Occurrence of the Bacteriochlorophyll-Binding Polypeptides B870α and B800−B850α in the Mutant Strains *Y5* and *A1a*$^+$ of *Rhodopseudomonas capsulata*, which are Defective in Formation of the Light-Harvesting Complexes B870 and B800−850, Respectively

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The mutant strains *A1a*$^+$ and *Y5* of *Rhodopseudomonas capsulata* are defective in formation of the light-harvesting complexes B800–850 and B870, respectively, but synthesize the large bacteriochlorophyll-binding polypeptides of the respectively complexes, shown by amino acid composition, N- or C-terminal sequence and immunoblotting. The stable assembly of the light-harvesting complexes in the membrane seems to be dependent from the coordinated synthesis of all pigment and protein components.

Introduction

Mutants of photosynthetic bacteria defective in the formation of the photosynthetic apparatus have been selected after chemical mutagenesis [1–4] irradiation with UV light [5] and transposon mutagenesis [6, 7]. Mutant strains blocked in bacteriochlorophyll (Bchl) synthesis are photosynthetically inactive and do not assemble Bchl-carotenoid (crt)-protein complexes into intracytoplasmic membranes [8, 9]. Mutants unable to synthesize colored carotenoids have been described as blue-green phenotypes. Some of them are photochemically active, have reaction center (RC) and light-harvesting complex B870 but lost the capacity to form the light-harvesting complex B800–850 [1, 10]. Blue-green mutants very often can be reconstituted easily or revert spontaneously to wild type cells, indicating that single point mutations may cause pleiotropic effects on pigment biosynthesis and pigment-protein complex formation. It has been shown that the synthesis of pigment-binding polypeptides stops immediately when the synthesis of Bchl was inhibited [11]. Bchl seems to be crucial for the assembly of RC and the light-harvesting (LH) complexes in the membrane. It is, however, unknown, whether all polypeptides of a complex must be present to allow formation of the respective pigment-protein complex. The Bchl-less mutant *YS* of *R. capsulata* was found to be capable to synthesize the Bchl-binding 10000 polypeptide of the B800–850 complex. But the polypeptide did not accumulate in the membrane, it was rapidly degraded [12]. In order to learn more about the role of pigments and polypeptides in formation and stabilization of RC and LH complexes we began to investigate the occurrence of polypeptides in mutant strains which synthesize Bchl but are defective in RC or one of the light-harvesting complexes. Here we describe the mutant strains *A1a*$^+$ and *Y5* which are defective in synthesis of the pigment complex B800–850 and RC-B870, respectively [13, 14].

Materials and Methods

Cell material, isolation of membranes and light-harvesting complexes

The mutant strains *A1a*$^+$ (*crt*$^-$, *B800–850*$^-$) and *Y5* (*RC*$^-$, *B870*$^-$, *pho*$^-$) of *Rhodopseudomonas capsulata* were grown phototrophically (*A1a*$^+$) and chemotrophically under semiaerobic conditions (*Y5*) as described recently [1, 13]. Fermentors inoculated with the strain *A1a*$^+$ were gassed for 10 min with purified nitrogen in order to remove oxygen before illumination was switched on. The culture vessels

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Abbreviations: Bchl, bacteriochlorophyll *a*; crt, carotenoids; RC, reaction center; LH, light-harvesting; *R.*, *Rhodopseudomonas;* SDS, sodium dodecyl sulfate.

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were irradiated with light intensities of about 2000 lx at the surface of the vessel. The light intensity was raised when the density of the culture increased.

The membrane fraction was isolated from the crude French-press homogenate by differential and sucrose density centrifugation [15]. The light-harvesting complexes B870 and B800–850 were solubilized from membranes with detergents and isolated by sucrose density centrifugation and column chromatography or polyacrylamide gel electrophoresis [13, 15, 16].

Isolation of polypeptides from freeze dried membranes or complexes

The polypeptides of the B870 complex were extracted with chloroform/methanol/ammonium acetate from freeze dried membranes of the mutant *Ala*$^+$ and separated by chromatography on Sephadex LH 60 (Pharmacia, Freiburg) as described [17]. Three fractions were obtained [17]. The peak fraction II was isolated and the volume reduced from 105 ml to about 1.6 ml by rotary evaporation. After addition of 6 ml chloroform/methanol (1:2 v/v) the extract was centrifuged (20 min, 15000 × *g*). The pellet was reextracted with the same solvent and again centrifuged. The supernatants were combined and rechromatographed on LH 60 with chloroform/methanol/ammonium acetate. The pellet was solubilized in chloroform/methanol/acetic acid (4.5:4.5:1, v/v/v) and applied to columns with LH 60 (3.6 × 140 cm) equilibrated with the same solvent. The fractions obtained after chromatography were dialyzed against distilled water, lyophilized and stored at −20 °C.

The polypeptides of the light-harvesting complex B800–850 were extracted from freeze-dried membranes of the mutant strain *Y5*. The methods of isolation and identification have been described [18, 19]. The chloroform methanol soluble polypeptide from the peak fraction II of the LH 60 chromatography has been shown to be the 10000 polypeptide. The pellet, which was obtained when the peak fraction II was pooled, concentrated, diluted with chloroform-methanol and centrifuged, was solubilized in chloroform:methanol:acetic acid (4.5:4.5:1, v:v:v) and applied to LH 60 chromatography columns equilibrated with the same solvent.

SDS polyacrylamide gel electrophoresis

Samples of purified polypeptides and membranes were solubilized in sample buffer [20] at 70 °C for 10 min and separated on SDS polyacrylamide gradient slab gel (11.5–16.5% acrylamide).

Amino acid analysis

The protein samples were hydrolyzed [18] and analyzed on an automatic amino acid analyzer (Durrum model D-500). Tryptophan and cystein were determined by Lin and Chany [21] and Hirs [22]. The amino acid sequence determination is described in [23].

For determination of the carboxy terminal amino acids digestion with carboxypeptidase Y (Boehringer, Mannheim) was used [19].

Immunochemistry

Immunoglobulines directed against single polypeptides were isolated from antibodies raised against intracytoplasmic membranes of light-harvesting complexes [23]. Antibodies against the large Bchl-binding polypeptide of the light-harvesting complex B800–850 were raised in rabbits using polymerized purified polypeptides [24]. Immunoblotting was conducted as described in Towbin *et al.* [25]. Tris buffer (25 mM, pH 8.3, 192 μM glycin, 20% (v/v) methanol) was used for transfer. The specific activity of ^{125}I was 2 μCi/μg IgG. The ^{125}I-labelled IgG was diluted to 10^{-6} cpm. The blots were exposed to Kodak X-Omat R-film.

Results

The mutant strain *Ala*$^+$ of *Rhodopseudomonas capsulata* does not synthesize colored carotenoids and does not express the light-harvesting complex B800–850 [1]. The SDS-polyacrylamide electropherograms of the solubilized membrane fraction showed in the low molecular weight region between the dominant 12000 and 7000 M_r protein bands of the B870 complex a faint band with an apparent M_r of 10000 (Fig. 1). At this position the large Bchl-binding polypeptide of the B800–850 complex banded. In order to identify this minor component of the *Ala*$^+$ membrane two methods were used.

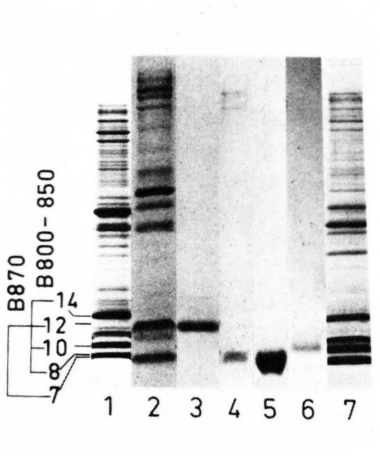

Fig. 1. SDS-Polyacrylamide gel electrophoresis. Polypeptides of light-harvesting complexes B870 (12000 and 7000) and B800-850 (14000, 10000 and 8000). Lane 1: membrane of strain *Y5;* 2: membrane of strain *A1a*$^+$; 3: 12000 polypeptide isolated from *A1a*$^+$; 4: 8000 polypeptide from strain *Y5;* 7000 polypeptide isolated from *A1a*$^+$; 6: 10000 polypeptide from strain *Y5:* 7: membrane of wild type strain St. Louis of *R. capsulata.*

Identification of the 10000 polypeptide in Ala$^+$ *as the large Bchl binding protein of the B800–850 complex*

Freeze dried membranes of the strain Ala$^+$ were extracted with chloroform-methanol-ammonium-acetate [18, 19]. The extract was subjected to chromatography on Sephadex LH 60. Three fractions were obtained. The fraction I contained reaction center polypeptides. The peak fraction II was not homogeneous by SDS-polyacrylamide gel electrophoresis and determination of the amino acid of the flanks of the peak (not shown here). The material was pooled and separated in chloroform-methanol- and chloroform-methanol-acetic-acid soluble material (see Methods) and again purified on Sephadex LH 60 columns. The major fraction, soluble in the more hydrophilic solvent was identified as the large Bchl-binding polypeptide of the B870 complex by the following criteria: The polypeptide has an apparent M_r of 12000 in SDS polyacrylamide gel electropherograms and ran to the same position as the large Bchl-binding polypeptide of the B870 complex (Fig. 1; [13, 14]). The amino acid composition of that polypeptide isolated from the B870 complex and the *Ala*$^+$ membrane were found to be identical (Table I). Antibodies against the B870 complex reacted with the organic solvent polypeptides isolated from the membranes of the mutant *A1a*$^+$ (Fig. 2, B2). Manual Edman degradation after deblocking [18] confirmed that the N-terminal amino acids are Met-Ser-Lys. No side sequence was detected. After repeated chromatography in chloroform/methanol/acetic acid the polypeptide fraction was homogeneous as shown by identical amino acid composition of both sides of the elution peak (not shown here). The minor fraction, soluble in chloroform-methanol, was also homogeneous and has an apparent M_r of 10000 (Fig. 1). The amount of the 10000 polypeptide relatively to the 12000 polypeptide was approx. 33% (w/w). The amino acid composition (not shown here) and the C-terminal amino acids (Ala-Gln-COOH) were the same as determined for the large Bchl-binding polypeptide of the B800–850 complex [19]. The protein fraction III from the LH 60 chromatography contained only one polypeptide which was found to be the small bacteriochlorophyll-binding polypeptide of the light-harvesting complex B870 [17]. The small Bchl-binding polypeptide of

Table I. Amino acid composition of the large bacteriochlorophyll binding polypeptide of light-harvesting complex B870 of *Rhodopseudomonas capsulata,* isolated from the mutant strains *A1a*$^+$ and *Y5.*

Amino acids	*A1a*$^+$ 25 min	*Y5*	
	mol/mol Peptide	25 min	50 min Hydrolysis Integer
Asp	2.67 (3)	2.70	2.69 (3)
Thr	2.77 (3)	2.85	2.63 (3)
Ser	2.14 (2)	2.26	1.81 (2)
Glu	2.56 (2–3)	2.57	2.51 (2–3)
Pro	2.15 (2)	2.10	2.35 (2)
Gly	3.39 (3)	3.60	3.50 (3)
Ala	8.00 (8)	8.00	8.00 (8)
Cys	0	0	0 0
Val	6.24 (6)	6.03	6.37 (6)
Met	0.79 (1)	0.64	0.92 (1)
Ile	2.82 (3)	2.77	2.96 (3)
Leu	8.49 (8–9)	8.28	8.45 (8–9)
Tyr	1.69 (2)	1.67	1.18 (2)
Phe	5.61 (6)	5.55	5.74 (6)
Trp	1.74 (2)	1.66	1.86 (2)
Lys	2.47 (3)	2.50	2.53 (3)
His	+	+	+ +
Arg	2.02 (2)	1.96	1.85 (2)

Protein samples were hydrolyzed as described in [27]; +, amino acid present but not quantified.
The values in paranthese are the most probable numbers of the residues.

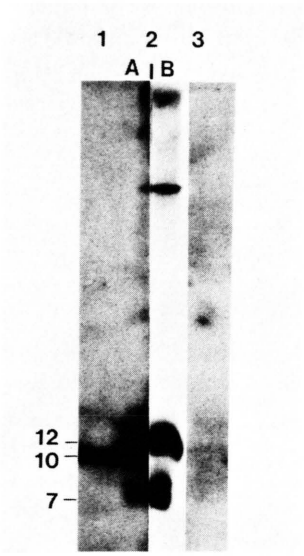

Fig. 2. Detection of pigment complex polypeptides in mutant strains of *R. capsulata* by immunoblotting. Organic solvent extractable polypeptides (see Materials and Methods) were separated on SDS-polyacrylamide gel electrophoresis. Lane 1: 10000 polypeptide of the B800–850 complex; lane 2: organic-solvent soluble fractions of mutants *A1a*$^+$ and lane 3 of *Y5*. The proteins were blotted and the blots incubated with immunoglobulines against the 10000 polypeptide of the B800–850 complex (A), and against the B870 complex (B). The blots were labeled with ^{125}I, dried and exposed to X-ray film for two days (lane 2) and 8 days (lanes 1 and 3). The numbers on the left side indicate the apparent $M_r \times 10^{-3}$.

the B800–850 complex was not detectable by SDS polyacrylamide gel electrophoresis or Edman degradation [17].

The light-harvesting polypeptides of the strain Y5

Chromatography on LH 60 of the organic solvent extract of freeze-dried membranes of the mutant strain *Y5* revealed three protein fractions which contained the three polypeptides of the B800–850 light-harvesting complex with apparent M_r of 14000, 10000 and 8000 as shown by Tadros *et al.* [18, 19]. The fraction II contained as contamination (about 20%) besides the 10000 polypeptide a polypeptide which was soluble in chloroform : methanol : acetic acid and ran in SDS polyacrylamide gel electrophoresis to the position of the 12000 polypeptide of the 870 complex (Fig. 1). Amino acid composition (Table I) N-terminal amino acid sequence (f-Met-Ser-Lys) and immunoblotting (Fig. 2) showed that the membrane of the mutant *Y5* containd the large Bchl-binding polypeptide of the B870 complex. The fraction III of the LH 60 chromatography contained the 8000 polypeptide of the B800–850 complex [18]. The 7000 polypeptide of the B870 complex was not detectable.

Discussion

In contrast to the wild type strain the mutant strains *A1a*$^+$ and *Y5* synthesize under low oxygen partial pressure only one light-harvesting complex. They can, however, synthesize one of the polypeptides of the missing complex, *i.e.* the 10000 polypeptide of the B800–850 complex in the mutant *A1a*$^+$ and the 12000 polypeptide in the mutant *Y5*. The amounts of these Bchl-binding polypeptides in the membrane relative to the proteins of the expressed complex are low (Fig. 1). Since we have not determined the complete amino acid sequence of both polypeptides isolated from both strains, differences in the primary structure cannot be excluded. It was observed that the 10000-polypeptide from *A1a*$^+$ but not from *Y5* was blocked on the N-terminus. The reasons have to be studied.

Although Bchl is present the respective spectral forms (B850 in *A1a*$^+$ and B870 in *Y5*) were not formed. The polypeptides seem to be rapidly degraded as described for the mutant *Y142* [23]. A defect in regulation of transcription of mRNA for the defective light-harvesting complex would be another possible explanation for the low concentrations of the polypeptides in the mutant membranes. All light-harvesting complexes of members of *Rhodospirillaceae* contain, so far as has been studied, two Bchl-binding polypeptides. Subfractions of these complexes which have native spectral forms but only one proteinous subunit have never been observed after detergent fractionation. Each Bchl-binding polypeptide spans the membrane once by one α-helix. Two polypeptides seem to be necessary to bind and stabilize 2–3 Bchl and 1 crt molecules. Oligomerization gives the basis for efficient exciton transfer [26].

Assembly and stable maintenance of light-harvesting complexes in the membrane seem to be dependent from the coordinated synthesis of all pigment and protein constituents of the complexes.

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