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ISOLATION AND PARTIAL CHARACTERIZATION OF A P870 REACTION CENTER COMPLEX FROM WILD TYPE *RHODOSPIRILLUM RUBRUM*

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SUMMARY

P870 was isolated from wild type *Rhodospirillum rubrum* by solubilization with dodecyldimethylamine oxide followed by ammonium sulfate precipitation. The particle contains some carotenoids, 0.3 mole of ubiquinone per mole of P870 and no cytochrome. The apparent particle weight is 140000. Electrophoresis in dissociating media containing 0.1 % sodium dodecyl sulfate shows the existence of at least two and more likely three protein bands. The complex is calculated to have a minimal molecular weight of 70600 ± 3200 and is supposed to be formed of three protomers.

INTRODUCTION

While great strides have been made in the last fifteen years towards unraveling their mechanism, the primary acts of photosynthesis are still poorly understood at the molecular level. Until recently progress was due mostly to spectroscopic and kinetic analysis of whole photosynthetic organisms or organelles and to the study of several model systems containing chlorophyll or its analogues in the liquid or the condensed state. However, very little information was available on the structural environment of the chlorophyll that was thought to be involved in the photochemical reaction center.

Today our best source of such knowledge is probably the so called "reaction center" preparations from photosynthetic bacteria. Following the work of Clayton[1] and of Kuntz *et al.*[2] which indicated the theoretical feasibility of isolating P870, two groups independently reported the preparation of this reaction center[3,4].

The two procedures were similar: chromatophores of carotenoidless mutants of *Rhodopseudomonas spheroides*[3,5] and of *Rhodospirillum rubrum*[4] were solubilized by the non-ionic detergent Triton X-100 and fractionated by density gradient centrifugation. However, Triton X-100 failed to solubilize the chromatophores of the wild type organisms, presumably because of the additional stability conferred on the membrane by the presence of unsaturated carotenoids.

More recently ionic detergents have been used with success by other authors: the anionic detergent sodium dodecylsulfate with *Rps. viridis* and *Chromatium*[6,7] and

Abbreviations: LDAO, dodecyldimethylamine oxide; CTAB, cetyl trimethylammonium bromide, TEMED, *N,N,N',N'*-tetramethylethylenediamine.

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with *Rps. spheroides*[8], and the cationic detergent cetyl trimethylammonium bromide (CTAB) with *Rps. spheroides* (strain Y)[9]. Most of these preparations involve fractionation by density gradient centrifugation[3,4,8] although adsorption chromatography or ammonium sulfate precipitation have also been used[6,7].

We report here the preparation and partial characterization of a P870 photochemical complex from a wild type strain of *Rsp. rubrum*. Our isolation procedure involves solubilisation of the chromatophores with the non-ionic detergent dodecyldimethylamine oxide (LDAO) followed by ammonium sulfate fractionation. It is simpler and less time consuming than the technique used previously with the G 9 mutant[1], and reproducibly yields a preparation which is stable and highly purified. Moreover it can be adapted to both wild type and to carotenoidless mutant organisms. A similar technique has been described recently for the isolation of P870 from the carotenoidless strain R 26 of *Rps. spheroides*[10].

MATERIALS AND METHODS

Growth conditions

Rsp. rubrum (American Type Culture Collection, strain No. 11170) was grown semi-anaerobically at 30 °C in the medium described by Cohen-Bazire *et al.*[11]. The growth chamber consisted of a glass cylinder (12 cm × 70 cm) illuminated by four 25 W "Daylight" fluorescent lamps and by four 2500 T3 Sylvania tubular infrared lamps operated at 115 V. The lamps were mounted parallel to the growth chamber axis, at 25 cm from its center in a cylindrical aluminium reflector. The bacteria were harvested after four or five days of culture (first two-thirds of the logarithmic growth phase) and collected by continuous flow centrifugation in the SS34 rotor of a RC 2B Sorvall centrifuge equipped with the Szent-Gyorgyi-Blum attachment. The bacteria were then washed with one-half volume of cold 0.05 M Tris–HCl buffer (pH 7.6) per volume of the initial culture and the pellet resuspended in the same buffer. The final suspension contained 500 mg of wet bacteria per ml and was used as such for the preparation of the chromatophores.

Extraction of the chromatophores

The crude chromatophore suspension used in this work was obtained by a procedure derived from that described by Gorchein for *Rps. spheroides*[12]. To each ml of the bacterial suspension described above are added 7.5 ml of 0.2 M Tris–HCl buffer (pH 7.0), 1.0 ml of a solution of lysozyme (1.0 mg per ml) in the same buffer and 1.0 ml of a solution of Na-EDTA (96 mg per ml) at pH 7.0. After a 30 min incubation at 37 °C, solid $MgCl_2$ is added (final concentration 50 mM) and 0.1 mg of bovine pancreas deoxyribonuclease (Sigma Chemical Co.). The suspension is allowed to stand at room temperature for 20 min and then centrifuged (3000 × *g* for 15 min). The protoplasts are washed once with 7.5 ml of 0.2 M Tris–HCl buffer (pH 7.0) and the last pellet resuspended in 10 ml of distilled water with a small clearance Dounce tissue grinder. Lysis is allowed to continue for 15 min at room temperature and the material centrifuged at 20000 × *g* for 20 min. The supernatants from three successive lyses are collected and pooled, buffered at pH 7.0 by addition of sodium phosphate (final concentration 50 mM) and recentrifuged for 15 h at 7000 × *g* in the G.S.A. rotor of a Sorvall RC 2B centrifuge. The pellet is resuspended in 50 mM phosphate buffer (pH

7.0) so as to obtain a final absorbance of 75 at 880 nm. The yield of the procedure is about 60 to 70 % on a pigment basis.

When the chromatophores obtained in this manner were subjected to a 19 h sucrose density-gradient centrifugation according to the method of Ketchum and Holt[13] a main band of pigmented material with a density of 1.147 and a minor band of density 1.198 were found. A small pellet was also present which presumably contained some ribosomes. These chromatophores usually contain about 12 mg of protein as determined by the method of Lowry *et al.*[14] per mg of bacteriochlorophyll.

The present article applies to P870 complex prepared from chromatophores obtained in the above manner. However, the P870 isolation procedure described here has also been used successfully with chromatophores liberated by mechanical means (alumina grinding).

Extraction of the P870 photochemical complex

The nonionic detergent dodecyldimethylamine oxide (LDAO) was used. The detergent was prepared from dodecyldimethylamine and H_2O_2 according to the method of Chadwick[15]. Our extraction procedure is described in Results. A solution of 30%, instead of 35%, H_2O_2 was used yielding a LDAO preparation containing some residual dodecyldimethylamine.

Spectroscopic methods

The absorption spectra were measured with a Cary 14 R Spectrophotometer. Photochemical P870 activity was assayed at 870 nm with the same apparatus under cross illumination from a tungsten lamp filtered by a $CuSO_4$ solution. Schott RG 8 filters were placed in front of the photomultiplier tube in the blank and in the sample compartment.

Gel filtration

Sephadex G-200 (10–40 μm) with a gel bed length of 40 cm was used in a Pharmacia K25/45 column. Ascending flow was maintained at the rate of 10.4 ml/h by means of a Buchler "Dekastaltic" peristaltic pump. Three successive filtrations were performed on the same column at 4 °C in 50 mM sodium phosphate buffer (pH 7.8) containing three different concentrations of LDAO. Before each filtration the gel was equilibrated by percolating with three times (600 ml) its volume of buffer containing the desired concentration of LDAO.

The protein markers for calibration of the gel were introduced two by two on the column under a layer of 10 % sucrose: (1) γ-globulin (Mann Research Corp.) and ovalbumin (Pharmacia Fine Chemicals Inc.), (2) bovine serum albumin (Sigma Chemical Co.) and chymotrypsinogen (Pharmacia Fine Chemicals Inc.). The void volume of the column was determined at the end of each filtration with Dextran blue 2000. The P870 complex was filtered in the dark and its elution volume determined by subsequent spectroscopic analysis.

Polyacrylamide gel electrophoresis

The P870 complex was electrophoresed on polyacrylamide gel according to two different methods (A and B). Electrophoresis was carried out at room temperature

in cylindrical gels (7.5 cm long, 6 mm in diameter) on an Ortec 4100 apparatus operated in the direct current mode until the tracking dye had traversed 7 cm of the gel length.

Method A. This was as described by Weber and Osborn[16]. Electrophoresis was carried out in 100 mM phosphate buffer (pH 7.0) containing 0.1 % sodium dodecylsulfate on a 10 % polyacrylamide gel with 0.28 % *N,N'*-methylene bisacrylamide. The average current density was 8 mA per tube.

Method B. Approx. 1 mg of protein was dissolved in 1.0 ml of Tris–acetate buffer (10 mM Tris, 5 mM acetic acid, pH 8.15)*. This solution was heated at 65 °C for 30 min. To 0.1 ml of this solution were added one drop of bromophenol blue (0.05 % in water) and one drop of glycerol. 0.01 ml of the latter mixture was placed on top of each gel under the electrode buffer. The gel contained 10 % acrylamide and 0.28 % *N,N'*-methylene bisacrylamide. After degassing, ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were added to a final concentration of 0.625 mg per ml and 0.05 %, respectively. The electrode and the gel buffer were the same: Tris–acetate (50 mM Tris; 25 mM acetic acid, pH 8.15) containing 0.1 % sodium dodecylsulfate. Electrophoresis was performed in an electrical field of 5.3 V per cm corresponding to an average current density of 2.5 mA per gel.

After a staining period of 5 h in a 0.2 % solution of Coomassie blue, the gels were soaked overnight under continuous stirring in a 7.5 % acetic acid, 4.5 % methanol solution. The excess stain was removed by electrophoresis. Molecular weight markers for sodium dodecylsulfate gel calibration were bovine serum albumin (Sigma Chemical Co.), aldolase (Pharmacia Fine Chemicals Inc.), chymotrypsinogen A (Pharmacia Fine Chemicals Inc.) and lysozyme (Sigma Chemical Co.).

Assays

Protein was measured by the method of Lowry *et al.*[14] with bovine serum albumin as the standard. Heme was determined on acetone-methanol residues by the pyridine hemochromogen method as described by Bartsch[17]. Quinone was determined by the method of Pumphrey and Redfearn[18].

RESULTS

Solubilisation technique

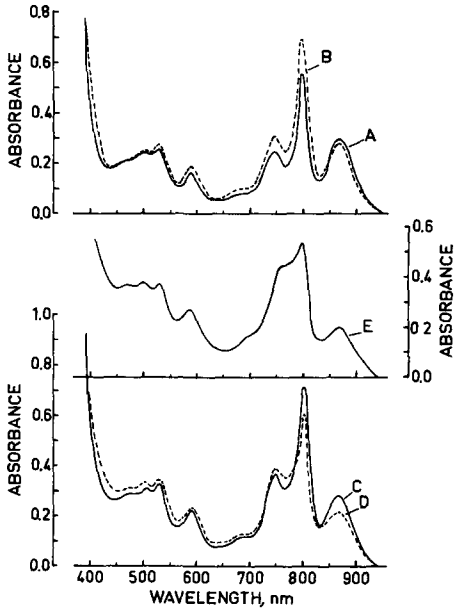
The method of Gingras and Jolchine[4] is inefficient with chromatophores isolated from wild type *Rsp. rubrum*. Partial success was obtained by the method of Jolchine *et al.*[9] but instability of the preparation in CTAB led us to develop the following technique.

The method consists of washing the chromatophores with a solution of LDAO and purifying the solubilized P870 complex. The chromatophore suspension ($A_{880\,nm}$ = 75) is diluted 2-fold with 50 mM phosphate buffer (pH 7.0) containing the proper (see next paragraph) detergent concentration. After standing in the dark at 4 °C for 1 h, the suspension is brought to a final LDAO concentration of 0.1 % (v/v) by dilution with cold buffer. A 1.5 h centrifugation at 105000 × *g* (rotor 40, Beckman L2-65 centrifuge) yields a pellet (P_0) of "washed" chromatophores and a supernatant (S_0) containing the solubilized P870 complex (see flow diagram).

* Containing 1 % sodium dodecyl sulfate.

Determination of the optimal LDAO concentration

The detergent concentration was varied from 0 to 0.5 % (v/v) and the absorbance spectra and P870 activity recorded in the S_0 supernatant fractions. The absorbance spectra (Fig. 1) of the fractions obtained with the lower detergent concentrations (0.2, 0.25 and 0.3%, v/v) are typical of P870 (ref. 1). Higher detergent concentrations yield higher amounts of material absorbing at 750 nm (bacteriopheophytin) and at 870 nm (B870).



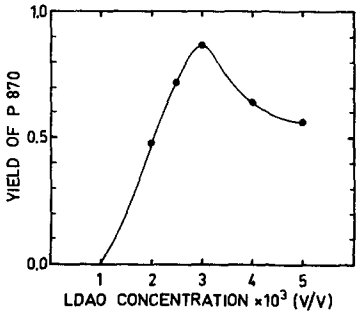


Fig. 1. Absorbance spectra of supernatant (S_0) obtained after treatment with various LDAO concentrations (v/v). Curve A, 0.2%; Curve B, 0.25%; Curve C, 0.3%; Curve D, 0.4%; Curve E, 0.5%.

Fig. 2. Yield of P870 extracted in the S_0 supernatant as a function of LDAO concentration. The P870 content of the starting chromatophore material is given the arbitrary value of 1.0. P870 activity was measured photochemically (see Materials and Methods).

Fig. 2 shows the yield of P870 in the S_0 supernatants as a function of detergent concentration. The highest yield (approx. 90%) is obtained with 0.3 % (v/v) LDAO. There is an apparent inactivation of P870 at higher concentrations accompanied with pheophytinization of the pigments. In view of these results we routinely use a concentration of 0.25 % (v/v) of LDAO which, while sacrificing yield, produces an S_0 fraction containing less bacteriopheophytin.

Purification procedure

A saturated aqueous solution of $(NH_4)_2SO_4$ is added slowly, with continuous stirring, to the S_0 supernatant until the final solution is 0.35 saturated. The solution is kept at room temperature for 30 min and then centrifuged at 10000 × *g* for 30 min. The precipitate (P_1) is rejected, the supernatant brought to 0.45 saturation in $(NH_4)_2SO_4$ and centrifuged as above after standing for 30 min at room temperature.

TABLE I

PIGMENT DISTRIBUTION AMONG THE AMMONIUM SULFATE FRACTIONS DESCRIBED IN THE TEXT

See flow diagram also B870 = absorbance at 870 nm not affected by illumination.

Fraction	$A_{755\ nm}$	$A_{800\ nm}$	*B870*	*P870*
S_0	100	100	100	100
P_1 (calculated)	23	48	100	0
S_2	43	28	0	15
P_2	34	24	0	85

The precipitates tend to float, probably due to the presence of LDAO, and are therefore collected on filter paper (Schleicher and Schuell, No. 595). Table I summarizes the pigment distribution typically obtained in fractions P_1, P_2 and S_2 (see flow diagram). The traces of B870 contaminating S_0 (see Fig. 1) are eliminated with P_1. The latter contains also most of the material absorbing at 800 nm but no P870. Most of P870 is found in P_2 although 15 % of it remains in S_2. The larger part of the absorbance at 755 nm (bacteriopheophytin) is left in S_2. Higher yields of P870 can be obtained in P_2 by increasing the $(NH_4)_2SO_4$ concentration. This, however, brings about the precipitation of more bacteriopheophytin with the P870 complex.

The last precipitate (P_2) is resuspended in 50 mM phosphate buffer (pH 7.8) and twice dialyzed for 20 h against 1 l of the same buffer. The photochemical complex precipitates after dialysis and is kept as a precipitate in the cold in the same buffer. No appreciable loss of activity occurs after several months in this state. Before use P_2 is redissolved by addition of the minimal amount of LDAO compatible with complete solubilization of the complex. This amount is rather critical: too low concentrations (0.01%) produce incomplete solubilization whereas too high concentrations (0.05%) induce instability and rapid pheophytinization of P870. Under our experimental conditions the optimal concentration of added LDAO is around 0.03 % (v/v) but varies slightly from one preparation to the next depending on the protein concentration. No special effort was made to determine the optimal LDAO to protein ratio.

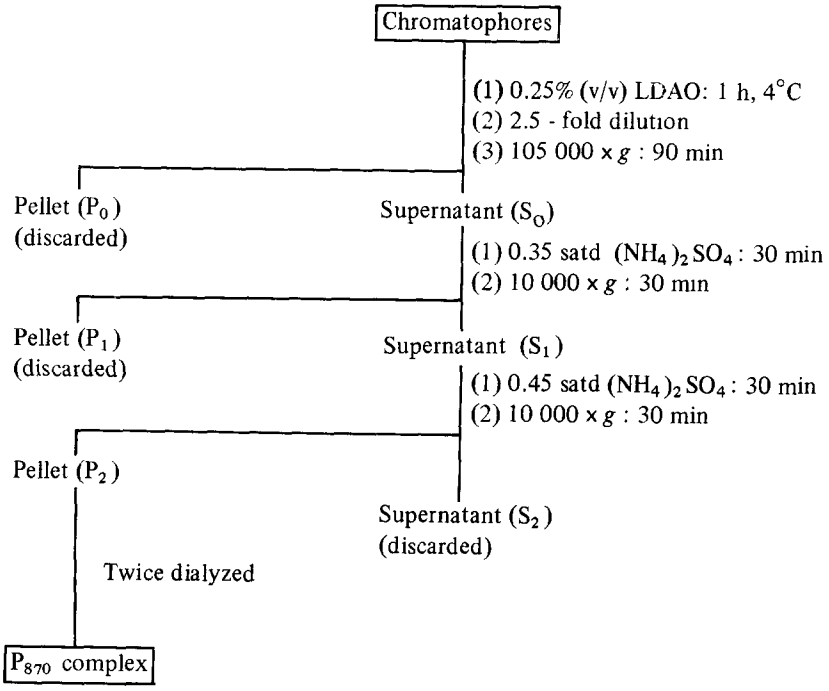
Alternatively P_2 is resuspended in 50 mM sodium carbonate buffer (pH 9.35) and dialyzed against the same buffer for about 15 h at 4 °C in the dark. This solution may be used as such and kept at 4 °C in the dark for several weeks without loss of activity.

In both procedures the solution is clarified before use by centrifugation at 20000 × *g* for 10 min. The pellet contains some insoluble pheophytinized material.

Absorbance spectra

The absorbance spectrum of the preparation in its reduced and in its oxidized state is shown on Fig. 3. The ratio of the absorbance at 800 nm to that at 865 nm in the reduced form is 2.3 in agreement with the spectrum of a similar preparation from the carotenoidless mutant G 9 of *Rsp. rubrum*[4]. In our experience higher values of this ratio are attributable to a slight oxidation of the preparation which can be reversed by adding small amounts of a reducing agent such as ascorbate.

The band at 755 nm is attributed to the presence of bacteriopheophytin which



Isolation of P_{870}. Flow diagram.

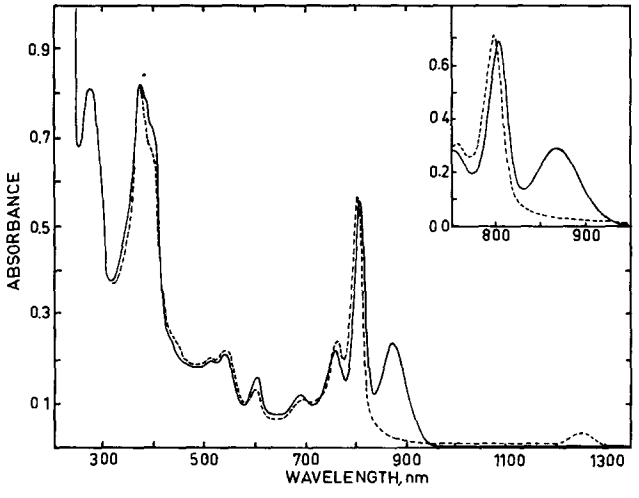


Fig. 3. Absorbance spectra of the reduced and of the oxidized P870 complex from wild type *Rsp. rubrum*. Main figure, ———, spectrum of the reduced form obtained in the presence of 2.5 mM sodium ascorbate; — — —, spectrum of the oxidized form obtained in the presence of 2.5 mM potassium ferricyanide. The buffer was 50 mM sodium carbonate (pH 9.35). Insert ———, near infrared spectrum of the reduced form obtained in the presence of 2.5 mM sodium ascorbate; -------, near infrared spectrum of the oxidized form obtained under cross-illumination (see Materials and Methods).

also accounts for some of the absorption at 535 nm[19]. The 680 nm band is probably due to a degradation product of bacteriochlorophyll. The bands at 535, 498 and 470 nm belong mainly to carotenoids. The 275 nm band is attributed to aromatic amino acids and to pigments, but not significantly to ubiquinone (see the following).

Ubiquinone determination

Rsp. rubrum chromatophores were found to contain one mole of ubiquinone per 3.3 moles of bacteriochlorophyll in good agreement with the values reported by Carr and Exell[21]. This is approximately equivalent to 16 moles of ubiquinone per mole of P870 *in situ*. The isolated P870 reaction center complex contains at most 0.3 mole of ubiquinone per mole of P870. This quantity of ubiquinone, if entirely in the reduced state, contributes about 1 % to the 275 nm absorption band of the complex assuming a millimolar extinction coefficient of 15.5 (ref. 18) at this wavelength.

Cytochrome determination

No heme could be detected in the complex we isolated. This finding is to be contrasted with the situation of the Triton X-100 preparations from carotenoidless mutants[3,4] and of that of the CTAB preparation from wild type *Rps. spheroides* strain Y (ref. 20); it is a confirmation, however, of the results reported for a similar LDAO reaction center complex isolated from the carotenoidless mutant R-26 of *Rps. spheroides*[10].

Molecular weight determination

When the P870 complex in 50 mM phosphate buffer (pH 7.8) is filtered on a G-200 Sephadex column the photochemical activity is eluted in the void volume of the column. The same is true in the presence of 0.025 % (v/v) LDAO in the buffer. With a LDAO solution of 0.05 % (v/v), the particle is retained on the column with an average retention coefficient of 0.22. Calibration of the column under these conditions (see Fig. 4) leads to an apparent molecular weight of 140000 for the reaction center complex. With the latter detergent concentration the eluate contains slightly more bacteriopheophytin, as judged by its absorbance at 755 nm, than the original material. With still higher LDAO concentrations (0.1 %, v/v) the eluate is completely pheophytinized but its retention coefficient is unchanged (0.22). The calibration curve remains the same within experimental error at the three detergent concentrations.

It is impossible to say from this experiment alone whether this molecular weight corresponds to the minimal size for photochemical activity. It is interesting however that the size of the particle is unaffected by detergent concentrations high enough to cancel the protective effect of the surrounding molecules against solvent attack on bacteriochlorophyll.

Electrophoretic studies

Either crude or purified (by gel filtration) P870 complex preparations were electrophoresed according to the method of Weber and Osborn[16] in 100 mM phosphate buffer (pH 7.0) containing 0.1 % sodium dodecylsulfate. Both preparations yielded identical results indicating that gel filtration does not remove important protein contaminants.

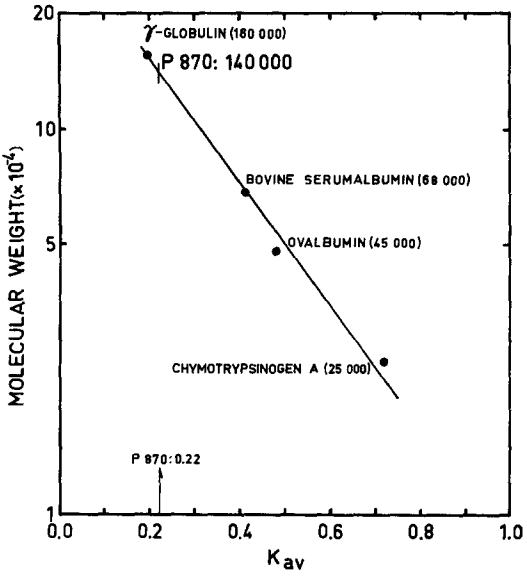


Fig. 4 Particle weight determination of the P870 complex by molecular sieve filtration. The gel was Sephadex G-200. The proteins were eluted with 50 mM, pH 7.8 sodium phosphate buffer containing either 0.05% or 0.1% (v/v) LDAO. The retention coefficient K_{av} of the various particles was the same with both concentrations.

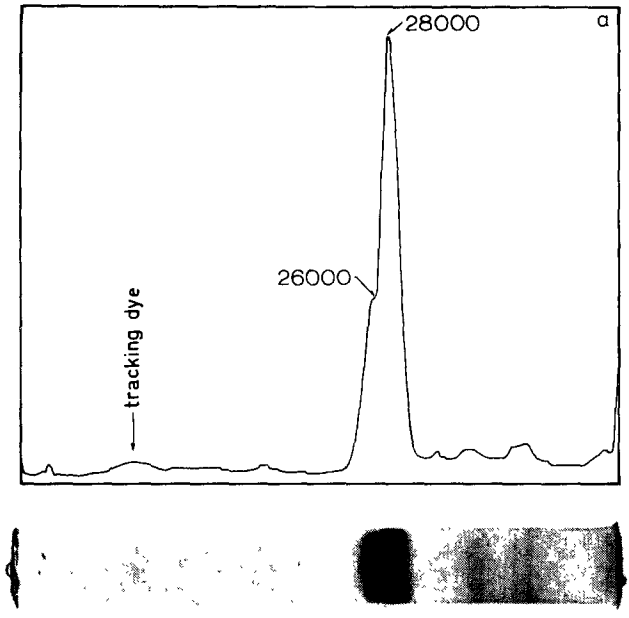
When the complex is electrophoresed in 100 mM phosphate containing 0.1 % sodium dodecylsulfate (Method A) two bands are obtained with apparent molecular weights of 26 000 ± 2000 and 28 000 ± 2000. The same preparation is resolved into three bands in Tris–acetate (Method B) containing 0.1 % sodium dodecylsulfate. The apparent molecular weights are 21 000 ± 2000, 24 000 ± 2000 and 31 500 ± 2000 (Fig. 5, a and b).

The faint satellite bands that are visible after electrophoresis according to Method A are not apparent on the electrophoretogram run according to Method B. We do not feel therefore that they should be attributed to contaminants. It appears more likely that they are due to higher forms of aggregation of the main complex.

Minimal molecular weight determination

As we have seen above the apparent molecular weight of our P870 particle is 140 000. Since its molar composition is not known yet, it is impossible to say whether it contains one or several units of P870. Theoretically this question can be answered by measuring the specific activity of the particle eluted from a Sephadex G-200 column. This is impractical however because the photochemical activity is partially lost and bacteriochlorophyll pheophytinized during the sieving process in the presence of LDAO concentrations equal to or higher than 0.05 % (v/v).

Fortunately, it is possible to determine a minimal molecular weight based upon the P870 and protein contents of the preparation. The P870 content was estimated from the extinction coefficient measured by Clayton on K_2IrCl_6-treated chromatophores[22]. Protein was assayed by the method of Lowry *et al.*[14]. The blanks and the standards were measured in the presence of LDAO concentrations equal to that of



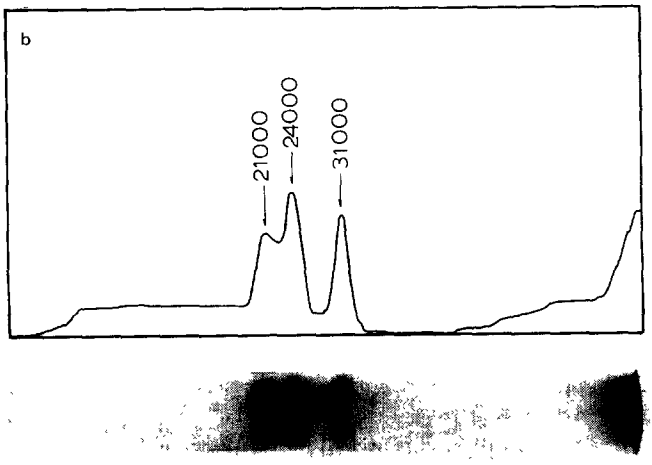


Fig. 5. Polyacrylamide gel electrophoresis of the P870 complex from *Rsp. rubrum*. The numbers in both (a) and (b) refer to the molecular weights. (a) Electrophoresis in 100 mM phosphate (pH 7.0) containing 0.1% sodium dodecylsulfate according to Weber and Osborn[16]. Described as Method A in the text. (b) Electrophoresis in Tris–acetate (50 mM Tris, 25 mM acetic acid, pH 8.15) containing 0.1% sodium dodecylsulfate Described as Method B in the text.

the unknown samples since the detergent was found to interfere with the development of the color reaction.

The following relationship was applied:

$$\text{Minimal molecular weight} = \frac{\text{protein content}}{\text{P870 molar content}}$$

since by definition:

$$\frac{\text{Protein content/molecular weight}}{\text{P870 molar content}} = 1$$

The average of six determinations was 70600 ± 3200 (standard deviation).

This estimation is based on the following assumptions: (i) P870 protein complex and bovine serum albumin have similar tyrosine contents (this is the basis of the Folin reaction), (ii) the extinction coefficient of Clayton is applicable to our preparation, (iii) the preparation contains no foreign proteins in sizeable amounts. Assumption (i) is justified by Feher's report of the amino acid composition of the complex he isolated from *Rps. spheroides* strain R-26 (ref. 10). Assumption (iii) seems to be justified by the electrophoretic behavior of our preparation.

The minimal molecular weight value obtained here is in remarkable agreement with that published by Feher[10] considering that he used also Clayton's extinction coefficient for P870 and that the protein content of his preparation was derived from the added molecular weights of its component amino acids.

DISCUSSION

The technique described in this report for the purification of P870 from wild type *Rsp. rubrum* is simpler and more efficient than the procedure using Triton X-100 solubilisation followed by density gradient centrifugation[4].

Spectroscopically the particle we obtain is comparable to those isolated so far from the Athiorhodaceae. Like the CTAB preparation from wild type *Rps. spheroides* strain Y (ref. 9) it contains carotenoids, mostly spirilloxanthin according to preliminary results. It is too early to propose a function for these pigments. There are a certain number of remarkable absorbance ratios common to our preparation and to those of Reiss-Husson and Jolchine[20] and of Feher[10]: $A_{802\,nm}/A_{865\,nm} = 2.3$, $A_{802\,nm}/A_{365\,nm} \simeq 0.7$ and $A_{365\,nm}/A_{275\,nm} \geqslant 1$.

This type of preparation contains no cytochrome as opposed to the Triton X-100 preparations which contain about one mole of cytochrome c_2 per mole of P870 (refs 3, 4). The CTAB preparation also contains cytochromes when it originates from "normal" cells but very small amounts when it originates from "low iron" cells (no *b*-type heme and 0.12 mole of *c*-type heme per mole of P870[20]). In no case therefore does cytochrome appear to be essential for photochemical activity.

The same is probably true of ubiquinone. Although it is of comparable abundance in the whole chromatophore and in the CTAB[20] or in the Triton X-100[5] P870 complex isolated from *Rps. spheroides* (about 10 moles of ubiquinone per mole of P870), it is nearly absent in our LDAO preparation from *Rsp. rubrum*. It would appear on these grounds alone that ubiquinone cannot be the primary electron acceptor of P870 as suggested earlier by Clayton[23].

It is noteworthy that despite the absence of cytochrome and the virtual absence of ubiquinone the photochemical activity of this preparation is unimpaired and that moreover it is reversible for an almost unlimited number of light–dark cycles. This probably indicates a recombination of charges between the electron acceptor (s) and P870 within the complex. If this interpretation is correct and applicable to the *in vivo* situation the slow rate of this reaction (half-life of several seconds) would account for the high quantum yield of bacterial photosynthesis, provided the rate of electron transfer from the acceptor to the chain is faster.

The following picture appears to emerge from the analysis of the size and of the properties of the protein moiety of the P870 complex. The protein is hydrophobic as evidenced by its insolubility in water except in the presence of detergents. It is oligomeric and has a minimal molecular weight of about 70000. The 70000 subunit is composed of at least two and more probably of three different protomers. We do not know yet whether all the protomers are essential for the photochemical activity.

It is premature to conclude that a dimer (2×70000) of molecular weight 140000 is obtained in 50 mM phosphate buffer (pH 7.8) containing 0.05 % (v/v) LDAO. This particle size determined by molecular sieve filtration may reflect the size of a micelle composed of the P870 complex, LDAO and lipids from the membrane.

It is interesting however that an apparent molecular weight of 150000 has recently been reported for the reaction center complex isolated with CTAB from wild type *Rps. spheroides* strain Y (ref. 20).

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