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ISOLATION AND CHARACTERIZATION OF LIGHT HARVESTING BACTERIOCHLOROPHYLL · PROTEIN COMPLEXES FROM *RHODOPSEUDOMONAS CAPSULATA*

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Summary

The isolation of two native light harvesting bacteriochlorophyl · protein complexes from *Rhodopseudomonas capsulata* is described. The light harvesting bacteriochlorophyll I (B 875) has been isolated from the blue-green mutant A1a$^+$ lacking both carotenoids and light harvesting bacteriochlorophyll II. Light harvesting bacteriochlorophyll I is associated with a protein (light harvesting band 2) of 12 000 molecular weight.

Light harvesting bacteriochlorophyll II complex has been isolated from the mutant Y5 lacking a reaction center and light harvesting bacteriochlorophyll I. Light harvesting bacteriochlorphyll II (B 800 + 850) together with carotenoids is associated with two polypeptides (light harvesting bands 3 and 4) having molecular weights of about 8000 and 10 000 (sodium dodecyl sulfate polyacrylamide gel electrophoresis). A third protein (light harvesting band 1) is in the purified light harvesting II fraction (mol. wt. approx. 14 000), but not associated with bacteriochlorophyll or carotenoids. The amino acid composition of the 3 antenna pigment II proteins is given. The polarity of these proteins was found to be 48%. From the amino acid composition the following molecular weights were calculated band 1: 17 350, band 3: 13 350 and band 4: 10 500.

Introduction

The photosynthetic apparatus of *Rhodopseudomonas (Rps.) capsulata* is localized on vesicular intracytoplasmic membranes [1]; it contains the photochemically active reaction center and two light harvesting Bchl-containing

Abbreviations: Bchl, bacteriochlorophyll; *Rps.*, *Rhodopseudomonas*; SDS, sodium dodecyl sulfate.

complexes. Light harvesting bacteriochlorophyll (Bchl) I has one infrared absorption maximum at 872 nm and is synthesized concomitantly with reaction center Bchl of a relatively constant ratio of about 30 : 1 [2,3,4]. Light harvesting Bchl II is characterized by two infrared absorption maxima at 802 and 855 nm [2,5]. It is the major Bchl component and varies considerably in the proportion to reaction center Bchl [2,3,4,6].

Reaction center Bchl molecules are associated with two polypeptides of 24 000 and 21 000 molecular weight, whereas the heavy protein of the reaction center complex (28 000) is not bound to Bchl [7,8].

Growth experiments have shown that the concentrations of the three Bchl spectral forms vary directly with the concentrations of certain major proteins of the intracytoplasmic membrane, i.e. one polypeptide of approximately 12 000 molecular weight possibly associated with light harvesting Bchl I [5,9] and at least two proteins concomitantly synthesized with light harvesting Bchl II [4]. Genetic transfer experiments confirmed that the formation of the three Bchl species depends on the simultaneous synthesis of their specific protein counterparts [5]. In studies reported here the native light harvesting Bchl · protein complexes were isolated and characterized. It will be shown that light harvesting Bchl I is associated with a single protein whereas light harvesting Bchl II is associated with two different protein species.

Material and Methods

Culture and analytical methods

Light harvesting Bchl I complex was isolated from the carotenoidless but photochemically active mutant strain A1a$^+$ of *Rps. capsulata* GSC 938 (German strain collection, Göttingen) = 37b4 [10,11]. Light harvesting Bchl II complex was isolated from the *Rps. capsulata* strain Y5 (a gift of Barry Marrs, St. Louis, Missouri) which has carotenoids and light harvesting Bchl II but is lacking reaction center and light harvesting Bchl I [5]. The strain A1a$^+$ was grown anaerobically in the light, the photosynthetically inactive strain Y 5 was cultivated semiaerobically in the dark at 30°C in the medium RÄH [6].

The isolation of intracytoplasmic membranes has been described elsewhere [6]. Polyacrylamide gel electrophoresis was carried out by the method of Laemmli [12] using a 10 to 15% polyacrylamide step gradient gel or, for preparative purposes, a 12.5% gel. The material was solubilized as described under Results.

Protein was determined by the modified method of Lowry [13]. Bchl and carotenoid concentrations were measured in the acetone-methanol extract using the molar extinction coefficients of 76 and 128 $mM^{-1} \cdot cm^{-1}$, respectively [14]. Absorption spectra were measured with a Cary 14 (Varian) spectrophotometer.

Isolation of light harvesting Bchl I complex

Intracytoplasmic membranes were solubilized at room temperature in the dark with sodium dodecyl sulfate (SDS) in 50 mM Tris · HCl buffer, pH 7.8, and 5 mM $MgCl_2$; SDS : Bchl = 10 : 1 (w/w). SDS was added dropwise as a 10% solution. The final concentration of Bchl was approximately 0.6 mg Bchl/ml.

The extract was applied to a hydroxyapatite column (35 × 65 mm) and separated by the method of Thornber [15]. The column was washed with 0.2 M NaCl in 10 mM phosphate buffer (pH 7.0). The Bchl · protein complexes were eluted with increasing concentrations of phosphate buffer at constant concentration of NaCl. Most of the light harvesting I complex was eluted at 0.7 M phosphate with 0.2 M NaCl. Fractions of 5 ml of the pigmented material were collected and concentrated by ammonium sulfate precipitation (50% saturation). The fractions were dialyzed and solubilized in Tris · HCl buffer (pH 6.8) containing 1 mg/ml SDS and 24 mg/ml glycerol (final concentrations) and subjected immediately to polyacrylamide gel electrophoresis without heating (2 mm slab gels). The SDS concentration in the gel was 0.05 or 0.10%. After electrophoresis the pigmented bands were spectroscopically analyzed and subsequently extracted with Tris · HCl buffer containing 0.05% SDS. The extract was concentrated by ammonium sulfate precipitation (50% saturation) heated in sample buffer (70°C, 10 min) and applied to polyacrylamide gel electrophoresis [12].

In another series of experiments light harvesting Bchl I was extracted with lauryl dimethyl amine oxide. The intracytoplasmic membranes were treated with ultrasonic in 10 mM Tris · HCl buffer, pH 7.5, containing 5 mM EDTA to remove external proteins. The membranes were spun down in the ultracentrifuge and resuspended in 10 mM Tris · HCl buffer, pH 7.5 to an absorbance of 50—55 (1.0 cm, 865 nm). An equal volume of 2% lauryl dimethyl amine oxide was added and the suspension stirred 15 min in the dark at room temperature. The mixture was centrifuged 90 min at 200 000 × *g* in the rotor Ti 60 (Spinco) at 4°C. The sediment was resuspended in Tris · HCl buffer and layered on a step gradient (0.5 → 1.0 M sucrose in 10 mM Tris · HCl buffer with 0.02% lauryl dimethyl amine oxide). After centrifugation at 200 000 × *g* for 180 min (4°C) the middle layer was separated and subjected to hydroxyapaptite chromatography [15]. After equilibration of the column with 0.2 M NaCl in 10 mM phosphate buffer, pH 7.0, the pigmented fractions were isolated with increasing concentrations of phosphate buffer up to 0.25 M under addition of 0.04% lauryl dimethyl amine oxide and 0.2 M NaCl.

Isolation of light harvesting Bchl II complex

Intracytoplasmic membranes were isolated as described previously [6]. Before solubilization of light harvesting Bchl II the membranes were sonicated three times for 10 s in 5 mM EDTA in Tris buffer to remove external proteins. The membranes were spun down and were resuspended in 10 mM Tris · HCl buffer, pH 7.6. This buffer was used for all isolation steps. The light harvesting Bchl II complex was solubilized by adding lauryl dimethyl amine oxide drop by drop under stirring to a final concentration of lauryl dimethyl amine oxide : Bchl = 20 : 1 (w/w). The solution was stirred for 30 min at room temperature. The solubilized membranes were loaded on a step gradient (1.2, 0.6, 0.3 M sucrose in Tris · HCl buffer with 0.5% lauryl dimethyl amine oxide) and centrifuged at 200 000 × *g* for 15 h at 5°C. The pigmented fractions were collected and dialyzed against Tris · HCl buffer.

The light harvesting Bchl fraction was precipitated with ammonium sulfate (50% saturation). The resulting floating pellet was resuspended in 3% Triton

X-100 and centrifuged 20 min at 10 000 × *g*. The supernatant was dialyzed against 10 mM Tris · HCl buffer and applied to a DEAE 52 column (14 × 2.5 cm). The column was washed with equilibration buffer (Tris · HCl buffer with 0.08% lauryl dimethyl amine oxide, pH 8.0) and eluted with a NaCl gradient in Tris buffer. The major light harvesting Bchl II fraction was dialyzed and further purified by hydroxyapatite column chromatography. The column was washed with 0.2 M NaCl in 10 mM phosphate buffer, pH 7.0. The adsorbed light harvesting Bchl was eluted with phosphate buffer of stepwise increased concentration up to 0.1 M PO_4^{3-} containing 0.04% lauryl dimethyl amine oxide.

For analytical purposes the pigment fraction was centrifuged 36 h at 160 000 × *g* at 15°C on a linear sucrose gradient 5—25% (w/w) in Tris · HCl buffer with 0.6% lauryl dimethyl amine oxide (equilibrium density centrifugation).

Analytic procedures

The protein bands which were found to be associated with light harvesting Bchl II were cut out of the slab gels after electrophoresis. The polypeptides were extracted with 50 mM Tris · HCl + 0.1% SDS, pH 8.0. The extract was dialyzed and the proteins precipitated by ammonium sulfate (65% saturation). The freeze dried material was hydrolyzed under nitrogen atmosphere in 6 N HCl at 100°C for 24, 48, 72 and 96 h, respectively. HCl was removed by repeated evaporation. The samples were dried by rotary evaporation at 40°C and then dissolved in 1 ml 0.2 M sodium citrate buffer, pH 2.2. Amino acid determination was carried out on a Biotronic Amino Acid Analyser LC 6000.

Results

The isolation of light harvesting bacteriochlorophyll I from strain A1a⁺

The mutant strain A1a⁺ from *Rps capsulata* was selected for the isolation of light harvesting Bchl I since this strain does not contain either carotenoids or light harvesting Bchl II (Fig. 1) or their respective protein components [5,7, 10]. Triton X-100, sodium dodecyl sulfate (SDS) and lauryl dimethyl amine

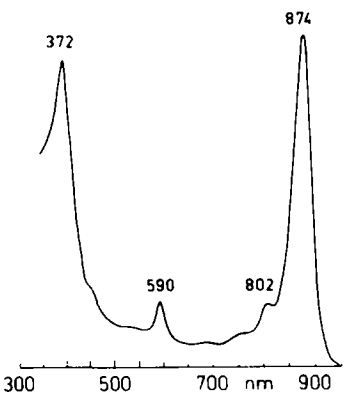

Fig. 1. Absorption spectrum of intracytoplasmic membranes isolate from *Rhodopseudomonas capsulata* strain A1a⁺.

oxide or a combination of these detergents were investigated for their ability to solubilize light harvesting Bchl I. High concentrations of all detergents tested solubilize Bchl as a monomeric form (absorption maximum at about 770 nm) which appears on the top of a sucrose gradient (10 to 30%) after 3 h centrifugation at 180 000 × *g*. Maximum solubilization of light harvesting Bchl under preservation of the characteristic infrared band at 872 nm was achieved at concentrations of SDS : Bchl = 10 : 1 or SDS : Bchl : lauryl dimethyl amine oxide = 11 : 1 : 2 or 17 : 1 : 17 or lauryl dimethyl amine oxide: Bchl = 20 : 1 (w/w/w). The pigmented fraction of the SDS-lauryl dimethyl amine oxide (11 : 2) extract appeared after 3 h of centrifugation at 180 000 × *g* between the 0.5 M and 1.0 M sucrose layers of a step gradient (0.3 → 1.2 M sucrose in Tris · HCl buffer, pH 7.6) which indicates that Bchl is still associated with macromolecular components of the membrane. This fraction was found to be enriched in light harvesting Bchl I and one low molecular weight protein but also contained some reaction center Bchl and higher molecular weight proteins (not shown here).

For most studies light harvesting Bchl I was solubilized with SDS : Bchl (10 : 1) and enriched by hydroxyapatite chromatography. This material showed a main infrared absorption peak at 870—872 nm, (Fig. 2). It contained approximately 75 μg Bchl per mg protein, and was enriched in light harvesting protein 2.

The concentrated light harvesting Bchl fractions were subjected to preparative gel electrophoresis under mild conditions (see Materials and Methods). In native gels without SDS the material precipitated. After completion of the run in SDS (0.1%) gels three pigmented bands were visible in the gel (Fig. 3). Material which migrated 40% of the distance between the slit and bromophenol blue front contained reaction center, the fast moving bands contained light harvesting Bchl I (fig. 3). The upper band of the light harvesting fractions has been found to be material from the late 0.7 M phosphate eluate of the hydroxyapatite column. Pigment and protein comigrate in this fraction.

It banded between the positions of cytochrome *c* (12 500) and chymotrypsinogen A (25 000). The material of this upper band absorbed at 778 nm

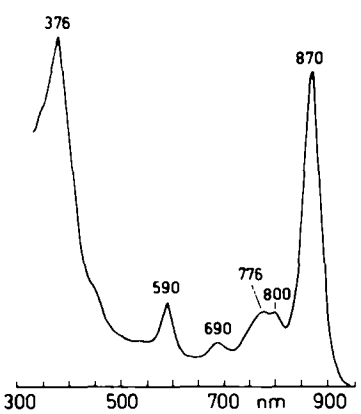

Fig. 2. Absorption spectrum of light harvesting bacteriochlorophyll complex I from strain A1a$^+$ after hydroxyapatite chromatography.

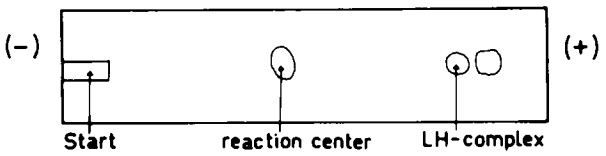

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis [12] of the light harvesting complex I under mild conditions. The fast moving band (LH-complex) was cut off the gel and used for measurement of absorption spectrum (Fig. 4). Afterwards the protein was extracted, heated in sample buffer [12] and applied to SDS polyacrylamide gels (Fig. 5).

(Fig. 4). This indicates that most of the Bchl is in the monomeric state [16]. The material was extracted from the gel, heated with SDS (see Material and Methods) and subjected to slab gel electrophoresis in 0.1% SDS gels. After electrophoresis and staining one characteristic polypeptide band was visible (Fig. 5). It comigrated with the light harvesting protein 2 from different wild type or phototrophic positive mutant strains of *Rps. capsulata* (Fig. 5 and ref. 5). The apparent molecular weight is approximately 12 000. A comparison of the migration of this fraction with the mildly treated light harvesting I complex shows clearly that the light harvesting I complex dissociates during electrophoresis in SDS-polyacrylamide gels in a low molecular weight form. Variation of the SDS concentrations in the gel or use of lithium dodecyl sulfate instead of SDS at about 5°C gave the same results, i.e. comigration of Bchl and protein, infrared absorption maximum at 770—778 nm, and run to a position where low molecular weight test proteins are banding (10—20 000 mol. wt.). However, at a concentration of 0.02—0.05% SDS in the gel an aggregated form banding at a position of about 100 000 mol. wt. with absorption maxima at 870 and 770 nm was observed in addition to the low molecular weight form. The other fast moving band (Fig. 3) contained mainly Bchl and traces of protein. It was not studied further. The results show that a dissociation of the light harvesting Bchl I complex during SDS polyacrylamide gel electrophoresis resulted in a shift of the main infrared absorption maximum of Bchl from 872 to 774 and the formation of monomeric light harvesting protein 2. After heating protein and Bchl degradation products run separately.

In a second procedure (see Material and Methods) the lauryl dimethyl amine oxide solubilized membranes were first separated by centrifugation from the bulk of reaction center and high molecular weight proteins. Subsequent gradient centrifugation yielded an upper layer containing reaction center and solubilized Bchl. The middle layer was separated and underwent hydroxy-apatite chromatography. The bulk of the light harvesting I Bchl was eluted

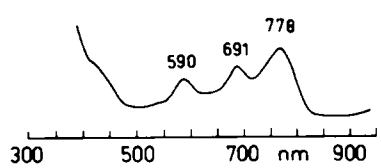

Fig. 4. Absorption spectrum of the light harvesting complex I in polyacrylamide gel after electrophoresis under mild conditions.

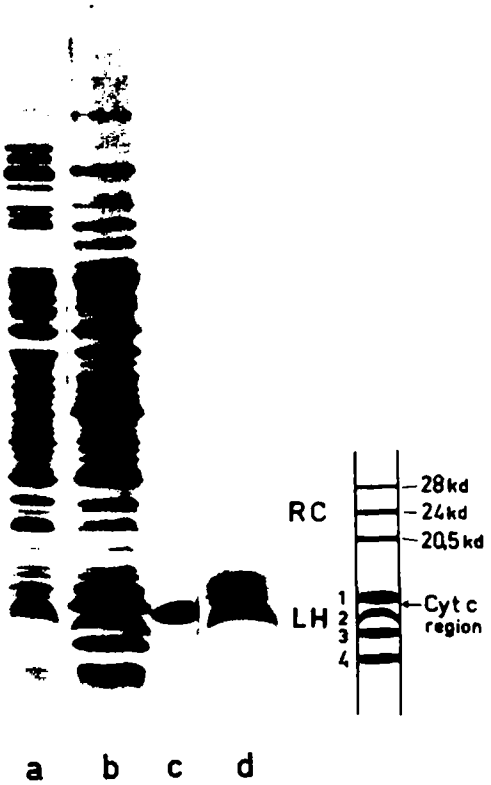

Fig. 5. Protein pattern of membranes or membrane fractions of *Rhodopseudomonas capsulata* after SDS polyacrylamide gel electrophoresis [12]. (a) strain A1a$^+$ (Bch$^+$Crt$^-$) showing three reaction center bands and one light harvesting band [2], (b) strain 37b4, wild type, has reaction center and light harvesting bands 1—4, (c) cytochrome *c* as marker protein, (d) purified light harvesting Bchl I complex, retrieved from polyacrylamide gel (Fig. 3) and subjected to electrophoresis after heating in SDS buffer [12]. The only protein band is light harvesting band 2.

with 0.25 M phosphate buffer containing lauryl dimethyl amine oxide and NaCl. The absorption spectrum of this effluent showed the characteristic infrared absorption maximum at 874 nm, (Fig. 6). The peak at 772 nm, indicating monomeric Bchl, is, however, clearly enhanced. The protein pattern in slab gel electrophoresis showed one dominating band in the same position as light harvesting I protein from other preparations.

Results from both experiments support the idea that light harvesting Bchl I is associated with a single major low molecular weight protein present in the photosynthetically active membrane. The role of lipids and of Bchl-Bchl interactions in this association requires further study.

The isolation of light harvesting bacteriochlorophyll II from strain Y5

Methods of solubilization and purification of light harvesting Bchl II were selected so as to ensure optimal conservation, based on the criteria of the in vivo spectrum of light harvesting Bchl II infrared maxima at 802 and 855 nm and an absorbancy ratio of 855/802 nm = 1.55; Fig. 7, Table I). Material

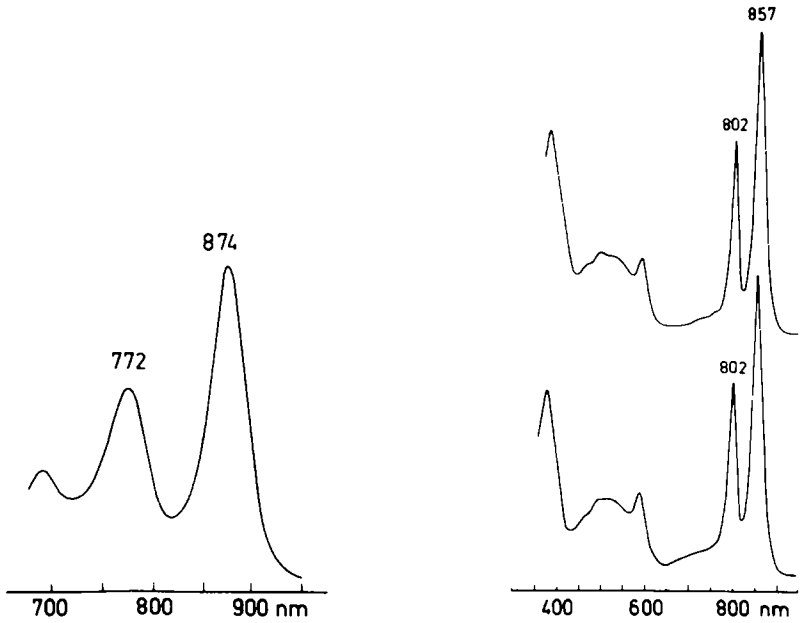

Fig. 6. Absorption spectrum of light harvesting Bchl complex I after hydroxyapatite chromatography and ultracentrifugation (procedure 2). In comparison with Fig. 2 the peak at 772 nm has increased, indicating the presence of monomeric Bchl.

Fig. 7. Absorption spectra of intracytoplasmic membrane of strain Y5 (upper curve) and of isolated light harvesting complex II after hydroxyapatite chromatography (lower curve).

solubilized by lauryl dimethyl amine oxide treatment of membranes was applied to a sucrose step gradient. This procedure separated an upper fraction A containing solublized Bchl and bacteriopheophytin (absorbing at 775 and 685 nm, respectively) and carotenoids (absorption maxima at 518, 488 and 454 nm) from the lower fraction B which showed the typical light harvesting Bchl II spectrum (Fig. 7). Polyacrylamide slab gel electrophoresis of fraction B showed that it contains three major polypeptides, light harvesting bands 1, 3 and 4 (Fig. 8e). The specific Bchl content of this fraction (based on protein) is not increased in spite of purification due to the loss of Bchl (Table I). Fraction

TABLE I

BACTERIOCHLOROPHYLL, CAROTENOIDS AND LIPID PHOSPHATE IN LIGHT HARVESTING COMPLEX DURING PURIFICATION

Purification step	Bacteriochlorophyll (μg Bchl/mg protein)	mol Bchl/ mol car	μg P/ mg protein	nmol Bchl/ nmol P
Crude membranes	63	2.0	18.8	0.11
Sucrose gradient	66.5	2.25	2.1	1.1
DEAE-chromatography	110	2.33	1.7	2.2
Isopycnic centrifugation	105	2.5	1.3—0.93	2.9—3.8

car = carotenoids; P = phosphorus.

Fig. 8. Protein pattern in sodium dodecyl sulfate polyacrylamide electrophoresis in a 10—15% acrylamide step gradient gel, stained with coomassie brilliant blue. All samples except sample i were heated in sample buffer 10 min at 50°C. (a): reaction center isolated from strain A1a$^+$ with traces of light harvesting complex I (lower band). (b): EDTA washed membranes of strain Y5, (c): cytochrome c, (mol. wt. 12 500), (d): myoglobin (mol. wt. 18 000), (e): light harvesting Bchl II complex after gradient centrifugation (fraction B), (f): light harvesting II complex after DEAE column chromatography, (g): light harvesting II complex after hydroxyapatite chromatography, (h): bovine serum albumine (mol. wt. 67 000), (i): purified light harvesting II complex, mild treated (room temperature, 0.05% SDS); the absorption spectrum of the upper high molecular weight gel fraction is seen in Fig. 9. In addition to the high molecular weight complex light harvesting protein 1 is present in the fraction (lower band), (j): the native pigment complex (upper band in i was extracted, heated in sample buffer (15 min, 60°C) and applied to electrophoresis, (k): protein pattern of intracytoplasmic membranes of *Rhodopseudomonas palustris* strain 1e5 showing reaction center and three light harvesting proteins as major proteins. The light harvesting bands run to a similar position as light harvesting bands 2, 3 and 4 of *Rps. capsulata*.

C (third fraction from the top) banding at the 0.6/1.2 M sucrose interface was found to contain predominantly two polypeptides with molecular weights of 50 000 and 55 000, but contained no pigments. The pellet fraction consists of ribosomes and cell wall fragments.

The light harvesting fraction B was concentrated and resolubilized in Triton X-100 as described under Methods. In contrast to the results reported by Clayton [17], the light harvesting Bchl material was soluble in Triton. The unsoluble material contained cell wall fragments similar to a P_i fraction described by Fraker and Kaplan [18].

Chromatography on DEAE-cellulose (see Methods) yielded a carotenoid fraction with absorption maxima at 515, 488 and 455 nm free of proteins and the light harvesting Bchl II fraction, which was eluted in the range of 0.09—1.2 M NaCl solution. This fraction was enriched in Bchl (Table I) and in three low molecular weight polypeptides (Fig. 8f).

A further purification of light harvesting II complex from high molecular weight proteins and solubilized Bchl has been achieved by hydroxyapatite

chromatography as described under Methods (Fig. 8g). In contrast to the isolated carotenoid free light harvesting Bchl I fraction, the enriched light harvesting Bchl II complex is stable even at room temperature in the light. The various purification steps increase slightly the molar ratio of Bchl to carotenoids (Table I).

Some of the Bchl is washed out of the membrane during purification by solubilization of pigment. Consequently the Bchl : protein ratio does not increase (Table I). During purification the phospholipid concentration of the preparation decreases strongly (Table I). This seems to support the idea that Bchl is associated with light harvesting proteins 3 and 4. The role of Bchl-Bchl interactions in this association requires further study.

The purified light harvesting Bchl II complex was analyzed by buoyant density gradient centrifugation in the presence of 0.6% lauryl dimethyl amine oxide. The material was detected after the run as a sharp band at a density of $\rho\ 15°C = 1.05$. The banding pattern of the proteins in the complex shows three bands in the same position as the bands 1, 3 and 4 of the purified complex II. A small amount of Bchl was solubilized.

It is known that reaction center Bchl is associated with the smaller proteins of reaction center and not with the heavy subunit [7, 8]. In order to determine which of the polypeptide components are associated with light harvesting Bchl II, a sample of purified light harvesting II complex was treated with low concentrations of SDS (0.05%) at room temperature and applied immediately to slab gel electrophoresis. The resulting high molecular weight pigment-containing fraction showed the same absorption spectrum as the purified complex (Fig. 9). Fast migrating pigmented material consisted of Bchl, bacteriopheophytin and carotenoids. An increase of the SDS concentration in the sample buffer led to a decrease in the amount of high molecular weight material with an in vivo spectrum, thereby increasing the amount of solubilized pigments and protein bands 3 and 4. Solubilized pigments always migrated

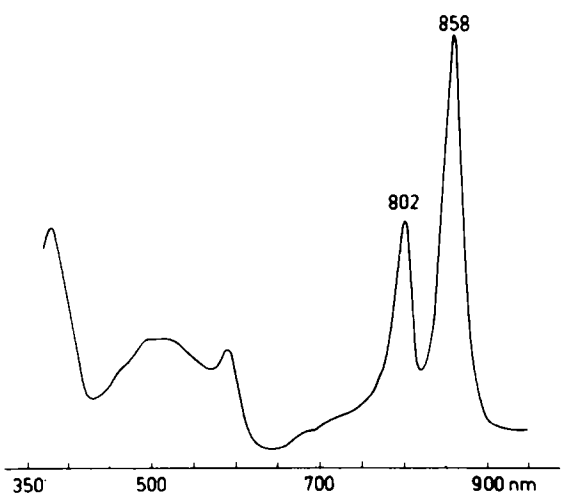

Fig. 9. Absorption spectrum of high molecular weight fraction of light harvesting complex II in the gel after SDS polyacylamide gel electrophoresis (Fig. 8i, upper band).

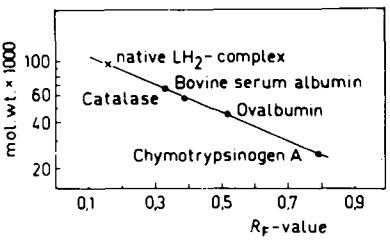

Fig. 10. Estimation of the molecular weight of the native light harvesting bacteriochlorophyll complex II by SDS polyacrylamide gel electrophoresis (10% acrylamide gel, 0.01% lauryl dimethyl amine oxide). Molecular weights and migration ratios of samples:

	R_F-value	Mol. wt.
Chymotrypsinogen	0.79	25 000
Ovalbumin	0.52	45 000
Catalase	0.39	58 000
Bovine serum albumin	0.33	67 000
Native light harvesting II complex	0.16	96 000

TABLE II

AMINO ACID COMPOSITION OF LIGHT HARVESTING II BACTERIOCHLOROPHYLL ASSOCIATED POLYPEPTIDES FROM *RHODOPSEUDOMONAS CAPSULATA*

Values are means of two samples. They are corrected for partial destruction of serine and threonine during hydrolysis.

Amino acid	mol%			No. of residues **		
	1 *	3 *	4 *	1	3	4
Asp	9.6	9.3	10.1	16.0	12.2	9.9
Thr	6.2	6.3	6.3	10.3	8.3	6.1
Ser	8.1	9.3	6.7	13.5	12.1	6.6
Glu	11.8	11.7	12.3	19.7	15.3	12.1
Lys	6.5	6.1	7.4	10.8	7.9	7.3
His	1.7	2.5	2.3	2.8	3.3	2.2
Arg	4.1	3.8	3.9	6.8	5.0	3.8
Gly	13.1	15.5	9.2	21.8	20.3	9.0
Ala	9.9	9.7	10.3	16.3	12.7	10.1
Val	6.6	6.2	7.3	11.0	8.1	7.2
Met	0	0	1.0	—	—	1.0
Ile	4.8	4.6	5.9	8.0	6.1	5.8
Leu	8.7	8.1	9.2	14.5	10.6	9.0
Pro	4.1	3.2	3.7	6.8	4.3	3.6
Tyr	0.6	0.8	1.0	1.0	0.9	1.0
Phe	4.1	2.9	3.3	6.8	3.8	3.2
Trp	n.d.	n.d.	n.d.			
Cys	n.d.	n.d.	n.d.			

* Bands of light harvesting Bchl II associated polypeptides, eluted from SDS-polyacrylamide gels.

** Values based on the calculated mean residue weight (MRW) and the assumed molecular weight of each polypeptide; 1, MRW = 104, mol.wt. = 17 350; 3 MRW = 102, mol.wt. = 13 350; 4 MRW = 107. mol.wt. = 10 500.

n.d., not determined.

faster than the low molecular weight polypeptides. The high molecular weight material was associated with protein (Fig. 8i). Band 1 of light harvesting proteins was also present in the gel, bands 3 and 4, however, were barely detectable. The native complex was extracted from the gel, treated with SDS sample buffer [12] for 15 min at 60°C and applied to polyacrylamide electrophoresis. By this procedure the complex was split into two proteins banding at positions 3 and 4 (Fig. 8j). We conclude from this result that light harvesting Bchl II is associated with two low molecular weight polypeptides forming a complex with a molecular weight of about 96 000 (Figs. 8, 10).

Rps. palustris has light harvesting Bchl II with similar spectral properties as *Rps. capsulata* [16]. The protein pattern of *Rps. palustris* membranes showed two bands which comigrate with the two low molecular weight bands of light harvesting Bchl II of *Rps. capsulata.* A protein corresponding to band 1 of LH II is not present (Fig. 8k).

Amino acid composition of light harvesting proteins

The purified light harvesting Bchl II complex was subjected to SDS polyacrylamide slab gel electrophoresis in a 12.5% gel (thickness 2 mm) in order to obtain a clear separation of the three light harvesting bands 1, 3 and 4. The bands were cut off from the gel and the proteins were eluted, concentrated and hydrolyzed. The results of the amino acid analysis are expressed as mole percent and as number of residues (Table II). Tryptophan and cysteine were not determined. The mole percent values for the proteins were calculated to total 100% omitting tryptophan and cysteine. SDS polyacrylamide gel electrophoresis with and without mercaptoethanol seems to indicate that no cystine is present. The polarity [19] of the three proteins was found to be 48 mole percent. The following molecular weights were calculated from mole percentage assuming one tyrosin per polypeptide: 17 350 (band 1), 13 350 (band 3) and 10 500 (band 4). The apparent molecular weights of these proteins estimated by SDS gel electrophoresis were 14 000 (band 1), 10 000 (band 3) and 8000 (band 4). Similar deviations in the estimations of molecular weights calculated from amino acid determination and mobility in SDS gel electrophoresis were described by Tonn et al. [20] and Fraker and Kaplan [18].

Discussion

The presence of three spectral forms of Bchl in *Rps. capsulata* [2,16] and the strong red shift of their infrared maxima were thought to be indicative of molecular interactions of bacteriochlorophyll or interaction of Bchl with the environment. The data on aggregated Bchl molecules in vitro [21,22] suggest that the individual absorption bands in vivo are attributed to various states of aggregation of Bchl molecules [23]. However, strong Bchl-protein interactions in vivo have been demonstrated by X-ray diffraction studies on the native Bchl a · protein complex from *Chlorobium limicola* [24]. Data indicating concomitant synthesis of native spectral forms of Bchl and major proteins of the photosynthetic apparatus provide further support for the idea that Bchl in vivo is associated with proteins [1,4,6,11,25–28]. Preparations enriched in light harvesting Bchl and one or more major proteins of intracytoplasmic membranes

have been isolated from *Rps. sphaeroides* [17], *Rhodospirillum rubrum* [29], *Chromatium vinosum* [15], and *Rps. palustris* [30]. Proteins thought to be associated with light harvesting Bchl (in vivo) have been previously isolated and purified from *Rps. sphaeroides* [18,31] and *Rhodospirillum rubrum* [20]. In this study the isolation and purification of two native Bchl-protein light harvesting fractions from *Rps. capsulata* are described for the first time. The isolated complexes show absorption spectra which were nearly identical with the spectral forms in the intracytoplasmic membrane, i.e. one major absorption maximum at 870 nm for light harvesting Bchl I (Fig. 2) and two major absorption maxima at 802 and 855 nm for light harvesting Bchl II (Figs. 7 and 9). The result of our studies shows that light harvesting Bchl I is associated with one polypeptide of an apparent molecular weight of 12 000. This is in accordance with results of kinetic [4] and genetic experiments [5].

The light harvesting Bchl II fraction contains three proteins of which the two smaller ones are associated with light harvesting Bchl II (Figs.8 and 9). In *Rps. palustris* the light harvesting Bchl II is associated with the bands corresponding to 3 and 4 of light harvesting proteins of *Rps. capsulata*, the band 1 is lacking (Firsow and Drews [38]). The function of the heavy protein in the light harvesting II Bchl complex of *Rps. capsulata* is unknown. During purification of the light harvesting II complex a carotenoid enriched fraction was isolated, which was free of proteins. We did not succeed in isolating a carotenoid protein from the light harvesting complex or membranes as described by Schwenker and Gingras [33]. Our kinetic [6,25] and genetic [5] experiments do not support the existence of a specific protein associated with carotenoids. We assume that the carotenoids are associated with the same proteins as light harvesting Bchl II.

It has been shown during this study and by other authors [17,29,30] that the native light harvesting Bchl II protein complex is a high molecular weight substance which sediments at 150 000 $\times g$ in an ultracentrifuge. A molecular weight of approximately 95 000 was estimated by gel electrophoresis (Fig. 10). An increased detergent concentration dissociates the Bchl from the proteins but does not effect a splitting of the native complex into subunits with the in vivo spectrum. The light harvesting II Bchl complex contained about 100 μg Bchl/mg protein. Assuming molecular weights of 8000 and 10 000 for the proteins which are bound to light harvesting Bchl II (Fig. 8i and 8j) and a molar ratio of 1 : 1 : 1 between the three proteins of the light harvesting II fraction a ratio of 3 molecules Bchl per proteins (8000 and 10 000) is calculated.

The assumption that 4 molecules of Bchl instead of 3 are associated with 2 protein subunits seems reasonable since about 25% of Bchl are lost by solubilization during isolation and purification (calculated on the basis of the Bchl : protein ratio of crude membranes and of purified complex and the estimated molar ratio of light harvesting proteins II/total membrane protein). On the basis of this assumption and the estimated molecular weight of the native complex (Fig. 10) it is proposed that the native light harvesting II complex of *Rps. capsulata* consists of 16 molecules of Bchl and 4 of each (8000 and 10 000 mol. wt.) proteins.

Antiserum against the native light harvesting Bchl II complex, solubilized in lauryl dimethyl amine oxide, forms one precipitation line in the immunoelectro-

phoresis with the native complex but not with the isolated denaturated proteins (Dierstein, personal communication). This result gives additional evidence that the native complex in the membrane consists of two proteins associated with Bchl.

The lability of light harvesting Bchl I complex under experimental conditions makes it more difficult to construct a model. In this case the molar ratio of Bchl to protein was found to be approximately 1 : 1. Bchl is associated with one protein of about 12 000 mol. wt.

The electrophoretic behavior of the partially dissociated complex (Fig. 3) indicates a molecular weight of about 20 000. Therefore it seems reasonable to assume that the native subunit of the light harvesting Bchl I complex consists of 2 polypeptides which are associated with 2 Bchl molecules.

Under steady state conditions 30 light harvesting Bchl I molecules are formed per reaction center [3,4]. The molecular mass of 30 molecules of Bchl and 30 molecules light harvesting I protein is approximately 390 000, about 2.6 times more than the mass of one reaction center [7]. The light harvesting Bchl II complex is the variable part of the photosynthetic unit [3,4,6,25,26]. The total molecular mass of the light harvesting II complex can be about 10 times greater than one reaction center. Molecular weights of membrane proteins calculated from migration velocity in SDS gels (refs. 18 and 19, and Figs. 5, 8 and 10) are somewhat smaller than those calculated from amino acid analysis (refs. 18 and 19, and Table II). This might be due to differences in the capacity of water soluble marker proteins and membrane proteins for binding SDS [20,34–36].

The polarity of the light harvesting II proteins (48%) is higher than the respective values determined for the organic solvent soluble polypeptide of *Rhodospirillum rubrum* (42%; ref. 20) and for band 15 of *Rps. sphaeroides* (38%; ref. 31). The density of the light harvesting II complex (1.05) is lower as the density of intracytoplasmic membranes of *Rhodospirillum rubrum* (1.165; ref. 37).

Antiserum specific for the light harvesting II complex precipitates in two dimensional immunoelectrophoresis the native light harvesting complex II. The antiserum, however, does not react with membranes which are dissociated by mild detergent treatment. These observations indicate that the light harvesting II protein bands 3 and 4 are associated with Bchl forming a complex localized in the hydrophobic internal region of the intracytoplasmic membrane.

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