The Nature of a Pigment–Protein Complex Excreted from Mutants of *Rhodopseudomonas sphaeroides*†

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ABSTRACT: Pigment–protein complexes excreted from three bacteriochlorophyll-less mutants (mutants 8, 8-29, and 8-47) of *Rhodopseudomonas sphaeroides* have been isolated and purified. In the absence of detergents, the complexes remained in an aggregated state, but were disaggregated by 0.2% Triton X-100. Sepharose 6B gel filtration indicated that the disaggregated complex from each of the mutants had a particle weight of about 165000, and contained 30 ± 3% protein. This complex was further dissociated by 1% sodium dodecyl sulfate. Sephadex G-100 gel filtration now indicated that the majority of the protein was present as a small polypeptide with a molecular weight of about 9000. The pigment–protein complex from one of the mutants was treated with a bacteriochlorophyll extract. The bacteriochlorophyll was converted to bacteriopheophytin and became bound to the protein, replacing the endogenous tetrapyrrole (a bacteriochlorophyll precursor). The red absorption maximum of the bacteriopheophytin was shifted during this process to 840–865 nm. These properties are consistent with the possibility that the pigment–protein complexes contain a protein normally associated with light-harvesting bacteriochlorophyll in the wide-type strain.

A great deal of work has been directed in recent years toward the isolation of pigment–protein complexes from the internal membranes of photosynthetic bacteria by the use of detergents. Most of this work has concentrated on obtaining photoreactive complexes. Use of Triton X-100 (Reed and Clayton, 1968; Reed, 1969), Triton X-100 in combination with an alkaline solution of urea (Loach et al., 1970; Hall et al., 1973), or sodium dodecyl sulfate (Segen and Gibson, 1971; Slooten, 1972) has yielded photoreceptor complexes from *Rhodopseudomonas sphaeroides* which contained a fully functional phototrap (or "reaction center"). In addition, variable amounts of light-harvesting (antenna) pigments were also present. Use of CTAB[1] (Reiss-Husson and Jolchine, 1972) or LDAO (Feher, 1971; Clayton and Wang, 1971; Jolchine and Reiss-Husson, 1974; Okamura et al., 1974; Steiner et al., 1974) has generally permitted the isolation of reaction center complexes from *R. sphaeroides* free from light-harvesting components, particularly when the carotenoid-less mutant (mutant R-26) was employed. The proteins of the reaction center complex have molecular weights of about 21, 24, and 28 × 10^3 as determined by dodecyl sulfate polyacrylamide gel electrophoresis (Okamura et al., 1974). Only the first two proteins appear to be associated with Bchl (Feher et al., 1971). Similar reaction center proteins appear to be present in *Rhodospirillum rubrum* (Hall et al., 1973; Wang and Clayton, 1973) and in *Rhodopseudomonas capsulata* and *Rhodopseudomonas palustris* (Clayton and Haselkorn, 1972).

Light-harvesting pigment–protein complexes have been less well characterized. Fraker and Kaplan (1972) have isolated a Bpheo-containing protein complex from *R. sphaeroides* chromatophores by treatment with 2-chloroethanol. The complex contained 59% protein, 39% phospholipid, and 6% Bpheo, and was felt to have been associated with the majority of the Bchl initially found in the chromatophores. The minimum molecular weight of the protein component was estimated to be between 7000 and 15000; however, dodecyl sulfate polyacrylamide gel electrophoresis yielded a value of 10000 (Fraker and Kaplan, 1972). Clayton and Clayton (1972) isolated a similar complex by the use of LDAO. The complex contained a protein component with a minimum molecular weight estimated by dodecyl sulfate polyacrylamide gel electrophoresis to be 9000. The complex had a typical "B800 + B850" light-harvesting spectrum. Hall et al. (1973) also found a correlation between the presence of small molecular weight proteins (between 9000 and 15000 as estimated by dodecyl sulfate polyacrylamide gel electrophoresis) and the presence of light-harvesting Bchl in both active photoreceptor and inactive pigmented fractions. Polypeptides with molecular weights between 10000 and 15000, apparently corresponding to light-harvesting proteins, have been observed in *R. rubrum* (Smith et al., 1972; Hall et al., 1973) and in four other species of *Athiorhodaceae* (Clayton and Haselkorn, 1972). A light-harvesting Bchl–protein complex has also been isolated from the green bacterium, *Chloropseudomonas ethylicum*,[2] by Olson and Romano (1962), and has been found to contain a protein with a minimum molecular weight of 35700 (Fenna et al., 1974).

Mutants of *Athiorhodaceae* unable to carry out photosynthesis have been the subject of several investigations. Mutant M46 of *R. rubrum* was found by Schick and Drews (1969) to excrete a complex containing 61% protein, 17.5% carbohydrate, and 21.5% Bpheo, and was thought to represent one of the light-harvesting forms in this organism. Pigment–protein complexes have also been found to be excreted by Bchl-less mutants of *R. rubrum* (Oelze and Drews, 1970) and *R. capuslata* (Drews et al., 1971; Drews, 1974). The former complex was found to contain 49% protein, 11% lipid, 3% carbohydrate, 30% pigment, and a trace of phos-

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[1] Abbreviations used are: CTAB, cetyltrimethylammonium bromide; LDAO, lauryldimethylamine oxide; Bchl, bacteriochlorophyll; Bpheo, bacteriopheophytin.

[2] This bacterium has been recently reclassified as *Chlorobium limicola* (Gray et al., 1973).

phorus. The minimum molecular weight of the protein component was estimated to be 16500 (Oelze and Drews, 1970). The latter complex was found to contain 38% protein, 13% fatty acids, 15.5% carbohydrates, 20% pigments, and a trace of phosphorus. It contained predominantly two proteins with molecular weights of 42000 and 45500 (Drews, 1974).

Mutant PM8 of *R. sphaeroides* contains Bchl but is unable to carry out photosynthesis. It was found by Clayton and Haselkorn (1972) to be devoid of reaction center proteins, but still contained an 11000 protein in the major Bchl-containing fraction. Lascelles (1966) and Lascelles and Altshuler (1967) have isolated several Bchl-less mutants of *R. sphaeroides* which excrete lipoprotein-bound tetrapyrroles into the growth medium. Takemoto and Lascelles (1973) could find no trace, in the internal membranes of several of these mutants, of a 6000–10000 protein thought to be associated with light-harvesting Bchl. The tetrapyrrole components of the pigment–protein complexes of several of these mutants have been determined (Lascelles, 1966; Richards and Lascelles, 1969; Pudek and Richards, 1975). Very little was known about the protein component(s), however. We have isolated and purified the pigment–protein complexes from three such mutants. We report herein some of the properties of the major protein component. Two preliminary reports of this work have already appeared (Shaw et al., 1973; Ho et al., 1974).

Experimental Procedures

Cultures and Maintenance. All mutants were derived from *Rhodopseudomonas sphaeroides* N.C.I.B. 8253 and were isolated and maintained on slope cultures as described by Lascelles (1966). The isolation of mutants 8-29 and 8-47 has been previously reported (Lascelles and Altshuler, 1967). The major pigment (P-662) produced by mutant 8-29 was found to be 2-devinyl-2-α-hydroxyethylchlorophyllide *a*, and the major pigment (P-720) produced by mutant 8-47 was found to be 2-desacetyl-2-α-hydroxyethylbacteriochlorophyllide (Richards and Lascelles, 1969). Mutant 8 is a previously unreported mutant also isolated by Lascelles. The major pigment (P-730) produced by this mutant has recently been characterized as 2-desacetyl-2-vinylbacteriochlorophyllide (Pudek and Richards, 1975).

Isolation of the Pigment–Protein Complexes. The Bchl-less mutants were incapable of photosynthetic growth and were grown and incubated semiaerobically in the dark as previously described (Pudek and Richards, 1975). The green-colored supernatants from the incubations were brought to 80% saturation with ammonium sulfate and allowed to stand for 24–48 hr at 4°. The pigment–protein complexes were collected by centrifugation for 30 min at 15000*g* and 4°. The colorless supernatants were discarded, and the pelleted pigment–protein complexes were dissolved in 10 ml of distilled water, dialyzed against distilled water for 24 hr at 4°, and freeze-dried. The complexes were stored in the freeze-dried state at −20°. A summary of the isolation and subsequent purification and analysis of the pigment–protein complexes is presented in Figure 1.

Sephadex and Sepharose Gel Filtration. All gel filtrations were run at room temperature in Sephadex K25/45 columns employing 30–35 cm of gel. The gels and buffers employed were as follows: Sephadex G-100 (0.1 *M* Tris-Cl (pH 6.8) plus 1% dodecyl sulfate) and Sepharose 6B (0.1 *M* Tris-Cl (pH 6.8) with or without 0.15–0.20% TRITON X-100). All buffers contained 0.01 *M* sodium azide. The eluent was monitored with an ISCO Model UA-2 analyzer at 254 nm. Protein standards employed for molecular weight calibration were as follows: Sephadex G-100, cytochrome *c*, chymotrypsinogen, and ovalbumin; Sepharose 6B, ovalbumin, bovine serum albumin, alcohol dehydrogenase, catalase, and β-galactosidase (some experiments); or bovine serum albumin, γ-globulin, and apoferritin (other experiments). Void volumes were determined with blue dextran 2000.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Three methods were employed. (1) The method of Weber and Osborn (1969), employing a current of 8 mA/gel and a running time of 4 hr. The samples (100 μg of protein) were digested according to the method of Segen and Gibson (1971); staining and destaining were accomplished by the method of Schnaitman (1969). Protein molecular weight standards employed were: chymotrypsinogen, aldolase, and ribonuclease. (2) The method of Fraker and Kaplan (1971), employing a current of 4 mA/gel and a running time of 4 hr. The sample (100–150 μg of protein) digestion and the staining and destaining techniques were also those of Fraker and Kaplan (1971). Protein molecular weight standards employed were: myoglobin, bovine serum albumin, γ-globulin, and β-galactosidase. (3) The method of Laemmli (1970) as modified by Clayton and Haselkorn (1972), employing a current of 2 mA/gel and a running time of 5 hr. Hematoporphyrin (12 μg) was employed as a dye marker instead of bromophenol blue. The samples (100 μg of protein) were digested in a slightly modified solution containing 0.0313 *M* Tris-Cl (pH 6.9) plus 1% dodecyl sulfate, 5% 2-mercaptoethanol, and 10% glycerol. Digestion was for 1 min at 100°. The staining and destaining techniques were also those of Clayton and Haselkorn (1972). Protein molecular weight standards employed were: cytochrome *c*, chymotrypsinogen, bovine serum albumin, and γ-globulin.

All gels were run in tubes of 6 mm i.d. and 10 cm length. Up to eight tubes were run at one time. Current was supplied by an ISCO Model 490 power supply. Coomassie Brilliant Blue was used in all of the stains and the gels were scanned at 595 nm in a Joyce Loebl Chromoscan Mk II double beam recording densiometer.

The Addition of Bacteriochlorophyll to the Pigment–Protein Complex. The pigment–protein complex from mutant 8-29 (isolated as shown in Figure 1) was further dialyzed against distilled water for 4 days at 4°. The resulting solution contained 5 nmol/ml of the endogenous tetrapyrrole pigment (P-662) and 1.6 mg/ml of protein. The wild-type strain of *R. sphaeroides* was extracted three times with acetone–methanol (7:2, v/v) by the method of Cohen-Bazire et al. (1957). The pigments were partitioned into petroleum ether (bp 60–110°) and the solvent was evaporated in vacuo at 30°. An ethanolic solution of the residue was prepared and 1 ml of this solution was added to 10 ml of the dialyzed pigment–protein complex solution described above. The final solution contained 1.5 mg/ml of protein and 67 nmol/ml of Bchl. Spectra of 1-ml aliquots (diluted to 3 ml with distilled water) were recorded after 10 min, 5 hr, and 15 hr. The remainder of the solution was dialyzed for 43 hr at 21° against distilled water and the spectrum of a similarly diluted aliquot was again recorded. Triton X-100 (final concentration 0.2%) was then added and the spectrum recorded an hour later. As a control, 0.5 ml of the ethanolic Bchl solution was added to 5 ml of an aqueous solution of bovine serum albumin. The final solution contained 12.2 mg/ml of protein and 67 nmol/ml of Bchl. The

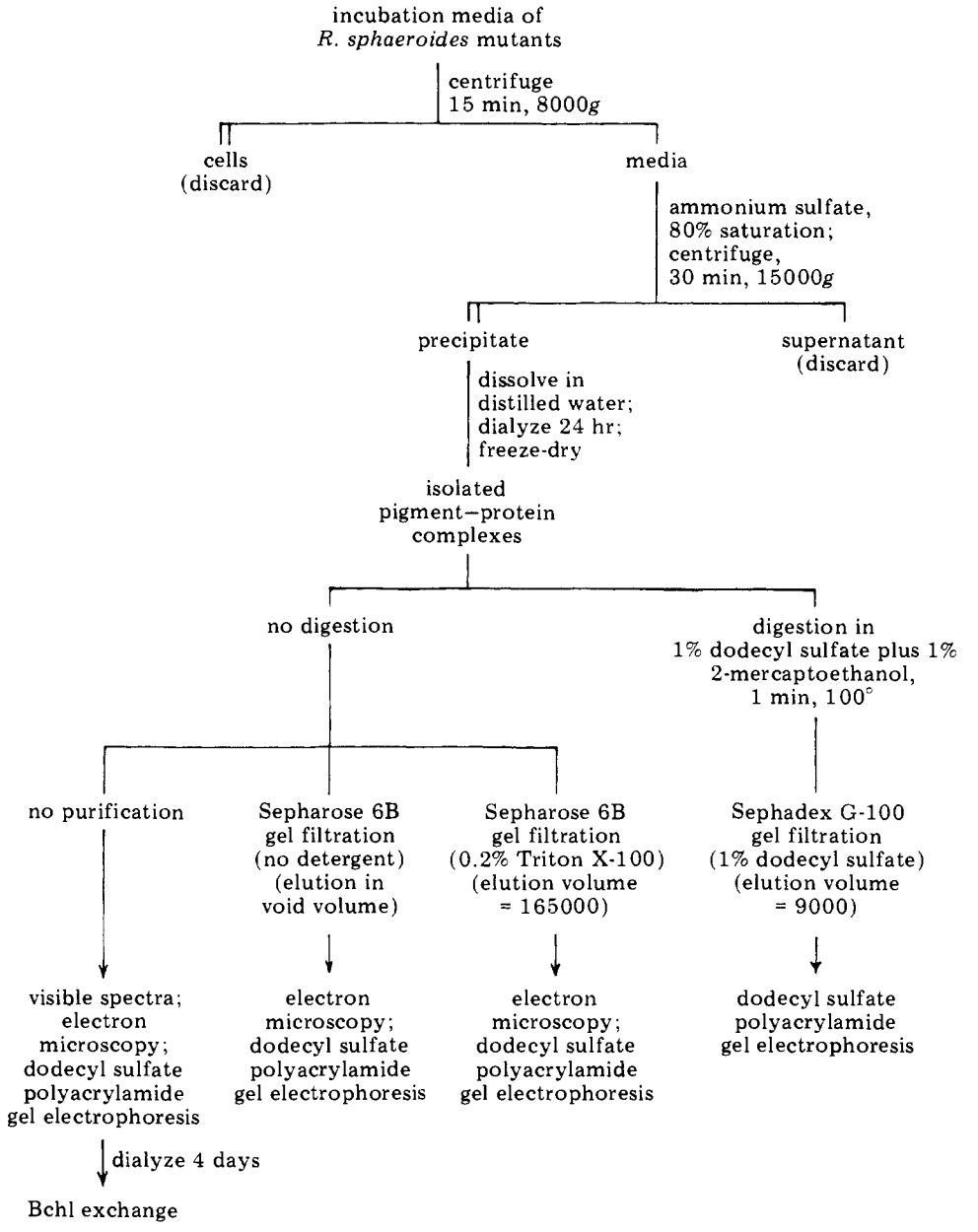

FIGURE 1: Flow diagram for the isolation, purification, and analysis of the pigment-protein complexes from mutants of *R. sphaeroides*.

spectrum of a 1-ml aliquot (diluted to 3 ml with distilled water) was recorded 5 hr later. Finally, 8 ml of the ethanolic Bchl extract was added to 66 ml of the same pigment-protein complex solution described above. The final solution contained 1.4 mg/ml of protein and 80 nmol/ml of Bchl. The mixture was left overnight at 4°, dialyzed for 2 days at 21° against distilled water, and freeze-dried. The residue was dissolved in 0.1 *M* Tris-Cl (pH 6.8) (with no detergent added) and the spectrum recorded. The sample was then run through a Sepharose 6B column, using the same buffer as eluent. The spectrum of the major pigmented band was recorded. Oxygen was not excluded during any of the above operations.

Materials and Instrumental Measurements. All chemicals were of reagent grade. Sucrose solutions were heated with activated charcoal and filtered before use. Acrylamide, bisacrylamide, and 2-mercaptoethanol were purchased from Eastman Kodak Co., Rochester, N.Y. Dodecyl sulfate was purchased from Fisher Scientific Co., Ltd., Montreal. Hematoporphyrin was purchased from Nutritional Biochemicals Co., Montreal. Triton X-100 was purchased from Sigma Chemical Co., St. Louis, Mo. Cytochrome *c*, myoglobin, chymotrypsinogen, ovalbumin, γ-globulin, and apoferritin were purchased from Schwarz/Mann, Orangeburg, N.Y. Alcohol dehydrogenase, catalase, and β-galactosidase were purchased from Worthington Biochemical Corp., Freehold, N.J. Aldolase, ribonuclease, Sephadex G-100, Sepharose 6B, and blue dextran 2000 were purchased from Pharmacia (Canada) Ltd., Dorval, Quebec. All spectra were recorded on a Cary Model 14 spectrophotometer. Protein was analyzed by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Samples from Sepharose 6B gel filtration employed for protein analysis were dialyzed 72 hr at 4° against distilled water and then freeze-dried. All samples were further dried at 60° over phosphorus pentoxide in a vacuum desiccator until constant weights were obtained. The pigment-protein ratios of the complexes were estimated by measuring the absorbance per milligram of protein per milliliter at 730, 720, and 662 nm, respectively, for the complexes from mutants 8, 8-47, and

Table I: Approximate Pigment–Protein Ratios in Complexes from Mutants of *R. sphaeroides*.

Mutant	Pigment–Protein Ratio[a] (nmol/mg of Protein) of Complexes after Various Stages of Purification			
	Crude Complex	After Dialysis	After Gel Filtration[b]	After Dialysis
8	5	4	5	5
8-29	7	2	5	1
8-47	7	5	10	7

[a] See Experimental Procedures for method of determining the pigment–protein ratios. [b] Gel filtration through Sepharose 6B in the presence of 0.2% Triton X-100.

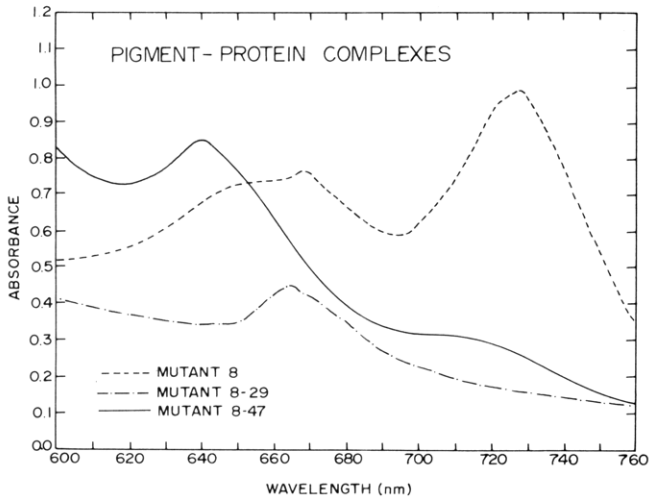

FIGURE 2: Visible spectra (in distilled water) of the pigment–protein complexes isolated from mutants of *R. sphaeroides* as shown in Figure 1.

8-29. The number of nanomoles of pigment was then estimated by assuming an extinction coefficient of 9×10^4 M^{-1} cm^{-1} for the pigments. Although completely unknown, the extinction coefficients of the pigments might be expected to be similar to either chlorophyll *a* or Bchl. Electron microscopy was performed on a Zeiss Model 9A. Latex spheres, 109 nm in diameter, were added and the samples were negatively stained with 1% potassium phosphotungstate. A small amount of bovine serum albumin was added to homogenize the stain.

Results

A summary of the isolation, purification, and analysis of the complexes is shown in Figure 1. The visible spectra of the complexes, isolated as described in the Experimental Procedures, are shown in Figure 2 for all three mutants. The Lowry protein analysis indicated that the complexes were approximately 50 ± 3% protein (on a dry weight basis). The approximate pigment–protein ratios were determined before and after dialysis and are shown in Table I. The results indicated that the pigment from mutant 8-29 (a chlorin, P-662) was more easily lost by dialysis, than pigments from mutants 8 and 8-47 (both tetrahydroporphyrins, P-730 and P-720, respectively). That the loss is due to dialysis and not chemical degradation is indicated by the fact that chlorins are more stable to air oxidation than are tetrahydroporphyrins. Electron micrographs of negative stains of the pigment–protein complexes before gel filtration showed numerous irregularly shaped particles, ranging in size up to 100 nm in diameter. Figure 3A and B show the complex isolated from mutant 8-47. The larger particles appear to be aggregates of numerous smaller particles. Many of the smaller particles can be seen singly or in smaller aggregates. In addition, the complexes isolated before gel filtration contained some obvious cellular debris (Figure 3A). A number of methods of purification of the pigment–protein complexes were attempted, including both ultracentrifugation and electrophoresis in sucrose density gradients, and gel filtration. Of these, the last method was found to be the most successful.

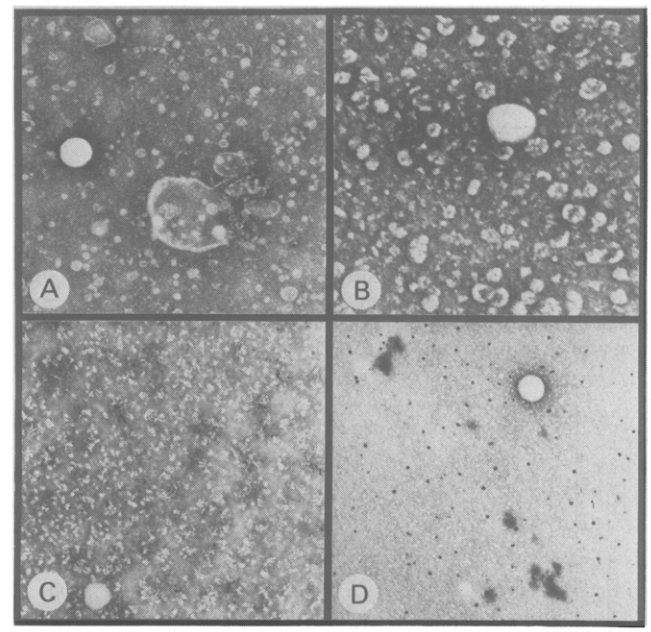

FIGURE 3: Electron micrographs of negatively stained pigment–protein complexes isolated from mutants of *R. sphaeroides* as shown in Figure 1. Latex spheres, 109 nm in diameter, were added to each sample and are visible as the large white spheres. A and B, unpurified complex from mutant 8-47; C, complex from mutant 8-47 after Sepharose 6B gel filtration; D, complex from mutant 8 after Sepharose 6B gel filtration (in the presence of 0.2% Triton X-100).

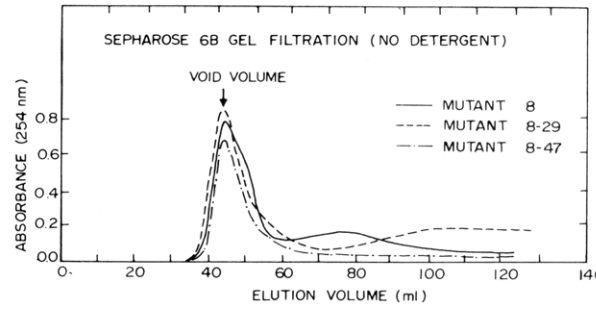

FIGURE 4: Sepharose 6B gel filtration (with no detergent added) of the pigment–protein complexes isolated from mutants of *R. sphaeroides* as shown in Figure 1.

Sephadex and Sepharose Gel Filtration. The majority of the pigment–protein complex from each of the three mutants was eluted in the void volume of Sepharose 6B when no detergent was included in the buffer (Figure 4), indicating that the aggregates had particle weights in excess of 4×10^6, the exclusion limit of Sepharose 6B. Electron micrographs of negative stains of the complexes after Sepharose 6B gel filtration showed fairly homogeneous aggregates of the smaller particles, with none of the larger aggregates or

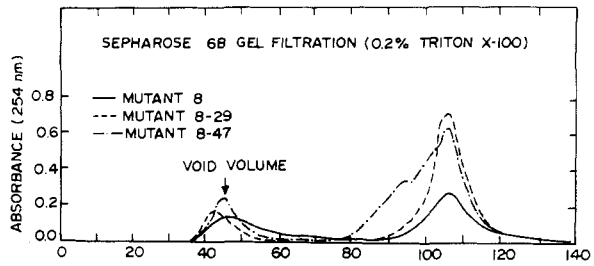

FIGURE 5: Sepharose 6B gel filtration (in the presence of 0.2% Triton X-100) of the pigment-protein complexes isolated from mutants of *R. sphaeroides* as shown in Figure 1. The major peak occurred at an elution volume corresponding to 165000.

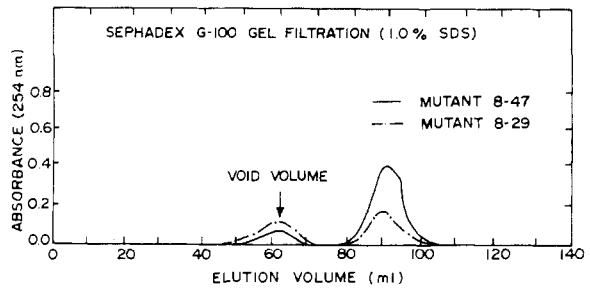

FIGURE 6: Sephadex G-100 gel filtration (in the presence of 1% dodecyl sulfate) of the pigment-protein complexes isolated from mutants of *R. sphaeroides* as shown in Figure 1. The major peak occurred at an elution volume corresponding to 9000.

Table II: Dodecyl Sulfate Polyacrylamide Gel Electrophoresis Analysis of the Polypeptides Contained in the Pigment–Protein Complexes from Mutants of *R. sphaeroides*.

Oligomer No.	Theor Mol Wt[a]	Apparent Molecular Weights ($\times 10^3$)[b] from Mutants: 8[c]	8[d]	8-29[d]	8-47[d]	8[e]	8-29[e]	8-47[e]	8-29[f]
1	9	11.5							*10*
2	18	23			23	21	22	21	20, 23
3	27		27	26	27	29			
4	36	*36*		38		38	36	37	
5	45			48					
6	54		*56*	*57*	*53*	*54*	*54*	*55*	57
7	63		62	64					
8	72								
9	81			83	82				
10	90				92				
11	99		97	95	97				
12	108			105					

[a] Theoretical molecular weight ($\times 10^3$) of the oligomer based on a minimum molecular weight of the monomer of 9000. [b] The major band is italicized. [c] Method of Weber and Osborn (1969) as modified by Segen and Gibson (1971); complex had no prior purification. [d] Method of Fraker and Kaplan (1971); complexes purified by Sepharose 6B gel filtration with no detergent. [e] Method of Laemmli (1970) as modified by Clayton and Haselkorn (1972); complexes purified by Sepharose 6B gel filtration with 0.2% Triton X-100. [f] Same as latter method, but complex purified by Sephadex G-100 with 1% dodecyl sulfate.

cellular debris visible. Figure 3C shows such a complex from mutant 8-47. When 0.15–0.20% Triton X-100 was included in the elution buffer, the complexes entered the Sepharose 6B gel (Figure 5). The elution volume of the major band indicated a predominant molecular weight of 165000. A shoulder in the elution profile of the complex of mutant 8-47 was at twice this weight, however (Figure 5). Electron micrographs of the complexes after Triton-Sepharose 6B gel filtration now showed only the completely dissociated smaller particles. Each particle appeared to be less than 10 nm in diameter. Figure 3D shows the complex from mutant 8. The approximate pigment-protein ratios were determined after the Triton-Sepharose 6B gel filtration (both before and after dialysis and freeze-drying) and are given in Table I. The Lowry protein analysis of the complexes of mutants 8 and 8-29 after Sepharose 6B gel filtration indicated that they were now approximately 30 ± 3% protein (on a dry weight basis), whether they had been run in the presence or absence of Triton X-100. Hence, some apparently loosely associated protein was removed. The pigment-protein complexes were then digested in 0.1 *M* Tris-Cl (pH 6.8) plus 1% dodecyl sulfate for 1 min at 100°, followed by gel filtration on Sephadex G-100 in the same buffer containing 1% dodecyl sulfate. The results for the pigment-protein complexes of mutants 8-29 and 8-47 are shown in Figure 6. This procedure removed all of the pigments from the complexes. The elution volume now indicated that the major fraction had a molecular weight of approximately 9000.

The Analysis of the Protein Components of the Pigment-Protein Complexes by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Three methods of dodecyl sulfate polyacrylamide gel electrophoresis (*cf.* Experimental Procedures) were employed with various samples of the pigment-protein complexes. The results are summarized in Table II. The pigment-protein complex from mutant 8 was examined (before gel filtration) by the method of Weber and Osborn (1969) as modified by Segen and Gibson (1971). The major polypeptide had an apparent molecular weight of 36000 (Table II). After Sepharose 6B gel filtration (but without added detergent in the buffer), the pigment-protein complexes from all three mutants exhibited a major polypeptide with an apparent molecular weight of 55000 ± 2000 (Table II) when examined by the method of Fraker and Kaplan (1971). When 0.2% Triton X-100 was included in the elution buffer during the Sepharose 6B gel filtration, the major polypeptide in the complexes from all three mutants still showed an apparent molecular weight of 55000 ± 2000 (Table II) when examined by the method of Clayton and Haselkorn (1972). There was, however, an increase in minor polypeptides of lower molecular weight, and a decrease in minor polypeptides of higher molecular weight (Table II). It was found that there was no effect on the protein pattern caused by variation of the 2-mercaptoethanol in the digestion solution from 0 to 10%, or by increasing the time of digestion to 5 min at 100°. Finally, the major polypeptide from the complex of mutant 8-29 after Sephadex G-100 gel filtration in the presence of 1% dodecyl sulfate (following digestion in 1% dodecyl sulfate for 1 min at 100°) was found to have an apparent molecular weight of 10000 (Table II) when examined by the method of Clayton and Haselkorn (1972).

The Addition of Bacteriochlorophyll to the Pigment-Protein Complex. Figures 7 and 8 show the results of the addition of a Bchl extract to the dialyzed pigment-protein complex of mutant 8-29 (cf. Experimental Procedures and Figure 1). Figure 7 (solid curve) shows the spectrum of the extract (in ether) containing Bchl (770, 580, 390, and 357 nm) plus some oxidized Bchl (680 and 425 nm) and carotenoids (520–480 nm). Figure 8 (solid curve) shows the spectrum of the control (the addition of the Bchl extract to a solution of bovine serum albumin). The major absorption

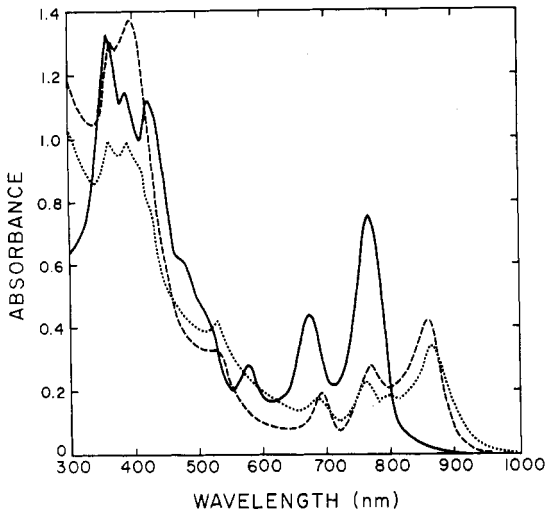

FIGURE 7: (—) The Bchl extract in ether; (- - -) the Bchl extract plus the pigment-protein complex isolated from mutant 8-29 as shown in Figure 1 (after dialysis, freeze-drying, and redissolution in 0.1 *M* Tris-Cl buffer (pH 6.8)); (• • •) the latter mixture after Sepharose 6B gel filtration in the absence of detergent.

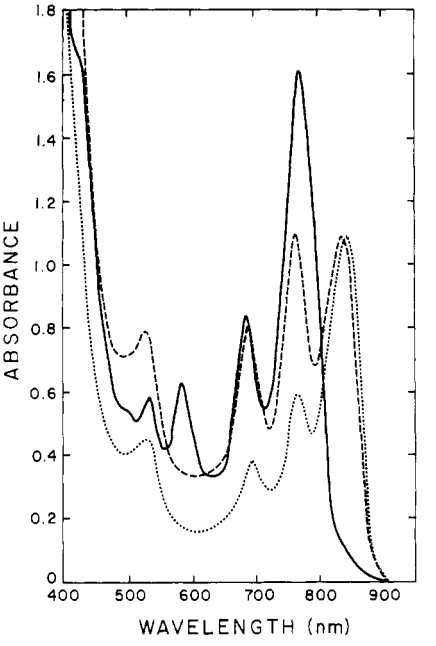

FIGURE 8: (—) The Bchl extract 5 hr after mixing with aqueous bovine serum albumin; (- - -) the Bchl extract 5 hr after mixing with the dialyzed aqueous pigment-protein complex isolated from mutant 8-29 as shown in Figure 1; (• • •) the latter mixture after dialysis for 43 hr at 21°.

maxima were at 770, 685, 585, and 530 nm. The relative ratios of absorption of these four peaks were not significantly different from those observed for the equivalent peaks in the ether extract (Figure 7, solid curve). The absorption in the carotenoid region (480–520 nm) was reduced in comparison with the ether extract, however. The 425-nm maxima was still visible as a shoulder; the remainder of the Soret region was not recorded. Upon addition of the Bchl extract to the dialyzed pigment-protein complex solution, some significant changes in the far-red region of the spectrum were observed. About 10 min after mixing, there was a slight drop in absorption at 770 nm, with the appearance of a shoulder at about 840 nm (not shown). After 5 hr, however, a new and distinct maximum had appeared at 840 nm (Figure 8, dashed curve). The 770-nm maximum was greatly reduced in intensity and had shifted to 765 nm. The 685-nm maximum was only slightly reduced but had shifted to 690 nm. The 585-nm maximum had completely disappeared while the 530-nm maximum had increased in intensity indicating conversion of the Bchl to Bpheo. The shoulder at 425 nm had also disappeared (Figure 8, dahsed curve). No further changes were observed after 15 hr (not shown). After dialysis of the sample for 43 hr at 21°, the far-red maximum had further shifted to 845 nm, but had not been reduced in intensity (Figure 8, dotted curve). The 765-, 690-, and 530-nm maxima had all been greatly reduced in intensity, however. The addition of Triton X-100 (to a final concentration of 0.2%) decreased the 845-nm peak and led to an increase in absorption at 760 and 530 nm (not shown), indicating a conversion of the pigment to free Bpheo.

A larger portion of the mixture was dialyzed for 2 days at 21° against distilled water and then freeze-dried. After redissolution, a complete spectrum revealed that the position of the maxima were now at 860, 770, 690, 530, 395, and 365 nm (Figure 7, dashed curve). The sample was run on a Sepharose 6B gel filtration column with no detergent added to the elution buffer. The complex was eluted in the void volume. A spectrum (Figure 7, dotted curve) revealed that the far-red maximum had been further shifted to 865 nm, with the development of a secondary maximum at 800 nm. All of the other maxima were still present, but there had been a shift of the 770-nm peak to 765 nm, and changes in the relative ratios of absorption of the 530 nm and Soret peaks (Figure 7, dotted curve).

Discussion

On the basis of these results, it seems most likely that the major protein component of the complexes from all three mutants has a minimum molecular weight of 9000–10000. Under all three of the conditions employed for sodium dodecyl sulfate polyacrylamide gel electrophoresis, this polypeptide may be forming a series of oligomers with apparent molecular weights which are multiples of 9000 (Table II). The prior removal of lipids and pigments by Sephadex G-100 gel filtration in the presence of 1% dodecyl sulfate evidently greatly reduced polypeptide association during sodium dodecyl sulfate polyacrylamide gel electrophoresis. Oelze and Drews (1970) also observed oligomer formation during sodium dodecyl sulfate polyacrylamide gel electrophoresis of the 16500 polypeptide component of a pigment-protein complex excreted by mutant F9 of *R. rubrum,* even though lipids and pigments had been previously removed by extraction. If oligomer formation is occurring, the major protein component in the complex examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis after Triton-Sepharose 6B gel filtration would exist predominantly as the hexamer (54000) with the remainder of the 165000 particle weight being composed of pigments, lipids, and/or detergent. The major protein component of the complexes thus appears to be similar in molecular weight to polypeptides thought to be associated with light-harvesting Bchl in *R. sphaeroides* (Fraker and Kaplan, 1972; Clayton and Clayton, 1972; Clayton and Haselkorn, 1972; Hall et al., 1973).

When the Bchl extract was added to the dialyzed pigment-protein complex of mutant 8-29, the Bchl was converted to Bpheo and underwent a spectral shift from 770 to 840–865 nm (Figures 7 and 8). These observations are simi-

lar to those made during chromatophore reconstitution experiments. Using *R. rubrum* chromatophores, Komen (1956) added acetone (up to 80%) and observed a spectral shift from 880 to 780 nm. Upon dilution with water back to 20% acetone, the 880-nm form could be partially regenerated, although some of the 780-nm form remained. Steffen and Calvin (1970), using chromatophores of *R. sphaeroides* mutant R-26, observed a spectral shift from 860 to 770 nm with 5 m*M* octanoic acid. Centrifugation and resuspension in fresh Tris buffer partially reversed the change. In both cases, the results were interpreted as recombination of the Bchl with a protein component of the chromatophores. In more concentrated octanoic acid (10 m*M*), Bpheo (absorption at 530 and 760 nm) was released instead (Steffen and Calvin, 1970). It was found that the Bpheo could regenerate a form with absorption at 530 and 850 nm upon resuspension, suggesting that magnesium was not necessary for the spectral shift.

It is possible, however, that the appearance of the longer wavelength band was due to the formation of colloidal Bchl or Bpheo by dilution of the ethanol solution with water. Similar spectral shifts have been obtained by diluting acetone solutions of Bchl with water or buffers (Komen, 1956; Goedheer, 1966, 1972). The resulting spectra exhibited two far-red maxima at about 785 and 840–850 nm, respectively. In the present work, both the 765- and 865-nm maxima were present in the void volume of the Sepharose 6B eluent along with the protein component (Figure 7). This result indicated that neither of these two absorption maxima represented monomeric Bpheo. However, if the two maxima were due to two different colloidal forms of Bpheo in equilibrium, then either both or neither would have been reduced in amount during dialysis. In fact, only the absorption at 765 nm was reduced whereas the absorption at 840 was stable during dialysis for 43 hr at 21° (Figure 8). The relative amount of the 840 nm could be reduced by the presence of 0.2% Triton X-100, however. Hence, the maximum at 845–865 was most likely due to Bpheo firmly bound to the protein, and at least some of the 765-nm form was less firmly bound. The possibility that the 765-nm form was more liable to air oxidation was not excluded, however.

It should be emphasized that we are not suggesting that the Bpheo has combined in a specific manner with the dialyzed pigment–protein complex (i.e., that found for Bchl in vivo). We present the result simply as an additional piece of evidence consistent with the possibility that the protein component is normally associated with light-harvesting pigment(s). If so, it is possible that the pigment–protein complexes of the mutants result from the combination of Bchl intermediates with such a protein. It is possible that this protein could somehow have been transported through the cell membrane into the periplasmic space. Peters (1970) has stated that he regards the periplasmic space to be the most likely site for Bchl synthesis in *R. sphaeroides*. The protein may have combined with the tetrapyrrole intermediates and subsequently been excreted through the cell wall into the growth medium where it aggregated. Such a process might be aided by the presence of detergents in the medium which would prevent the complexes from prematurely aggregating. It is difficult to imagine how such a large complex could otherwise have been excreted without extensive cell lysis. In the case of the wild-type strain, the protein would combine with Bchl (instead of the intermediates) and be incorporated into the developing photosynthetic apparatus. This might occur by entry into one of the cell membrane invaginations, budding of the invagination, and development into a mature chromatophore. In this respect, a membrane fraction (termed the "prephore" fraction) tentatively identified as a chromatophore precursor (Shaw and Richards, 1971, 1972) has been found to contain a protein pattern very similar to that of the pigment–protein complexes of the mutants (Shaw, 1974). It has been found that Bchl first appeared in the prephore fraction in cultures adapting from aerobic to phototrophic growth conditions (Shaw and Richards, 1971, 1972).

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Studies on IgA and IgG Monoclonal Proteins Derived from a Single Patient. Evidence for Identical Light Chains and Variable Regions of the Heavy Chain†

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ABSTRACT: Two immunoglobulins, IgA(κ) and IgG(κ), were isolated from the serum of a single patient with two monoclonal components (biclonal proteins). After chain separation, the light chains from each molecule were found to be identical by the following criteria: electrophoretic mobilities under various pH and dissociating conditions, amino acid composition, fingerprint analysis of tryptic peptides and of ^{14}C-succinylated chymotryptic peptides, and amino acid sequence of the N-terminal 40 residues. The heavy chains were indistinguishable for the N-terminal 45 amino acid residues. These data are consistent with the hypothesis that a single heavy chain variable (V_H) region may be associated with two different heavy chain constant (C_H) genes.

Multiple myeloma is a neoplasm of antibody-producing cells which may result in the appearance of a monoclonal immunoglobulin in the serum. Biclonal myeloma, on the other hand, shows an increase in two distinct immunoglobulins. This condition, which occurs in a much higher frequency than what would be expected for two independent neoplastic events, may reflect the normal differentiation process of antibody-producing cells (Sledge et al., 1975). Therefore, immunoglobulins from patients with biclonal myeloma may be used as a model to investigate the nature of genetic control during clonal differentiation of these cells.

There are three pieces of evidence which suggest a shared structural relationship between the individual members of biclonal immunoglobulin pairs. First, antigenic sites located within the H and L chain variable (V)[1] regions appear to be unique for a given immunoglobulin and are termed idiotypic determinants. The existence of shared idiotypic determinants between the individual members of a pair of biclonal proteins suggests that the V region genes of both the H and

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[1] Abbreviations used are: H chain, heavy chain; L chain, light chain; V region, variable region; C region, constant region; Tos-PheCH$_2$Cl, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone; Pth, phenylthiohydantoin.