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RELATIONS BETWEEN PIGMENTS AND PROTEINS IN THE PHOTOSYNTHETIC MEMBRANES OF *RHODOPSEUDOMONAS SPHEROIDES*

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SUMMARY

We have isolated from *Rhodopseudomonas spheroides* a pigment–protein complex of apparent weight 9 kdaltons that bears more than 60 % of the light harvesting bacteriochlorophyll. The isolation procedure involved exposure to 1 % lauryl dimethyl amine oxide (LDAO). The purified 9-kdalton fraction showed the light harvesting bacteriochlorophyll components B800 and B850, *plus* carotenoids. The ratio of bacteriochlorophyll to protein was 17 %. This protein is probably the same as the "band 15" protein of Fraker and Kaplan. It may exist *in vivo* as characteristic aggregates of higher molecular weight. LDAO added to *Rps. spheroides* chromatophores converted the bacteriochlorophyll component B870 to a form absorbing at 770 nm but had little effect on the "B800 + B850" system, causing only a reversible shift of the 850-nm band to 845 nm. Anti-reaction center serum, added to subcellular fractions from *Rps. spheroides* with 1 % LDAO, precipitated reaction center chromoprotein unaccompanied by light harvesting bacteriocholorophyll. Other antisera precipitated light harvesting components and left the reaction center chromophores in solution. A major protein of apparent weight 45 kdaltons was found in relatively nonpigmented fractions from *Rps. spheroides*, associated with cell wall fragments. The 45-kdalton protein showed considerable interstrain variability, whereas the 9-kdalton and reaction center proteins appeared constant.

INTRODUCTION

The photosynthetic apparatus of *Rhodopseudomonas spheroides* contains bacteriochlorophyll and carotenoid pigments that serve a light harvesting function, and lesser amounts of bacteriochlorophyll and bacteriopheophytin associated with photochemical activity[1]. The light harvesting bacteriochlorophyll shows absorption maxima near 800, 850, and 870 nm ("B800", "B850", and "B870") attributable to different physical environments. Photochemical activity resides in reaction centers, which have been isolated and characterized as hydrophobic chromoproteins[2,3]. The

Abbreviation: LDAO, lauryl dimethyl amine oxide.

pigments of this reaction center preparation are bacteriopheophytin and bacteriochlorophyll, the latter showing absorption maxima near 803 and 867 nm ("P800" and "P870", respectively)[4].

When the reaction center protein is analyzed electrophoretically following dissolution with sodium dodecylsulfate and mercaptoethanol, it shows three components of apparent weights about 28, 22, and 20 kdalton, respectively[5,6]. The pigments of the reaction center and everything needed for photochemical activity, are associated with the two components of lower molecular weight[5]. Electrophoretic and serological analyses[6] (also W. R. Sistrom, unpublished experiment) of the pigmented membranes ("chromatophores") of *Rps. spheroides* show that the reaction center proteins make up about 20 to 30 % of the total protein. Other major proteins found by electrophoresis of chromatophore preparations have apparent weights of 45 and 9 kdaltons*; in addition one can distinguish about 20 lesser protein components[6–8].

Loach *et al.*[9,10], noting that chromatophores of both *Rps. spheroides* and *Rhodospirillum rubrum* could be dissociated into fragments of low molecular weight by a combination of detergent (Triton X-100), alkali and urea ("AUT treatment"), proposed the existence of a "photoreceptor subunit": a particle that combines the light harvesting and photochemical functions. He did not specify the protein composition of such a particle, nor has he shown that his preparations contain only one kind of pigment–protein particle. Meanwhile, Fraker and Kaplan[7,8] have found evidence that the light harvesting pigments are associated principially with the major 9-kdalton protein, and not with the reaction center protein.

We have found two examples in which the light harvesting pigments are bound to proteins other than those of the reaction center. First, the isolation of reaction center preparations from carotenoidless mutant *Rps. spheroides*, using the detergent Triton X-100, yields as a byproduct a "light harvesting pigment–protein" fraction in which the bacteriochlorophyll remains in its native state, judging from the nearly unchanged absorption band near 860 nm[11]. Second, in Sistrom's nonphotosynthetic mutant strain PM-8 of *Rps. spheroides* the light harvesting bacteriochlorophyll and carotenoids are present and bound to proteins in seemingly the same way as in the parent strain, again judging from absorption spectra[12]. This mutant lacks the reaction center proteins[6].

To explore further the relations between pigments and proteins of *Rps. spheroides* we have made a variety of biochemical separations of subcellular fractions, some with the help of antisera, and have analyzed the various fractions spectroscopically, serologically, and by electrophoresis in polyacrylamide gels. We will show that in the presence of 1 % lauryl dimethyl amine oxide (LDAO, a zwitterionic detergent) the light harvesting pigments are bound not to reaction center proteins, but mainly to the major 9-kdalton protein fraction. The major 45-kdalton protein fraction is most abundant in nonpigmented fractions associated with cell wall fragments. The 45-kdalton protein fraction shows great variability among independent strains of *Rps. spheroides*, whereas the reaction center and 9-kdalton proteins appear to be constant.

* This was identified as an 11-kdalton protein in an earlier communication. The present work showed an apparent molecular weight of 9 kdaltons. The molecular weight had probably been overestimated because it was based on a band moving near the ion front in 10% acrylamide gels.

MATERIALS AND METHODS

Bacteria

Photosynthetic bacteria included 15 strains of *Rps. spheroides*: van Niel's strain ATH 2.4.1 and a carotenoidless mutant strain R-26 derived from it[13], and 13 strains (TS-1, TS-2, *etc.*) isolated by us in 1969 from various sources in Ithaca, N.Y. We also isolated three strains of *Rhodopseudomonas capsulata* (TC-1, TC-2, and TC-3) from mud in Ithaca. Four of the *Rps. spheroides* strains were nutritionally like *Rps. capsulata* in that they grew well with propionate as the main carbon source, but not with tartrate[14]. In all other respects they appeared to be *Rps. spheroides*, and were classified as such. This classification was supported by serological experiments to be described. In all the strains identified as *Rps. spheroides*, the main absorption peak of bacteriochlorophyll was at 850 nm. In the three strains classified as *Rps. capsulata*, the peak was at 857 nm.

Cultures were grown photosynthetically or aerobically as described earlier[15], using modified Hutner medium with either malate or succinate as principal carbon source (the choice seemed immaterial).

Fractionations

Bacteria were broken either by sonic disruption or by passage through a French Pressure Cell. Various subcellular fractions were then derived by differential centrifugation, sometimes through sucrose density steps or gradients; and by fractional precipitation with $(NH_4)_2SO_4$. These operations were performed in the presence or absence of EDTA and the detergents LDAO and Triton X-100. The basic suspension medium in all steps was 0.01 M Tris-HCl, pH 7.5, hereafter called "buffer". Details are given in Results.

Certain components were precipitated more or less selectively by adding rabbit antisera which had been prepared in collaboration with R. L. Berzborn and W. R. Sistrom. The following antisera proved useful:

Ab No. 88; the antigen was a purified reaction center preparation from *Rps. spheroides*. This serum showed a strong precipitin reaction with reaction center proteins and a much weaker ("impurity") reaction with other proteins. The major and lesser components could be distinguished through immunoelectrophoresis.

Ab No. 92; the antigen was "light harvesting pigment–protein" derived as a byproduct in purifying reaction centers from *Rps. spheroides*[2]. This antigen contained a mixture of proteins, but no reaction center protein. The serum was multivalent; it showed a particularly strong precipitin reaction with fractions that were rich in the major 45-kdalton protein. It showed no reaction with purified reaction center preparations.

Ab No. 66; the antigen was a suspension of "light chromatophores" prepared by the method of Worden and Sistrom[16], probably similar to Fraker and Kaplan's "purified chromatophores"[7,8]. This antigen contained the reaction center proteins and the major 9-kdalton protein, but relatively little of the major 45-kdalton protein (see later). When tested against crude extracts of *Rps. spheroides*, Ab. No. 66 showed precipitin activity with at least three distinct proteins, but not with purified reaction centers. The lack of anti-reaction center activity was surprising, but it probably has a trivial quantitative explanation in terms of amounts of antigen given to the rabbit.

The antigenic determinants of reaction center proteins are accessible at the exterior of chromatophores judging from the reaction between chromatophores and the anti-reaction center component of Ab No. 88.

Analyses

Protein was assayed by the method of Lowry *et al.*[17]. Bacteriochlorophyll and bacteriopheophytin were extracted with acetone–methanol (7:2, v/v), the bacteriochlorophyll converted to bacteriopheophytin by acidification, and the bacteriopheophytin estimated from the extinction coefficient[18], ε (525 nm) = 23 $mM^{-1} \cdot cm^{-1}$. Serological precipitin tests were made by the agar-diffusion technique of Ouchterlony[19]. Disc-gel electrophoresis of proteins in polyacrylamide followed the method of Laemmli[20], as described earlier[6]. Unless stated otherwise, the samples were prepared for electrophoresis by boiling them for 1 min with 1.5 % sodium dodecylsulfate and 3.3 % 2-mercaptoethanol.

Apparent molecular weights were estimated from electrophoretograms with the following standards: bovine serum albumin, 68 kdaltons; bovine deoxyribonuclease, 31 kdaltons; bovine trypsin, 23.5 kdaltons; galline lysozyme, 14.4 kdaltons; equine cytochrome *c*, 12.4 kdaltons. In 10 % acrylamide gels the lysozyme and cytochrome moved with the ion front and were not resolved. In this study the molecular weights of the three reaction center proteins appeared to be 29, 24, and 21 kdaltons($\pm$ about 2) whereas earlier investigations yielded values of 28, 23, and 21 kdaltons[5] and 27, 22, and 19 kdaltons[6].

All the reagents required specifically for sodium dodecylsulfate–acrylamide electrophoresis were obtained from Bio-Rad Laboratories, Richmond, Ca. Standard proteins were from Sigma Chemical Co., St. Louis, Mo. LDAO was a gift from the Research Department of Onyx Chemical Co., Jersey City, N.J., through the courtesy of R. Sorrentino. Catalase, 10 mg/l, was added to the LDAO to dispose of a trace (about 5 mM) of H_2O_2.

Absorption spectra were measured with a Cary 14R spectrophotometer. Spectra of samples in strong white light were obtained by using the "IR-2" mode; in the "IR-1" mode the samples were exposed only to weak monochromatic illumination.

Optical scanning of the electrophoretograms was done with a slit-and-carriage device that we fabricated as an accessory to a "home-made" split-beam absorption spectrometer described elsewhere[21].

RESULTS

Some fractionation procedures

We have used three schemes to separate pigment and protein components of *Rps. spheroides*. In all three we first made twice-washed chromatophores by breaking cells suspended in buffer, centrifuging 20 min at 10000 $\times$ *g* to remove debris, centrifuging the supernatant at about 200000 $\times$ *g* for 90 min to precipitate the pigmented material, and washing the precipitate twice by dispersal and recentrifugation. The final pellet was redispersed in buffer and designated "crude chromatophores" (CC).

In the first of three basic schemes, the crude chromatophores were adjusted to a concentration of 15 mg protein per ml; 1 % (v/v) LDAO was added, and the material was layered over a two-step sucrose "gradient": 0.6 M and 1.2 M. After 2 h centri-

fugation at 200000 × *g* there was an upper phase (U) containing all of the reaction centers and some other material, a lower phase (L) at the 0.6/1.2 M sucrose interface bearing much of the light harvesting pigment, and a relatively nonpigmented pellet (P). This is the first step in purifying reaction centers from carotenoidless mutant R-26 of *Rps. spheroides*[2], completed by subjecting the upper phase to $(NH_4)_2SO_4$ fractionation and agar gel column filtration. With wild type *Rps. spheroides* (ATH 2.4.1) the upper phase retained considerable light harvesting pigment and the 9-kdalton protein (see Plate IV of ref. 6), and the foregoing method of purifying reaction centers failed to remove these constituents.

Electron microsopy with negative staining showed that both the lower phase and the pellet, but not the upper phase, contained an abundance of cell wall fragments. The major 45-kdalton protein showed the same distribution as the cell wall fragments. Reaction center protein was found only in the upper phase. The 9-kdalton protein was concentrated in the lower phase and the pellet with strain R-26, but was distributed fairly evenly through all phases with wild type *Rps. spheroides* (Plates III and IV, ref. 6).

Following a different plan, crude chromatophores were treated with 0.01 M EDTA (pH 7.5 with NaOH), layered over 1.0/1.2 M sucrose, and centrifuged at 200000 × *g* for 2 h. This was equivalent to one step in Fraker and Kaplan's procedure[7,8] for purifying chromatophores, and was similar to the procedure of Worden and Sistrom[16] for obtaining "light chromatophores". Most of the pigmented material went to the 1.0/1.2 M sucrose interface; there was a copious but relatively non-pigmented pellet (Pe). The material at the interface, designated "purified chromatophores"

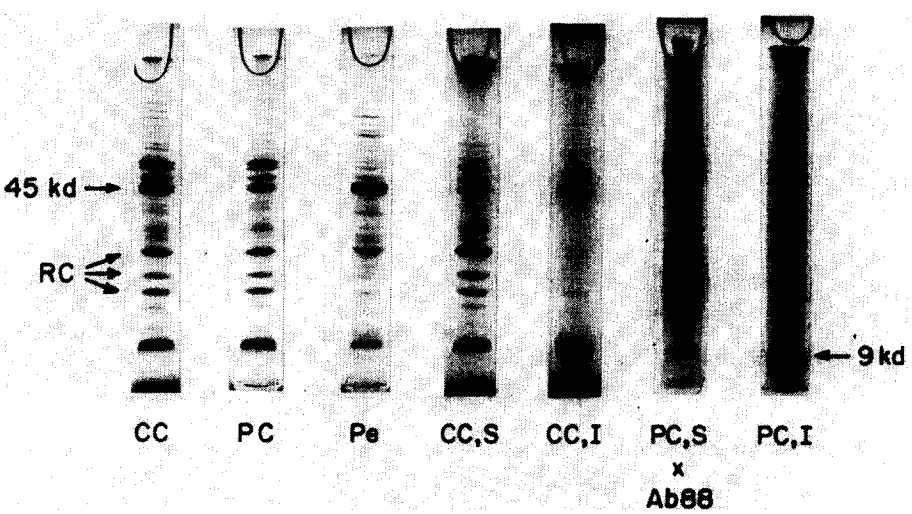


Fig. 1. Analysis of proteins in fractions prepared from *Rps. spheroides*, wild-type strain ATH 2.4.1, by electrophoresis in polyacrylamide with sodium dodecylsulfate. Details of fractionations are in the text. CC, crude chromatophores; PC, purified chromatophores; Pe, pellet (byproduct in purifying chromatophores); CC, S, Triton X-100-soluble fraction from crude chromatophores after exposure to LDAO and precipitation with $(NH_4)_2SO_4$; CC,I, Triton-insoluble fraction from crude chromatophores; PC,S × Ab88, precipitate derived by mixing anti-reaction center serum with Triton-soluble fraction from purified chromatophores; PC,I, Triton-insoluble fraction from purified chromatophores. Positions of major proteins (45 kdaltons, reaction center (RC) components, and 9 kdaltons) are indicated. Proteins were stained with Coomassie Brilliant Blue.

(PC), was diluted with buffer, collected as a pellet by centrifugation, and redispersed in buffer. These purified chromatophores had lost most of the 45-kdalton protein and most of the cell wall fragments, both of which could be found in the pellet. The pellet also contained some 9-kdalton protein. Protein components of crude and purified chromatophores, and of the pellet fraction, are shown in Figs 1 and 2 (CC, PC and Pe). Amounts of protein and bacteriochlorophyll recovered in these fractions are shown in Table I, top section. Note that about 90 % of the bacteriochlorophyll of crude chromatophores was recovered in the purified chromatophores.

TABLE I

PROTEIN AND BACTERIOCHLOROPHYLL RECOVERED IN VARIOUS FRACTIONS FROM *Rps. spheroides*, WILD-TYPE STRAIN ATH 2.4.1

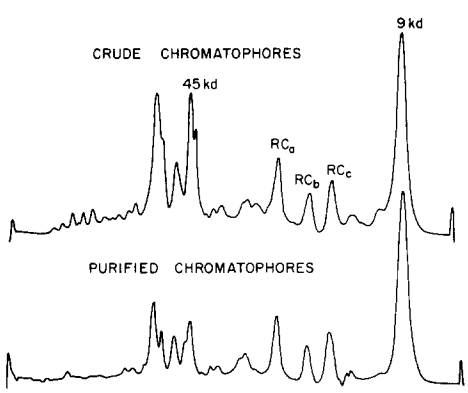
Cells were broken by sonication. Each section pertains to one fractionation experiment. Details of fractionations are given in the text.

Fraction	*Protein (mg)*	*Bacteriochlorophyll (mg)*	*Bacteriochlorophyll (% of protein)*
Crude chromatophores	48	2.4	5.1
Purified chromatophores	34	2.2	6.5
Pellet (Pe)	7.4	0.053	0.7
Crude chromatophores	175	8.3	4.7
Insoluble phase (CC,I)	88	4.6	5.2
Soluble phase (CC,S)	60	2.3	3.8
Purified chromatophores	130	8.0	6.2
Insoluble phase (PC, I)	76	6.9	9.1
Soluble phase (PC,S)	40	1.22	3.1
Insoluble phase after further purification (PC, I:LDAO, L)	33	5.5	16.7

One might hope that purified chromatophores would be better than crude chromatophores as starting material for the isolation of reaction centers by the first of the foregoing procedures (from LDAO, upper phase U). Actually the reverse was found to be true. Not only were conditions unimproved with wild type *Rps. spheroides*; even with the carotenoidless mutant, the reaction center fraction now contained a tenacious contamination by light harvesting pigment and the 9-kdalton protein. It was as if certain impurities in the crude chromatophores, by adhering to the light harvesting components and rendering them denser, facilitated their removal from the upper (reaction center-rich) phase during centrifugation over sucrose.

A third fractionation procedure took advantage of differential solubilities in the presence of LDAO, Triton X-100, and $(NH_4)_2SO_4$. Crude chromatophores, 15 mg/ml, were treated with 1 % LDAO and then diluted 3-fold with buffer. An equal volume of saturated $(NH_4)_2SO_4$ (pH about 7 with NaOH) was added, and the mixture centrifuged 10 min at 10000 × *g*. The floating mass of insoluble material, containing all of the pigment, was collected and dispersed in buffer containing 0.5 % Triton X-100, to a volume equal to the starting volume of crude chromatophores. This dispersion contained an insoluble component (I) which was collected by centrifuging 5 min at 10000 × *g*. The supernatant (S) was especially rich in reaction centers.

Recoveries of protein and bacteriochlorophyll in this procedure are shown in the second section of Table I. The protein components are shown by gels "CC,I" and "CC,S" in Fig. 1. Note that "CC,I" contained principally the 45-kdalton and 9-kdalton proteins, and no reaction center protein (this was confirmed serologically).



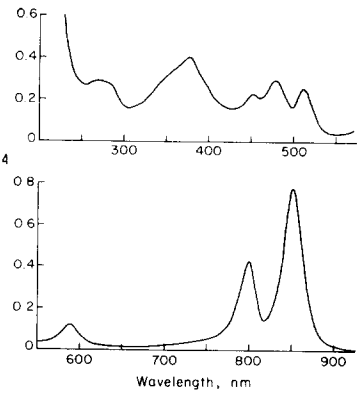


Fig. 2. Densitometer scans, at 550 nm, of two of the gels (CC and PC) shown in Fig. 1.

Fig. 3. Absorption spectrum of the Triton-insoluble fraction from purified *Rps. spheroides* chromatophores, purified further by density gradient centrifugation after addition of LDAO (fraction PC,I:LDAO,L; see the text).

The 9-kdalton protein could be isolated in nearly pure form* by either of two methods. First, the insoluble fraction obtained as above from crude chromatophores (CC,I) could be redissolved by adding 1 % LDAO, layered onto 0.6/1.2 M sucrose and centrifuged 2 h at 200000 × *g*. Most of the pigmented material went to the 0.6/1.2 M interface and remained soluble when collected and diluted with buffer. This preparation, which could be called "CC,I:LDAO,L", contained little protein other than the 9-kdalton one. Alternatively the insoluble fraction (I) could be made, starting with purified chromatophores rather than crude chromatophores. The resulting "PC,I" was again highly enriched for the 9-kdalton protein, as can be seen in Fig. 1. Finally, "PC,I" could be treated with 1 % LDAO and centrifuged into 0.6/1.2 M sucrose; the interface fraction "PC,I:LDAO,L" contained almost exclusively light harvesting pigments and 9-kdalton protein, with an exceptionally high bacteriochlorophyll: protein ratio of 16.7 % (bottom of Table I). An absorption spectrum of "PC,I:LDAO,L" is shown in Fig. 3. Note the relatively small absorbance in the neighborhood of 260 nm, and the intact "B800 + B850" spectrum of bacteriochlorophyll.

Separations using antisera

Special pigment–protein components could be removed from detergent-treated chromatophores and other fractions by precipitation with antisera. One experiment utilized the reaction center-rich fraction PC,S derived from purified chromatophores

* Our only present criterion for homogeneity of the 9-kdalton protein is the occurrence of a single band under sodium dodecylsulfate–acrylamide gel electrophoresis. However, Fraker and Kaplan[7,8] have shown that the major 9-kdalton ("band 15") protein isolated by their method, which included preparative electrophoresis, is a single protein by the criteria of serology, end-group analyses and sedimentation equilibrium.

of wild type *Rps. spheroides* as described earlier. To this fraction we added 1 % LDAO and some Ab No. 88, "anti-reaction center". The amount of antiserum was chosen for serological equivalence with respect to reaction center protein, after a preliminary titration. The mixture was incubated 90 min at 37 °C and then 15 h at 3 °C. The antigen–antibody precipitate was then collected and washed by 4 successive centrifugations. It was redispersed in buffer with 1 % LDAO the first three times and in buffer the last time. Electrophoresis of this material, PC,S × Ab88 in Fig. 1, showed the characteristic reaction center proteins, together with other components that we assume were mainly fragments of immunoglobulin.

The foregoing antigen–antibody precipitate was suspended in buffer containing 30% bovine serum albumin to reduce its turbidity, and absorption spectra were measured with opal glass behind the cuvettes so as to reduce further the effects of scattering. Spectra under strong and weak illumination are shown in Fig. 4. They show the typical appearance and reversible light-induced alteration of purified reaction centers; compare Fig. 1 of ref. 2. These spectra show the presence of some carotenoid pigment but no light harvesting bacteriochlorophyll (as B850).

Fig. 4 shows that under suitable conditions, preparations from wild type *Rps. spheroides* can be made to yield reaction centers entirely free of light harvesting bacteriochlorophyll. We made the same experiment, using purified chromatophores instead of the PC,S fraction, and with varying amounts of detergent present. With 1 % LDAO or 2 % Triton X-100 the antigen–antibody precipitate contained a little light harvesting bacteriochlorophyll, accounting for about 30 % "non-bleachable" absorption at 850 nm. With 0.3 % LDAO the non-bleachable component at 850 nm was about 70 %, and with no detergent added, the antibody appeared to precipitate the entire chromatophore.

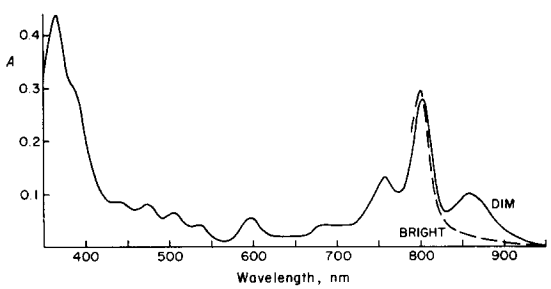


Fig. 4. Absorption spectra of the antigen–antibody precipitate "PC,S × Ab88" shown in Fig. 1 and described in the text. The IR-2 mode of a Cary 14R spectrophotometer was used for the spectrum of the sample exposed to bright light. The light-induced change was reversible.

As a converse to the foregoing experiment we added Ab No. 66 ("anti-chromatophore") in excess, followed by Ab No. 92 ("anti-light harvesting component"), to a suspension of purified chromatophores containing 1 % LDAO. After removing the resultant precipitates, we examined absorption spectra of the soluble phase with the results shown in Fig. 5. Evidently the antibodies had removed most of the light harvesting bacteriochlorophyll, leaving the soluble phase enriched for the reaction center chromophores. A trace of B850 is evident in the "bright-light" spectrum. These spectra show that much carotenoid pigment remained in the soluble phase.

We have not determined the state (association with protein?) of this carotenoid pigment. We did not attempt electrophoretic analysis of this soluble phase because it contained very large amounts of rabbit serum proteins.

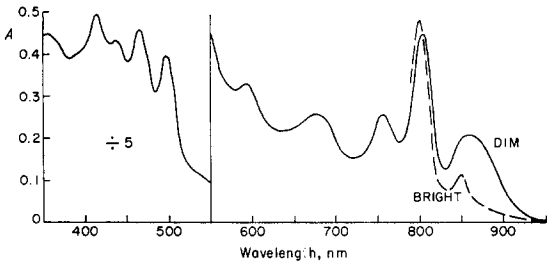


Fig. 5. Absorption spectra of LDAO-treated purified chromatophores after excess antisera had been added and the resulting pigmented antigen–antibody precipitates had been removed. The sera, Ab. No. 66 and Ab. No. 92 (see the text), were chosen so as to remove light harvesting components and leave reaction centers in the soluble phase.

The 9-kilodalton protein

The 9-kdalton protein described here is probably identical to the "band 15" protein of Fraker and Kaplan[7,8]. From the data in Table I we can infer that more than half of the bacteriochlorophyll of crude chromatophores can ultimately be found in association with the 9-kdalton protein in fraction PC,I:LDAO,L. With improved techniques for isolating this protein it may become possible to show that it binds essentially all of the light harvesting bacteriochlorophyll in *Rps. spheroides*.

Examination of acrylamide gels prior to fixing and staining revealed that a green color and absorption at 800 nm coincided with the position of the 9-kdalton protein, even when the sample had been boiled with 1.5 % sodium dodecylsulfate. If the sample had not been boiled, there was less 9-kdalton material in the gel, and two green bands could be seen near the top of the gel. With a sample exposed to 0.2 % sodium dodecylsulfate rather than 1.5 %, and without 2-mercaptoethanol, there was even less material in the 9-kdalton region. These results are shown in Fig. 6. The two upper green bands, seemingly due to an aggregated form of the 9-kdalton protein, require further analysis. Their positions correspond to molecular weights greater than 100 kdaltons. Heat and a high concentration of sodium dodecylsulfate evidently combined to convert the aggregate into the dissociated 9-kdalton form.

The 45-kilodalton protein

The 45-kdalton protein was abundant in fractions CC, L, P, Pe, and CC,I, all of which contained cell wall fragments. A lesser amount of 45-kdalton material seen in purified chromatophores may or may not be the same protein.

The major 45-kdalton protein showed variability among strains of *Rps. spheroides*, correlated with the ability to react with Ab No. 92. When this antiserum was tested against crude chromatophores from *Rps. spheroides* ATH 2.4.1 in agar diffusion plates we observed one dense precipitin band and two lesser ones. The dense band was seen with all preparations that contained the major 45-kdalton protein. We tested crude chromatophores and CC,I from 14 strains of *Rps. spheroides* and three strains of *Rps. capsulata* in this way. The dense precipitin band was present in only five strains of *Rps. spheroides* and in none of the *Rps. capsulata* strains. Presence of the dense

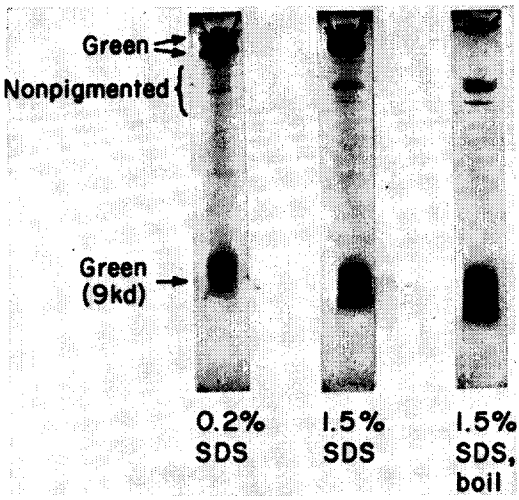


Fig. 6. Electrophoretic analyses of fraction PC,I:LDAO,L; see Fig. 3 and the text. The three gels show how aggregates of higher molecular weight were dissociated into the 9-kdalton protein as the sample was exposed to a higher concentration of sodium dodecylsulfate (SDS) and to boiling. The descriptions "green" and "nonpigmented" pertain to the gels prior to fixing with trichloroacetic acid and staining with Coomassie Brilliant Blue. The green zones coincided with regions of absorption at 800 nm due to bacteriochlorophylll.

TABLE II

SOME PROPERTIES OF 14 STRAINS OF *Rps spheroides*.

Strain	*Weight (kdaltons) of "45-kdalton" protein or of its apparent counterpart*	*Precipitin reaction with Ab No. 92*	*Growth with tartrate as carbon source*
ATH 2.4.1	45	+	+
TS-1	44.5	+	+
TS-4	44.5	+	+
TS-7	45.5	+	+
TS-9	45	+	+
TS-2	43 (faint)	−	+
TS-5	45	−	+
TS-6	45	−	+
TS-14	44.5	−	−
TS-8	46 (faint)	−	+
TS-15	47	−	−
TS-12	48 (one of several faint bands)	−	−
TS-11	49	−	−
TS-10	missing	−	+

precipitin band with Ab No. 92 was correlated with an apparently normal 45-kdalton protein. In many of the strains that failed to show the precipitin band, this protein appeared to have an altered molecular weight or was deficient in amount or missing entirely. Molecular weights of the proteins were estimated in gels made from both crude chromatophores and fraction CC,I with bovine serum albumin added as an

auxiliary marker. Results of these experiments are given in Table II. All of the strains that could not grow with tartrate as carbon source (see Materials and Methods) lacked the precipitin reaction, but this correlation could have been coincidental.

In all of these strains of *Rps. spheroides* the 9-kdalton and reaction center proteins appeared not to vary. Chromatophores of all strains reacted equally well with Ab No. 88 and with Ab No. 66, at least with respect to the major precipitin components.

Rps. capsulata gave lesser but not major precipitin bands with these antisera. Thus *Rps. capsulata* and *Rps. spheroides* have some common antigenic determinants, but these are not in the reaction center proteins and probably not in the 45-kdalton and 9-kdalton proteins, which we suspect are responsible for the major precipitin bands with Ab No. 92 and Ab No. 66 respectively.

DISCUSSION

Referring to Table I, and combining numerical data for crude chromatophores, purified chromatophores, and the PC,I:LDAO,L fraction (which was nearly pure 9-kdalton protein), we find that more than 60 % of the bacteriochlorophyll of crude chromatophores could be recovered as a complex with the 9-kdalton protein. The absorption spectrum of this bacteriochlorophyll protein (Fig. 3) corresponded to the B800 + B850 light harvesting system.

The absence of B870 in these preparations can be explained by the prior exposure to 1 % LDAO. When 1 % LDAO was added to purified chromatophores, we found that the absorption band of B870 was replaced by one at 770 nm, probably signifying monomeric bacteriochlorophyll. The 800-nm band was unaltered and the 850-nm band was shifted to 845 nm. Fraction PC,I showed bands at 800 and 850 nm; all B870 present in the purified chromatophores had been altered and separated from PC,I. When 1 % LDAO was added to the PC,I fraction the 850 nm band shifted to 845 nm. It was found restored to 850 nm in fraction PC,I:LDAO,L. These alterations of the spectrum of light harvesting bacteriochlorophyll are mild compared with the derangement suffered in the procedure of Fraker and Kaplan[7,8], in which the material was dissolved in acidified chloroethanol. Fraker and Kaplan showed that in their final "PII" fraction, band 15 was the only component that carried much pigment (probably bacteriopheophytin). They did not try to compare the amount of this pigment with the amount of bacteriochlorophyll in their starting material.

In order to relate our electrophoretograms to those of Fraker and Kaplan we dissolved a purified reaction center preparation and also some "PC,I:LDAO,L" in acidified 2-chloroethanol, dialyzed these solutions against aqueous Tris buffer, washed the resulting precipitated proteins with buffer, and subjected them to electrophoretic analysis. The 9-kdalton protein from PC,I:LDAO,L was indistinguishable from Fraker and Kaplan's band 15. Of the three reaction center proteins, the stained band reflecting reaction center$_b$ (see Fig. 2) was attenuated moderately, and reaction center$_c$ was attenuated severely, by the chloroethanol treatment. We can probably relate reaction center$_a$ to Fraker and Kaplan's band 13, reaction center$_b$ to band 14, and reaction center$_c$ to a small unlabeled prominence between bands 14 and 15.

These experiments have shown that with enough detergent (1 % LDAO), the light harvesting bacteriochlorophyll is disengaged from the reaction center protein

but much remains attached to the 9-kdalton protein. Possibly all of the light harvesting pigment, or all of the B800 and B850 components, are bound to the 9-kdalton protein in the living cell. We do not know whether in the absence of detergent, the light harvesting pigments are also bound directly to reaction center proteins, or only indirectly because the reaction center and 9-kdalton proteins are parts of a larger lipoprotein structure. If we follow Loach's conception[9,10] of a photoreceptor subunit, a constellation of molecules combining the light harvesting and photochemical functions, then we might suppose that the subunit contains the 9-kdalton protein as well as the reaction centers. Alternatively a photoreceptor subunit could be composed of reaction center protein bearing the reaction center chromophores and also the B870 component of light harvesting bacteriochlorophyll. In our experiments, with 1 % LDAO present, B870 was converted to an apparently monomeric form absorbing at 770 nm; it might well have been disengaged by the detergent from an association with reaction center protein. In this view the system "B800 + B850 + carotenoids + 9-kdalton protein" could be an accessory light harvesting chromoprotein intercalated with a photoreceptor subunit based on reaction centers and B870. This model is attractive in the light of experiments by Sistrom[22] and Aagaard and Sistrom[23] on the ratios of bacteriochlorophyll components in photosynthetic bacteria. Aagaard and Sistrom[23] have shown that in *Rps. spheroides* the ratio of B800 to B850 is constant, and the ratio of B870 to reaction centers (that is, to P870) is constant, whereas the amount of "B800 + B850" can vary, subject to physiological regulation, relative to the amount of "B870 + RC". In *Rhodospirillum rubrum* the only light harvesting bacteriochlorophyll is B880, which bears a constant ratio to the reaction centers (about 50:1). Perhaps *R. rubrum* has a photoreceptor subunit "reaction center + B880" but no auxiliary light harvesting system. However, Fraker and Kaplan (verbal communication) have found in *R. rubrum* a low-molecular weight protein similar to the "band 15" protein of *Rps. spheroides*. Furthermore, the 9-kdalton protein is present[6], (also observed in this study) in carotenoidless mutant *Rps. spheroides*, which lacks "B800 + B850" and has an invariant ratio of B870 to reaction centers. Finally, B870 occurs in reaction center-less mutant PM-8 of *Rps. spheroides*, bound to proteins other than reaction center protein[6,12].

The existence and possible significance of distinctive higher molecular weight aggregates of the 9-kdalton protein remain to be explored.

The major 45-kdalton protein, because of its association with cell wall material, may be part of a relatively nonpigmented outer membrane that adheres to the cell wall in broken cell preparations. We do not suppose that this protein is part of the cell wall, since in known cases the walls of bacteria are composed exclusively of small peptides and aminated carbohydrates. The 45-kdalton protein, but not the 9-kdalton or reaction center proteins, shows striking variations among different strains of *Rps. spheroides* (Table II). It will be interesting to see whether these variations are related to any functions associated with cell walls or limiting membranes. There was no apparent correlation with bacteriophage lysogenicity or sensitivity: strains TS-2, 5, and 7 are phage donors while ATH 2.4.1 and TS-6, 7, 8, 10, 12, and 15 are susceptible to phage infection (unpublished experiments with W. R. Sistrom).

We observed no striking differences between the protein composition of fractions from *Rps. spheroides* grown anaerobically in the light, and of corresponding fractions from cells grown in darkness under limited aeration so as to have abundant pigments.

The ratio of B850 to B870 was about 20 % greater in the light-grown cells than in the dark-grown cells.

Future studies along these lines will be aided by the preparation of antisera against purified 9-kdalton and 45-kdalton proteins.

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