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MEMBRANE PROTEINS OF *RHODOPSEUDOMONAS SPHEROIDES*

IV. CHARACTERIZATION OF CHROMATOPHORE PROTEINS

JIUNN WONG HUANG and SAMUEL KAPLAN

Department of Microbiology, University of Illinois, Urbana, Ill. 61801 (U.S.A.)

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SUMMARY

Less than 5% of the protein isolated from *Rhodopseudomonas spheroides* chromatophores (designated Fraction P_I) is insoluble in 2-chloroethanol. Electrophoresis of these proteins on dodecyl sulphate–polyacrylamide gels reveals a gel pattern similar to those obtained from anaerobic and aerobic cell envelope proteins. Chromatophore P_I is shown to be part of the chromatophore structure and its presence in the chromatophore is not due to contamination from the cytoplasmic membrane.

Preparative dodecyl sulphate–polyacrylamide gel electrophoresis was performed to purify chromatophore P_{II} proteins, which comprise 95% of the total chromatophore protein. These proteins contain approximately 60–65 mole% non-polar amino acids. Comparison studies of the amino acid compositions, tryptic and chymotryptic maps, molecular weights, and antigenic reactivity of chromatophore proteins demonstrate the existence of protein heterogeneity in chromatophores. These investigations lead us to suggest that chromatophore-specific proteins do not appear in other particulate or soluble fractions derived from either aerobic or anaerobic-grown cells.

INTRODUCTION

Using the simplified procedures developed by Fraker and Kaplan[1], batch preparations of chromatophores can be isolated and purified from the facultative photosynthetic bacterium *Rhodopseudomonas spheroides* as homogeneous particulate vesicles. These workers demonstrated that the 2-chloroethanol-soluble chromatophore fraction (P_{II}) represented approximately 95% of the total chromatophore protein and could be resolved into 5–6 major bands by dodecyl sulphate–polyacrylamide gel electrophoresis. The question arises as to whether these dodecyl sulphate–acrylamide-resolvable bands are heterologous proteins, or whether they are aggregations of a lower molecular weight component. The most direct way to answer this question involves the isolation and characterization of the individual P_{II} proteins. The resolution of this question will help us to build a more complete picture of the chromatophore architecture.

The 2-chloroethanol-insoluble chromatophore proteins (P_I) represent less than 5% of the total chromatophore protein, and could be resolved into at least 10 components by dodecyl sulphate–polyacrylamide gel electrophoresis (Fraker and Kaplan[1],

see Paper III, (ref. 2 of this series). Chromatophore P_I has a dodecyl sulphate–ge electrophoretic pattern similar to those of the anaerobic and aerobic cell envelop membrane proteins[2]. The question then remains, is the presence of chromatophore P (less than 5%) due to contamination by the anaerobic cell envelope fraction or is P an actual part of the chromatophore structure?

The present study is concerned with (1) comparison of the protein compositio of chromatophore P_{II} and cell envelope membranes; (2) chemical characterizatio and immunochemical studies of chromatophore P_{II} proteins; (3) determination o the structural role of chromatophore P_I and (4) studies on the origin of the chroma tophore and chromatophore proteins.

METHODS

Organism and medium

Rhodopseudomonas spheroides strain 2.4.1. was grown in Medium AGSu o Sistrom[3], supplemented with 0.2% casamino acids.

Growth

Anaerobic cultures were grown as described by Fraker and Kaplan[1]. 800 ml o semi anaerobic cultures were grown at 130 ft-candles and sparged with different mix tures of O_2–N_2 (*e.g.* 90% N_2:10% O_2, 95% N_2:5% O_2, 99% N_2:1% O_2, *etc.*) b connecting two 7480 Rotameters (Ace Glass Incorp. Vineland, N. J.) to N_2 and O_2 sources. The flow rate of the gassing mixture was maintained at 600 ml/min. Cells were harvested at a cell density of $2 \cdot 10^9$ cells/ml.

Preparation of chromatophore P_{II} proteins

The method of Fraker and Kaplan[1] was used to purify chromatophores from anaerobically grown cells. Chromatophore P_{II} proteins, which are insoluble in 2-chloroethanol, were fractionated employing preparative dodecyl sulphate–polyacryl-amide gel electrophoresis[4].

The isolation and purification of the individual chromatophore P_{II} proteins was as described previously[1].

Chemical characterizations

Amino acid analysis and tryptic fingerprints were performed as describec previously[2]. The procedures used for chymotryptic and tryptic digestions are identical Paper electrophoresis was conducted at 1000 V (200 V/cm) on Whatman 3 MM pape (46 × 57 cm) in pyridine–acetic acid–water (200:7:793, by vol.; pH 6.6) for 90 min The second dimension was developed in a descending chromatographic tank equili-brated with *n*-butanol–pyridine–acetic acid–water (60:40:12:48, by vol.) for 12–13 h The dried paper was sprayed with 0.1% ninhydrin in acetone or absolute alcohol anc heated at 80 °C for 10 min or until the colour developed.

Preparation of antisera

Antisera against total aerobic or anaerobic cell envelope proteins were preparec as described[1]. For the preparation of antiserum against individual purified protein 2 mg of protein were injected into the rabbit at two hip positions on 3 successive

occasions at intervals of 2 weeks. The micro-ouchterlony method of Korngold[5] was used.

Assay methods

Protein concentration was determined as described by Lowry *et al.*[6]. Dodecyl sulphate concentration was determined according to Reynolds and Tanford[7]. *In vivo* chlorophyll estimation was according to Cohen-Bazire *et al.*[8].

RESULTS

Chromatophore P_{II}

The question arises as to whether each of the P_{II} components shown in Fig. 1 and ref. 1 is a unique protein or an aggregate of a faster moving component(s). Preparative dodecyl sulphate–polyacrylamide gel electrophoresis was performed to purify Bands 12, 13, and 15. The molecular weights as determined by dodecyl sulphate–polyacrylamide gel analysis against marker proteins are 44000, 27000 and 9700 for Bands 12, 13, and 15, respectively. Evidence will be presented showing that these three proteins are not only unique proteins but also chromatophore-specific proteins. Each isolated protein was rerun on analytical acrylamide gels in order to verify the presence of a single component.

Amino acid composition of chromatophore P_{II} proteins

The average mole % values of duplicate amino acid determinations of Bands 12, 13, 15, and whole chromatophore protein hydrolyzed for 24, 48 and 72 h are shown in Table I. The amino acid compositions of all three purified proteins as well as that of whole chromatophores are similar in that all appear high in hydrophobic residues. However, differences do exist in many amino acids.

Since the three proteins have distinct amino acid compositions the possibility that the higher molecular weight components are aggregates of the lower molecular weight components is unlikely. To further establish this conclusion and in an effort to fully characterize these proteins, the tryptic and chymotryptic maps of each were determined.

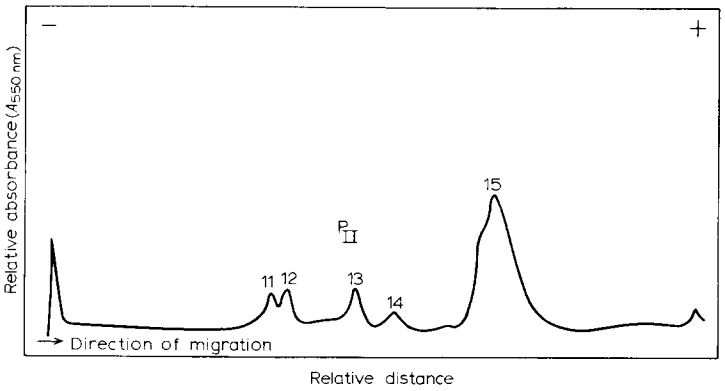


Fig. 1. Dodecyl sulphate–polyacrylamide gel electrophoretic pattern of chromatophore P_{II}, 2-chloroethanol-soluble protein, scanned at 550 nm.

Tryptic peptide fingerprints

Conventional two-dimensional paper electrophoresis and chromatography was performed on trypsin-digested Band 15. Two-dimensional thin-layer electrophoresis and chromatography was performed on tryptic digests of Bands 12 and 13. Based on the specificity of trypsin, the theoretical number of tryptic peptides from Bands 12, 13 and 15 are 29, 18 and 10, respectively. As shown in Figs 2, 3 and 4, Band 12 reveals 14–16 peptides; Band 13 reveals 21 peptides; and Band 15 reveals 10–14 peptides. The number of tryptic peptides of Band 12 is below the theoretical value, this may be due to the presence of undigested core or incomplete digestion of Band 12, or Band 12 may be a dimer. As to Band 15, the inconsistent appearance of T1a, T1b, T5a and T5b as well as the amino acid composition analyses of these peptides (Huang and Kaplan[11]) suggest that T1a and T1b may be derived from T1, and T5a and T5b may be derived from T5.

A comparison of the tryptic peptide fingerprints of the three purified proteins shows that each protein has its own distinct and unique tryptic peptide fingerprint.

TABLE I

AMINO ACID COMPOSITION OF THE PURIFIED CHROMATOPHORE PROTEINS OF *RHODOPSEUDOMONAS SPHEROIDES*

N.D., not determined.

Amino acid	*Mole %*			
	Band 12	*Band 13*	*Band 15**	*Whole chromatophore***
Lys	4.57	4.36	4.27	3.93
His	1.52	1.72	0.00	2.08
Arg	3.55	3.94	3.97	3.41
Asx	8.29	7.56	5.79	5.85
Thr††	5.36	5.57	5.83	5.88
Ser††	4.56	5.19	7.15	5.09
Glx	9.01	8.40	7.30	8.00
Pro	6.58	7.18	4.70	5.40
Gly	10.96	9.86	7.30	8.09
Ala	13.98	12.47	13.31	14.68
Val	8.37	8.65	11.17	8.54
Met	2.14***	2.52***	2.72†	3.18†
Ile	4.55	4.81	5.72	4.77
Leu	8.47	10.21	11.37	10.76
Tyr	2.88	2.70	2.82	2.96
Phe	5.21	4.87	5.12	4.78
Trp	N.D.	N.D.	1.54†	2.21†
Cys	N.D.	N.D.	0.00†	0.39†

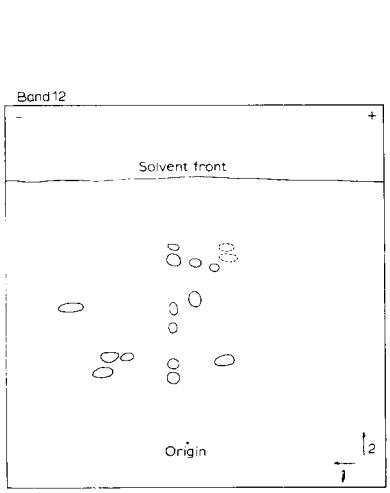
* Values obtained from Fraker and Kaplan[1].

** Values obtained from Fraker and Kaplan[1].

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

† Methionine was determined as methionine sulphone (Moore[9]); cysteine and cystine were determined as cysteic acid (Moore[9]); tryptophan was determined spectroscopically (Edelhoch[10]).

†† Threonine and serine values are corrected for losses by extrapolation to zero time.



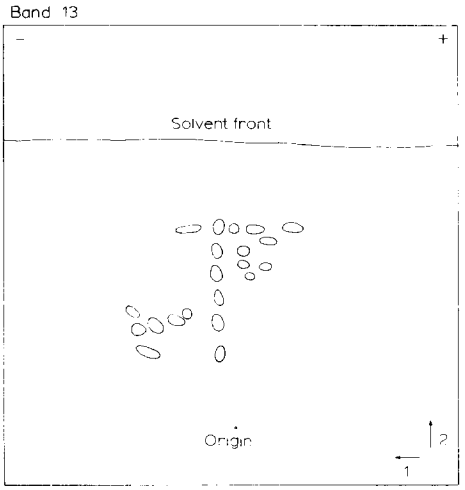
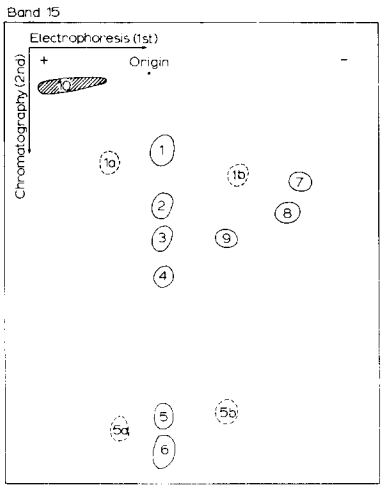


Fig. 2. Tryptic peptide map of purified chromatophore P_{II} protein, band 12. Separation of trypsin-digested Band 12 by two-dimensional thin-layer electrophoresis (1) and chromatography (2) on powdered cellulose sheet.

Fig. 3. Tryptic peptide map of purified chromatophore P_{II} protein, Band 13. Separation of trypsin-digested Band 13 by two-dimensional thin-layer electrophoresis (1) and chromatography (2) on powdered cellulose sheet.



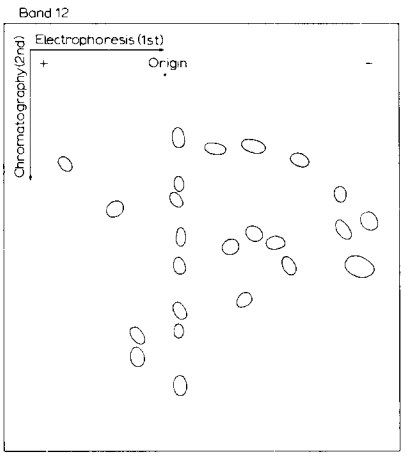
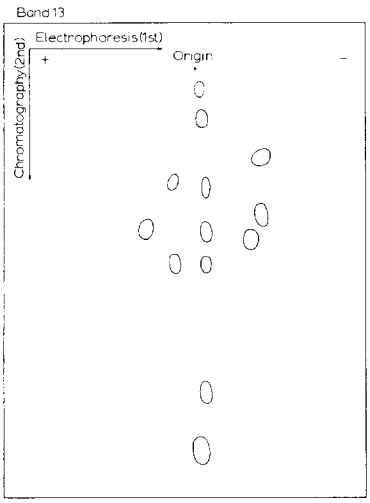


Fig. 4. Tryptic peptide map of purified chromatophore P_{II} protein, Band 15. Separation of trypsin-digested Band 15 by two-dimensional paper electrophoresis (1) and chromatography (2).

Fig. 5. Chymotryptic peptide map of purified chromatophore P_{II} protein, Band 12. Separation of chymotrypsin-digested Band 12 by two-dimensional paper electrophoresis (1) and chromatography (2).

A similar analysis of Bands 12, 13, and 15 employing chymotrypsin further reveals that each of these proteins represents a distinct chromatophore species (see Figs 5, 6 and 7). Because of the uncertainty involving the use of chymotrypsin it is impossible to make rigorous predictions regarding the number of peptides expected.



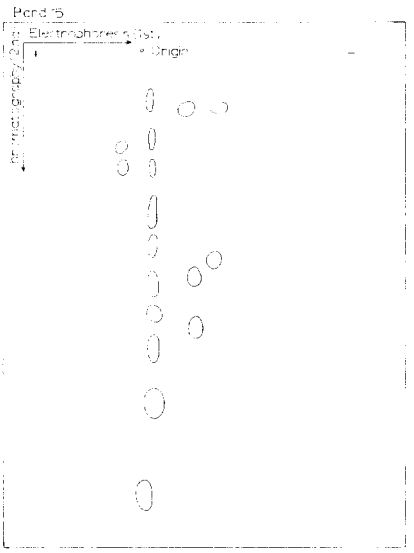


Fig. 6. Chymotryptic peptide map of purified chromatophore P_{II} protein, Band 13. Separation of chymotrypsin-digested Band 13 by two-dimensional paper electrophoresis (1) and chromatography (2).

Fig. 7. Chymotryptic peptide map of purified chromatophore P_{II} protein, Band 15. Separation of chymotrypsin-digested Band 15 by two-dimensional paper electrophoresis (1) and chromatography (2).

Immunochemical studies

An immunochemical analysis was used to define the structure of each of these three purified proteins further. In Fig. 10, A-1, A-2 and A-3 are micro-ouchterlony plates containing antigen in the centre well and antisera in the peripheral wells; B-1, B-2 and B-3 designate antiserum in the centre well and antigens in the peripheral wells.

Band 12 (centre well of A-1) cross-reacts with only anti-band 12, but anti-band 12 (centre well of B-1) cross-reacts with Bands 12, 15, and P_{II}; the cross-reaction suggests the contamination of Band 15 in Band 12, (see below).

Band 13 (centre well of A-2) cross-reacts with only anti-band 13, and anti-band 13 (centre well of B-2) cross-reacts with band 13, as well as suggesting a slight contamination of Band 15 in Band 13. Note also a slight reaction with P_I. This reaction depends upon the specific P_I preparation and we interpret this to mean that some contamination of P_{II} in P_I is possible. Anti-band 15 (centre well of B-3) cross-reacts only with Band 15 and chromatophore P_{II}, but Band 15 (centre well of A-3) cross-reacts with antisera against all three chromatophore proteins and chromatophore P_{II}. Band 12 and Band 13 are probably contaminated with Band 15. The dilution experiment indicates a slight contamination of Bands 12 and 13 with Band 15 (see below).

The fractionation of chromatophore P_{II} proteins includes preparative dodecyl sulphate–polyacrylamide gel electrophoresis. Bands 12 and 13 move behind pigmented Band 15 which comprises more than 50% of the total chromatophore protein. Trailing of the pigmented band is undoubtedly responsible for contamination of Bands 12 and 13 with Band 15. Less than a 10% level of contamination would be expected to have no effect on the amino acid composition and peptide maps of the

individual proteins. Because the immunodiffusion technique is so sensitive, antibodies produced against Bands 12 and 13 containing even trace amount of Band 15 would cross-react with Band 15. However, dilutions of the antisera prepared against Bands 12 and 13 result in loss of anti-band 15 activity without loss of anti-band 12 or 13 activity.

A comparison of the amino acid composition, peptide maps, and antigenic properties of these three chromatophore proteins to proteins derived from the aerobic-enriched cytoplasmic membrane does not enable us to single out any protein which has characteristics similar to the chromatophore-specific species, namely Bands 12, 13 and 15. We conclude, from the evidence presented, that these three chromatophore proteins are not only unique but are also chromatophore-specific proteins. However, until all cytoplasmic membrane proteins (aerobic and anaerobic) are analyzed using similar criteria, this statement must remain for the present, preliminary.

Chromatophore P_I

Less than 5% of the chromatophore protein is insoluble in 2-chloroethanol.

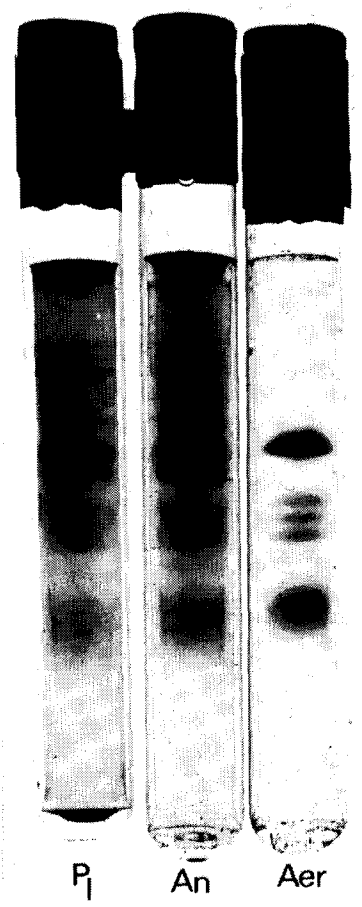


Fig. 8. Dodecyl sulphate–polyacrylamide gel patterns of chromatophore P_I (P_I), anaerobic cell envelope proteins (An), and aerobic cell envelope proteins (Aer).

Chromatophore P_I shows a dodecyl sulphate–polyacrylamide gel profile almost identical to those obtained from the aerobic cell envelope and the anaerobic cell envelope (Fig. 8). This observation leads to the question as to whether chromatophore P_I is actually part of the chromatophore or whether it represents contamination from the envelope membrane fraction.

In order to answer this question, the following experiment was performed. A culture of *R. spheroides* was grown anaerobically in Sistrom's medium containing 20 μCi of [U-^{14}C]phenylalanine and 10 μg/ml of carrier phenylalanine. The chromatophores were isolated and purified as described[1]. During each purification step, 1 mg of crude or purified chromatophore protein was extracted using 2-chloroethanol and separated into a soluble P_{II} fraction and insoluble P_I fraction. The radioactivity of each fraction was then determined. The P_I content present in the chromatophore proteins is represented as the cpm ratio of P_I to $P_I + P_{II}$. As shown in Table II, chromatophore P_I content decreases as the purification procedure proceeds. The combined first Sepharose 2B column chromatography and sucrose gradient centrifugation result in a 10-fold depletion of chromatophore P_I. The application of additional shearing forces by passage through the French pressure cell, and further purifications as indicated under the second Sepharose 2B column chromatography and second sucrose gradient centrifugation demonstrate no further depletion of chromatophore P_I. This result is consistent with the interpretation that a small but discrete amount of chromatophore P_I is part of the chromatophore. This leads to the question as to its structural role in the chromatophore.

Origin of chromatophore proteins

In order to monitor the biogenesis of the chromatophore under varying conditions of growth, a 400-ml culture of *R. spheroides* was grown aerobically under 100% O_2 in the dark in Sistrom's medium containing 30 μCi of ^{14}C-labelled amino acid mixture (100 μCi/ml, Schwartz BioResearch) and 1.5 μg/ml of the appropriate carrier amino acids. The cells were harvested at a very low cell density ($A_{680\,nm}=0.3$) to ensure high aerobiosis. The washed ^{14}C-labelled aerobic cells were inoculated into 800 ml of fresh Sistrom's medium containing 80 μCi of ^{3}H-labelled amino acid

TABLE II

PURIFICATION OF CHROMATOPHORES AND CHROMATOPHORE P_I CONTENT

Purification procedure	*P_I cpm/mg chromatophore protein*	*P_{II} cpm/mg chromatophore protein*	*$P_I + P_{II}$ cpm/mg chromatophore protein*	*P_I% = (P_I +*
First french pressure cell	9326	31 550	40 876	22.8
First Sepharose 2B	2250	24 380	26 640	8.5
First sucrose gradient	492	20 560	21 052	2.3
Second french pressure cell*	99	5 770	5 869	1.7
Second Sepharose 2B	156	6 910	7 066	2.2
Second sucrose gradient	85	5 320	5 425	1.6

* Unlabelled purified chromatophores were added as carrier.

mixture (500 μCi/ml, Schwartz BioResearch) and 20 μg/ml of the appropriate carrier amino acids. The culture was grown anaerobically under N_2 in the light (130 ft-candles). During growth, cell mass was determined at $A_{680\ nm}$, *in vivo* chlorophyll was determined at $A_{855\ nm}$, and the incorporation of ^{3}H-labelled amino acids into the culture was also determined. The synthesis of pigments and the incorporation of ^{3}H-labelled amino acids into cells follow the growth curve of *R. spheroides* (Fig. 9). Samples were removed and cells harvested at three stages: A, early logarithmic phase; B, late logarithmic phase; and C, stationary phase. Chromatophores were isolated and purified as described[1].

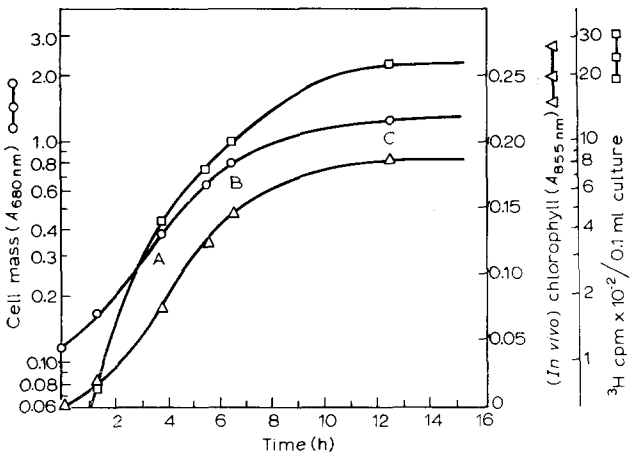


Fig. 9. Washed, aerobic ^{14}C-labelled *R. spheroides* were adapted to anaerobic conditions, and labelled with ^{3}H-labelled amino acids. The cell mass was determined at $A_{680\ nm}$ and the *in vivo* chlorophyll was determined at $A_{855\ nm}$. The ratio of chlorophyll and chromatophore isolated from three different stages of cell growth was as follows.

Stage	*Whole cells* $A_{855\ nm}$	*Chromatophores*			
		$A_{260\ nm}$	$A_{280\ nm}$	$A_{855\ nm}$	$A_{855\ nm}/A_{280\ nm}$
A	0.075	0.136	0.134	0.115	0.86
B	0.145	0.406	0.407	0.650	1.60
C	0.185	1.460	1.483	2.350	1.59

When the ^{14}C and ^{3}H cpm per mg protein of chromatophores at Stages A, B and C were compared (Table III), it is apparent that the increase of ^{3}H counts is due to incorporation into new chromatophore material and the decrease of ^{14}C counts is due to dilution following resumption of growth under anaerobic conditions. The rate of ^{3}H incorporation into chromatophores is faster than into total cell protein, indicating that the differential rate of chromatophore biosynthesis greatly exceeds the rates of synthesis of most other cell proteins.

Almost all ^{3}H counts go into the 2-chloroethanol-soluble P_{II} fraction, indicating that either no more chromatophore P_I is synthesized during the anaerobic phase of growth or the level is so low as to be unobservable. At early logarithmic

TABLE III

THE ORIGIN OF CHROMATOPHORE P_I

Washed, ^{14}C-labelled aerobic cells were adapted to anaerobic conditions in a medium containing ^{3}H-labelled amino acids. The incorporation of both ^{3}H and ^{14}C into whole cells, chromatophores, chromatophore P_I (2-chloroethanol-insoluble fraction) and chromatophore P_{II} (2-chloroethanol-soluble fraction) was followed employing these fractions obtained from three different stages of cell growth (Fig. 9). The cell mass was determined at $A_{680\ nm}$ and *in vivo* bacteriochlorophyll was determined at $A_{855\ nm}$.

Stage	*Relative amount of cell growth based upon* $A_{680\ nm}$	*Relative amount of bacterio-chlorophyll based upon* $A_{855\ nm}$	*cpm incorporated per 0.1 ml of culture*		*cpm per mg of chromatophore*		*cpm per mg of* P_I *protein*		*cpm per* P_{II} *prot*	
			^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{1}
A	1.00	1.0	1430	10 000	344	1306	30	17 540	314	4
B	2.10	2.7	3300	10 000	608	675	16	720	592	6
C	3.27	4.7	7400	10 000	849	512	18	400	831	4

phase, Stage A, the bulk of the membrane-localized ^{14}C fractionates with the 2-chloroethanol-insoluble P_I fraction and we conclude that chromatophore P_I appears to be synthesized only during aerobic growth. The absence of ^{14}C counts in chromatophore P_I at Stages B and C, and the presence of low levels of ^{14}C counts in chromatophore P_{II} at Stages A, B and C can be explained as follows: the turnover of ^{14}C-labelled protein, during the period of transition from aerobic to anaerobic growth is considerable (Kaplan, unpublished results; with a 6-h growth lag during aerobic to anaerobic transition, nearly 20% of previously trichloroacetic acid-precipitable ^{14}C-labelled amino acid becomes soluble), and the breakdown of ^{14}C-labelled protein does reincorporate into chromatophore P_{II} whose differential rate of synthesis is high relative to other cell protein. The absence of ^{14}C in P_I at Stages B and C probably reflects levels too low to detect. We should further point out that the amount of ^{14}C contained in the P_{II} fraction per mg protein is less than 1% of that found in Fraction P_I per mg protein at Stage A of growth following transition. Because of this and the existence of some turnover, we feel that the level of ^{14}C in P_{II} is inconsequential. Chromatophore P_I probably represents the link between the chromatophore and the cytoplasmic membrane. Although this experiment is not conclusive and other interpretations are possible it is compatible with the idea that P_I is the attachment site of chromatophore and cell membrane.

Whether there is a concurrent biosynthesis of chromatophore P_{II} proteins and pigment systems could be answered by determining if there is a constant chlorophyll to chromatophore protein ratio throughout chromatophore formation. Although the ratio of chlorophyll ($A_{855\ nm}$) to protein content ($A_{280\ nm}$) at Stage A is much lower than the values at Stages B and C (Fig. 9), new chromatophore P_{II} proteins are not necessarily synthesized at a greater differential rate than is chlorophyll at the initial stage of chromatophore differentiation. As indicated by their insolubility in 2-chloro-

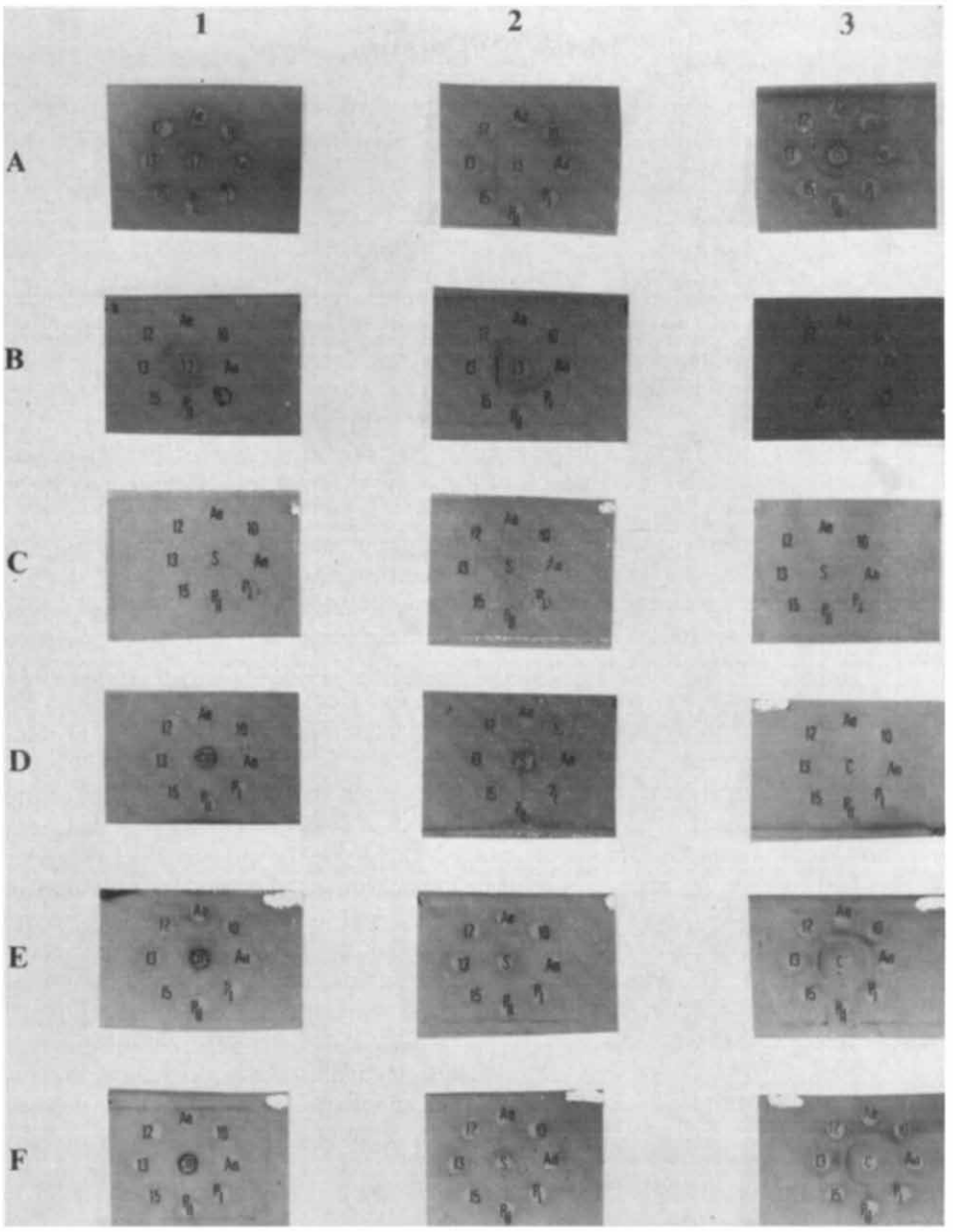


Fig. 10. Immunodiffusion test of cross-reactivity. Section C-1, supernatant isolated from Stage A; Section C-2, supernatant isolated from Stage B; Section C-3, supernatant isolated from Stage C (Fig. 9); Section D, cell envelope membrane fraction, supernatant, and chromatophore fraction isolated from cells grown under 5% O_2; Section E, cell envelope membrane fraction, supernatant, and chromatophore fraction, isolated from cells grown under 2.5% O_2; Section F, cell envelope membrane fraction, supernatant, and chromatophore fraction isolated from cells grown under 1% O_2. Centre wells, antigens; outer wells, antisera (except Section B: centre wells, antisera; outer wells, antigens). The symbols are: aerobic cell envelope (Ae); aerobic cell envelope protein, Band 10 (10); anaerobic cell envelope membranes (An); chromatophore P_I (P_I); Chromatophore P_{II} (P_{II}); chromatophore P_{II} protein, Band 15 (15); supernatant, S; cell envelope membranes, cm; chromatophores, C.

ethanol and high ^{14}C content, the chromatophores isolated at Stage A (Table III) are unusually high in chromatophore P_I which may represent the special site of chromatophore formation. At Stages B and C, both chromatophore protein and chlorophyll are continuously synthesized and their constant ratio indicates a co-ordinate biosynthesis of chromatophore protein and chlorophyll.

Biogenesis of chromatophore-specific proteins and environmental influences

It has been known that the assembly of chromatophores requires the concurrent biosynthesis of both bacteriochlorophyll and chromatophore proteins, but the sequence of synthesis of chromatophore proteins is not yet clear. Using antisera against the three chromatophore-specific proteins (Bands 12, 13 and 15) we are in a position to monitor the sequence of biosynthesis of these three proteins. We are interested in knowing (1) at what O_2 tension does the biogenesis of chromatophore protein begin; and (2) if any preferential biosynthesis of a specific chromatophore protein exists at different O_2 tensions.

Cultures of *R. spheroides* were grown in the light under different oxygen tensions (10% O_2, 5% O_2, 2.5% O_2 and 1% O_2). Each culture was harvested at a cell density of $2 \cdot 10^9$ cells/ml. The supernatant, cell envelope membranes, and chromatophores were isolated as described. The protein concentrations of all three fractions were adjusted to 1 mg/ml. Sodium dodecyl sulphate was added to a final concentration of 0.1% to solubilize the cell envelope membrane and chromatophore proteins. No fraction corresponding to chromatophores was isolated from cells grown at O_2 tensions of 5% or greater.

In Fig. 10 (Sections D-3, E-3, and F-3), we see that chromatophores (centre wells), isolated from cells grown at 2.5% O_2 tension or below, cross-react with antisera against all three chromatophore proteins (E-3 and F-3); the cross-reaction suggests that the chromatophore proteins are formed synchronously at least in a qualitative manner. In Fig. 10 (Sections D-1, E-1, and F-1), cell envelope (centre wells) cross react with antisera against cell envelope proteins but not with antisera against chromatophore proteins; the simplest interpretation of this result is that the formation of chromatophores has no effect on the nature of the cytoplasmic membranes. In Fig. 10 (Sections D-2, E-2, and F-2; Sections C-1, C-2, and C-3), supernatant fraction (centre wells) shows no cross-reactivity with antisera against the cell envelope proteins and chromatophore proteins; once the chromatophore proteins are synthesized they are rapidly incorporated into structures and little or no free pool of these proteins is present in the cell.

DISCUSSION

Approximately 95% of the chromatophore protein (P_{II}) can be resolved on dodecyl sulphate–polyacrylamide gels into 5–6 major bands with molecular weights ranging from 10000 to 50000[1]. The question of whether a single structural protein is responsible for the organization of the chromatophore is still unanswered. Biochemical studies of cell envelopes and chromatophores demonstrate protein heterogeneity and therefore supports the idea that many proteins are involved in the architecture of the membrane structure. Pigmented Band 15 is a predominant component of chromatophores and must perform a very important structural role in the chromatophores.

This does not rule out possible structural roles played by other chromatophore proteins. With the help of electron microscopic studies using native and reassembled membranes, we may be able to assess the contribution of each chromatophore protein to chromatophore membrane structure. With the help of biochemical assays and reconstitution experiments, we may also be able to elucidate the functional role of a specific chromatophore protein.

All the chromatophore-specific proteins contain approximately 65 mole % non-polar amino acids and 35 mole % polar amino acids. These are more hydrophobic than the cell envelope proteins which contain only 50–60 mole % non-polar residues. The higher ornithine lipid concentration of chromatophores is thought to accommodate the more hydrophobic proteins and pigments (Gorchein[12]). The high content of non-polar residues of the chromatophores is reflected by their low solubility in aqueous systems.

The chromatophore proteins of wild-type *R. spheroides* consist of three reaction centre (RC) proteins, RC_a, RC_b and RC_c, as well as two additional proteins, a larger protein with molecular weight of 46000 and a smaller protein with molecular weight of 11000[13]. Reaction centre proteins account for 20–30% of total chromatophore protein, and contain 65% non-polar residues (Feher[14], Clatyon and Haselkorn[13]). From the carotenoidless mutant strain, reaction centre proteins could be separated from the light harvesting component by lauryldimethylamino oxide treatment, centrifugation, and $(NH_4)_2SO_4$ fractionation.

Because of the similarities in the molecular weights of the reaction centre proteins (Clayton and Haselkorn[13]) and our chromatophore-specific proteins, our chromatophore-specific protein, Band 13 (mol. wt 27000) may be equivalent to RC_a; chromatophore-specific protein, Band 14 (mol. wt 20000) may be equivalent to either RC_b or RC_c. Clayton's other two major components with molecular weights of 46000 and 11000 do not carry out photochemical reactions; they may be equivalent to our chromatophore-specific proteins, Band 12 (mol. wt 44000) and Band 15 (mol. wt 10000), respectively. Whether reaction centre proteins also play a structural role in constructing the chromatophore membranes is still unknown.

The problem of the mechanism of chromatophore biogenesis has been discussed (Flexer *et al.*[15], Boatman[16], Gorchein *et al*,[17], Oelze and Drews[18]). Considering the structure of the chromatophores, which lack a specific DNA and genetic system, it is doubtful that chromatophores arise by autonomous self-replication.

When aerobic cells are adapted to anaerobic photosynthetic conditions, the isolated anaerobic cytoplasmic membrane proteins still have the same electrophoretic mobilities on dodecyl sulphate gels as that of aerobic cell envelope proteins; this fact suggests a common membrane structure based on similar protein components. Likewise, immunochemical studies suggest a similar relationship. As shown here, newly synthesized chromatophores contain more than 95% chromatophore-specific protein P_{II}, which is different from the cell envelope proteins; and less than 5% chromatophore protein, P_I, which shows an electrophoretic pattern on dodecyl sulphate gels similar to those of cell envelope proteins (Fig. 8).

Our results strongly suggest that chromatophore P_I is a part of native chromatophores. Furthermore, the results of shift-labelling experiments suggest that P_I represents the site of the growth of chromatophores or the link between the chromatophore and the cytoplasmic membrane. The results certainly do not rule

out the possibility that P_i is diluted throughout the chromatophore membrane by the incorporation of chromatophore-specific proteins. We are in the process of providing an unambiguous answer to this very important question. The synthesis of bacteriochlorophyll is correlated with protein synthesis, and the addition of protein inhibitors stops both bacteriochlorophyll and chromatophore formation (Bull and Lascelles[19], Burnham and Lascelles[20], Drews[21]). In the bacteriochlorophyll mutant strain of *R. rubrum* no chromatophores are formed when the synthesis of bacteriochlorophyll is totally or partially blocked (Oelze *et al.*[22]). Also, the inhibition of bacteriochlorophyll synthesis by O_2 will stop chromatophore formation (Cohen-Bazire *et al.*[8], Oelze and Drews[23]). Similarly, in the mutant PM-8 of *R. spheroides*, the formation of chromatophore membrane is coupled with the synthesis of reaction centre proteins (Sistrom and Clayton[24]).

Our experiments substantiate both the suggestion that the adaptation of non-pigmented aerobic cells to form chromatophores involves the incorporation of chromatophore-specific proteins and pigments onto the cytoplasmic membrane, and that the continued formation of chromatophores is then independent of the cytoplasmic membrane. This represents a slight modification of the mechanism proposed by Segen and Gibson[25]. These conclusions are in contrast to the suggestion that chromatophore membranes are formed by the growth of an identical, preexisting membrane structure[18]. We do not agree with the proposal of Oelze and Drews[18,26] that the disappearance of chromatophores from the pigmented anaerobic cells adapting to aerobic condition occurs by the reversible differentiation of the chromatophore membrane to the cytoplasmic membrane. Because there exists differences in the protein composition of the chromatophore and cytoplasmic membranes, we suggest that the disappearance of chromatophores is due to dilution through cell division and the turnover of the chromatophore.

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