ISOLATION AND CHARACTERIZATION OF A BACTERIOCHLOROPHYLL-ASSOCIATED CHROMATOPHORE PROTEIN FROM *RHODOSPIRILLUM RUBRUM* G-9

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1. Introduction

In addition to photochemically active reaction centers, a variety of bacteriochlorophyll (BChl)–protein complexes presumed to serve in a light-harvesting (LH) capacity have been described for several species of photosynthetic bacteria [1–4]. In these complexes LH-BChl seems to be associated mainly to one or two protein components with molecular weights around 10 000. For *Rhodopseudomonas spheroides* there is immunological evidence that these LH-BChl-containing proteins are specific for chromatophore membranes and distinct from the reaction center proteins [1,5].

In this report we describe (a) the isolation of a BChl–protein–lipid complex (tentatively called LH-complex) from reaction center-depleted chromatophores of the carotenoidless mutant G-9$^+$ of *Rhodospirillum rubrum* and (b) the characterization of the protein part of this complex. This protein which was found to be soluble in a chloroform/methanol mixture, was also compared to the organic solvent soluble protein extracted from chromatophores of the wild type bacterium according to the method of Loach and Tonn [6,7].

2. Materials and methods

All reagents were of analytical grade. Ammonyx LO (lauryldimethylamine *N*-oxide, LDAO) Onyx Chemicals, Jersey City, NJ, was freeze-dried and recrystallized from ethylacetate. The reaction center-depleted chromatophores were obtained from the carotenoidless mutant G-9$^+$ of *R. rubrum* and from the wild type of this bacterium by isolating reaction center complexes first, according to the method of Okamura et al. [8] modified as described by Snozzi [9].

All the following purification steps were performed in the dark at 4°C. Remaining reaction centers were completely removed from 1 g frozen membranes through incubation in 20 ml 0.1% deoxycholate in 10 mM barbital buffer pH 9 for 25 min. After centrifugation at $2.8 \cdot 10^5 \times g$ for 60 min the blue-colored, reaction center-containing supernatant was discarded. The membrane pellet was then extracted twice with 20 ml 0.1% LDAO in 10 mM Tris–Cl buffer, pH 7.5, for 25 min. After recentrifugation LH-complexes were obtained in the supernatants. When the first LDAO extract still contained traces of reaction center complexes it was discarded. The green-colored, LH-complex-containing extracts were precipitated with ammonium sulfate at 60% saturation. The pelleted complexes were directly used for characterization or stored in a freeze-dried state after dialysis against water.

The organic solvent soluble protein was extracted with a mixture of chloroform/methanol (1:1) either from freeze-dried LH-complexes or directly from freeze-dried reaction center-depleted chromatophores. As described by Loach and Tonn [6,7] the protein was separated from pigment and lipid by gel filtration on Sephadex LH 20 in chloroform/methanol.

Analytical SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on 12% gels according to the method of Laemmli [10]. The gels were stained

Abbreviations: BChl-, bacteriochlorophyll-; LH-, light harvesting; LDAO, lauryldimethylamine *N*-oxide; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin

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with Coomassie Blue for proteins or with the periodic acid-Schiff stain method [11] for glycoproteins. For electrophoresis in the presence of 0.1% LDAO the same system was used except that the acrylamide concentration was 5%. Gels were run at 4°C in the dark at 80 V (6 tubes). The complex was checked for integrity by gel filtration on Sepharose 6 B in 0.1 M Tris–Cl pH 7.5 containing 0.1% LDAO.

Molecular weights were assigned from SDS–PAGE employing cytochrome *c* (Sigma) and a BDH marker mixture (mol. wt range 14 300–71 500, BDH Chemicals, England) as standards. Other molecular weight estimations were obtained from sedimentation equilibrium analysis on a MSE analytical centrifuge equipped with an ultraviolet scanning system. These experiments were carried out in 60% formic acid. The partial specific volume used for LH-protein (0.82 cm^3/g) was determined with a digital precision densitometer DMA 10 (Anton Paar K.G., Graz, Austria).

Protein was assayed by the method of Lowry et al. [12] using BSA as a standard with SDS present in the samples.

The total lipid phosphorous was determined by the method of Bartlett [13]. Assuming av. mol. wt 675 for phospholipids the concentration of lipid phosphorous was converted to micrograms of phospholipid. BChl was determined by the spectroscopic method of Smith and Benitez [14]. Absorption spectra were measured on a Cary 17 spectrophotometer. Amino acid analysis of hydrolysates in 6 N HCl and 4 N methanesulfonic acid [15] were carried out on a Beckman 121 C amino acid analyzer.

3. Results and discussion

After complete removal of the reaction center complexes, LDAO-solubilized LH-complexes showed an absorption spectrum similar to the one of reaction center-depleted chromatophores as shown in fig.1. Precipitated LH-complexes contained all the BChl present in the LDAO-extracts. Upon resuspension of the complexes in the presence of 0.1% LDAO the 863 nm absorption maximum (fig.1c) was shifted to 820 nm (fig.1d). Subjected to gel filtration on Sepharose 6B the latter LH-complex solution yielded a single pigmented protein peak showing near-infrared absorption maxima at 820 nm and 770 nm (fig.1e).

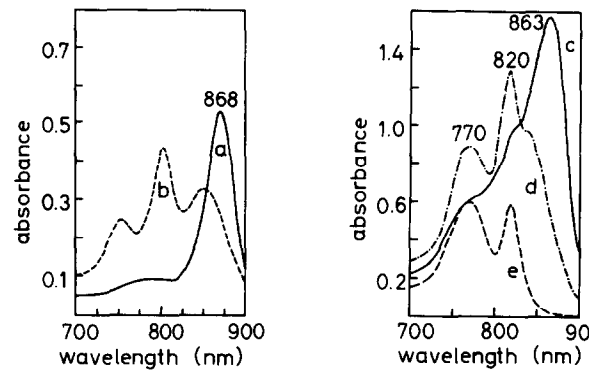

Fig.1. Near-infrared absorption spectra: (a) Reaction center-depleted chromatophores of *R. rubrum* G-9$^+$. (b) Supernatant after deoxycholate extraction. (c) LH-Complexes solubilized with LDAO. (d) LH-Complexes resuspended after ammonium sulfate precipitation. (e) LH-Complex solution of (d) after filtration on Sepharose 6B.

The elution volume of this peak indicated mol. wt 68 000.

Quantitative determinations of the protein, phospholipid and BChl contents of the complex yielded approximately 66% protein, 29% phospholipid and 5% BChl on the basis of dry weight.

SDS–PAGE of the LH-complex revealed one major band with an apparent mol. wt 14 000 (fig.2). Under modified electrophoresis conditions, e.g., lower SDS content in the sample or SDS–PAGE 48 h after sample denaturation, the formation of aggregates of higher molecular weight was observed. Electrophoresis in 12% acrylamide gels has shown that with 2% SDS in the sample BChl is removed from the protein: the observed faster moving BChl band did not coincide with the 14 000 protein band. On the other hand, BChl remained attached to the protein during LDAO–PAGE as reported before [16].

All the protein present in the complex dissolved in chloroform/methanol. Gel filtration on Sephadex LH-20 yielded a single protein component which also ran at 14 000 on SDS–PAGE (fig.2). The formation of aggregates observed during electrophoresis prior to the removal of pigment and lipid was greatly reduced. Since the 14 000 component could be stained with the periodic acid Schiff reagent as well as with Coomassie Blue it seems likely that the LH-complex contains a glycoprotein.

Sedimentation equilibrium analysis showed a high

Table 1
Amino acid composition of the organic solvent soluble polypeptide isolated from LH-complexes and reaction center-depleted chromatophores from G-9$^+$ and wild type *Rhodospirillum rubrum*

	LH-Complex			G-9 Chromatophores			Wild type chromatophores		
	Mol%	Average No. residues[e]	Nearest integer	Mol%	Average No. residues[e]	Nearest integer	Mol%	Average No. residues	Nearest integer
Lysine	2.45	2.92	3	2.3	2.82	3	2.2	2.74	3
Histidine	1.1	1.30	1	0.9	1.14	1	1.1	1.39	1
Arginine	6.5	7.76	8	6.2	7.60	8	6.2	7.55	8
Aspartic acid	4.4	5.26	5	4.05	4.97	5	3.7	4.59	5
Threonine[a]	8.1	9.72	10	8.0	9.76	10	7.9	9.71	10
Serine[a]	6.65	7.95	8	6.5	7.93	8	6.3	7.78	8
Glutamic acid	11.2	13.46	13	10.9	13.33	13	10.6	13.03	13
Proline	5.1	6.13	6	4.8	5.84	6	4.8	5.86	6
Glycine	4.9	5.88	6	4.8	5.88	6	4.7	5.86	6
Alanine	7.6	9.13	9	7.4	9.04	9	7.6	9.26	9
Valine	5.65	6.77	7	5.5	6.67	7	5.3	6.48	6
Methionine	1.9	2.27	2	2.5	2.6 /3.05[c]	3	1.8	2.22	2
Isoleucine[b]	4.65	5.57	6	5.45	6.68	7	6.0	7.31	7
Leucine[b]	16.3	19.50	20	17.4	21.26	21	18.1	22.2	22
Tyrosine	0.25	0.32	0	0.25	0.30	0	0	0	0
Phenylalanine	8.0	9.62	10	8.2	10.06	10	8.9	10.87	11
Cysteine	0	0	0	0	0/0[c]	0	0	0	0
Tryptophan[d]	5.25	6.27	6	4.9	6.09	6	4.8	6	6
Total (and min. mol. wt)			120(14 200)			123(14 500)			123(14 300)

[a]Extrapolated to zero time of hydrolysis
[b]Values after 72 h of hydrolysis only
[c]Determined as cysteinesulfonic acid and as methionine sulfone respectively, according to Hirs [17]
[d]Determined after hydrolysis in methanesulfonic acid, according to the method of Liu and Chang [15]
[e]Based on the presence of one histidine per polypeptide. The values are the averages of 24-, 48- and 72 h hydrolyses

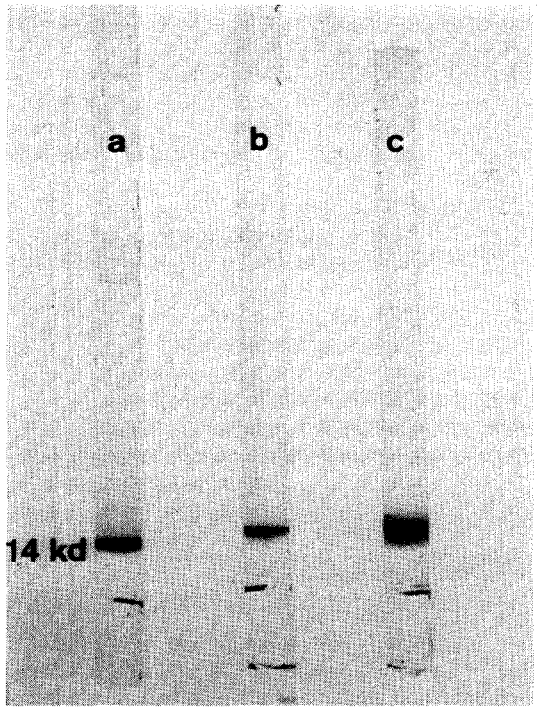

Fig.2. SDS-PAGE of (a) LH-complexes and of the chloroform/methanol-extracted proteins from G-9$^+$ (b) and wild type (c) *R. rubrum* chromatophores. Coomassie Blue staining.

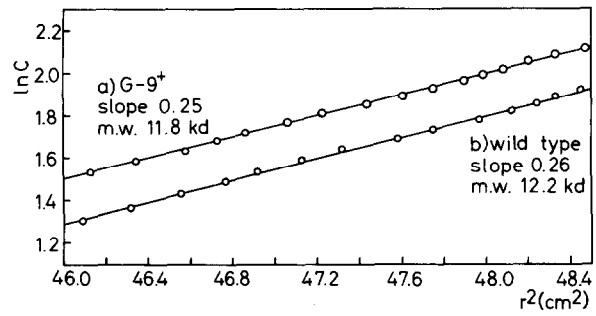

Fig.3. Equilibrium sedimentation experiment on chloroform/methanol soluble proteins from (a) G-9 and (b) wild type *R. rubrum* chromatophores. Protein concentration 1 mg/cm^{-3}, rotor speed $4 \cdot 10^4$ rev/min, temperature 20°C. Centrifugal equilibrium was reached after 45 h. From the continuous lines apparent mol. wt 11 800 (a) and 12 200 (b) were obtained.

degree of purity of our preparation. From linear plots of ln C versus r^2 an apparent mol. wt 12 000 was obtained (fig.3).

The proteins extracted into organic solvent from reaction center-depleted chromatophores of the mutant G-9$^+$ and the wild type bacterium showed identical properties on SDS–PAGE and in equilibrium sedimentation.

The amino acid analyses of the chloroform/methanol soluble proteins from LH-complexes and reaction center-depleted chromatophores are given in table 1. It is evident that these preparations have an almost identical amino acid composition. Thus it seems very likely that the same protein which is found to be associated with BChl in the mutant G-9$^+$ of *R. rubrum* is also present in reaction center-depleted chromatophores of the wild type bacterium. Based on the presence of one histidine/polypeptide a minimum mol. wt approx. 14 000 is obtained from the three amino acid compositions in table 1. In contrast to the reports of Loach and Tonn [6,7] this value is of the same order as the apparent molecular weights obtained by SDS–PAGE and equilibrium sedimentation.

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