into two fractions. Fraction P_I contained 3 to 5% of the total chromatophore protein and could be resolved into 10 electrophoretic components. The second fraction, P_{II}, contained five electrophoretic components. One of these components had associated with it all of the pigment and phospholipid present in P_{II}. Preliminary immunochemical studies on these fractions are also reported.

In their early studies on the ultrastructure of the purple bacteria, Vatter and Wolfe (34) demonstrated the existence of chromatophores in the cytoplasm of cells grown anaerobically in the light (phototrophic). These "organelles" (subcellular structures possessing a specialized function) appeared to be absent during aerobic, dark growth (chemotrophic). Examination of thin sections of *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* by Cohen-Bazire (5) confirmed that chromatophores were either rare or absent in chemotrophic cells. The transition of *R. spheroides* from chemotrophic to phototrophic growth with the concurrent biosynthesis of chromatophores provides an opportunity to study the induction and development of this complex membranous organelle. In addition, such studies should enable us to gain insight into the molecular architecture of organelle structure as well as structure-function relationships. A complete understanding of the components of the chromatophore, namely, protein, pigment, and lipid, and their relationships to one another would be essential for achieving these goals.

We have chosen to begin our studies by iso-

lating and purifying the proteins composing the chromatophore. Once purified, individual proteins may be used biochemically and immunologically to determine which chromatophore proteins are newly synthesized during the induction period and which, if any, are components of the chemotrophic cell membrane from which the chromatophore is believed to be derived (16). Furthermore, the isolation and characterization of chromatophore-specific proteins would make feasible accurate determinations of the molecular weight and chemical composition of the various components of the chromatophore, as well as the relationship and degree of homology between them. To carry out these studies, it is essential that the isolated chromatophores be free of all non-chromatophore proteins whether they are nonspecifically adsorbed soluble proteins or particulate contaminants. Previous purification attempts (11, 37) consisted of either numerous cycles of density gradient centrifugation involving considerable expense and time, which yield only small amounts of material, or batch preparations of material contaminated with other cellular proteins (2, 29). The method presented here was developed for the rapid, large-scale purification of chromatophores. In addition, methods are presented for the fractionation of purified chromatophore proteins.

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MATERIALS AND METHODS

Organisms. *R. spheroides* strain 2.4.1 supplied to us by R. Y. Stanier was used throughout these investigations.

Medium and growth of the organism. All cultures were grown on medium A of Sistrom (31) supplemented with 0.2% Casamino Acids (Difco). To obtain uniform growth conditions, batch cultures of *R. spheroides* were grown anaerobically by gassing with CO₂-N₂ (5:95) in one of two ways. In the first method, photosynthetic growth was achieved by using a submerged quartz illuminator set at 500 ft-c with a New Brunswick Fermenter at 33 C. The second method involved the use of carboys maintained at 30 C. An incident light intensity of 300 ft-c was supplied by a bank of 75-w flood lights. Cells were harvested at 2×10^9 cells/ml with a Sharples centrifuge.

Chemotrophic cells devoid of chromatophores were grown for six generations in complete darkness and vigorously sparged with 100% oxygen to maintain strict conditions of aerobiosis. After two successive subcultures, the aerobic cells were harvested at a low cell density of 4×10^8 cells/ml to insure high aerobiosis for all the cells of the culture.

Electron microscopy. The chromatophores were examined at each step in the isolation and purification procedure by using an electron microscope (Siemens Elmiskop 1A) at 60-kv gun potential. The visual observations served as a monitor for the homogeneity of our final preparation and as a guide for determining the extent of non-chromatophore particulate debris. Each sample was mixed 1:1 with 1% phosphotungstic acid (pH 7.6). A drop of the mixture was placed on a 200-mesh copper grid lightly coated with 0.25% Formvar and carbon. The grids were examined after 5 min of staining.

Amino acid analysis. Pigment and lipid were removed from purified chromatophores by three successive extractions using acetone-methanol (7:2, v/v). The residual protein was lyophilized and then hydrolyzed by using constantly boiling hydrochloric acid for 24, 48, and 72 hr at 110 C in evacuated Pyrex vials. The amino acid analysis was determined on an analyzer (Beckman Spinco model 120). Separate analyses were made for cysteine and cystine as cysteic acid and methionine as methionine sulfone by the method of Moore (25). Tryptophan was determined spectroscopically by the procedure of Edelhoch (7). Serine and threonine were corrected for hydrolysis losses by extrapolation to zero time. The total amino acid composition data were averaged and expressed as mole per cent.

Polyacrylamide gel electrophoresis. Samples to be analyzed by polyacrylamide gel electrophoresis were first suspended at a concentration of 1 mg of protein/ml in 0.01 M tris(hydroxymethyl)amino-methane (Tris; pH 9.0), containing 0.005 M sodium acetate, 1.0% sodium dodecylsulfate (SDS), 0.001% disodium dihydrogen ethylenediaminetetraacetate dihydrate (EDTA), 0.5 M urea (ion free), and 1% mercaptoethanol, and heated for 0.5 hr in a water bath at 50 C. The sample was then dialyzed overnight against a buffer solution identical to the one listed above but containing 0.1% SDS. Electrophoretic separations were performed by using 7% acrylamide gels (7 by 0.6 cm) polymerized with 0.23% bisacrylamide, 0.07% ammo-

nium persulfate, and 0.04% *N,N,N',N'*-tetramethylethylene diamine in 0.1 M Tris buffer (pH 9.0) containing 0.05 M sodium acetate, 0.1% SDS, 0.001% EDTA, and 0.5 M urea. The electrophoretic buffer consisted of 0.1 M Tris (pH 9.0) with 0.05 M sodium acetate, 0.1% SDS, 0.01% EDTA, and 0.1% mercaptoethanol. The samples (0.1 to 0.2 ml) were made 10% with respect to glycerol and layered onto gels which were electrophoresed at 2 ma/gel for 2.5 hr.

After electrophoresis, the gels were soaked for 14 hr at 25 C in 20% sulfosalicylic acid. The gels were then stained for 6 hr in 0.25% Coomassie blue in 0.01 M sodium citrate buffer (pH 3.0; reference 6).

Destaining was accomplished by repeated washing in 7% acetic acid. The gels were scanned by the methods of Marrs and Kaplan (22) at 550 nm with an Instrument Specialties Co. (ISCO) model UA visible-light analyzer.

Preparation of antisera. The appropriate protein fraction (6 to 8 mg) for the preparation of each anti-serum was suspended in 2 ml of 0.01 M Tris buffer (pH 7.6) and emulsified with an equal volume of Freund's complete adjuvant. A 1.5-ml amount was injected into each hip muscle of a white albino rabbit whose pre-immune serum had been shown to have no natural antibody against the antigen. Twelve days after the primary injection, 50 ml of blood was drawn from each rabbit, and the primary immune serum was collected. Three weeks after the first injection, a booster was given; 12 days later, secondary immune serum was collected. The capillary precipitin test was used to determine the relative extent of cross reactivity between chromatophores and fractions P_I and P_{II} (18).

Chemicals. 2-Chloroethanol grade 131 was purchased from Eastman Organic Chemicals (Rochester, N.Y.). 3-¹⁴C-L-phenylalanine (specific activity, 50 mCi/mole) was obtained from Schwarz BioResearch (Orangeburg, N.Y.). Sepharose 2B was purchased from Pharmacia (Piscataway, N.J.), and Coomassie blue R250 was obtained from Colab Laboratories Inc. (Chicago, Ill.).

Proteins used as markers for the determination of molecular weights were as follows: catalase, lysozyme, and ovalbumin from Sigma Chemical Co. (St. Louis, Mo.), cytochrome *c* from Nutritional Biochemicals Corp. (Cleveland, Ohio), pepsin and trypsin from Worthington Biochemical Corp. (Freehold, N.J.).

Assay methods: protein. The method of Lowry et al. (21) was used to determine proteins, with bovine serum albumin as the standard.

Pigment. The pigments were extracted with acetone-methanol (7:2, v/v), and the optical density was determined at 775, 510, and 456 nm. The extinction coefficient given by Clayton (4) was used for the determination of the bacteriochlorophyll content.

Phospholipid. The total lipid phosphorus of the chromatophore was determined by using the method of Bartlett (1). By using the phospholipid composition data for *R. spheroides* given by Gorchein (13) and the fatty acid composition given by Hands and Bartley (15), the average molecular weight of a phospholipid in *R. spheroides* was determined to be approximately 770 g. By using this value, the number of micromoles of lipid phosphorus was converted to micrograms of total phospholipid.

DNA. Samples of deoxyribonucleic acid (DNA) were treated with 5% trichloroacetic acid at 0 C and

centrifuged. Lipids and pigments were extracted from the pellet by using acetone-methanol. The DNA content of the pellet was determined by the method of Cerriotti (3).

RNA. Ribonucleic acid (RNA) was determined by the orcinol method (24) on an extracted trichloroacetic acid pellet as described above.

Protein-bound hexose. This was determined by the method of Winzler (36).

PHB. Poly- β -hydroxybutyric acid (PHB) was obtained by the method of Gorchein (13) and assayed by the method of Slepecky and Law (32).

RESULTS

Chromatophore isolation: crude preparation of chromatophores. Twelve grams (wet weight) of anaerobically grown cells were resuspended at 4 C in 30 ml of 0.1 M sodium phosphate buffer (pH 7.6) containing 0.01 M EDTA. This buffer was found to be very effective in preventing aggregation of chromatophores and the resulting inclusion of non-chromatophore cell material (Zablen and Kaplan, *unpublished data*). Previous investigators (19) routinely employed Tris buffers and a source of Mg^{2+} , both of which, either singly or together, can cause aggregation of the chromatophores. Therefore, all subsequent procedures were performed at 4 C with phosphate-EDTA buffer. Cells were broken by three passages through a French pressure cell (Aminco) at 15,000 psi. Unbroken cells, cell wall, and debris were removed by two successive centrifugations at $27,000 \times g$ for 15 min. The supernatant fluid was then centrifuged at $150,000 \times g$ for 1.5 hr. Eighty to 90% of the pigment, as determined by the optical density at 775 nm, was found in the pellet which was designated crude chromatophores.

Chromatophore isolation: chromatophore purification. The crude chromatophores were resuspended in 10 ml of buffer by homogenization in a Tenbroeck tissue grinder and centrifuged at $27,000 \times g$ for 10 min to remove large aggregates. The supernatant fluid was layered onto a Sepharose 2B column (5×60 cm) which has a molecular sieving range of 2×10^6 to 25×10^6 . Fractions of approximately 10 ml were collected every 6 min. Fractions containing the pigmented chromatophores, which elute in the column void, were pooled and concentrated by centrifugation at $150,000 \times g$ for 1.5 hr. The resulting chromatophores were designated as partially purified chromatophores.

A maximum of 40 mg (protein) of the partially purified chromatophores were resuspended into 2 ml of buffer by homogenization and layered onto a 56-ml 20 to 40% (w/v) linear sucrose gradient which was centrifuged at $66,000 \times g$ for 10 hr in an ultracentrifuge (Beckman model L-2)

with an SW 25.2 rotor. The gradients were fractionated from the top by using a density gradient fractionator (ISCO model 180) and monitored at 420 nm by using a visible-light analyzer (ISCO model UA) during fractionation.

The fractions obtained from the single pigmented zone located approximately one-third of the way from the top of the gradient were pooled and dialyzed against 0.01 M Tris (pH 7.6). The resulting material was designated purified chromatophores.

With the exception of the buffer employed, the isolation of the crude chromatophores, given above, is similar to that used by other workers (10). However, electron microscopy revealed that the crude chromatophores (Fig. 1) were

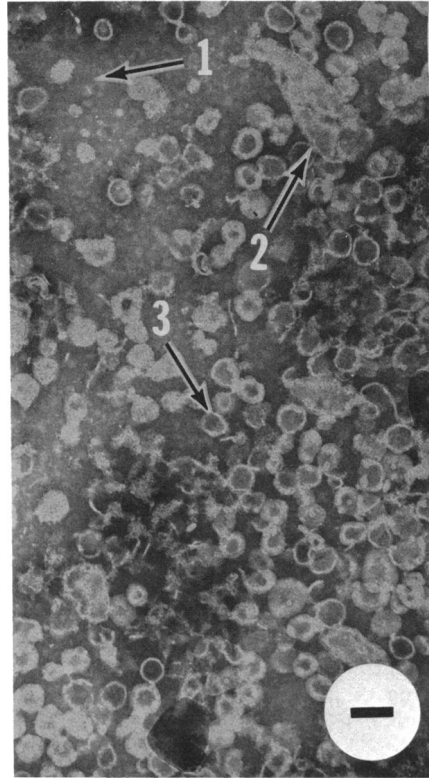


FIG. 1. Electron micrograph of a crude chromatophore preparation from *Rhodospseudomonas spheroides*. Considerable quantities of ribosomes (1) and membrane fragments (2) are present with the chromatophores (3). (See step 1, Table 1.) Marker on this and subsequent micrographs represents 0.1 μ m.

contaminated with considerable quantities of what appeared to be large membrane fragments and ribosomes. The use of Sepharose 2B column chromatography served to reduce both ribosomal and other particulate contamination as well as nonspecifically adsorbed soluble protein contami-

nation (Table 1). Although most of the larger membrane fragments elute before the chromatophores, Fig. 2 shows that there is still some non-chromatophore particulate material present in the chromatophore fraction after chromatography. Figure 3 shows that essentially all non-chromatophore particulate material was removed during sucrose gradient centrifugation, leaving a homogeneous chromatophore preparation.

To quantitate the extent of nonspecific protein contamination at each step of the isolation procedure, ^{14}C -L-phenylalanine-labeled chemotrophic cells were mixed with unlabeled photosynthetically grown cells, and the mixture was used as a source of chromatophore material following the scheme outlined above. Since the chemotrophic cells were grown under strict conditions of aerobiosis in the dark, radioactivity was only associated with protein of non-chromatophore origin and therefore served as a measure of the extent of contamination of the chromatophore preparation. Chemical determinations of RNA, protein, and chlorophyll were also performed during the purification. From Table 1, we see that after column chromatography the chromatophores are contaminated with 10% of the original radioactivity and 2% of the RNA. Less than 1% of the radioactivity and no detectable RNA are present in the final chromatophore preparation after sucrose gradient centrifugation (Table 1). Virtually all nonspecific soluble and particulate protein was removed, including ribosomes.

Characterization of the purified chromatophores. Cesium chloride density gradient centrifugation of these purified chromatophores indicated that they have a density of 1.18 g/cm^3 , the same value reported by Gibson (9) for preparations purified by three successive cycles of CsCl gradient centrifugation.

The chemical composition of pure lyophilized chromatophores is shown in Table 2 and is not significantly different from the values given by Gorchein et al. (12) for pure chromatophores. As can be seen, our chromatophores are com-

posed primarily of protein (64%), phospholipid (25%), and bacteriochlorophyll (4.6%). Only trace amounts of nucleic acid, carbohydrate, or PHB are present.

To characterize the total chromatophore protein and to gain insight into the forces that promote protein interactions within the chromatophore, total amino acid analyses were performed. The results of the analyses of purified chromatophores are presented in Table 3.

Solubilization and fractionation of the chromatophore. Purified chromatophores were pelleted by centrifugation at $150,000 \times g$ for 1.5 hr and resuspended by homogenization in 100% 2-chloroethanol at a concentration of 1 mg of protein/ml. It is important for solubilization that the chloroethanol be sufficiently acidified so that a 1/30 dilution in distilled water gives a pH of approximately 2.2. If necessary, a few drops of concentrated hydrochloric acid can be added to the chloroethanol to give the proper level of acidity. After homogenization, the clear green solution was allowed to stand at 25 C for 0.5 hr before centrifugation at $27,000 \times g$ for 15 min. The clear gelatinous pellet which contained less than 5% of the total chromatophore protein was designated P_1 . The green supernatant solution was dialyzed overnight against 0.01 M Tris (pH 7.6). A brown precipitate formed during dialysis, was removed by centrifugation at $27,000 \times g$ for 15 min, and was designated P_{II} . The remaining supernatant fluid contained less than 5% of the P_{II} protein. This supernatant fluid was concentrated and found to have the same properties and composition as P_{II} .

Disc-gel electrophoresis of P_1 , the chloroethanol insoluble protein fraction, revealed the presence of approximately 10 components (Fig. 4). P_{II} , the major protein fraction containing all the pigment of the purified chromatophores, consisted of approximately five components (Fig. 5). Weber and Osborn (35) have shown that SDS gels can be used to estimate the molecular weight of polypeptide chains. By using the appropriate marker proteins in our system we estimate that

TABLE 1. Purification of chromatophores from *Rhodospseudomonas spheroides*

Purification step	Per cent recovery ^a				Chlorophyll: counts/min (protein)
	Protein	^{14}C counts/min ^b	RNA	Chlorophyll	
1. Crude	100	100	100	100	1.2×10^{-5}
2. Sepharose column	45	10	2	63	5.0×10^{-5}
3. Sucrose gradient	32	1	Undetectable	51	50×10^{-5}

^a One hundred per cent equivalents were: protein, 835 mg; ^{14}C , 2×10^6 counts/min; RNA, 42 mg; chlorophyll, 24 mg.

^b ^{14}C -L-phenylalanine.

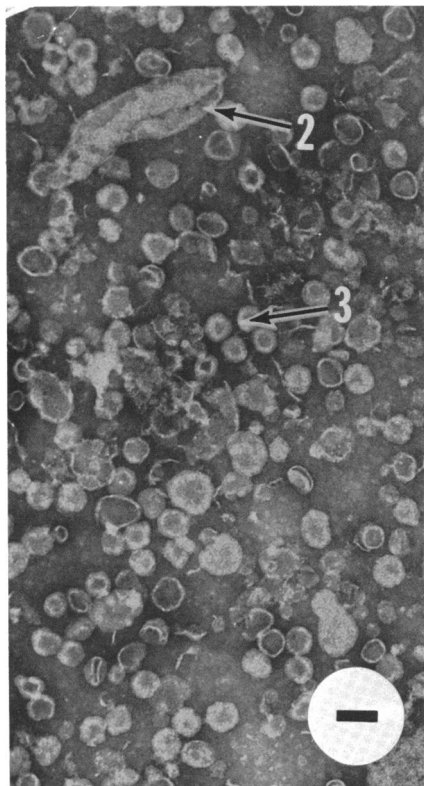


FIG. 2. Crude chromatophores after being passed over a Sepharose 2B column. Some membrane fragments (2) are still found with the chromatophores (3). (See step 2, Table 1.)

the chromatophore proteins range in molecular weight from approximately 10,000 to 130,000 daltons (Fig. 6). Qualitatively similar results were obtained with the phenol-acetic acid system of Takayama et al. (33).

Of special interest is band 15 which contains all of the chromatophore pigment as shown by a scan of the gel containing fraction P_{II} at 365 nm (an absorption maximum of bacteriochlorophyll) before staining the gel (Fig. 7). In an experiment where ^{32}P was used to follow the fractionation of phospholipid, all of the chromatophore-associated ^{32}P was found to be associated with band 15. For this particular experiment, photosynthetic cells were labeled with ^{32}P -orthophosphate, and chromatophores were isolated according to the scheme outlined above. Extraction of the purified chromatophores with acetone-methanol indicated that all of the radioactivity was in the lipid fraction. The chromatophores were then fractionated into P_I and P_{II} . All of the radioactivity was located in P_{II} . After gel electrophoresis of P_{II} was performed, the gel was frozen and taped to an X-ray film and allowed to expose the film for 6 hr.

At the end of this time the gel was thawed and stained. Band 15 is coincident with the exposed part of the film (Fig. 8). No other exposed areas can be seen except for the small spot of ^{32}P placed at the top of the gel for alignment purposes.

The estimated molecular weight of band 15 on SDS gels is 9,700 daltons (Fig. 6). The validity of assigning a molecular weight by this procedure to a protein that has so much pigment and lipid attached to it is questionable. However, a minimum molecular weight calculated from the amino acid analysis data of band 15 indicates that this value is reasonably accurate (Fraker and Kaplan, unpublished data).

Immunochemical studies. Antisera were prepared against fractions P_I , P_{II} , and whole purified chromatophores as described under methods. Preliminary immunological studies (Table 4) indicate little cross-reactivity between P_I and P_{II} , although antisera prepared against either fraction cross-reacted well with whole chromatophores. Antibody obtained from secondary bleedings against P_{II} and whole chromatophores as antigen gave similar results. This suggests that there are few if any common antigenic determinants between P_I and P_{II} , but both share determinants with the whole chromatophore.

DISCUSSION

The question of how subcellular organelles are formed represents a major area of investigation. Several systems, particularly those dealing with mitochondria and chloroplasts, are being extensively studied. The size and complexity of these organelles and their semiautonomous existence make biochemical and biological studies difficult. However, the study of chromatophore formation in the facultatively photosynthetic bacterium *R. spheroides* does not suffer from these difficulties. These organelles are small and anatomically relatively simple by comparison to other systems.

To date, most of the work done on chromatophore development has been primarily electron microscopy (11, 16, 17). A more rigorous biochemical and genetic approach seems appropriate if one is to understand the molecular events which surround the induction and biosynthesis of these organelles. For such studies, it is essential that these organelles be isolated in large quantities, free from other cellular material, particularly protein. The development of a procedure for the isolation of large quantities of chromatophores with less than 1% non-chromatophore protein contamination makes possible the fractionation of the chromatophore into its individual protein components so that studies can be conducted to determine the nature and means of

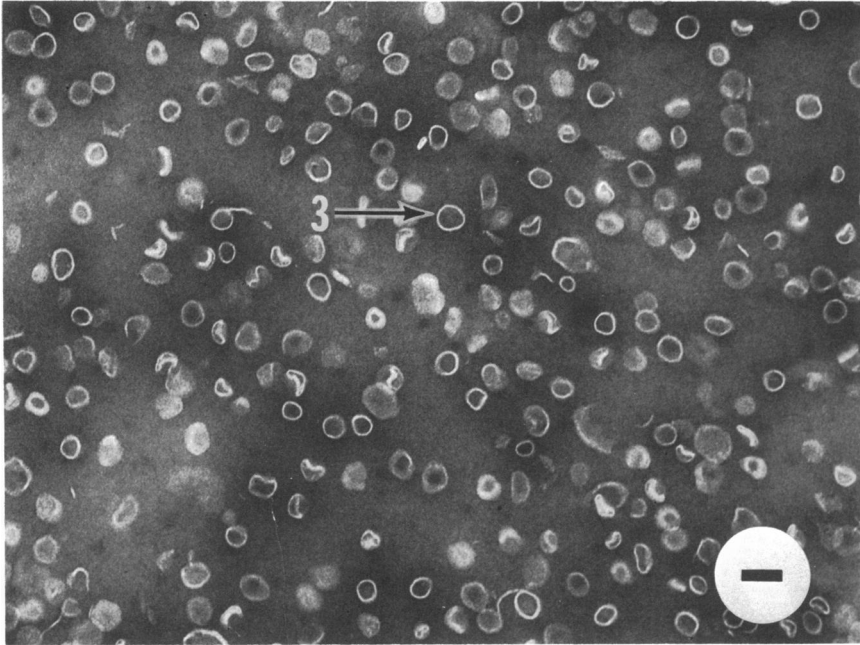


FIG. 3. Purified chromatophores (3) from sucrose gradient. (See step 3, Table 1.)

TABLE 2. Composition of purified chromatophores from *Rhodopseudomonas spheroides*

Component	Per cent of dry weight
Protein	64
Phospholipid	25
Bacteriochlorophyll	4.6
DNA	0.13
RNA	0.25
Protein-bound hexose	0.15
Poly- β -hydroxybutyric acid	0.03
Carotenoids	Not determined

production of chromatophore-specific proteins.

The purification scheme which we have outlined is noteworthy in several respects. It is rapid and capable of being "scaled up or down," depending on the needs of the investigator. The final product obtained in high yields appears by several criteria to be free of both ribosomal and cell envelope material. From the electron micrographs, we conclude that the final preparation is very homogenous.

The amino acid composition of purified chromatophore proteins is very similar to that given for chloroplast lamellar protein (20), mitochondrial structural proteins (14), *Mycoplasma* membrane (26), and erythrocyte membranes (23). Many of the organelle and membrane proteins that have been analyzed have in common a high content of hydrophobic amino acids, a rela-

tively high proline content, low half cystine, and a low level of the charged amino acids. Such an amino acid composition is in marked contrast to many of the soluble proteins that have been analyzed (28), and we would suggest that hydrophobic interactions rather than ionic or disulfide

TABLE 3. Amino acid composition of the whole chromatophore protein of *Rhodopseudomonas spheroides*

Amino acid	Mole per cent
Lysine	3.93
Histidine	2.08
Arginine	3.41
Methionine sulfone ^a	3.18
Aspartic acid	5.85
Threonine ^b	5.88
Serine ^b	5.09
Glutamic acid	8.00
Proline	5.40
Glycine	8.09
Alanine	14.68
One-half cystine ^a	0.39
Valine	8.54
Isoleucine	4.77
Leucine	10.76
Tyrosine	2.96
Phenylalanine	4.78
Tryptophan ^a	2.21

^a Determined by separate analysis.

^b Corrected for hydrolysis losses by extrapolation to zero time.

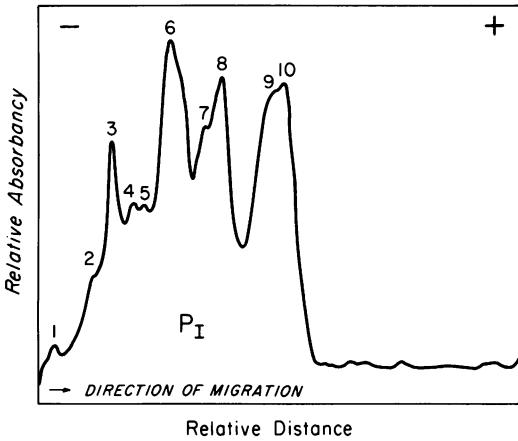


FIG. 4. Sodium dodecylsulfate polyacrylamide gel pattern of the chloroethanol-insoluble chromatophore fraction, P_I , scanned at 550 nm.

bonds are predominant in maintaining the structural integrity of the chromatophore. This would explain why much of the chromatophore protein is insoluble in a variety of reagents and solvents that are commonly used to solubilize other proteins, but soluble in 2-chloroethanol which has a low dielectric constant. The chloroethanol insoluble fraction P_I is an exception, but only comprises 3 to 5% of the chromatophore protein.

Since P_I was less than 5% of the chromatophore protein, nonpigmented, and insoluble in chloroethanol, it might be thought to be non-chromatophore protein which had not been completely removed by the purification procedure outlined. ^{14}C -L-phenylalanine-labeled, purified chromatophores were passed a second time over the Sepharose column and sucrose gradient. The

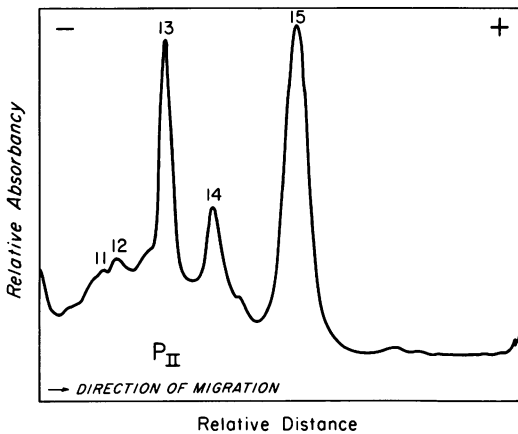


FIG. 5. Sodium dodecylsulfate polyacrylamide gel pattern of the chloroethanol-soluble chromatophore fraction, P_{II} , scanned at 550 nm.

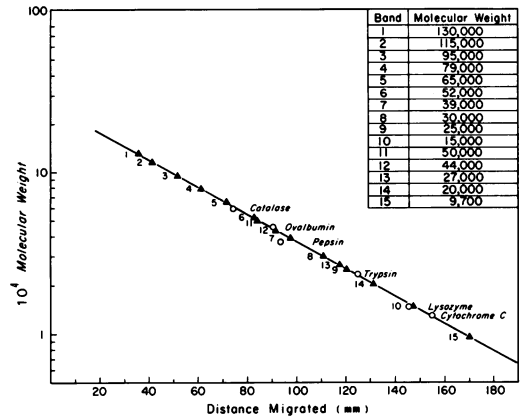


FIG. 6. Determination of the molecular weights of the proteins of the chromatophore fractions P_I and P_{II} according to the method of Weber (35). Symbols: marker proteins of known molecular weight, \circ ; bands from P_I and P_{II} , \blacktriangle .

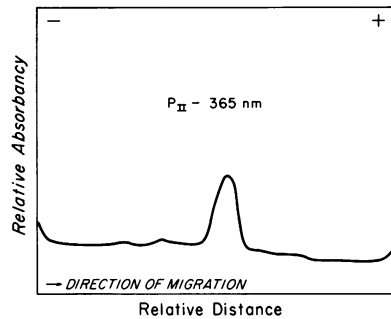


FIG. 7. Scan of the P_{II} -containing gel at 365 nm before staining, showing that the bacteriochlorophyll peak is coincident with band 15 (see Fig. 5).

resulting chromatophores contained exactly the same amount of P_I , as determined by radioactivity and Lowry protein estimation, as the original purified chromatophores, thus ruling out the possibility that P_I might simply be protein not normally associated with the chromatophore (J. Huang, unpublished data). Disc-gel electrophoresis of P_I indicated that it is composed of 10 electrophoretic bands. Oelze et al. (27), using the methods developed by Takayama, also found a similar number of proteins in partially purified membranes from *R. rubrum* grown semiaerobically. Electrophoresis of crude aerobic and non-pigmented anaerobic membrane isolated from *R. spheroides* yielded a band pattern nearly identical to that of P_I (J. Huang, unpublished data). Since P_I seems to be an integral but small portion of the total chromatophore protein and since it behaves in a similar manner to the membrane proteins, we are tempted to postulate that P_I is a

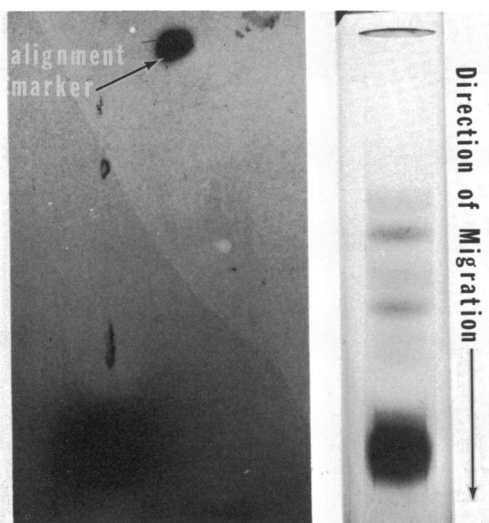


FIG. 8. ^{32}P chromatophore phospholipid was found to be associated entirely with fraction P_{II} which was electrophoresed and allowed to expose an X-ray film. The exposed area of the film is coincident with band 15 (the leading gel band), indicating that the chromatophore phospholipid is also associated with this protein.

portion of the site on the cell membrane from which the chromatophores are formed. On the other hand, P_I may simply be those proteins that are common to both the chromatophore and the cell membrane.

P_{II} , which is chloroethanol-soluble, comprises over 95% of the chromatophore protein and displays three major and two minor protein components after electrophoresis. The fastest migrating protein (band 15) has a molecular weight of approximately 9,700 daltons and is associated with all of the chromatophore pigment and phospholipid. This observation assigns to band 15 a key role in the chromatophore structure, making it of prime interest for future study. It should be

TABLE 4. Immunological studies of the cross-reactivity between the whole chromatophore and its fractions

Antiserum	Antigen		
	Whole chromatophore	P_I	P_{II}
Whole chromatophore	+++ ^a	++ ^b	+++
P_I	+ ^c	++	± ^d
P_{II}	+++	+	+++

^a Very strong reaction.

^b Strong reaction.

^c Moderate reaction.

^d Weak reaction.

noted that Oelze et al. (27) also observed a "fast migrating" membrane protein but only in those strains of *R. rubrum* which synthesized bacteriochlorophyll. This may or may not be analogous to band 15 found in *R. spheroides*.

Although P_I and P_{II} have not yet been shown to contain any common antigenic components, preliminary immunochemical data tend to support a physical relationship of fractions P_I and P_{II} to the chromatophore and show an apparent lack of cross contamination between these fractions.

A similar number and size distribution of proteins as observed for the chromatophore has been observed for *Mycoplasma* membranes (26), chloroplast membranes of *Chlamydomonas* (8), and the outer membrane as well as the smooth and rough microsomal membrane of rat liver (30).

To continue our chemical, physical, and immunochemical characterization of chromatophore-specific proteins, we are in the process of isolating each of the proteins in quantity. The pigmented protein (band 15) has already been isolated and purified and is presently being characterized.

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