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Deficiencies of Chromatophore Proteins in Some Mutants of *Rhodopseudomonas spheroides* with Altered Carotenoids

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Chromatophore proteins of a wild type and three mutant strains of *Rhodopseudomonas spheroides* were examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The mutants consisted of a green and a blue-green one, whose phenotypes were essentially the same as those of known mutants, and a brown one, which may be a double mutant and represents a new phenotype. Wild-type chromatophores contained at least six major and seven minor protein bands, with molecular weights ranging from 10,000 to 65,000. The green mutant contained the same protein bands in the same relative quantities. The brown mutant had one protein completely missing and no other alterations. The blue-green mutant was deficient in a different protein, and had reduced quantities of all proteins with molecular weights less than 25,000. Chromatophores were separated into a fraction containing the reaction centers and a fraction containing the light-harvesting bacteriochlorophyll by treatment with sodium dodecyl sulfate. Eight of the proteins were found only in the reaction center fraction, one was only in the light-harvesting fraction, and the remainder were present in both fractions. The protein missing from the brown mutant was found to be a component of the reaction center fraction, whereas the proteins which were missing from the blue-green mutant were all components of the light-harvesting fraction. Some implications for the structure and biogenesis of chromatophores are discussed.

Mutants of photosynthetic bacteria with an altered carotenoid content have been known since the classical work of Cohen-Bazire et al. (4). Subsequent experiments by these workers and others (11, 13, 14, 25) have led to the view, now generally accepted, that all the changes observed in the chemistry and physiology of such mutants can be explained as direct or indirect results of deficiencies in certain enzymes responsible for carotenoid biosynthesis. Thus, the green mutant of *Rhodopseudomonas spheroides*, which lacks the two major carotenoids found in the wild type, contains instead neurosporene and chloroxanthin, and this has been taken as evidence that this mutant is deficient in an enzyme which methylates chloroxanthin. Similarly, the blue-green mutant, which lacks colored carotenoids and accumulates phytoene, is usually thought to lack the enzyme which converts phytoene to phytofluene (13).

We present here the results of a study of the proteins from chromatophores by gel electrophoresis, in which we have compared the patterns obtained from a wild-type strain *R. spheroides* with those obtained from three mutants. The mutants are a green mutant (G), phenotypically identical to the original green mutant of Cohen-Bazire et al., a blue-green mutant (BG) which resembles the blue-green mutant isolated by Griffiths and Stanier (11), and a brown mutant (B) which appears to represent a phenotype that has not been reported previously. The first one shows a normal spectrum between 700 and 900 nm, whereas the last two have altered spectral characteristics in this region. There is no detectable difference in the pattern of protein bands from wild type and from mutant G; however, in the other two mutants, one or more protein bands are missing or greatly reduced in quantity. In mutant B, the missing band appears to be one of the major components of the reaction centers; by contrast, in mutant BG all the components of the reaction centers are present in normal amounts, but the remaining protein bands are greatly reduced or absent.

MATERIALS AND METHODS

Production of mutants. The wild-type organism used in this study was *R. spheroides* NCIB 8327. Mutants

were isolated by picking colonies from plates which had been seeded with cells treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (16). Mutants G and B were maintained in stabs, and mutant BG was kept on slopes with periodic reisolation of a single clone from a plate.

Growth of cells and preparation of chromatophores. Cells were grown semianaerobically as described previously (6). Under our conditions, bulk cultures of the different mutants were never contaminated with more than 1 to 3% of wild-type organisms. Chromatophores were isolated by using gradients of CsCl as described previously (7), and stored at 4 C in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.5.

Electrophoresis of chromatophore proteins. Chromatophore proteins were dissolved in sodium dodecyl sulfate (SDS)-phosphate buffer for gel electrophoresis in two ways. (i) Chromatophores were heated for 10 min at 100 C in 0.01 M sodium phosphate (*p*H 7.2) containing 1% SDS and 1% β-mercaptoethanol; after cooling, the solution was dialyzed against 0.01 M sodium phosphate-0.1% SDS-0.1% β-mercaptoethanol. In some experiments, lipid was extracted from the chromatophores beforehand (8), and the resulting pellet of protein was treated as described above. (ii) An aqueous suspension of chromatophores was extracted twice with phenol, and the combined phenol extracts were subjected to the dialysis procedure of Viñuela et al. (27), which changes the solvent in several steps to 0.01 M sodium phosphate (*p*H 7.2)-0.1% SDS-0.14 M β-mercaptoethanol. It was found that SDS had to be included at a concentration of 1% at all steps of this procedure except the first and the last two; if the SDS was omitted from the solvents used in these steps the chromatophore proteins formed insoluble aggregates which did not redissolve.

Electrophoresis was carried out in 10% polyacrylamide gels using the phosphate-SDS buffer system of Weber and Osborn (29). All the experiments reported were performed in 60-mm tubes, but we have obtained the same results with 120-mm tubes. The amount of protein applied to each gel was 100 to 150 μg. Gels were fixed and stained by the procedure of Schnaitman (23).

Determination of molecular weights. The following proteins were used for calibrating gels for molecular weight determinations: bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.); hemoglobin, myoglobin, ovalbumin, and pepsin (Mann Research Co., New York, N.Y.); chymotrypsinogen and α-chymotrypsin (Boehringer and Soehne, Mannheim, Germany). The proteins were dissolved in phosphate-SDS before use (29). Gels were standardized by determining the mobilities of these proteins relative to chymotrypsinogen, and plotting these relative mobilities against the logarithm of their molecular weights. The usual linear relation was observed, with least squares correlation coefficient in the range −0.995 to −1.0. Molecular weights of unknowns were calculated from their relative mobilities, under identical conditions, in tubes to which chymotrypsinogen (10 μg) had been added as an internal standard.

Preparation of reaction centers. Chromatophores from the strains of *R. spheroides* used in this study can be split cleanly into two fractions which contain, respectively, reaction centers and light-harvesting bacteriochlorophyll, as evidenced by spectral properties, by brief treatment with SDS at low concentrations, in a manner similar to that described by Thornber et al. (26) for *R. viridis*. Typically, a suspension of chromatophores, in 0.1 M sodium phosphate (*p*H 7.2) containing 4.5 mg of protein per ml, was mixed with SDS to give a final concentration of 0.45 mg/mg of protein. After 30 min at 20 C, the mixture was centrifuged through a discontinuous sucrose gradient essentially as described by Reed and Clayton (22). Alternatively, the mixture of chromatophores and SDS was allowed to stand for 30 min at 20 C, and then centrifuged in the cold for 2 hr at 30,000 × *g*. The supernatant solution consisted of almost pure reaction centers. The pellet, which contained only light-harvesting bacteriochlorophyll, was washed once by resuspension in 0.01 M Tris-hydrochloride (*p*H 7.5) followed by centrifugation, and finally resuspended in this buffer. The basis for this separation appears to be the strong tendency for the light-harvesting fraction to reaggregate after it has been detached from the reaction centers. For this reason, the concentrations of protein and SDS are rather critical; at lower concentrations of SDS the two fractions do not become completely detached, and at higher concentrations of detergent the light-harvesting fraction does not reaggregate. The fractions from the chromatophores were prepared for electrophoresis by heating with SDS as described above.

Analytical methods. Protein and pigment were assayed as described previously (6). Spectra were obtained with a Cary model 14R recording spectrophotometer, operated in the IR1 or IR2 mode (2).

RESULTS

Characterization of mutants. Spectra of chromatophores from the wild type and the three mutants are presented in Fig. 1. The absorption peaks due to bacteriochlorophyll in the spectrum of mutant G are virtually identical to those of the wild type. The differences from the wild type are all found in the carotenoid region; the spectrum in this region is the same as the spectrum of the green mutant isolated by Cohen-Bazire et al. (4). By contrast, chromatophores from mutants B and BG show spectral differences from wild-type chromatophores in the far red as well as in the carotenoid region. The blue-green mutant virtually lacks peaks due to carotenoids and shows a typical shift of the major peak due to bacteriochlorophyll from 850 to 870 nm. The minor peak at 800 nm is also greatly reduced in height relative to the major one. In the brown mutant, the effects are much less marked. The carotenoid peaks are all shifted towards the blue by 5 nm, whereas the major bacteriochlorophyll peak is shifted the other way, from 850 to 855 nm. In addition, the peak at 800 nm is reduced in height relative to the major peak. These changes are not due to alterations of the bacteriochlorophyll itself, since spectra of acetone-methanol extracts of chromatophores show normal peaks.

The carotenoids of the mutants were extracted by the method of Jensen et al. (14) and subjected to thin-layer chromatography on Silica Gel G with benzene used as solvent. The green mutant lacked the major yellow and red carotenoids of the wild type, and contained, instead, two yellow carotenoids with different mobilities. We have not made a definitive identification of these carotenoids, but the evidence from the spectrum and chromatography indicate that mutant G is phenotypically identical with the green mutant of Cohen-Bazire et al. On the other hand, the brown mutant B does not seem to be identical with the brown mutant described by Griffiths and Stanier (11). Mutant B has one major carotenoid which behaves chromatographically like the yellow carotenoid, spheroidene, of the wild type. It also has a small amount of a red carotenoid, which however, is more polar than the wild type red pigment spheroidenone; unlike the brown mutant of Griffiths et al., it does not contain detectable amounts of neurosporene or chloroxanthin. Further, the absorption spectrum of the carotenoids is quite dissimilar from the spectrum found by Nakayama (19). For these reasons we believe that mutant B represents a different phenotype from the original brown mutant found by Griffiths et al. (11).

The green mutant had a significant rate of spontaneous reversion to wild type, which we estimate to be at least 10^{-6}; however, its growth rate under semianaerobic conditions was the same as that of the wild type. The other two mutants both grew more slowly under semianaerobic conditions (doubling time greater by a factor of two), although aerobic growth was as vigorous as wild type. We have not been able to detect reversion from the brown mutant to wild type, although we have occasionally seen green colonies on plates of this mutant. The blue-green mutant also reverted to wild type at a significant rate. Cultures of the blue-green mutant frequently contained small amounts of colored carotenoids, which were spectrally similar to wild-type carotenoids. This is not due to the presence of wild-type organisms in these cultures, but to the fact that the mutant is leaky; thus, a high proportion of pale pink colonies, but few red ones, appear on plates. For these reasons we believe that mutant BG arose by a single point mutation. On the other hand, the brown mutant may be a double mutant.

Electrophoresis of chromatophore proteins. Figure 2 shows the electrophoresis pattern of wild-type chromatophores which had been solubilized by heating with SDS. The left-hand frame is a photograph of an actual run, and the right-hand frame is an idealized drawing containing all the protein bands we have been able to distinguish reproducibly in this and other experiments. The pattern obtained from chromatophores solubilized by heating with SDS with prior extraction of lipid is identical to that shown in Fig. 2, except that the four fastest moving bands (J, K, L, and M) are less diffuse. Solubilization of chromatophores by the method of Viñuela et al. (27), modified as described above, also leads to an identical but slightly less clear-cut pattern. The fact that there is no significant difference between the electrophoretic patterns obtained by such different methods of solubilization leads us to believe that the protein bands we have observed are not artefacts.

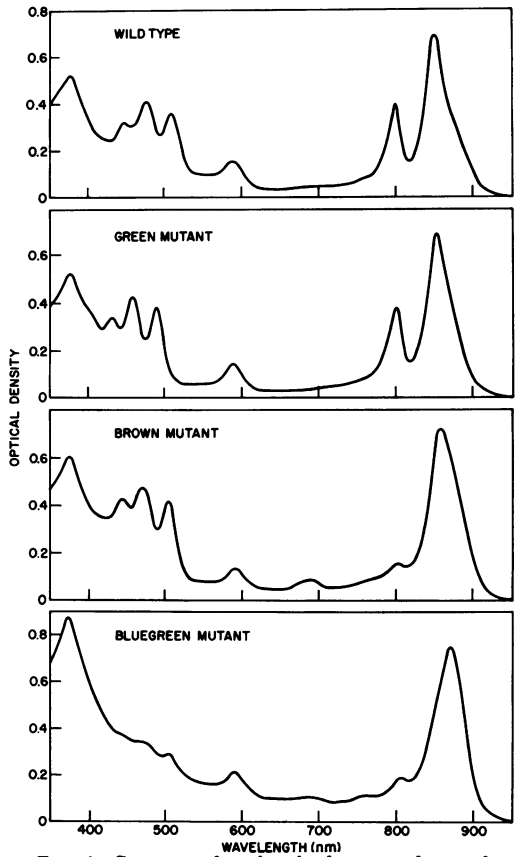

FIG. 1. *Spectra of isolated chromatophores from wild type and mutants. Top frame, wild type; second frame, green mutant; third frame, brown mutant; bottom frame, blue-green mutant.*

Table 1 lists the molecular weights of the protein bands shown in Fig. 2, as determined from approximately 20 electrophoresis patterns. The accuracy of these molecular weights is within 5% in most cases; however, the error is nearer 10% for the three smallest proteins. From scans of stained gels it was found that the proteins in bands H, L, and M were present in largest

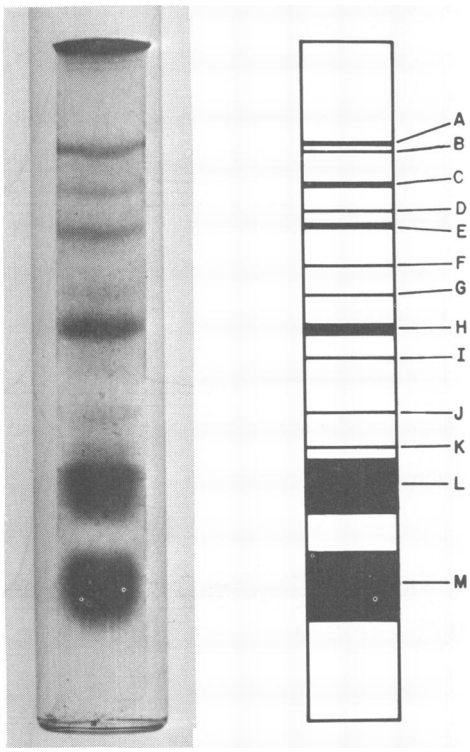

FIG. 2. *Electrophoresis pattern of wild-type chromatophore proteins solubilized by heating with SDS. Left frame, photograph of an actual run; right frame, idealized drawing showing all bands that have been distinguished. The direction of migration was from top to bottom.*

amounts; those in bands A, C, and E were also present in relatively large amounts, whereas the remainder were present as minor components. It is probable that there are two proteins in band C, and at least two in band L; however, we have not been able to resolve these bands consistently, and have therefore reported them as single bands.

Electrophoretic patterns from the three mutants are shown in Fig. 3. The pattern from mutant G is not significantly different from the wild-type pattern. However, there are distinct differences in the patterns from the other two mutants. In the case of mutant B, there was only one observable difference; this was the absence of band E, corresponding to a molecular weight of 47,000, which is one of the major components of wild-type chromatophores. No other difference could be detected. Mutant BG, on the other hand, showed alterations in more than one band; thus, band C of molecular weight 55,000 was virtually absent, whereas bands K, L, and M with molecular weights 10,000 to 16,000 were all reduced in amount (at least 50% in the case of band M, as determined from scans of the gels). The apparent increase in some of the other bands is due to the fact that the proteins which are deficient in this mutant are major components of the chromatophores.

Electrophoresis of chromatophore subfractions. Chromatophores were split into two fractions containing, respectively, reaction centers (RC fraction) and light-harvesting bacteriochlorophyll (LH fraction). The spectra of the two fractions from the wild type (Fig. 4) show that the RC fraction contained only reaction center bacteriochlorophyll, whereas the LH fraction contained only light-harvesting bacteriochlorophyll. The recovery of protein and the specific bacteriochlorophyll content of each fraction are shown in Table 2. The total recovery of bacteriochlorophyll was 82%; thus, most of the light-harvesting bacteriochlorophyll remains bound to the LH fraction during the isolation procedure. Electrophoresis patterns of the two fractions are shown in Fig. 5. All but one of the protein bands with molecular weight greater than 16,000 are associated with the RC fraction; the exception is band C, which is found only in the LH fraction. The remaining bands of molecular weight 10,000 to 16,000 appeared to be associated with both fractions. The bands found in the LH fraction, namely C, K, L, and M, are the ones which were either absent or reduced in amount in whole chromatophores from mutant BG.

Chromatophores from the three mutants were split into RC and LH fractions by the technique used with the wild type. The specific bacterio-

TABLE 1. *Molecular weights of chromatophore proteins*

Protein band[a]	Molecular wt[b]	Location[c]
A	64,500 ± 2,600	RC
B	60,100 ± 2,300	RC
C	55,200 ± 1,500	LH
D	50,500 ± 2,700	RC
E	47,300 ± 2,200	RC
F	38,600 ± 900	RC
G	35,800 ± 900	RC
H	31,200 ± 1,000	RC
I	27,700 ± 1,000	RC
J	21,000 ± 600	RC, LH
K	16,100 ± 1,100	RC, LH
L	13,500 ± 700	RC, LH
M	9,900 ± 800	RC, LH

[a] Designations of bands correspond to Fig. 1.

[b] Mean ± standard error, determined from runs of chromatophore proteins solubilized by heat or phenol treatment. Number of determinations for each band varied between 4 and 21.

[c] Location in chromatophore: RC, fraction containing reaction centers; LH, fraction containing light-harvesting bacteriochlorophyll.

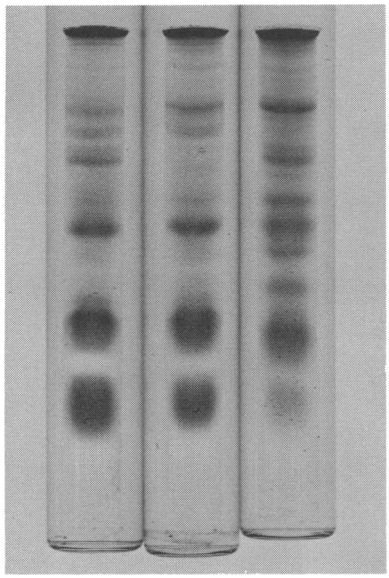

FIG. 3. *Electrophoresis of chromatophore proteins from mutants. Proteins were solubilized by heating with SDS. Gels, from left to right, contain chromatophore proteins from mutant G, mutant B, and mutant BG, respectively. The direction of migration was from top to bottom.*

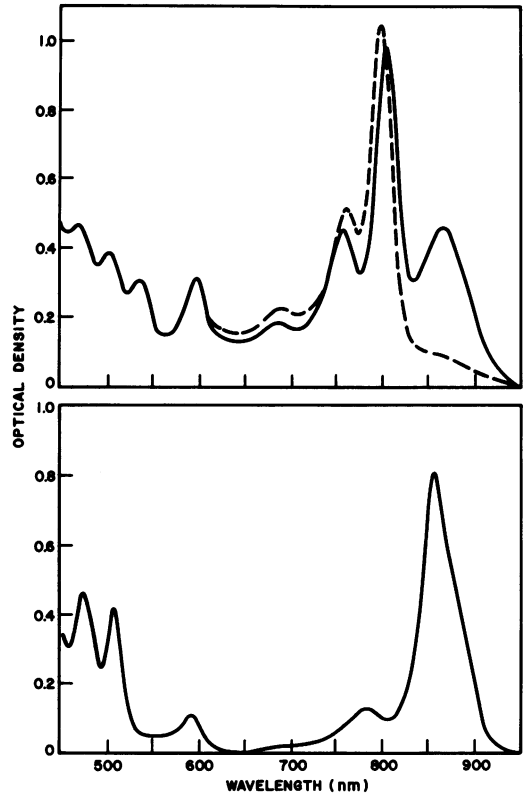

FIG. 4. *Spectra of reaction centers and light-harvesting fraction from wild-type chromatophores. Top frame, RC fraction, in 0.1 M phosphate, pH 7.2, + 0.001 M dithiothreitol + 0.001 M KCN; solid line, IR1; dashed line, IR2 modes. Bottom frame, LH fraction in 0.01 M Tris-hydrochloride, pH 7.5; solid line, IR1 or IR2 mode.*

chlorophyll contents of the RC fraction were about the same for all three mutants (Table 2). The specific bacteriochlorophyll contents of the LH fractions from mutants G and B were almost identical with that of the LH fraction from the wild type, whereas the bacteriochlorophyll content of the LH fraction from mutant BG was about 30% lower. The recovery of protein in the two fractions from mutants G and B was almost the same as for the wild type; however, in the case of mutant BG, the RC fraction accounted for 61% of the total chromatophore protein, rather than 25 to 35% as in the other mutants (Table 2). Together with the results of electrophoresis of whole chromatophores, this suggests strongly that chromatophores from mutant BG have a reduced amount of the whole LH fraction. The infrared spectrum and electrophoresis pattern of the RC fraction from this mutant were indistinguishable from those of the wild type.

The RC fraction from mutant B had a spectrum that was very similar to that shown in Fig. 4 (upper frame), except that the peak at 805 nm was shifted to 800 nm. The light-induced changes in all peaks were virtually identical to those observed in wild-type reaction centers. Electrophoresis of this fraction confirmed that band E, which is a component of wild-type reaction centers (*see* Table 1), was absent from reaction centers of mutant B. The LH fraction from this mutant gave an electrophoretic pattern which was identical to that of the wild type; however, the absorption peak due to bacteriochlorophyll was at 860 nm, rather than 855 nm as in the wild type. Thus, the shift in absorbance which is seen

TABLE 2. *Specific bacteriochlorophyll content of chromatophores and subfractions*

Organism	Specific bacteriochlorophyll content[a]			
	Chromatophore	RC fraction	LH fraction	Per cent of chromatophore protein in RC fraction
Wild type ...	0.155	0.017	0.147	26
Mutant G ...	0.159	0.010	0.169	27
Mutant B ...	0.142	0.010	0.141	34
Mutant BG .	0.039	0.008	0.108	61

[a] Expressed as milligrams of bacteriochlorophyll per milligram of protein.

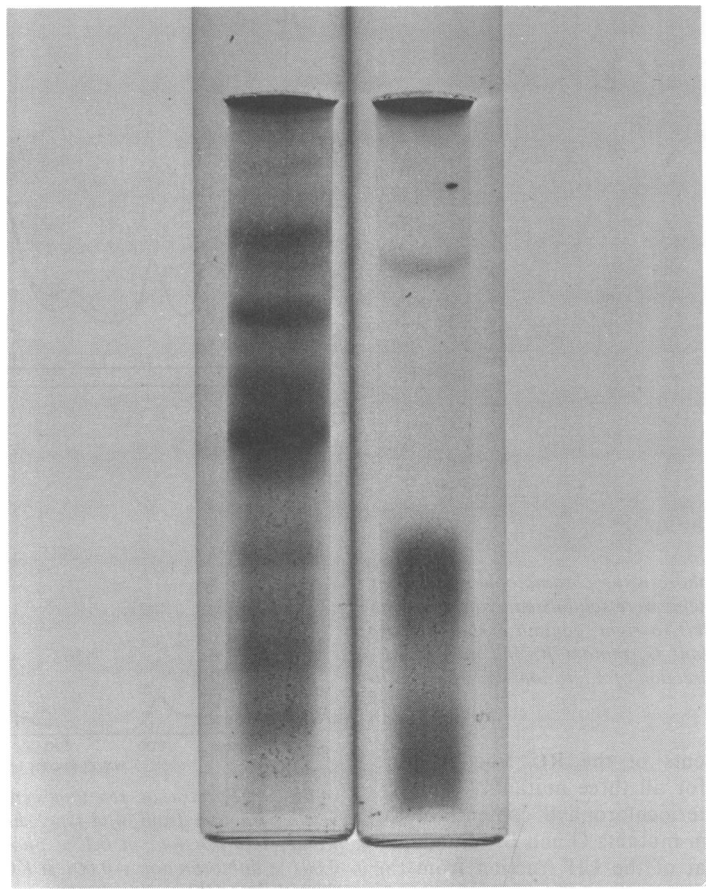

FIG. 5. *Electrophoresis of proteins from reaction centers and light-harvesting fraction of wild-type chromatophores. Proteins were solubilized by heating with SDS. The left gel contains the RC fraction, and the right one the LH fraction. The direction of migration was from top to bottom.*

in whole chromatophores of this mutant is also found in the LH fraction, even though the latter appears to have a normal complement of proteins.

DISCUSSION

The significance of chromatophores as distinct morphological entities is a matter of debate, although probably most workers in the field accept the view that they are derived from invaginations of the cytoplasmic membrane (3). From the point of view of the work presented here, the question is not really important. It has been established by many workers (7, 12, 15, 20, 30) that, whatever the nature of the structure in vivo from which they are derived, chromatophores can be isolated from French pressure cell extracts of many photosynthetic bacteria as a rather homogeneous particulate fraction which is free from ribosomes or cells envelope fragments. The preparations of chromatophores used in this work were of this type, namely, homogeneous [a preparation of wild-type chromatophores (0.16 mg of protein/ml) in 0.01 Tris-hydrochloride, *p*H 7.5–0.175 M CsCl, sedimented as a single homogeneous species with $S_{20,w}$ equal to 146 svedbergs, in a Spinco model E analytical ultracentrifuge equipped with a photoelectronic scanner and scanned at 280 nm] and virtually free from contamination by other components of the cell (9). Therefore, the observed electrophoretic patterns probably give a true representation of the proteins that are actually present in the photosynthetic membranes of *R. spheroides*.

Electrophoresis of chromatophore proteins has been performed by other workers (1, 15) using techniques in which the chromatophores were dissolved in phenol-acetic acid or phenol-formic acid and electrophoresed at low *p*H. In our hands these techniques were not reproducible, probably

because of varying degrees of aggregation between the chromatophore proteins (*unpublished data*). Poor reproducibility has been observed by other workers who have attempted to use these techniques for separating the proteins of membranes (23). By contrast, the use of proteins solubilized by SDS led to consistent results, which are open to clear-cut interpretation. The conditions required for solubilization of chromatophore proteins are somewhat more drastic than are normally used; however, the fact that the same patterns are observed with preparations solubilized in two very different ways indicates that the resulting pattern of protein bands is genuine.

Electrophoresis of the chromatophore proteins from the green mutant showed no differences from the wild-type pattern. From its high reversion rate, it is clear that this mutant arose as the result of a single point mutation; also, it was phenotypically identical to the green mutant of Cohen-Bazire et al. (4). This mutant is thought to differ from the wild type solely in its inability to convert chloroxanthin to spheroidene (13), which is presumed to be the result of a deficiency in a single enzyme. Our results support this conclusion, by showing that the difference between the mutant and the wild type does not reside in any of the protein components of chromatophores that are present in significant quantities.

The results with the brown mutant present a different picture. In addition to the changes in its carotenoids, this mutant definitely lacks one of the major protein components of the chromatophore. As mentioned earlier, the mutant does not readily revert to wild type, as far as we can tell. The simplest explanation at present is that it is a double mutant, which both lacks this particular protein and has a defective pathway for carotenoid synthesis.

Although our results with the green mutant are consistent with the accepted interpretation of this mutant as being defective in a single enzyme, our findings with the blue-green mutant require more explanation. The mutant is definitely leaky and forms a small amount of wild-type carotenoids, suggesting that it arose by a single point mutation. Also, its reversion rate is too high for it to be a double mutant. Apart from the leakiness, it resembles phenotypically the blue-green mutant of Griffiths and Stanier (11), which has been thought to be deficient only in the enzyme which converts phytoene to phytofluene (13). However, chromatophores from mutant BG are definitely deficient in all proteins of the LH fraction, which contains the light-harvesting bacteriochlorophyll, and appear to lack at least one of these proteins completely. The question arises whether the carotenoid deficiency is responsible for the changes in the protein bands or vice versa. In our view, there are two reasonably likely explanations for these phenomena. (i) The original explanation of the phenotype is correct, and the LH portion of the chromatophore is a self-assembling system which does not assemble properly unless all its components are present. The absence of carotenoids interferes with the assembly of the other components and gives rise to a defective chromatophore. This hypothesis was advanced by Sistrom et al. (24) as a possible explanation for the shifts in the infrared spectrum observed in the blue-green mutant. (ii) The biosynthesis of carotenoids is subject to a feedback inhibition system analogous to the one postulated to control the biosynthesis of bacteriochlorophyll in this organism (10, 17, 18). The feedback is mediated by free carotenoids, and is removed when the carotenoids are incorporated into the chromatophore. Mutant BG is unable to assemble the LH portion of the chromatophore correctly, either because it lacks protein C or because it has a mutation in a regulatory gene; therefore, small concentrations of free carotenoids accumulate and inhibit the enzyme which oxidizes phytoene. It is not possible to choose between these alternatives on the basis of current evidence.

There are three further implications of our results. The first concerns the basis for the shift in the spectrum of bacteriochlorophyll when it is incorporated into the photosynthetic membrane system of *R. spheroides*. Wassink et al. (28) originally proposed that this shift is due to combination of bacteriochlorophyll with proteins. Later, Sistrom et al. (24) suggested that the spectral changes were due to complexing with carotenoids rather than proteins. More recently still, Crounse et al. (5) concluded that the original suggestion of Wassink et al. was correct, on the basis of an analysis of the spectrum of a mutant whose carotenoid content could be varied experimentally. Our results offer considerable support for the view that the spectral shifts are the result of complexing with proteins rather than carotenoids. With one exception, every fraction whose electrophoresis pattern was normal also had a normal bacteriochlorophyll spectrum even though its carotenoid content was abnormal, whereas fractions whose proteins were abnormal also had abnormal spectra. The exception is the LH fraction from mutant B, whose proteins appear normal even though the spectrum is not. It is possible that there is an undetected abnormality in the proteins of this fraction; however, this is unlikely in view of the fact that this mutant is already lacking a protein from the reaction centers, as well as having altered carotenoids. A more plausible explanation is that the spectrum

depends to some extent on the way in which the components of the membrane fit together, and not solely on the binding of the pigment to one membrane component. In this way, the absence of one of the protein components from the RC fraction might well cause a small spectral shift in the light-harvesting bacteriochlorophyll.

The second point to emerge from this study concerns the bacteriochlorophyll-protein complex which Olson (21) proposed to be a common feature of all photosynthetic systems. This complex is postulated to contain subunits consisting of a single polypeptide chain with molecular weight 40,000, to which the light-harvesting chlorophyll is bound at a molar ratio of 5:1, or about 0.12 mg of bacteriochlorophyll per mg of protein. Our results show that *R. spheroides* does not contain a complex of this type. From the specific bacteriochlorophyll content of wild-type chromatophores (Table 2; and reference 30), it is evident that, if such a complex were present, it would have to account for at least 75% of the total chromatophore protein. However, the only proteins in chromatophores of this organism whose molecular weights approach 40,000 are distinctly minor components (bands F and G, Fig. 1). By labeling chromatophores with ^{14}C-leucine or phenylalanine, we found that the protein bands in Fig. 1 account for more than 90% of the total chromatophore protein (Gibson, Segen, and Niederman, *unpublished data*); hence, there is no possibility that we have missed a major chromatophore protein. Also, the LH fraction, which contains all the light-harvesting bacteriochlorophyll in the chromatophore, does not contain any protein with molecular weight between 20,000 and 55,000. We conclude that, if there is a distinct bacteriochlorophyll-protein complex in *R. spheroides*, it contains no peptide chain with molecular weight close to 40,000.

Our final point concerns the origin of chromatophores. The view that chromatophores are slightly modified invaginations of the cytoplasmic membrane to which photosynthetic pigments have been added has been partially abandoned lately, although they are still thought to *originate* from this membrane (3, 12, 15). Our observations make it seem likely that the process is rather complex. The blue-green mutant appears to have an RC fraction which is normal in every way, but its LH fraction is grossly abnormal in amount and probably also in composition. This suggests that the chromatophore consists of at least two, and perhaps several, parts which are assembled independently. Although it is still probable that assembly of each part takes place at the cytoplasmic membrane, it is clear that the assembly of at least one part would have to be under separate regulatory control from the biogenesis of the cytoplasmic membrane as a whole, and that a mechanism must exist for joining the various parts together after they are assembled.

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