Biochimica et Biophysica Acta, 511 (1978) 70—82

BBA 78070

MEMBRANES OF *RHODOPSEUDOMONAS SPHAEROIDES*

V. IDENTIFICATION OF BACTERIOCHLOROPHYLL *a*-DEPLETED CYTOPLASMIC MEMBRANE IN PHOTOTROPHICALLY GROWN CELLS *

LAWRENCE C. PARKS ** and ROBERT A. NIEDERMAN
Department of Microbiology, Rutgers University, New Brunswick, N.J. 08903 (U.S.A.)
(Received November 4th, 1977)

Summary

The separation of membrane fragments was investigated in extracts of phototropically grown *Rhodopseudomonas sphaeroides* to determine if the plasma membrane contains discrete regions. A highly purified fraction of bacteriochlorophyll *a*-deficient membrane fragments was isolated by differential centrifugation, chromatography on Sepharose 2B, reaggregation, and isopycnic sedimentation on sucrose gradients. Significant levels of *b*- and *c*-type cytochromes and succinate dehydrogenase were demonstrated in the isolated membrane fragments and their appearance in electron micrographs, their polypeptide profile in dodecyl sulfate-polyacrylamide gel electrophoresis, and overall chemical composition were essentially identical to a similar fraction isolated from aerobically grown cells. Their polypeptide profiles were distinct from those of the intracytoplasmic chromatophore and outer membranes, and on the basis of bacteriochlorophyll content the phototrophic fraction was contaminated with chromatophores by <9%. The membrane fragments contained no diaminopimelic acid or glucosamine. It is concluded that the membrane fragments isolated from phototrophically growing *Rp. sphaeroides* have arisen from photosynthetic pigment-depleted regions of the plasma membrane structurally and functionally differentiated from the intracytoplasmic chromatophore membrane. These regions represent conserved chemotrophic cytoplasmic membrane whose synthesis continues under photoheterotrophic conditions.

---

* Paper IV in this series is ref. 11.

** Present address: Department of Microbiology and Immunology, Temple University, School of Medicine, 3400 North Broad St., Philadelphia, Pennsylvania 19140, U.S.A.

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate.

Introduction

The photosynthetic apparatus of many *Rhodospirillaceae* is localized within an extensive system of bacteriochlorophyll *a*-rich intracytoplasmic membranes [1] designated as the chromatophore membrane [2]. When *Rhodopseudomonas sphaeroides* is grown under appropriate photoheterotrophic conditions, the chromatophore membrane appears in electron micrographs as an internal system of vesicles which fills the cytoplasm, and in some cases, as invaginations of the peripheral cytoplasmic membrane [3] *. When *Rp. sphaeroides* is grown chemoheterotrophically at high aeration, the formation of the chromatophore membrane and associated photosynthetic pigments is repressed and the cytoplasmic membrane is observed in close apposition to the cell wall [4,5]. During adaptation from aerobic to phototrophic growth, the synthesis of the chromatophore membrane is derepressed and much of the membrane becomes intracytoplasmically localized [3,4]. Such morphological observations led to the suggestion [3] that the chromatophore membrane arises from the intrusions of the peripheral cytoplasmic membrane into which bacteriochlorophyll *a* and other chromatophore-specific components are inserted.

A critical test of this hypothesis has been precluded by the inability to isolate a cytoplasmic membrane fraction from phototrophically grown cells. Several groups [6—8] have isolated cytoplasmic membrane fractions from aerobic *Rp. sphaeroides* but were unsuccessful in the isolation of cytoplasmic membrane with phototrophic cells because of the association of the peripheral structure with both chromatophore and outer membranes. This communication describes a procedure for the isolation of photosynthetic pigment-depleted portions of the cytoplasmic membrane from photosynthetically grown *Rp. sphaeroides*. The physicochemical properties of these highly purified preparations were essentially identical to a similar fraction isolated from aerobic cells. It is concluded that chemotrophic cytoplasmic membrane is conserved during photoheterotrophic growth. (A preliminary report of these studies was presented at the 77th Annual Meeting of the American Society for Microbiology, New Orleans, Louisana, U.S.A. May 1977).

Materials and Methods

Growth of organism. Rp. sphaeroides NCIB 8253 was grown phototrophically in the medium of Cohen-Bazire et al. [9] at 30°C with a light intensity of 800 ft-candles. Cultures were maintained to a final absorbance of 2.5—3.0 as measured at 680 nm (1 cm light path). Cultures were grown aerobically on a gyrotory shaker at 350 rev./min in 2800-ml Fernbach flasks filled maximally

---

* The cytoplasmic membrane as observed in thin sections of phototrophically grown *Rp. sphaeroides* is thought to represent a continuous structure. It consists of intracytoplasmic portions believed to be the site of light-driven cyclic electron flow and peripheral regions that closely appose the cell wall. We prefer to designate the former as the chromatophore membrane since the term intracytoplasmic membrane is non-specific and has been used to describe a variety of structures found in non-photosynthetic bacteria. We have designated photosynthetic pigment-depleted membrane fragments isolated in the present study as "cytoplasmic membrane". Although they have been separated from chromatophores and cell walls and presumably arise from peripheral regions of the plasma membrane, definitive designation awaits in situ electron microscopic evidence.

with 700 ml of the same medium. Aerobic cultures were maintained until an A_{680nm} between 0.8 and 1.0 was reached.

Membrane isolation and purification. All isolation procedures were performed at 0—4°C. Cell-free extracts were prepared by passage through a French pressure cell essentially as described previously [10,11] except that the buffer was 0.1 M sodium phosphate (pH 7.5), containing 0.01 M EDTA [12]. The extracts were centrifuged at 10 000 × *g* for 10 min, and the supernatant fraction was then centrifuged at 160 000 × *g* for 90 min. The supernatant fraction from this ultracentrifugation was recentrifuged at 160 000 × *g* for 60 min. The final supernatant fraction was pumped onto a column of Sepharose 2B (Pharmacia) equilibrated with phosphate/EDTA buffer. Eluted column fractions were assayed for absorbance [11] at 260, 280 and 850 nm (the absorption maximum of *Rp. sphaeroides* bacteriochlorophyll in vivo) and for succinate dehydrogenase (succinate : phenazine methosulfate oxidoreductase, EC 1.3.99.1) activity [13]. Membrane fragments were pooled from fractions with the highest ratio of succinate dehydrogenase activity to absorbance at 850 nm and concentrated to approximately one-third of their original volume by ultrafiltration with a PM-30 membrane (Amicon). Concentrated membrane suspensions were dialyzed against 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.5) and aggregated by sedimentation at 115 000 × *g* for 18 h. Pellets were resuspended in HEPES buffer and layered onto preformed linear gradients of 20—55% (w/w) sucrose in the same buffer. Gradients were centrifuged at 96 000 × *g* for 13.75 h and collected by upward flow displacement [10,14]. Appropriate fractions were pooled and washed exhaustively with distilled-deionized water. Chromatophore and cell wall fractions were purified from the 160 000 × *g* · 90 min pellet by rate-zone sedimentation [10]. The gradients were prepared in 1 mM Tris · HCl buffer (pH 7.5). The cell wall fraction was resuspended in HEPES buffer, and further purified by the isopycnic centrifugation procedure described above.

Physical characterization. Electron microscopic and thin sectioning procedures have been described [14]. Dodecyl sulfate-polyacrylamide disc slab gel electrophoresis was performed as described by Laemmli and Favre [15], modified as follows. The separating gel contained 10% acrylamide. Samples (2 mg protein/ml) in 62.5 mM Tris (pH 6.8) were maintained at 100°C for 75 s, and 75 μg sample protein were applied to the gel. Gels were fixed, stained and destained electrophoretically. The fixing and destaining solutions contained 25% isopropanol and 10% acetic acid; staining solution contained 0.02% Coomassie Brilliant Blue in 7% acetic acid. Molecular weights of the polypeptide bands were determined by comparison of their relative mobilities in the gel with those of proteins of known molecular weight. Standards employed were: albumin (bovine serum, Miles); catalase, carbonic anhydrase and myoglobin (all from Sigma); γ-globulin (human, Schwartz/Mann); and albumin (hen egg, Calbiochem).

Chemical characterization. The procedures employed for dry weight, protein, phosphorus, carbohydrate, and nucleic acid determinations, and lipid extraction were described previously [14]. The procedures for determination of bacteriochlorophyll, spheroidenone, and spheroidene contents were also described [11]; the extinction coefficient of Clayton [16] was employed for

bacteriochlorophyll. Diaminopimelic acid and glucosamine were determined on a Beckman Model 120B amino acid analyzer [17] equipped with a System AA computing integrator. Membrane suspensions containing 150 μg protein were hydrolyzed with constant-boiling HCl at 100°C for 24 h. Cytochromes were determined from sodium hydrosulfite reduced minus oxidized difference spectra as described by Collins and Niederman [14]. Relative cytochrome levels were determined from the Soret peaks of such spectra. The α-bands of the chromatophores could not be quantified with accuracy due to the interference by overlapping carotenoid bands in this region of the spectra.

Results

Succinate dehydrogenase serves as a marker for both the cytoplasmic and chromatophore membranes of phototrophically grown *Rp. sphaeroides* [13,18]; however, the specific activity of the enzyme in the cytoplasmic membrane is much higher than that of chromatophores [6]. When crude extracts from phototrophically grown cells were subjected to the differential centrifugation procedure described above, 43% of the succinate dehydrogenase activity * remained unsedimented after the first centrifugation at 160 000 $\times g$. After the second sedimentation at this speed, 53% of the activity still remained in the supernatant fraction, while much additional chromatophore material was sedimented. In contrast with aerobic extracts, 80% of the succinate dehydrogenase activity partitioned with the supernatant fraction after the first centrifugation at 160 000 $\times g$; the second ultracentrifugation yielded 94% of the activity in the supernatant fraction. It has been shown previously [6] that the succinate dehydrogenase activity in these supernatant fractions is not due to a soluble cytoplasmic form of the enzyme but rather results from fragmentation of the cytoplasmic membrane into small bits that do not sediment at 160 000 $\times g$ in 90 min. The differences in the relative distributions of activity between the aerobic and phototrophic extracts is due to the succinate dehydrogenase activity that sedimented with the chromatophore and cell envelope fractions in the latter [13].

Niederman et al. [6] have shown that when the small membrane fragments from the supernatant fraction of phototrophic extracts were aggregated by prolonged centrifugation and purified by rate-zone sedimentation, the resulting membranes had bacteriochlorophyll contents that were about 25% of those observed in isolated chromatophores [6]. Reconstruction experiments suggested that about 65% of this bacteriochlorophyll could be accounted for by contaminating chromatophores [6]. To purify the membrane fragments further, the supernatant fraction obtained after the second centrifugation at 160 000 $\times g$ was chromatographed on a Sepharose 2B column (Fig. 1). Phototrophic small membrane fragments depleted in bacteriochlorophyll as shown by their high succinate dehydrogenase/A_{850nm} ratio were pooled from the last half of the succinate dehydrogenase profile. Although this usually resulted in

* These percentages represent the distribution of activity between the supernatant and pellet fractions after the respective ultracentrifugations. Typically, 90—95% of the total starting activity survived these centrifugation procedures.

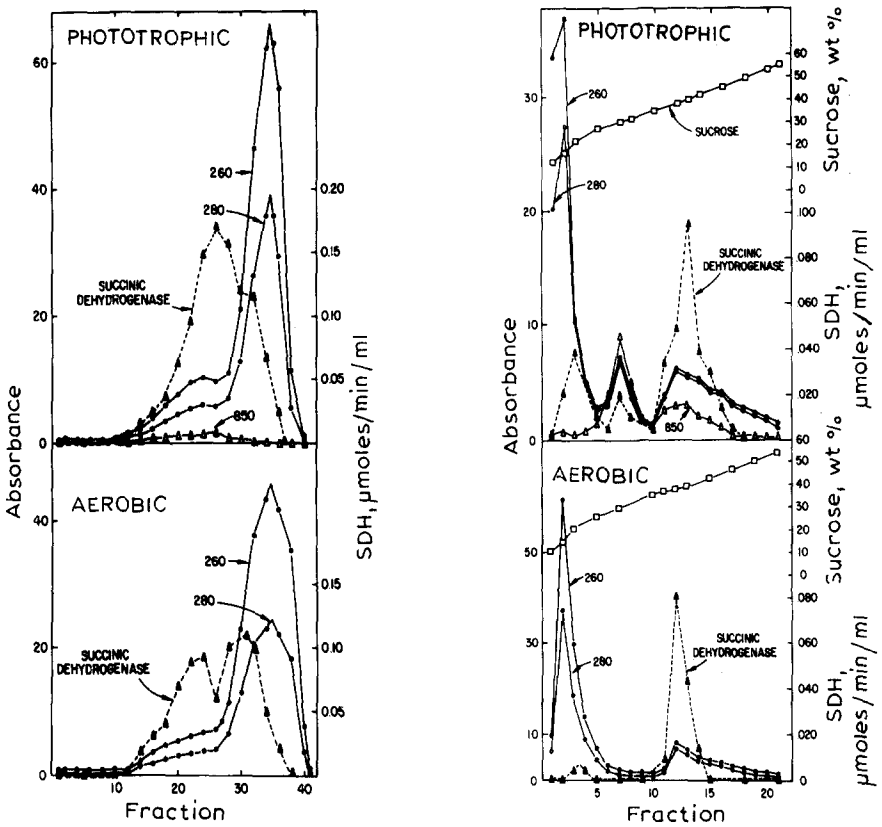

Fig. 1. Elution profiles of 160 000 X *g*, 60-min supernatant fractions on a 2.6 X 36 cm Sepharose 2B column. The supernatant fractions which were applied to the columns were derived from 10 and 17 g of wet phototrophic (upper panel) and aerobic (lower panel) cells, respectively. The small membrane fragments were pooled from fractions 30—37 from both the phototrophic and aerobic extracts. Other experimental details are presented in Materials and Methods. SDH, succinate dehydrogenase.

Fig. 2. Resolution of reaggregated membrane fragments from ribosomal material on isopycnic sucrose gradients. Fractions 30—37 from the Sepharose 2B columns were concentrated by ultrafiltration, dialyzed, and reaggregated by centrifugation for 18 h at 115 000 X *g*. The resuspended pellets were layered on 20—55% (w/w) sucrose gradients and centrifuged to equilibrium in a Beckman SW 27 rotor. Sedimentation was to the right. Sucrose concentrations were determined refractometrically. Upper panel, extract from phototrophically grown cells; lower panel, extract from aerobically grown cells. Reaggregated membrane fragments, fractions 11—15; ribosomal material, fractions 1—5; chromatophore material (phototrophic extract), fractions 6—9. SDH, succinate dehydrogenase.

recovery of about half the succinate dehydrogenase activity in the pooled fractions, preparations with a lower final bacteriochlorophyll content resulted. Membrane fractions as pooled from the column are contaminated with ribosomal material as evidenced by the co-elution of the ultraviolet absorbance peak of high A_{260nm}/A_{280nm} ratio. Although no significant quantities of larger cell envelope or chromatophore material appeared in the early portions of the elution profile, fractions with membranes of intermediate size appeared as a shoulder of ultraviolet absorbances that preceded the small membrane fragments. The elution profile of succinate dehydrogenase-rich membrane fragments from aerobic cells was similar to that of the photrophic extracts.

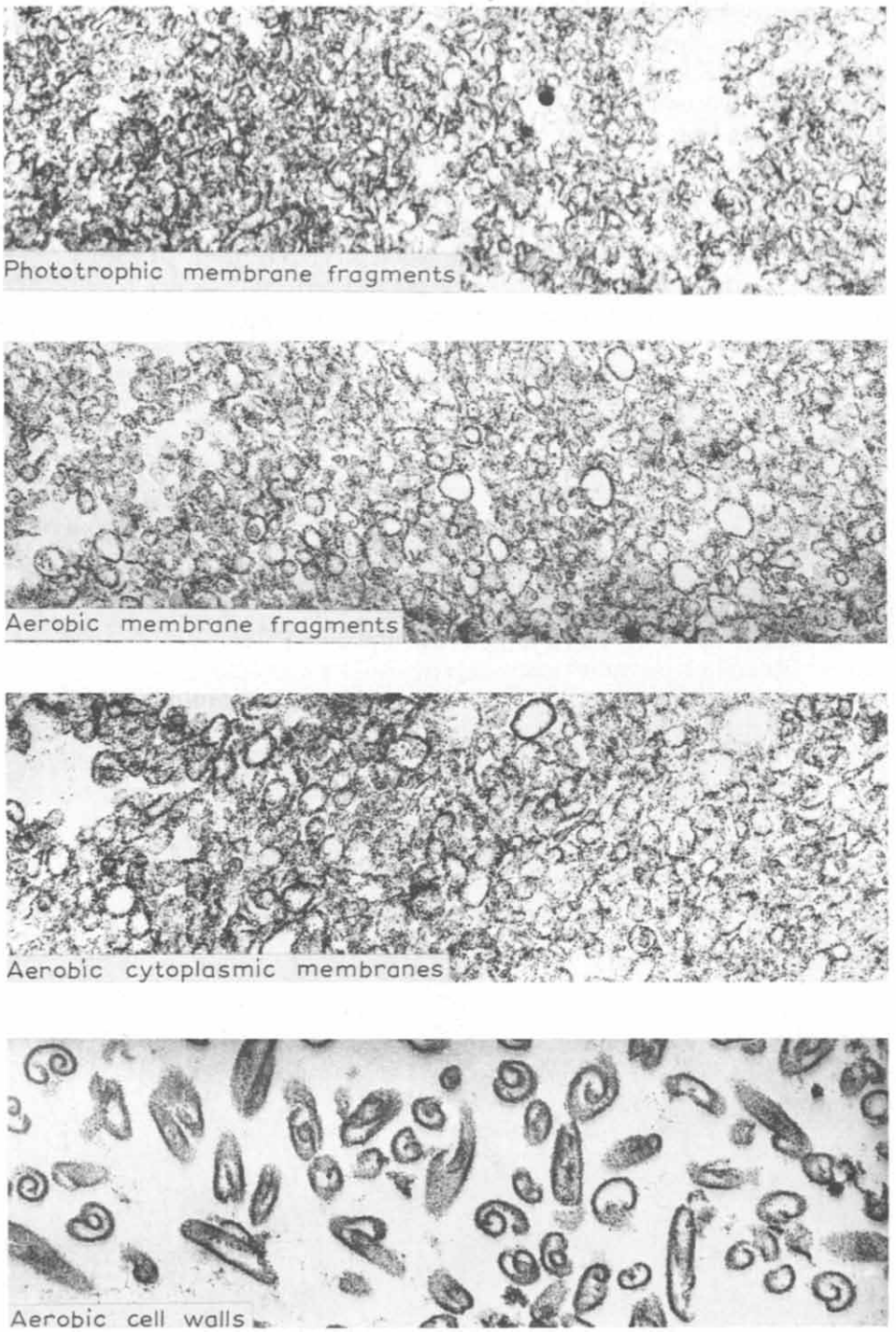

Fig. 3. Electron micrographs of thin sections of isolated membrane fractions. From top to bottom: reaggregated phototrophic and aerobic membrane fragments, respectively, isolated from 160 000 × *g* supernatant fraction by gel filtration and isopycnic sucrose gradient centrifugation; aerobic cytoplasmic membrane and cell wall fractions, respectively, isolated by the procedure of Schnaitman [19]. Magnification: approx. ×53 000.

Aggregation of pooled membrane fragments by prolonged centrifugation was necessary prior to their resolution from ribosomes [6]. This converted the small fragments into larger structures which banded two-thirds of the way into a linear isopycnic sucrose gradient (Fig. 2). Although this aggregation procedure causes the bulk of the membrane fragments to band at an anomalously high equilibrium density, they are cleanly separated from the ribosomal material which has remained at the top of the gradient. The majority of the succinate dehydrogenase activity has banded with the reaggregated membrane fragments. Chromatophore-like material (fractions 6—9) with a high A_{850nm}/A_{280nm} ratio was also released from phototrophic small membrane fragments. The reaggregated fragments, and those from aerobic cells, migrated to the same position in the gradients (fractions 11—15) and contained high levels of succinate dehydrogenase activity, which is consistent with the similarity of these fractions.

Electron micrographs of stained thin-sections from isolated membrane preparations are seen in Fig. 3. Both phototrophic and aerobic membrane fragments appeared as small, mainly vesicular structures with approximate average diameters of 52 nm. The appearance of these fractions corresponded closely to that of aerobic cytoplasmic membranes prepared by the method of Schnaitman [19]. Aerobic cell walls that consist of both the peptidoglycan layer and outer membrane of the cell envelope are shown in the lower panel. They stained more densely and assumed coiled and sheet formations typical of preparations isolated from other gram-negative bacteria [14,19].

The overall chemical composition of the phototrophic and aerobic cytoplasmic membrane fragments was virtually identical, aside from small amounts of photosynthetic pigments in the former (Table I). The specific bacteriochlorophyll content of the phototrophic membrane fragments was 6.4 μg bacteriochlorophyll per mg protein while that for the chromatophores purified from the same extract was 70.6. These data and data with other preparations suggested that isolated phototrophic fragments were 91—97% free of contamination by chromatophores. Neither diaminopimelic acid nor glucosamine

TABLE I

CHEMICAL COMPOSITION OF ISOLATED MEMBRANE FRACTIONS

Constituent	Dry weight (%)			
	Membrane fragments		Cell wall	
	Phototrophic	Aerobic	Phototrophic	Aerobic
Protein	65.0	65.0	70.0	67.0
Phospholipid	25.0	22.0	31.0	26.0
Carbohydrate *	4.5	4.2	8.8	9.1
Bacteriochlorophyll	<0.55	<0.03	<0.55	0
Carotenoid	<0.25	<0.02	<0.16	0
Total nucleic acid	<0.38	<0.39	<0.19	<0.28
Diaminopimelic acid	0	0	8.8	8.2
Glucosamine	0	0	0.32	0.32

* Expressed as glucose equivalents.

were detected in the membrane fragments. The cell wall fractions contained substantial levels of diaminopimelic acid and glucosamine; this indicates that the peptidoglycan layer remained attached to the outer membrane during the isolation procedures. The insignificant levels of nucleic acid in all preparations demonstrate that they were free from contamination by ribosomes and DNA. Protein, phospholipid and carbohydrate values were comparable to those found in the cytoplasmic membrane and cell wall material isolated from other gram-negative bacteria [20,21]. The amino acid composition of the phototrophic and aerobic membrane fragments were essentially identical [2]; they contained 46.7 and 47.2% hydrophobic residues, respectively.

The relative cytochrome content of phototrophic membrane fragments has been estimated from reduced-minus-oxidized difference spectra (Fig. 4). The α-peaks at 551 and 558 nm indicated the presence of both *c*- and *b*-type cytochromes, respectively [22]. Cytochrome levels were assessed from the magnitude of the Soret peak by absorbance differences between 428 and 407 nm. The dashed line reflects the contribution to the Soret peak that may be made by chromatophores; the chromatophore and membrane fragment samples contained equivalent amounts of bacteriochlorophyll. On this basis, 80% of the cytochrome content of the membrane fragments could not be accounted for by contamination with chromatophores. This suggests that the fragments have arisen largely from bacteriochlorophyll-depleted cytoplasmic membrane.

A comparison of the polypeptide profiles of the various membrane fractions after solubilization and electrophoresis on SDS-polyacrylamide slab gels is seen in Fig. 5. The multi-banded profiles of the aerobic and phototrophic membrane

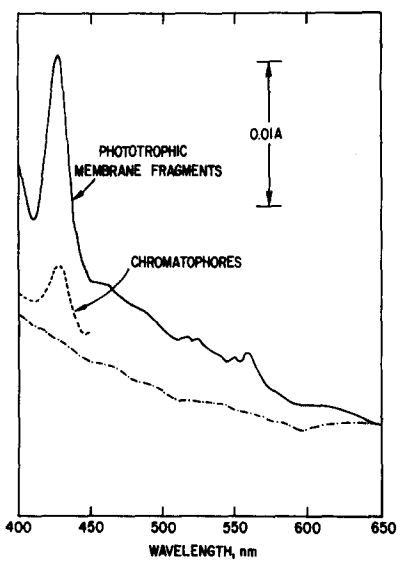

Fig. 4. Reduced-minus-oxidized difference spectra of membrane fraction from phototrophically grown cells. Membrane fragments, 0.2 mg protein per ml; chromatophores, bacteriochlorophyll level equivalent to that in membrane fragment sample. The lower trace is the oxidation baseline. Other experimental details are provided in the text.

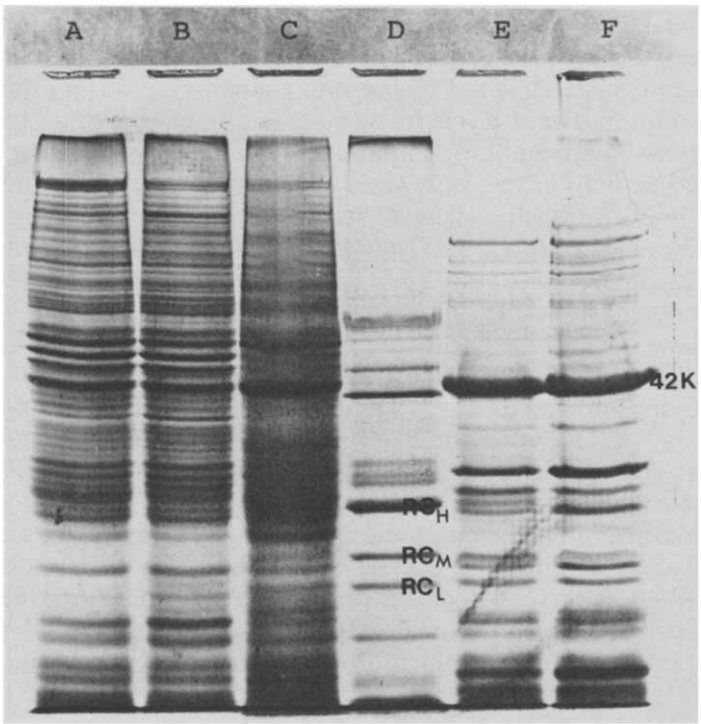

Fig. 5. Dodecyl sulfate-polyacrylamide slab gel electrophoresis of isolated membrane preparations. The method of Laemmli and Favre [15] was employed. A, aerobic membrane fragments; B, phototrophic membrane fragments; C, aerobic cytoplasmic membrane fraction; D, chromatophores; E, phototrophic cell walls; F, aerobic cell walls. The isolation procedures for the membrane fragments, the cytoplasmic membranes, and cell walls are indicated in the legend of Fig. 3. Chromatophores were isolated as described previously [10]. RC_H, RC_M, and RC_L, polypeptide subunits of the photochemical reaction center [23,24]; 42 k, major 42 000 dalton outer membrane polypeptide band [6].

fragments (A and B) were identical both qualitatively and quantitatively. This is to be contrasted to the results of co-electrophoresis reported previously with less purified preparations of membrane fragments from aerobic and phototrophic cells grown with ^{3}H- and ^{14}C-labeled amino acids, respectively [6]. These preparations shared all of the higher molecular weight bands; the differences in the quantities of the lower molecular weight bands can now be ascribed to contaminating chromatophore material. No such differences are observed here even with the higher order of resolution afforded by the slab gel electrophoresis procedure. In Fig. 5 it can also be seen that aerobic cytoplasmic membrane (C), isolated by the method of Schnaitman [19] was typical of that from other gram-negative bacteria [14,19,21] and closely corresponded to the profiles of the membrane fragments with only a few differences in the relative amounts of low molecular weight polypeptides. However, chromatophores exhibited a distinct profile (D) with much fewer bands than the cytoplasmic membrane or cell wall profiles, (E, F) and the prominent photochemical reaction center-associated polypeptides observed by others [23,24]. Molecular weights of these polypeptides were calculated as 28 000, 24 500 and 21 000 for reaction center subunits H, L, and M, respectively. These bands were absent

from membrane fragments of phototrophic as well as aerobic cells. Cell wall preparations displayed a prominent band of molecular weight 42 000 which has been found in large quantity in the outer membrane profiles of other gram-negative bacteria [19,25] and resolved into several subcomponents [26,27]. Although this band appeared to be present in the membrane fragment profiles, scans of stained gel slabs with an integrating densitometer suggested that it represented less than 3% of the total protein of these fractions. A 42 000 dalton band was absent from chromatophore profiles.

Discussion

The procedures described here have resulted in the isolation of highly purified fragments of bacteriochlorophyll-depleted membrane from phototrophically grown *Rp. sphaeroides.* They were identified as small unit-membrane vesicles with a primary lipoprotein composition and were essentially identical to membrane fragments isolated by the same procedures from aerobically grown cells by every parameter tested (gel filtration and sedimentation behavior, appearance in thin sections, chemical composition, and polypeptide profiles in SDS-polyacrylamide gel electrophoresis). The phototrophic fraction contained significant levels of succinate dehydrogenase activity and cytochromes; 80% of the *b*- and *c*-type cytochrome could not be accounted for by their small contamination with chromatophores. Moreover, the physicochemical properties of the highly purified fragments were typical of cytoplasmic membrane preparations isolated by more conventional techniques from aerobically grown *Rp. sphaeroides* (refs. 7 and 8, and present study) and *Rhodospirillum rubrum* [14,28,29] and other gram-negative bacteria [19—21]. Based upon all the preceding evidence, it is concluded that the reaggregated membrane fragments of phototrophically grown *Rp. sphaeroides* have been derived from bacteriochlorophyll-depleted cytoplasmic membrane. Not only are these membrane regions depleted in photosynthetic pigments, but their polypeptide profiles indicate that they lack the protein components specific to the chromatophore membrane.

The presence of photosynthetic pigment-deficient regions of the cytoplasmic membrane in phototrophically grown *Rp. sphaeroides* had been suggested previously [6,13]. A dense cell envelope band of low bacteriochlorophyll content was isolated by differential and zonal ultracentrifugation [13]. Although, as shown in the present study, a substantial portion of the cytoplasmic membrane is fragmented and lost from the envelope, the isolated envelope material contained significant levels of succinate dehydrogeanse activity and cytochromes that could not be accounted for by chromatophore contamination. These components were attributed to bacteriochlorophyll-depleted cytoplasmic membrane [13]. When crude cell extracts were applied directly to sucrose gradients and subjected to rate-zone sedimentation, about half the succinate dehydrogenase activity appeared in a bacteriochlorophyll-depleted band near the top of the gradient after rate-zone sedimentation (Niederman, R.A. and Mallon, D.E., unpublished observations). In the present study, this increment of succinate dehydrogenase activity apparently remained unsedimented with small membrane fragments during the initial differential

ultracentrifugation procedure described above; this activity was then isolated largely with the cytoplasmic membrane. Much of the sedimented cytoplasmic membrane and residual chromatophore material previously isolated with the cell wall, was separated in this study from the latter fraction by subsequent isopycnic sucrose gradient centrifugation. The small membrane fragments recognized previously [6,30] in phototrophically grown cells were isolated in a previous study [6] by reaggregation and rate-zone sedimentation; they consisted of a mixture of pigmented and non-pigmented membrane. Here, additional differential centrifugation, Sepharose column chromatography [12,31] and isopycnic centrifugation have resulted in a more effectual separation of both cell wall and chromatophore material from the non-pigmented cytoplasmic membrane fragments. Care was taken to avoid divalent cations during the isolation procedure since they have been shown to prevent effective membrane separation [13,32]. Based upon their bacteriochlorophyll content, the isolated cytoplasmic membrane fragments were contaminated <9% by chromatophore material; their polypeptide profile suggests <3% contamination with outer membrane, and the absence of diaminopimelic acid and glucosamine suggests that they have been separated completely from the peptidoglycan layer of the cell wall.

The results presented here suggest that portions of the cytoplasmic membrane of phototrophically grown cells remain largely undifferentiated during chromatophore development and represent conserved aerobic cytoplasmic membrane. The outer membranes of phototrophic and aerobic cells were also identical based upon the parameters examined. In contrast, the chromatophore membrane is differentiated from other cellular membranes by the incorporation of bacteriochlorophyll, carotenoids, and specific proteins involved in the photosynthetic process within a typical lipoprotein membrane structure. Since the peripheral cytoplasmic and intracytoplasmic membranes are thought to be continuous [3], it is suggested that structurally and functionally differentiated regions exist within the plasma membrane of *Rp. sphaeroides*. The pigment-depleted cytoplasmic membrane fraction may have originated from the cell periphery because its physicochemical properties were equivalent to those of the fraction derived from aerobic cells in which the cytoplasmic membrane is observed [4,5] closely apposed to the cell wall. Unlike the chromatophore membrane whose formation is only observed under conditions of low oxygen tension [4,5,11], the present results demonstrate that the formation of aerobic cytoplasmic membrane continues irregardless of the oxygen tension to which the cells are exposed.

A precedent for structurally and functionally differentiated regions within a continuous procaryotic plasma membrane is provided by cell envelope disaggregation studies in *Halobacterium halobium* [33–35]. After dialysis against distilled water and ultracentrifugation, the plasma membrane was resolved into two distinct fractions: small fragments of orange-red membrane; and large purple membranous sheets [34]. Discrete patches of purple membrane continuous with the orange-red membrane were identified in electron micrographs of whole cells [35]. The orange-red membrane contains α-bacterioruberin and electron transport components and is thought to be the site of oxidative phosphorylation. The purple membrane contains bacteriorhodopsin, a retinal · pro-

tein complex [36] that mediates a unique photophosphorylation mechanism at low oxygen tensions in which a light-generated transmembrane proton gradient [37] is coupled to ATP synthesis [38]. During repigmentation of aerobically grown *Rp. sphaeroides* at reduced oxygen tension, the components responsible for photochemical activity also appear to be inserted into the cytoplasmic membrane at discrete sites [2,4]. As the synthesis of the new membrane continues, it invaginates and results in the formation of the chromatophore membrane. The aerobic cytoplasmic membrane is conserved and upon cell disruption gives rise to small fragments similar in size to those of the aerobic membrane of *H. halobium*.

The invaginations of the cytoplasmic membrane observed in cell sections have suggested alternative morphogenetic relations between the peripheral and intracytoplasmic membranes [39]. In the first, the chromatophore and peripheral cytoplasmic membranes are thought to consist of the same components but in different relative proportions. Alternatively, it was suggested that although the specialized chromatophore membrane is anchored to the peripheral cytoplasmic membrane, the two membranes are discontinuous with respect to composition. The first of these hypotheses was elaborated by Oelze and Drews [1]. They suggested that during derepression of chromatophore membrane formation the cytoplasmic membrane is differentiated and a gradient of protein components becomes established within the continuous membrane system. The alternative hypothesis derives support from the studies of Kaplan and his collaborators [40,41]. They proposed that although initially chromatophore membrane growth occurs at the periphery of the cell, new material is condensed onto the plasma membrane and continued growth becomes independent of the peripheral cytoplasmic membrane. The findings presented here provide considerable support for the second of these models. In previous studies [42], unresolved small membrane fragments appeared to contain precursors of chromatophore proteins. It was not known whether this was due to bacteriochlorophyll-depleted cytoplasmic membrane, or a pigmented precursor particle. Further pulse-chase studies support the latter possibility (Niederman, R.A., Mallon, D.E. and Parks, L.C., unpublished observations). The bacteriochlorophyll-depleted cytoplasmic membrane fragments isolated as described here did not exhibit precursor activity in phototrophically growing cells. Instead, pigmented material partially resolved from the cytoplasmic membrane showed the behavior expected of a precursor of chromatophore protein components. The basis for this observation is currently under investigation.

Acknowledgements

This work was supported by National Science Foundation grants PCM74-18151 and PCM76-24142 and Public Health Service grant GM20367 from the National Institute of General Medical Sciences. Support from Rutgers University was provided from a Public Health Service Biomedical Research Support Grant and the Charles and Johanna Busch Memorial Fund Award to the Bureau of Biological Research. R.A.N. is the recipient of Public Health Service Research Career Development Award GM00093-03 from the National Institute of General Medical Sciences. We thank R.D. Poretz for amino acid analyses.

References

1 Oelze, J. and Drews, G. (1972) Biochim. Biophys. Acta 265, 209—239
2 Niederman, R.A. and Gibson, K.D. in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), Plenum Publishing Corp., New York, in the press
3 Cohen-Bazire, G. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 89—110, Antioch Press, Yellow Springs, Ohio
4 Peters, G.A. and Cellarius, R.A. (1972) J. Bioenerg. 3, 345—359
5 Brown, A.E., Eiserling, F.A. and Lascelles, J. (1972) Plant Physiol. 50, 743—746
6 Niederman, R.A., Segen, B.J. and Gibson, K.D. (1972) Arch. Biochem. Biophys. 152, 547—560
7 Guillotin, J. and Reiss-Husson, F. (1975) Arch. Microbiol. 105, 269—275
8 Ding, D.H. and Kaplan, S. (1976) Prep. Biochem. 6, 61—79
9 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell. Comp. Physiol. 49, 25—68
10 Niederman, R.A. and Gibson, K.D. (1971) Prep. Biochem. 1, 141—150
11 Niederman, R.A., Mallon, D.E. and Langan, J.J. (1976) Biochim. Biophys. Acta 440, 429—447
12 Fraker, P.J. and Kaplan, S. (1971) J. Bacteriol. 108, 465—473
13 Niederman, R.A. (1974) J. Bacteriol. 117, 19—28
14 Collins, M.L.P. and Niederman, R.A. (1976) J. Bacteriol. 126, 1316—1325
15 Laemmli, U.K. and Favre, M. (1973) J. Mol. Biol. 80, 575—599
16 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 312—323
17 Spackman, D.H., Stein, W.H. and Moore, S. (1958) Anal. Chem. 30, 1190—1206
18 Gorchein, A., Neuberger, A. and Tait, G.H. (1968) Proc. R. Soc. Lond. Ser. B. 170, 319—329
19 Schnaitman, C.A. (1970) J. Bacteriol. 104, 890—901
20 Miura, T. and Mizushima, S. (1968) Biochim. Biophys. Acta 150, 159—161
21 Osborn, M.J., Gander, J.E., Parisi, E. and Carson, J. (1972) J. Biol. Chem. 247, 3962—3972
22 Bartsch, R.G. (1968) Annu. Rev. Microbiol. 22, 181—200
23 Clayton, R.K. and Haselkorn, R. (1972) J. Mol. Biol. 68, 97—105
24 Okamura, M.Y., Steiner, L.A. and Feher, G. (1974) Biochemistry 13, 1394—1403
25 Schnaitman, C.A. (1970) J. Bacteriol. 104, 1404—1405
26 Bragg, P.D. and Hou, C. (1972) Biochim. Biophys. Acta 274, 478—488
27 Schnaitman, C.A. (1974) J. Bacteriol. 118, 442—453
28 Simon, S.R. and Siekevitz, P. (1973) in Mechanisms in Bioenergetics (Azzone, G.F., Ernster, L., Papa, S., Quagliariello, E. and Silliprandi, N., eds.), pp. 3—31, Academic Press, Inc., New York
29 Oelze, J., Golecki, J.R., Kleinig, H. and Weckesser, J. (1975) Antonie van Leeuwenhoek J. Microbiol. Serol. 41, 273—286
30 Gorchein, A. (1968) Proc. R. Soc. Lond. Ser. B 170, 279—297
31 Takacs, B.J. and Holt, S.C. (1971) Biochim. Biophys. Acta 233, 258—277
32 Gibson, K.D. (1965) Biochemistry 4, 2042—2051
33 Stoeckenius, W. and Rowen, R. (1967) J. Cell Biol. 34, 365—393
34 Stoeckenius, W. and Kunau, W.H. (1968) J. Cell Biol. 38, 337—357
35 Blaurock, A.E. and Stoeckenius, W. (1971) Nat. New Biol. 233, 152—155
36 Oesterhelt, D. and Stoeckenius, W. (1971) Nat. New Biol. 233, 149—152
37 Oesterhelt, D. and Stoeckenius, W. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2853—2857
38 Danon, A. and Stoeckenius, W. (1974) Proc. Natl. Acad. Sci. U.S. 71, 1234—1238
39 Drews, G., Biedermann, M. and Oelze, J. (1969) Prog. Photosynth. Res. 1, 204—208
40 Huang, J.W. and Kaplan, S. (1973) Biochim. Biophys. Acta 307, 317—331
41 Kosakowski, M.H. and Kaplan, S. (1974) J. Bacteriol. 118, 1144—1157
42 Gibson, K.D., Segen, B.J. and Niederman, R.A. (1972) Arch. Biochem. Biophys. 152, 561—568