INVESTIGATION OF LIGHT-HARVESTING COMPLEX *RHODOPSEUDOMONAS SPHAEROIDES*

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1. Introduction

The investigation of LH complexes is of great interest since the main bulk of bacteriochlorophyll in purple bacteria is associated with them. Similar complexes have been isolated from different types of bacteria [1–5]. Two kinds of polypeptides have been found in the LH complexes from *Chr. minutissimum* [6], *Rh. palustris, T. roseopersicina, Ect. shaposhnikovii* [7] and *R. rubrum* [8,9]; however, only one has been discovered in the LH complex from *Rh. sphaeroides* [5]. This discrepancy may result from the use of two different electrophoretic systems [10]. In the present study the separation of two polypeptides from the LH complexes in two different electrophoretic systems containing SDS is compared. In contrast with [5], the LH complex from the wild strain *Rh. sphaeroides* is now shown to contain two polypeptides (9000 and 12 000 daltons). These polypeptides were not separated by electrophoresis in 10% polyacrylamide gel with SDS as in [13].

2. Materials and methods

The LH complexes were isolated from chromatophores of *Chr. minutissimum* and *T. roseopersicina* treated with Triton X-100 by the electrophoretic technique in a 7% polyacrylamide gel as in [11]. The LH complex of *Rh. sphaeroides* was isolated as in [5] with slight modifications.

Several systems of electrophoresis in polyacrylamide gel were used. System I [12]: the gel contained 7% acrylamide, 0.14% bis, 10 mM Tris, 5.6 mM glycine and 0.1% Triton X-100 (final pH 9.2). The electrode buffer contained 41 mM Tris, 22.4 mM glycine (final pH 9.2). System II [13]: the lower gel contained 10% acrylamide, 0.26% bis, 0.1% SDS, 375 mM Tris–HCl buffer (final pH 8.8). The upper gel contained 3% acrylamide, 0.08% bis, 0.1% SDS and 125 mM Tris–HCl (final pH 6.8). The electrode buffer, 25 mM Tris, 192 mM glycine and 0.1% SDS (final pH 8.3). System III [14]: the gel contained 10% acrylamide, 0.27% bis, 0.1% SDS, 50 mM Tris–H_3PO_4 (final pH 7.0). The electrode buffer contained 50 mM Tris–H_3PO_4 (final pH 7.0) and 0.1% SDS.

Gels were polymerized in glass tubes with an inner diameter of 6 mm (systems II, III) or 13 mm (system I). All the reagents used in electrophoresis (except SDS) were purchased from Reanal (Hungary); SDS was from Serva (FRG).

Electrophoresis was carried out in a home-made apparatus at 3.76 mA/cm^2 in system I and 17.7 mA/cm^2 in systems II and III. This amounted to 5 mA/gel.

The samples for electrophoresis were treated with SDS and mercaptoethanol for 90 s at 100°C as in [6]. Bovine serum albumin, ovalbumin and cytochrome *c* from horse heart were used as standard proteins.

Gels were fixed in 50% methanol–glacial acetic acid (9.8:1) and were stained with Coomassie blue as in [14].

Abbreviations: LH, light-harvesting; SDS, sodium dodecylsulfate; *Chr., Chromatium; Rh., Rhodopseudomonas; T, Thiocapsa, Ect., Ectothiorhodospira; R, Rhodospirillum;* kd, kilodalton; bis, *N,N'*-methylene bisacrylamide

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The densitograms were scanned with the microphotometer G-II and the recorder GIBI (GDR). The absorption spectra were measured on the modified spectrophotometer SF-14 (USSR) within 410–900 nm.

3. Results and discussion

We have compared the ability of systems II and III to separate low molecular polypeptides from the LH complexes *Chr. minutissimum* and *T. roseopersicina*. Earlier it was shown that these complexes consisted of two types of polypeptides [3,4]. There is no separation of such polypeptides in system II (fig.1). We obtained similar results in attempting to separate polypeptides of the LH complex *Chr. minutissimum* in other electrophoretic systems [3]. Unlike a similar system containing phosphate buffer [15], system III affords a good separation of reaction center proteins [7].

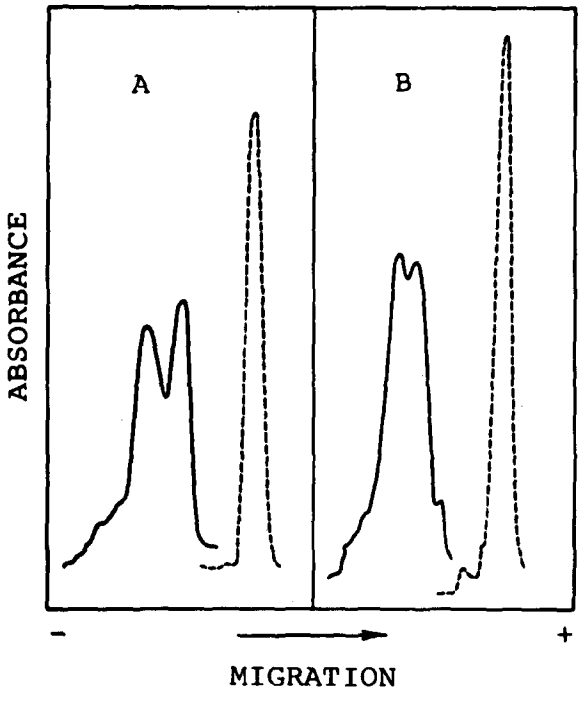

Fig.1. Gel scans of low molecular polypeptides from the LH complexes of *Chr. minutissimum* (A) and *T. roseopersicina* (B): solid line, system III; dashed line, system II. Absorbance in arbitrary units.

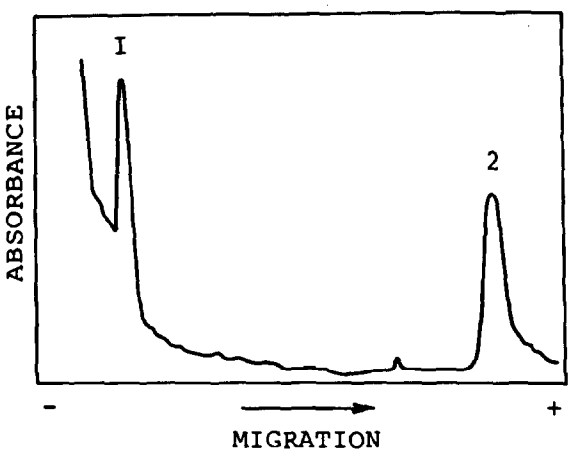

Fig.2. Gel scan of the LH complex from *Rh. sphaeroides* isolated as in [5] in system I: (1) LH complex; (2) low molecular protein.

Electrophoresis in system I also separated a low molecular protein from the LH complex of *Rh. sphaeroides* which had been isolated as in [5] (fig.2). Therefore the complex was further purified by electrophoresis in system I (gel diameter 13 mm) in the presence of Triton X-100. Its absorption spectrum changed very slightly after purification (fig.3).

According to the technique in [5] only one type of the LH complex can be isolated from chromato-

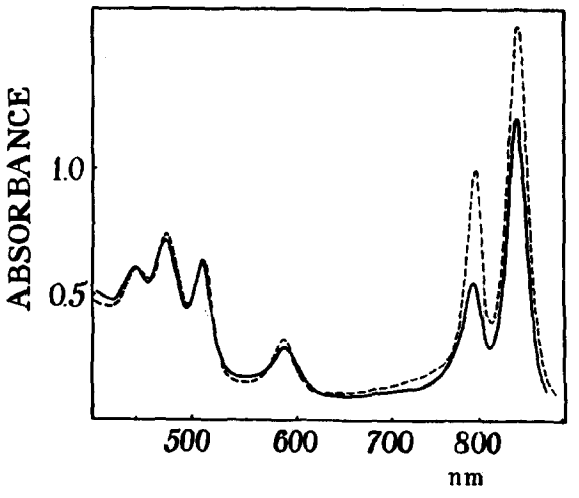

Fig.3. Absorption spectrum of the LH complex from *Rh. sphaeroides* before (dashed line) and after (solid line) additional purification by electrophoresis.

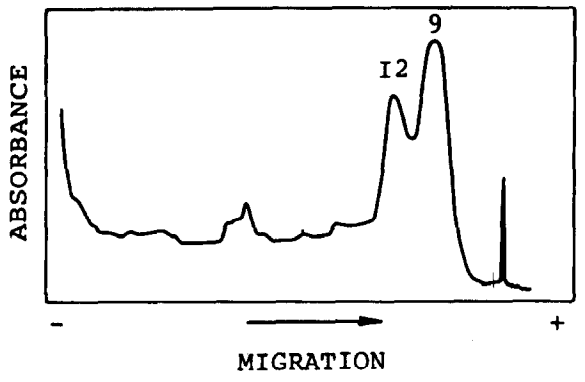

Fig.4. Gel scan of the LH complex from *Rh. sphaeroides* in system III. The numbers indicate the apparent molecular weights (kd).

phores of *Rh. sphaeroides* whereas chromatophores treated with Triton X-100 yield two LH complexes. One of them resembles the complex [5] and the other differs by the shape of the absorption bands within 400–500 nm region. The LH complex of *Rh. sphaeroides* isolated by electrophoresis as in [6] and determined after slight SDS treatment (30 min, 24°C) in a 10% gel is 80 kd. The LH complexes from the other bacteria are in the 60 – 100 kd region, as measured in 10% gel [7].

The purified LH complex from *Rh. sphaeroides* treated with SDS and mercaptoethanol for 90 s at 100°C was resolved into two polypeptides, 9 kd and 12 kd, respectively (fig.4). Similar results were obtained for the LH complex from *Rh. sphaeroides* isolated by electrophoresis. These data are in good agreement with the results obtained recently in our laboratory: two polypeptides of low molecular weight have been found in the LH complexes from *Chr. minutissimum, T. roseopersicina, Ect. shaposhnikovii, Rh. palustris* [6,7] and *R. rubrum* [8]. The existence of two kinds of polypeptides (13.5 kd and 9.9 kd) has also been observed [16] in studying proteins from chromatophores of a wild species of *Rh. sphaeroides.*

The 9 kd polypeptide is possibly similar to 'fraction 15' [17,18]. This fraction, a complex of 9 kd polypeptide and bacteriopheophytin, is probably a result of a secondary association of protein and pigment during elution since both components have identical electrophoretic mobility in the electrophoretic system used; both possess hydrophobic properties also. This assumption is supported by the data on separation of polypeptides of a complex similar to 'fraction A' [4]. We separated bacteriopheophytin and polypeptides of low molecular weights by electrophoresis in the discontinuous buffer system (A.A.M., unpublished data).

These results suggest a common principle of arrangement of the LH complexes isolated from wild types of purple bacteria: they contain two polypeptides of low molecular weight.

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