Biochimica et Biophysica Acta, 307 (1973) 301–316

BBA 76285

MEMBRANE PROTEINS OF *RHODOPSEUDOMONAS SPHEROIDES*

III. ISOLATION, PURIFICATION, AND CHARACTERIZATION OF CELL ENVELOPE PROTEINS*

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(Received October 23rd, 1972)

SUMMARY

Cell envelopes (cell wall and cell membrane) from aerobically grown cells of *Rhodopseudomonas spheroides* were isolated and purified by a combination of differential centrifugation and centrifugation through 40% sucrose. Cell envelope protein from aerobically grown cells was resolved by dodecyl sulphate–polyacrylamide gel electrophoresis. Biochemical characterization of selected envelope membrane proteins demonstrated heterogeneity between different protein species. Amino acid analyses of individual proteins revealed between 50–60 mole% nonpolar residues.

Envelope membranes derived from anaerobically grown cells were also isolated and purified by a combination of differential centrifugation, column chromatography on Sepharose 2B, and centrifugation in 40% sucrose. The dodecyl sulphate–polyacrylamide gel patterns of anaerobic and aerobic envelope membrane proteins were very similar and the results suggest a common protein structure.

INTRODUCTION

Chromatophores from the facultative photosynthetic bacterium *Rhodopseudomonas spheroides* are the site of primary light entrapment and photophosphorylation (Frenkel and Hickman[1], Geller and Lipmann[2]). *R. spheroides* is able to "turn on" or "off" chromatophore biosynthesis as well as to regulate the final cellular level of chromatophores. Such control is directly dependent upon the growth conditions employed (Van Neil[3]). Aerobically grown cells, whether in the dark or light, produce little or no chromatophores[3]. Cells grown anaerobically in the light have abundant chromatophores[3] and the amount is inversely related to the light intensity (Cohen-Bazire *et al.*[4]). Chromatophore development and differentiation is considered to be physically associated with the cytoplasmic membrane (may include outer as well as inner membrane) as well as with the concurrent accumulation of the appropriate pigment systems and proteins (Frenkel and Hickman[1], Cohen-Bazire and Kunisawa[5], Boatman[6], Holt and Marr[7]).

In order to fully understand the architecture and the biogenesis of the bacterial

* For Papers I and II of this series, see refs 11 and 16, respectively.

chromatophore as well as their relationships to other cell membrane components, more information about the variety and nature of chromatophore and envelope membrane proteins is required. The present paper will (1) describe the methods used to isolate and purify aerobic and anaerobic envelope membranes (greatly enriched for the inner membrane) and (2) compare the protein composition and fine structure of selected proteins derived from aerobic and anaerobic envelope membranes. In subsequent studies (see Papers IV (ref. 8) and V (ref. 9) of this series) we have characterized chromatophore-specific proteins, compared the protein composition of chromatophore and envelope membranes, and we have investigated the origin of various chromatophore components.

METHODS

Organism

R. spheroides strain 2.4.1. was used throughout.

Medium

Medium AGSu of Sistrom[10] supplemented with 0.2% casamino acids (Difco) was used.

For ^{32}P-labelling experiments, Medium AGSu of Sistrom[10] containing Tris and low phosphate (Sis–Tris medium) was used. Sis–Tris medium was similar to Medium AGSu of Sistrom[10] with the following modifications: the concentration of Na_2HPO_4 was 0.0525 g/l instead of 3.48 g/l; solid Tris instead of KOH was used to bring the pH to 6.8–7.0.

Growth

Anaerobic and aerobic cultures were grown as described by Fraker and Kaplan[11].

Electron microscopy

The envelope fractions were examined using a Siemens Elmiskop IA electron microscope with 200 μm condenser aperture and 50 μm objective aperture at 60 kV gun potential. The membrane fraction (1 mg/ml) was diluted with 1% phosphotungstic acid (pH 7.6). A drop of the mixture was immediately put on a 200-mesh copper grid coated with 0.3% Formvar and carbon. After 5 min the excess stain was drawn off by touching the grid with filter paper. The air-dried grids were examined under the microscope.

Analytical dodecyl sulphate–polyacrylamide gel electrophoresis

Analytical dodecyl sulphate–polyacrylamide gel electrophoresis was performed using gels of 5% acrylamide–0.17% bisacrylamide. The constitutions of the electrophoretic and sample buffers as well as that of the gels has been described by Kiehn and Holland[12]. The gels were either 6 mm × 70 mm with a volume of 2 ml or 6 mm × 100 mm with a volume of 2.8 ml.

Proteins were dissolved at 1 mg/ml in 0.01 M Tris–0.005 M sodium acetate (pH 9.0) sample buffer containing 1% dodecyl sulphate, 0.001% EDTA, 0.5 M urea (ion free), 1% β-mercaptoethanol, and 10% glycerol. The samples were heated in a 50 °C

water bath for 10 min. Approximately 0.1 ml of protein sample (1 mg/ml) was layered on top of the gel and electrophoresis was performed at 4 mA/gel tube for 3.5 h for 6 mm × 70 mm gels and 6 h for 6 mm × 100 mm gels.

Two different methods of staining and destaining were used. (1) Gels were fixed and stained for 1 h in a filtered mixture of 50% methanol (454 ml) and glacial acetic acid (46 ml) containing 0.25% Coomassie blue. Destaining was performed for 2 h by several changes in a solution containing 35 ml of acetic acid, 250 ml of methanol, 720 ml of distilled water. The gels were destained further in 7% acetic acid. (2) The gels were fixed in 20% sulfosalicylic acid for 16 h at room temperature. The gels were stained for 6 h in 0.01 M citrate buffer (pH 3) containing 0.25% Coomassie blue. Destaining was performed by several changes in 7% acetic acid (De St. Groth *et al.*[13]). The stained proteins were scanned according to the method of Marrs and Kaplan[14] at 550 nm using an ISCO UA visible-light analyzer.

Preparative dodecyl sulphate–polyacrylamide gel electrophoresis

Preparative dodecyl sulphate–polyacrylamide gel electrophoresis was performed according to Fogel and Sypherd[15]. The only modification was a cooling jacket which was installed on the electrophoretic apparatus (Fraker and Kaplan[16]). Electrophoresis was carried out at 20 mA for 60 h.

Amino acid analysis

Protein (0.5 mg) was hydrolyzed in 6 M HCl in evacuated and sealed Pyrex tubes at 110 °C. Duplicate samples were removed at 24, 48, and 72 h. The HCl was removed by flash evaporation or by drying over P_2O_5 and NaOH pellets under vacuum The amino acids were determined quantitatively according to Spackman *et al.*[17] using a Beckman/Spinco Model 120 amino acid analyzer.

Tryptic peptide mapping

Trypsin (3 times crystallized, Worthington Biochemical Corp.) was dissolved at 10 mg/ml in 0.06 M HCl and incubated at 37 °C for 12 h in order to reduce contaminating chymotryptic activity.

The sample protein was dissolved in 1% NH_4HCO_3 at 5 mg/ml in a screw-capped conical tube. Trypsin was added to the protein solution in two portions, at zero time and after 12 h, such that the final weight of trypsin to that of sample protein was 2–5%. The mixture was incubated in a 37 °C water bath for a total of 24 h. The sample was lyophilized to dryness, washed in distilled water, and the washing–drying process repeated four times.

Thin-layer electrophoresis was performed using Eastman Chromagram Sheets (cellulose, 20 cm × 20 cm) at 400 V (10 mA) in pyridine–acetic acid–water (200:7:793, by vol.; pH 6.6) for 30 min. The second dimension was developed in a chromatographic tank equilibrated with *n*-butanol–pyridine–acetic acid–water (60:40:12:48, by vol.). The dried thin-layer sheet was sprayed with 0.1% ninhydrin in acetone or absolute alcohol, and heated in the 80 °C oven for 10 min or until the peptides appeared.

Assay methods

Protein concentration was determined by the method of Lowry *et al.*[18]. Hexo-

samine concentration was determined as described by Winzler[19]. Protein-bound dodecyl sulphate or free dodecyl sulphate in solution was determined by the method of Reynolds and Tanford[20].

RESULTS

Isolation and purification of aerobic and anaerobic cell envelope membranes

Untreated envelopes from aerobic cells. To isolate and purify the non-lysozyme-treated, aerobic cell envelope, 50 g wet weight of aerobically grown cells were resuspended in 150 ml of 0.1 M sodium phosphate buffer (pH 7.6) containing 0.01 M EDTA and fractionated according to the procedure outlined in Fig. 1.

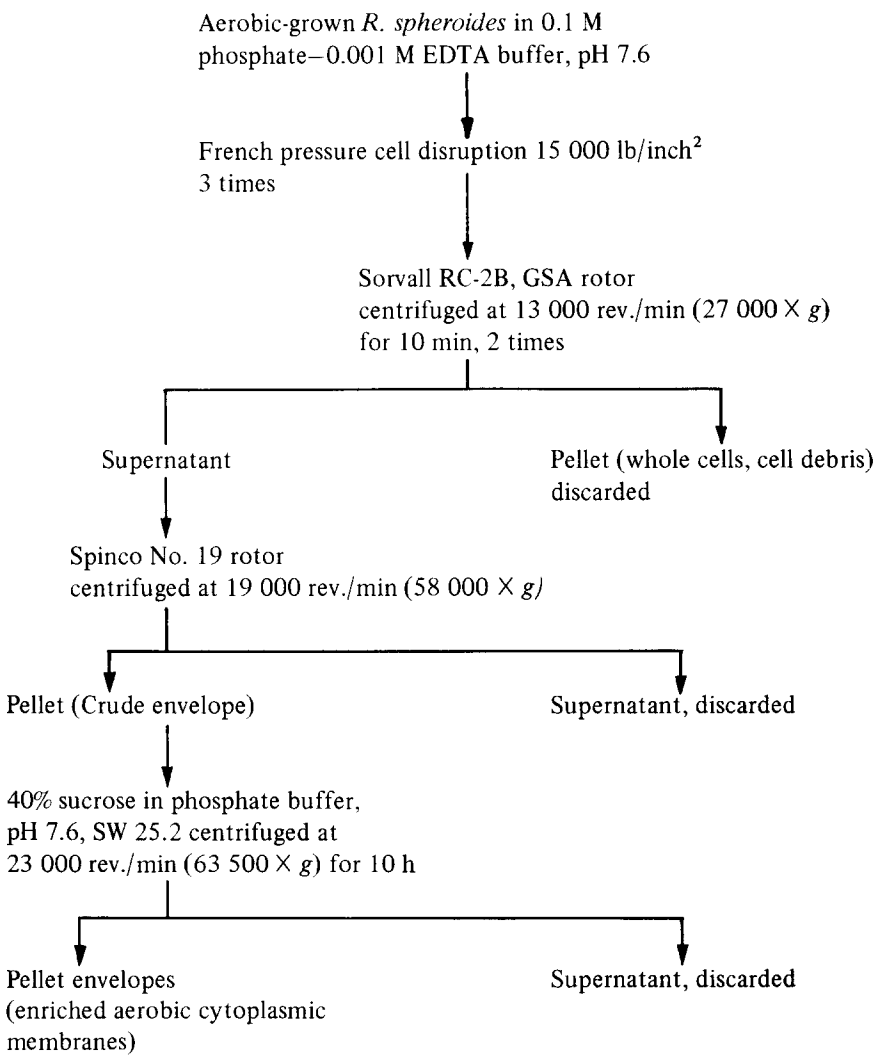


Fig. 1. Outline of the purification procedure for aerobic cell envelopes from *R. spheroides*.

Lysozyme-treated aerobic membranes. To isolate and purify cell wall-depleted aerobic cytoplasmic membranes, cell walls of *R. spheroides* were digested by treatment with lysozyme. The cells were resuspended at 250 mg wet weight/ml in 0.1 M Tris buffer (pH 7.6) containing 0.25 M sucrose. Lysozyme was added at 1.25 mg/ml. Deoxyribonuclease and ribonuclease were added at 2 μg/ml. The mixture was gently stirred at room temperature for 1 h. The membrane fractions were purified following the procedure in Fig. 1. Tris buffer (pH 7.6) was used throughout the purification procedure in place of phosphate buffer.

The procedure we have employed to isolate an enriched cytoplasmic membrane fraction is similar to that outlined by Schnaitman[21] and Osborn *et al.*[22], although several major differences are essential to our scheme. In addition, the density of the enriched aerobic cytoplasmic membrane fraction is 1.180 g/cm^3 similar to the inner cytoplasmic membrane fraction obtained from *Salmonella* by Osborn *et al.*[22].

Hexosamine content and electron microscopy of treated and untreated aerobic envelope preparations

The non-lysozyme-treated membrane fractions were found to contain 6.7 mg of hexosamine per 100 mg of protein; electron microscopy (Fig. 2) reveals a rigid membrane fraction with intact cell walls and β-hydroxybutyrate granules which accumulate in the aerobically grown cells.

No hexosamine is detected in the lysozyme-treated, aerobic envelope fractions. Electron microscopy (Fig. 3) confirms that the rigid layer of cell wall is removed after

Fig. 2. Electron micrograph of non-lysozyme-treated, aerobic cell envelopes of *R. spheroides*. The marker represents 0.1 μm.

lysozyme treatment. The cytoplasmic membranes are collapsed to form homogeneous sacs.

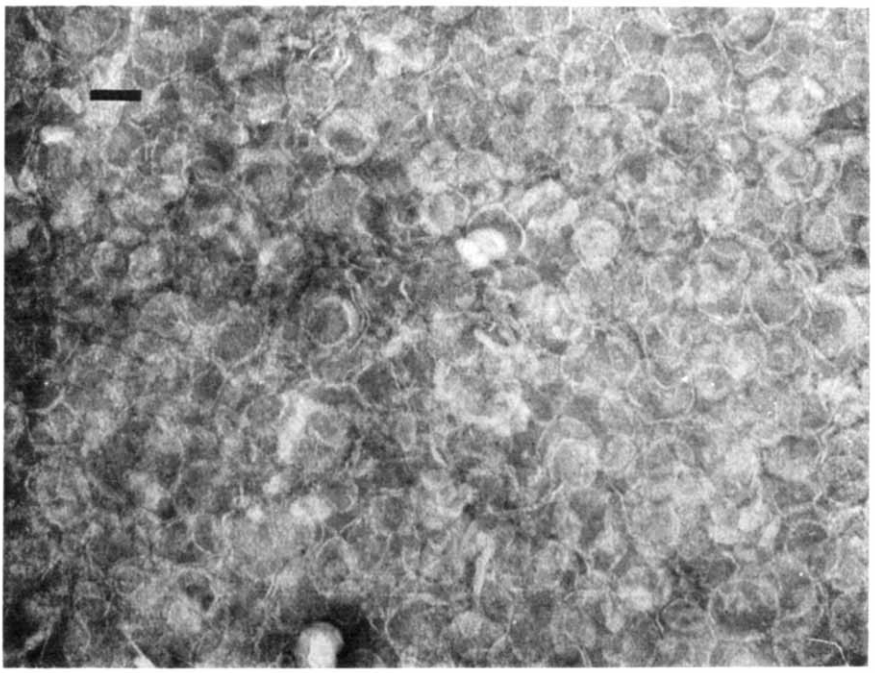


Fig. 3. Electron micrograph of lysozyme-treated, aerobic cytoplasmic membranes of *R. spheroides*. The marker represents 0.1 μm.

Nucleic acid content of the purified untreated aerobic cell envelopes

To characterize the purified membrane preparation further, cells were grown aerobically in medium containing 0.75 mCi of $^{32}P_i$. The radioactivity of ^{32}P-labelled RNA and DNA was determined by subtracting the hot 5% trichloroacetic acid-precipitable counts from the 5% cold trichloroacetic acid precipitable counts.

As shown in Table I, the radioactivity of ^{32}P-labelled RNA and DNA in the purified membrane fraction is less than 4% of that in the crude envelope. An im-

TABLE I

PURIFICATION OF AEROBIC ENVELOPES FROM *RHODOPSEUDOMONAS SPHEROIDES*

Purification step	*^{32}P cpm in DNA and RNA per mg protein*
Crude envelopes*	256 140
Purified envelopes**	69 524

* Prior to centrifugation in 40% sucrose.
** Following centrifugation through 40% sucrose.

portant source of potentially contaminating protein is from ribosomes and our removal of nucleic acid from the envelope preparations could suggest the removal of a major source of contaminating protein.

Anaerobic cell envelopes

The isolation and purification of non-lysozyme-treated, anaerobic cell envelope is similar to that of the aerobic cell envelope, with the exception that chromatography on Sepharose 2B was performed prior to centrifugation through 40% sucrose. The "milky" fractions, eluting ahead of the chromatophore fraction from the column, were collected and designated anaerobic cell envelope.

Bacteriochlorophyll content of the purified anaerobic cell envelope

To compare the pigment content of purified chromatophores with the anaerobic cell envelope, *R. spheroides* was grown anaerobically in the presence of 15 μCi of δ-amino[4-^{14}C]levulinic acid (37.4 mCi/mole). The amount of chlorophyll per mg protein of the anaerobic cell envelope is less than one-fifth that of the chromatophores. However, these membranes are still highly pigmented in comparison to the purified aerobic cell envelope (Table II). Our purified chromatophores contain more than 70 μg bacteriochlorophyll per mg protein and the enriched anaerobic cell envelope contains less than 10 μg bacteriochlorophyll per mg protein. Considering that chromatophores most likely originate from the cytoplasmic membrane and because of their proximity within the cell, it is not surprising to find that some pigments are associated with the anaerobic cell envelope.

We have shown (Fraker and Kaplan[11], Huang and Kaplan[8]) that 2-chloroetha-

TABLE II

DISTRIBUTION OF ^{14}C-LABELLED PIGMENT INTO 2-CHLOROETHANOL-SOLUBLE AND -INSOLUBLE FRACTIONS

Fraction	*cpm/mg protein**	*Relative pigment content* (%)
Anaerobic cell envelope	$1.30 \cdot 10^5$	100
Anaerobic cell envelope** (2-chloroethanol insoluble)	$0.05 \cdot 10^5$	4
Anaerobic cell envelope (2-chloroethanol soluble)	$1.25 \cdot 10^5$	96
Chromatophores	$6.00 \cdot 10^5$	100
Chromatophore P_I*** (2-chloroethanol insoluble)	$0.005 \cdot 10^5$	0.1
Chromatophore P_{II} (2-chloroethanol soluble)	$6.00 \cdot 10^5$	99.9

* Represents either cpm/mg anaerobic cell envelope protein or cpm/mg chromatophore protein.

** Represents more than 50% of the anaerobic cell envelope protein.

*** Represents less than 5% of the chromatophore protein.

nol can distinguish between two classes of chromatophore proteins, namely (1) P_I, 2-chloroethanol insoluble, and (2) P_{II}, 2-chloroethanol soluble. The 2-chloroethanol-soluble fraction appears to be unique to the chromatophore (Fraker and Kaplan[11], Huang and Kaplan[8] and unpublished observations) whereas Fraction P_I is similar to the aerobic and anaerobic cell envelope proteins. Therefore, 2-chloroethanol solubility represents a convenient tool for distinguishing chromatophore-specific protein. In the experiment presented in Table II we have used 2-chloroethanol extraction to determine the extent of contamination of our anaerobic cell envelope with chromatophores. As we can see, whereas 96% of the pigments remain in solution following 2-chloroethanol extraction of anaerobic cell envelope, more than 90% of the protein is insoluble in 2-chloroethanol. In the case of the chromatophores, approximately 95% of the chromatophore protein (P_{II}) and all of the pigments are 2-chloroethanol-soluble whereas less than 5% of the chromatophore protein (P_I) is precipitated. The gel profiles obtained reveal no differences between the chloroethanol-insoluble anaerobic envelope proteins, and non-chloroethanol-treated anaerobic envelope proteins (Huang and Kaplan, unpublished results).

Fractionation of cell envelope proteins

Analytical dodecyl sulphate–polyacrylamide gel electrophoresis was performed on the treated and untreated, aerobic cell envelope membrane proteins as well as on the untreated, anaerobic cell envelope membrane proteins. Fig. 4 shows the scan of a dodecyl sulphate–polyacrylamide gel electrophoretic profile. It reveals approximately 10 major bands using the untreated, aerobic cell envelope fractions. In Fig. 5 the scan of dodecyl sulphate–polyacrylamide gel electrophoretic profile of the lysozyme-treated, aerobic cell envelope membrane fraction is shown, which reveals more than 10 bands. Although both electron microscopy and hexosamine content indicate that the lysozyme-treated, aerobic cell envelope fraction represents enriched cytoplasmic membranes, the gel profile reveals many additional bands which are not present in the non-lysozyme-treated material. These components may result from the release of cytoplasmic membrane proteins from the lipopolysaccharide–membrane aggregate composing the unfractionated cell envelope. Upon removal of significant portions of

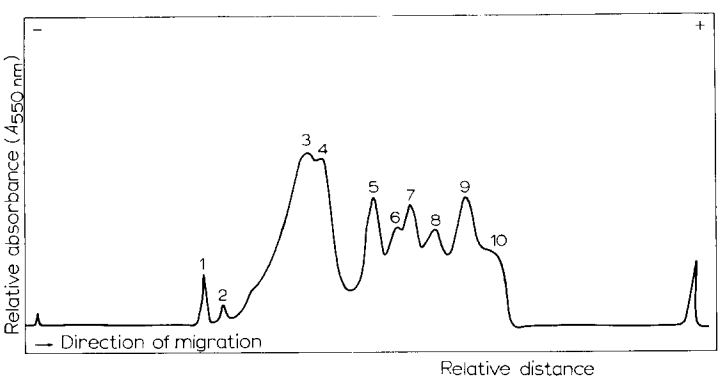


Fig. 4. Dodecyl sulphate–polyacrylamide gel electrophoretic pattern of non-lysozyme-treated, aerobic cell envelope proteins, scanned at 550 nm. The gel was 6 mm × 100 mm.

the wall material in the treated preparation these cytoplasmic membrane proteins are free to enter the gel.

The gel scan of the anaerobic envelope proteins is shown in Fig. 6. The gel profile of anaerobic envelope proteins is similar to that derived from the enriched aerobic membrane fraction.

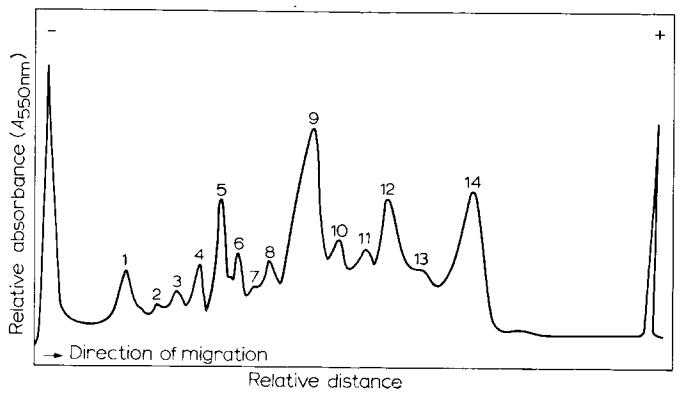


Fig. 5. Dodecyl sulphate–polyacrylamide gel electrophoretic pattern of lysozyme-treated, aerobic cell envelope proteins, scanned at 550 nm. The gel was 6 mm × 100 mm.

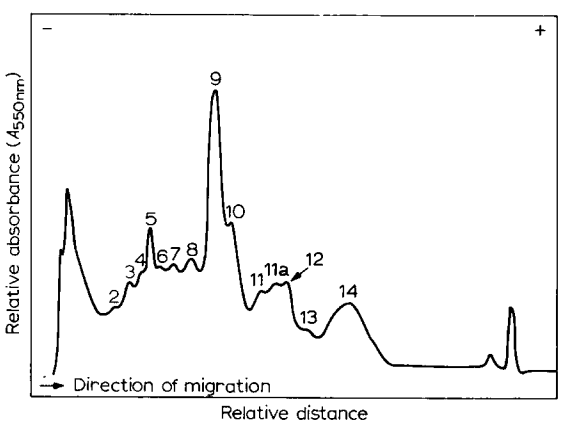


Fig. 6. Dodecyl sulphate–polyacrylamide gel electrophoretic pattern of anaerobic cell envelope proteins, scanned at 550 nm. The gel was 6 mm × 70 mm.

Relationship between phospholipids and the aerobic envelope proteins

The untreated aerobic cell envelope proteins were fractionated by analytical dodecyl sulphate–polyacrylamide gel electrophoresis. Duplicate gels were used. One gel was stained with Coomassie blue and scanned at 550 nm to locate the protein bands. The other gel was sliced into 1-mm segments, 10 ml of scintillation fluid was added to each slice and the radioactivity determined. Nearly all of the ^{32}P migrates as a sharp moving band which is coincident with Band 10 (Fig. 7) and we tentatively conclude that the ^{32}P is associated with Band 10 either as lipoprotein or as phosphoprotein.

Molecular weight determination

The molecular weights of the aerobic cell envelope proteins were determined by dodecyl sulphate–polyacrylamide gel electrophoresis using bovine serum albumin, pepsin, trypsin and cytochrome *c* as marker proteins having molecular weights of 66500, 35000, 23200 and 14000, respectively. The relative mobility of each protein was averaged from triplicate samples. When the mobilities of the proteins are plotted against the logarithm of the molecular weights, the aerobic cell envelope proteins are calculated to have molecular weights ranging from 10000 to 100000 (Fig. 8).

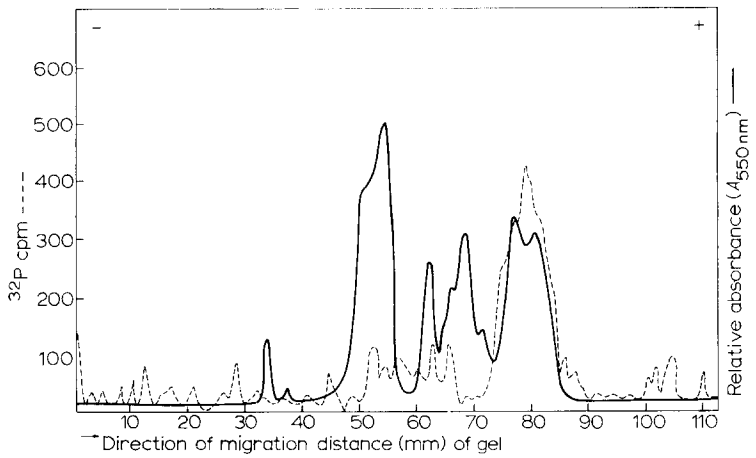
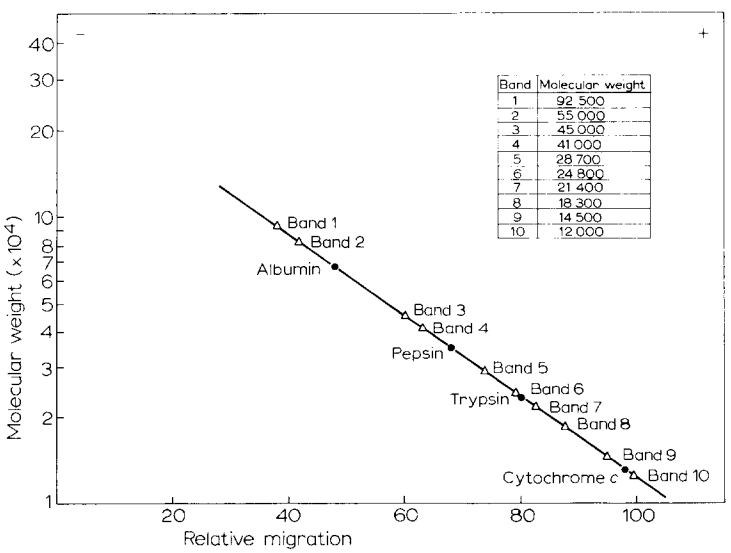


Fig. 7. Dodecyl sulphate–polyacrylamide gel electrophoretic pattern of the untreated cell envelope proteins, scanned at 550 nm (———). ^{32}P (-------) is associated with the fast moving component of the aerobic cell envelope proteins. The gel was 6 mm × 100 mm.



Band	Molecular weight
1	92 500
2	55 000
3	45 000
4	41 000
5	28 700
6	24 800
7	21 400
8	18 300
9	14 500
10	12 000

Fig. 8. Determination of the molecular weights of 10 major aerobic cell envelope proteins. Standard proteins (●); aerobic cytoplasmic membrane proteins (△).

Isolation and purification of individual membrane proteins

Preparative dodecyl sulphate–polyacrylamide gel electrophoresis was performed in order to fractionate the cell envelope proteins derived from the non-lysozyme-treated, aerobic cell envelope. The proteins were eluted from gel slices by electrophoresis at 60 mA for 12 h in diluted (1:50) electrophoretic buffer[12]. The eluate was filtered through glass wool. The filtrate was lyophilized, resuspended in water, and dialyzed against distilled water to remove excess salts, and lyophilized a final time. The protein was suspended in distilled water and layered onto a Sephadex G-25 column (2.8 cm × 40 cm) in order to remove dodecyl sulphate and residual salts (Bishop *et al.*[23]). Residual dodecyl sulphate could be removed by cold acetone (9 parts acetone and 1 part water) extraction (Weber and Osborn[24]). Analytical gel electrophoresis was performed to determine the purity of the individual protein preparations and each isolated species was shown to consist of a single electrophoretic component.

Amino acid composition

The values of duplicate analyses for 24, 48, and 72 h hydrolysis of Bands 3, 5, 8, 9, 10, and total aerobic cell envelope protein are averaged and shown in Table III.

TABLE III

AMINO ACID COMPOSITION OF AEROBIC CELL ENVELOPE PROTEINS OF *RHODOPSEUDOMONAS SPHEROIDES*

N.D., not determined.

Amino acid	*Mole %*					
	Band 3	*Band 5*	*Band 8*	*Band 9*	*Band 10*	*Total protein*
Lys	2.76	6.34	6.48	9.12	10.23	4.24
His	1.60	2.02	1.78	1.84	1.83	1.40
Arg	2.53	7.14	6.44	7.89	8.53	4.66
Asx	13.55	9.33	8.77	7.32	7.71	11.24
Thr*	5.37	5.40	5.57	5.76	5.79	5.72
Ser*	4.18	6.39	7.81	6.87	6.18	5.29
Glx	8.71	9.35	10.55	10.87	9.93	8.33
Pro	2.18	5.18	4.50	4.99	4.34	2.68
Gly	16.29	14.30	12.43	10.75	9.40	12.18
Ala	12.73	9.13	10.52	10.00	11.12	11.24
Val	6.54	7.34	7.75	8.60	7.12	7.24
Met	2.51***	1.62***	1.20***	1.18***	1.46***	2.95***
Ile	4.22	4.11	4.41	4.20	4.17	4.03
Leu	7.26	7.24	7.42	6.07	7.07	6.94
Tyr	3.75	2.27	1.79	2.15	2.14	2.89
Phe	5.83	2.86	2.58	2.40	3.01	4.03
Trp	N.D.	N.D.	N.D.	N.D.	N.D.	3.19**
Cys	N.D.	N.D.	N.D.	N.D.	N.D.	1.73**

* Serine and threonine were corrected for hydrolysis losses by extrapolation to zero time.

** Cysteine and cystine were determined as cysteic acid; methionine was determined as methionine sulphone (Moore[25]). Tryptophan was determined spectroscopically (Edelhoch[26]).

*** Methionine was determined as sum of methionine sulphone and methionine sulphoxide.

There is a general resemblance between the amino acid composition of the selected individual proteins and that of the total aerobic cell envelope protein (Table III). However, each protein has its distinctive amino acid composition. These data render it improbable that some of the higher molecular weight components are aggregates of the lower molecular weight components or that the purified proteins are artifacts produced by simple chemical modification, such as deamination or methionine oxidation. However, in order to further establish this conclusion tryptic maps of some selected purified proteins were prepared.

Tryptic peptide fingerprints

Two-dimensional thin-layer electrophoresis and chromatography was performed on protein Bands 3, 5, and 10. Figs 9–11 are the tracings of the tryptic peptide fingerprint patterns of Bands 3, 5 and 10, respectively. As shown in Figs 9–11, Band 3 reveals 20 peptides; Band 5 reveals 11 peptides; Band 10 reveals 14 peptides. The

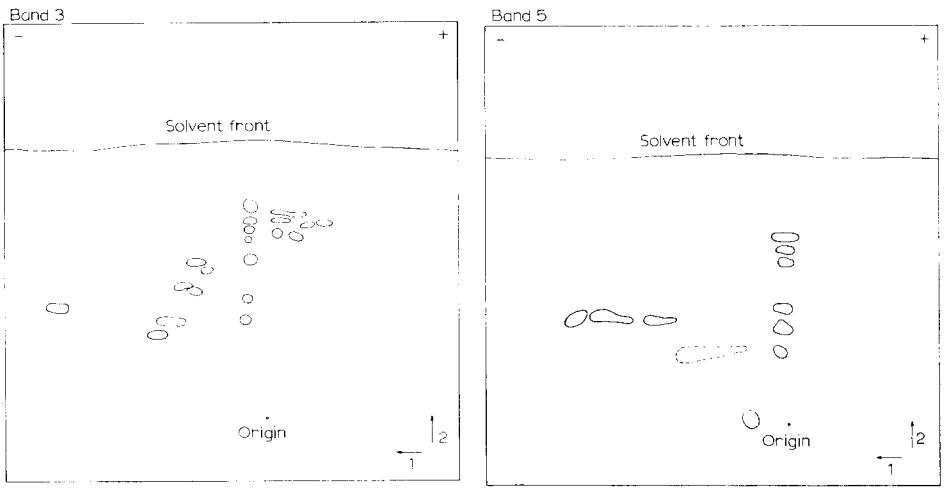


Fig. 9. Tryptic peptide map of purified aerobic cell envelope protein, Band 3. Separation of trypsin-digested Band 3 by two-dimensional thin-layer electrophoresis (1) and chromatography (2) on powdered cellulose sheet.

Fig. 10. Tryptic peptide map of purified aerobic cell envelope protein, Band 5. Separation of trypsin-digested Band 5 by two-dimensional thin-layer electrophoresis (1) and chromatography (2) on powdered cellulose sheet.

exceptionally low number of peptides from our tryptic digestion data of Band 5 could be due to the presence of insoluble core protein, incomplete digestion, or protein subunits.

Although all these proteins have a few neutral peptides in common, the majority of the peptides from each digest appear to be unique to that digest. These selected proteins show differences in their tryptic peptide maps and amino acid compositions, and therefore are concluded to be absent from purified chromatophores[8] and to be unique aerobic membrane proteins.

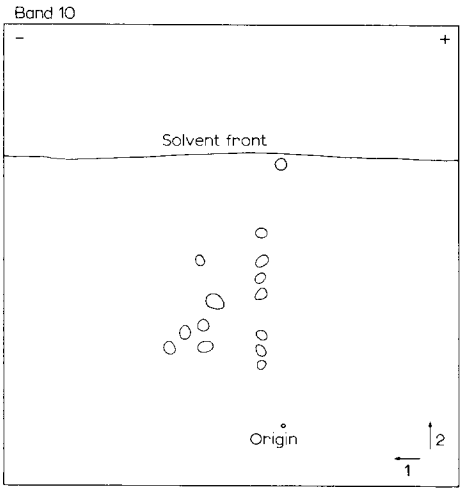


Fig. 11. Tryptic peptide map of purified aerobic cell envelope protein, Band 10. Separation of trypsin-digested Band 10 by two-dimensional thin-layer electrophoresis (1) and chromatography (2) on powdered cellulose sheet.

DISCUSSION

We have used a variety of procedures in order to isolate and solubilize envelope preparations as well as purified chromatophores from *R. spheroides*: (1) chaotropic ions, (Hatefi and Hanstein[27]); (2) *n*-butanol extraction (Lockshin and Burris[28]); (3) aqueous pyridine solubilization (Blumenfeld[29]); and (4) chloroethanol solubilization (Fraker and Kaplan[11]), but only dodecyl sulphate has been found to be suitable for solubilization and disaggregation of the cell envelope proteins. Although a number of different fractionation procedures were employed (Reisfield *et al.*[30], Takayama *et al.*[31], chromatography, gradient centrifugation, $(NH_4)_2SO_4$ and ethanol fractionation, as well as others), only the dodecyl sulphate–polyacrylamide gel electrophoresis system seems to offer the most convenient, fast, and reproducible method for resolving and determining the molecular weight and band composition of cell envelope proteins.

Multiple components

Dodecyl sulphate–polyacrylamide gel electrophoresis of untreated cell envelope proteins demonstrates 10 major protein bands with molecular weights ranging from 10000 to 100000. Although it has been demonstrated that purified chromatophores contain a major component (Fraker and Kaplan[16]) our studies using treated aerobic membranes and isolated anaerobic membranes demonstrate that no single protein serves as the dominant component of these membrane structures, (see Figs 5 and 6, also Schnaitman[21]). The aerobic and anaerobic envelope preparations are shown by dodecyl sulphate–polyacrylamide gel electrophoresis to be similar and to be composed of a number of proteins with different molecular weights. These results together with the amino acid composition data, and tryptic fingerprints of selected proteins strongly suggest the existence of protein heterogeneity in these fractions.

The demonstration of an association between a low molecular weight membrane protein, Band 10, with phospholipids is interesting. Chromatophore protein, Band 15 of *R. spheroides* co-migrates with all of the phospholipids present in the chromatophore (Fraker and Kaplan[11]). Whether or not these associations exist *in vivo* remains to be determined. However, it seems improbable that phospholipids bind to only one of 10 or more protein components comprising the membrane structure, especially when Band 10 is not a predominant component of this structure.

Of the membrane proteins studied here, all contain a relatively high proportion of nonpolar residues. Aerobic cell envelope proteins contain 50–60 mole% nonpolar amino acids. The low solubility of the cell envelope proteins in aqueous solvents is undoubtedly explained by the high content of nonpolar residues.

For many soluble proteins, such as cytochrome *c* (Dickerson *et al.*[32]), lysozyme (Blake *et al.*[33]), and haemoglobin (Perutz *et al.*[34]) the charged groups are on the surface of the protein, and the nonpolar groups are packed in the centre. Vanderkooi and Green[35] proposed that the hydrophobic membrane proteins have a larger proportion of nonpolar side chains on the surface in addition to the polar ones. The higher proportion of nonpolar amino acids on the surface can account for the low solubility of the membrane proteins in aqueous media.

Although our studies have led to a great deal of information concerning the composition and subunit structure of the chromatophore and cell envelope membranes from *R. spheriodes*, more remains to be learned about the localization of the proteins within the membranes, as well as the arrangement and interactions of the proteins and lipids of the membranes.

These studies and those presented later (Huang and Kaplan[8]) as well as those previously published suggest a re-examination of the thesis that the chromatophore proteins and the cytoplasmic membrane proteins are identical. Immunochemical studies employing the micro-ouchterlony diffusion technique indicate weak cross-reactions between anti-aerobic membrane and either aerobic membrane, anaerobic membrane, or chromatophore P_1. Anti-anaerobic membrane also shows weak reactivity towards either aerobic membrane, anaerobic membrane, or chromatophore P_1. Anti-chromatophore P_1 is also cross-reactive against either aerobic membrane, anaerobic membrane, or chromatophore P_1. The weakness of these reactions may be explained by the high insolubility of the antigens in an aqueous medium. Our preliminary studies suggest that there are similarities between aerobic membrane, anaerobic membrane, and chromatophore P_1 proteins[8]. However, additional experiments are necessary to further establish this conclusion.

Furthermore, our extensive chemical investigations of both chromatophore proteins and of proteins derived from the aerobic and chromatophore-depleted anaerobic membrane fractions strongly indicate that the bulk (greater than 75%) of the chromatophore proteins share no identity with any proteins observed to be present in these other membrane preparations.

However, we must point out that our immunochemical criteria and chemical analyses are not yet complete, and until they are, we cannot state that all chromatophore proteins are unique to this structure.

As Oelze and Drews[36] point out, all parts of the intracytoplasmic membrane are interconnected as well as connected to the cytoplasmic membrane. This obviously implies that all membranes are continuous, but in our opinion it does not mean that

all components are identical. This is a critical question, which must be answered if one is to consider the origin of the chromatophore membrane structure. Similarly, further studies relating to control and regulation of chromatophore biosynthesis must await an extensive and detailed analysis of the proteins comprising the chromatophore membrane on the one hand and the aerobic and anaerobic cytoplasmic membranes on the other. To date, no such investigation has been completed and we feel no unqualified proof is yet available in order to answer this question.

However, the approach set forth in this and other communications[8,9,11,16] has suggested a difference in protein composition for these membrane structures. As we have shown here, at least three proteins derived from the enriched aerobic membrane are not present in purified chromatophores. The presence of these same proteins in the anaerobic cell membrane is suggested although not yet firmly established.

ACKNOWLEDGEMENTS

We wish to thank Miss Judy R. Murphy for preparing the electron micrographs for this paper. This work was supported by Public Health Service research grant GM-15590. J. W. H. was supported by a Public Health Service traineeship in Cell Biology research grant GM-941.

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