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A new possible binding site for bacteriochlorophyll *b* in a light-harvesting polypeptide of the bacterium *Ectothiorhodospira halochloris*

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Whole cells from *Ectothiorhodospira halochloris* were extracted with an organic solvent mixture. At least five small hydrophobic polypeptides representing most probably the light harvesting polypeptides were purified by gel filtration and consecutive FPLC-RP chromatography. The complete amino acid sequence of a 7.4 kDa polypeptide was determined. The polypeptide shows a three domain structure, indicative of an integral membrane protein, similar to the structure of the light-harvesting polypeptides from purple non-sulfur bacteria. Sequence homologies to the β-LHPs of purple bacteria range from 23.1% to 36.4%. The conserved intramembrane located histidine residue of the antenna polypeptides of purple non-sulfur bacteria, assigned as the possible binding site for bacteriochlorophyll, was found to be replaced by asparagine.

Purple bacteria; Bacteriochlorophyll *b*; Light-harvesting polypeptide; Amino acid sequence; (*Ectothiorhodospira halochloris*)

1. INTRODUCTION

Ectothiorhodospira halochloris is a slightly thermophilic, purple bacterium, which was isolated from extremely saline and alkaline soda lakes in Egypt [1]. *E. halochloris* is one of the bacteriochlorophyll *b* containing bacteria like *Rhodopseudomonas viridis* and shows the typical red shifted absorption maximum around 1020 nm and additional maxima at 800 and 830 nm. The photosynthetic apparatus is located in the intracytoplasmic membranes, which are organized as stacks of thylakoidal sacs and appear as the dominant internal structure. A three dimensional model of a complete thylakoidal stack has been reconstructed by Wanner et al. [9]. The photosynthetic unit consists of a central core, the reaction center, surrounded by a ring of light harvesting complexes. It has been shown by electron microscopy and digital image analysis that the photosynthetic complexes are arrayed hexagonally in the membrane with lattice spacings of approx. 13 nm [2,3], similar to *R. viridis* [22,23]. A B800/1020 antenna complex has been isolated and characterized [5] as consisting of three polypeptides differing in size [4,5]. In the case of purple non-sulfur bacteria a great number of light-harvesting polypeptides from various species have been isolated and their primary structures have been determined [13–20]. These antenna polypeptides exhibit a histidine residue in the central hydrophobic domain, assigned as the fifth ligand to the Mg atom of bacteriochlorophyll. Here we wish to report on the isolation, purification and sequence analysis of a 7.4 kDa polypeptide from *E. halochloris* revealing a new possible binding site for bacteriochlorophyll.

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Abbreviations: LHP, light-harvesting polypeptide; Bchl, bacteriochlorophyll; PTH, phenylthiohydantoin; RP, reverse phase; DTT, 1,4-dithio-DL-threitol

2. MATERIALS AND METHODS

Lyophilized cells of *E. halochloris* were extracted in the dark

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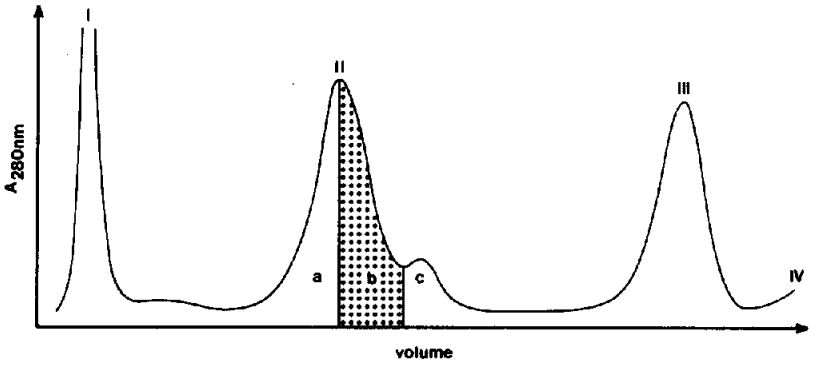

Fig.1. Gel filtration on Sephadex LH-60 in chloroform/methanol/ammonium acetate (1:1, v/v, 0.1 M) of the polypeptide extract from lyophilized cells into organic solvents. Peak I, large polypeptides; peak II, at least 4 polypeptides (7.5–5 kDa); peak III, small hydrophobic polypeptide(s); peak IV, pigments and lipids.

at 4°C several times with chloroform/methanol/ammonium acetate (1:1, v/v, 0.1 M) containing 5 mM octadecanthiol (in later experiments chloroform was replaced by methylene chloride). In order to separate antenna polypeptides from larger polypeptides and pigments, the extract was applied to a Sephadex LH-60 column (4 × 150 cm). Further purification was achieved by FPLC RP chromatography (Pharmacia, ProRPC HR 10/10) with a linear water/acetonitrile gradient, containing 0.1% TFA at constant concentration.

For reductive alkylation [6], 600 μg purified protein were dissolved in 1 ml buffer (570 mg guanidine hydrochloride, 60 mg Tris, 10 mg phenol, 0.5 mg EDTA, pH 8.6), 15 mg DTT were added and, after an incubation for 24 h at room temperature under Freon, 36 mg iodoacetamide were added. After 4 h in the dark, the reaction was stopped with 1 ml formic acid. Desalting was achieved by gel filtration on a P-2 column (BioRad, +200 mesh) in 50% formic acid.

For amino acid analysis, polypeptide samples (1–10 nM) were hydrolyzed in constant boiling HCl for 24 h at 110°C in vacuo and analyzed on a Biotronic LC 6000 E analyzer. The

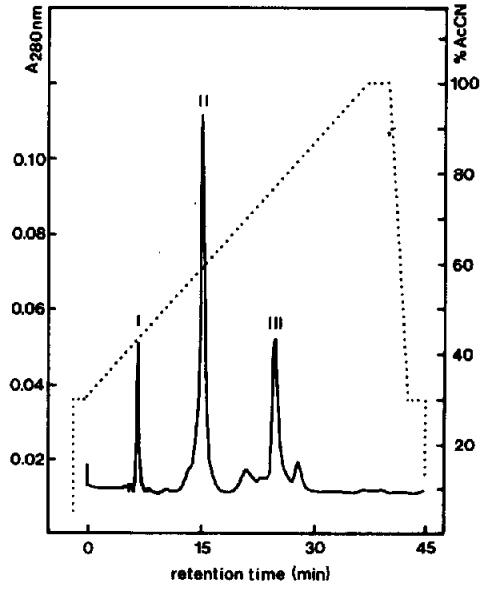

Fig.2. FPLC chromatography of pooled material of peak IIb from LH-60 on ProRPC: peak I, salt; peak II, the 7.4 kDa polypeptide; peak III, possibly another antenna polypeptide.

Table 1

Amino acid composition (mol/mol) of the 7.4 kDa antenna polypeptide

Amino acid	A	B	C
Asx	7.02	7	7
Thr	3.84	4	4
Ser	1.95	2	2
Glx	7.37	7	7
Pro	2.11	2	2
Gly	4.98	5	5
Ala	8.16	8	8
Cys	0.41	1	1
Val	1.05	1	1
Met	2.86	3	3
Ile	3.71	4	4
Leu	5.88	6	6
Tyr	2.77	3	3
Phe	1.1	1	1
His	3.54	4	4
Lys	–	0	0
Arg	4.73	5	5
Trp	nd	–	2

A, 24 hydrolysis; B, nearest integer; C, number of residues derived from amino acid sequence

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     5        10        15        20        25        30        35
TDIRTGLTDEECQEIHEMNMLGMHAYWSIGLIANA

     40        45        50        55        60        65
LAYAWRPFHQGRAGNRLEDHAPDYVRSALT
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Fig.3. Amino acid sequence of the 7.4 kDa polypeptide of *E. halochloris*. (——) Carboxy-terminal amino acids confirmed by carboxypeptidase digestion.

amino acid sequence was determined by the automated Edman degradation procedure on an Applied Biosystems 470 A protein sequencer. The PTH-amino acids were identified on a HPLC system (Milton Roy), which resembles the systems of Lottspeich [11] and Tsmasawa et al. [12]. For determination of the carboxy-terminal amino acid residues, the polypeptide (100 nM) was digested with a combination of carboxypeptidase A and B (Boehringer) at pH 8.5 [7,8]. The liberated amino acids were identified by amino acid analysis.

3. RESULTS

The organic solvent extract of the lyophilized cells was separated on a Sephadex LH-60 column (fig.1). Peak I possibly contains reaction centers and other large polypeptides, peak II + III include small hydrophobic polypeptides ranging from 3.5 to 7.5 kDa, peak IV contains the intensively coloured pigments. FPLC RP chromatography of peak II material revealed at least four polypeptides (not shown). For purification of the 7.4 kDa polypeptide only the pooled fractions of peak IIb were applied on a Pro-RPC column and separated with a linear gradient from 30% acetonitrile in water containing 0.1% TFA at constant concentration to 100% acetonitrile (fig.2). Peak I contains salt, peak II the 7.4 kDa polypeptide and peak III possibly another antenna polypeptide.

The amino acid composition of the 7.4 kDa polypeptide is depicted in table 1. No lysine was found. For the first time a cysteine residue was detected in a bacterial antenna polypeptide and as a consequence the polypeptide was alkylated before Edman degradation. The polarity of this peptide defined by Capaldi and Vanderkooi [24] is 44.6%.

The complete amino acid sequence of the 65 amino acid long polypeptide was determined in a single sequencer run (fig.3). In order to confirm the carboxy-terminal amino acids, digestion with carboxypeptidase A and B was performed. The five last amino acid residues were identified accurately by the kinetics of their liberation (fig.3).

According to its significant homology to the known sequences of β-antenna polypeptides (fig.4), the 7.4 kDa polypeptide was assigned as β-chain. Based on the amino acid sequence the 7.4 kDa polypeptide has a true molecular mass of 7415 Da.

4. DISCUSSION

E. halochloris is a representative of the Ectothiorhodospiraceae, a family which has recently been placed between the Rhodospirillaceae and the

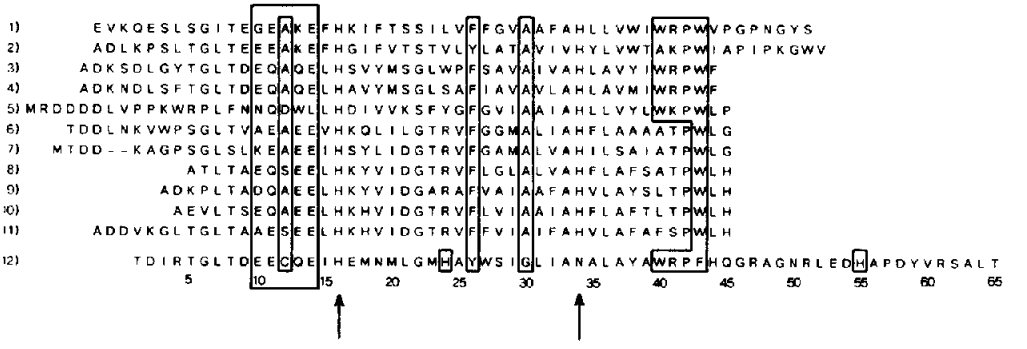

Fig.4. Sequence homologies between the 7.4 kDa β-polypeptide of *E. halochloris* and the antenna polypeptides of purple non-sulfur bacteria and the green bacterium *Chloroflexus aurantiacus*. Aligned amino acid sequences of β-polypeptides of (1) *Rhodospirillum rubrum* B890, (2) *Rhodopseudomonas viridis* B1015, (3) *Rhodobacter sphaeroides* B890, (4) *Rhodobacter capsulatus* B890, (5) *Chloroflexus aurantiacus* B806–866, (6) *Rhodobacter sphaeroides* B800–850, (7) *Rhodobacter capsulatus* B800–850, (8) *Rhodopseudomonas acidophila* 7750 B800–850, (9) *Rhodopseudomonas acidophila* 7750 B800–820, (10) *Rhodopseudomonas acidophila* 7050 B800–850, (11) *Rhodopseudomonas acidophila* 7050 B800–820, (12) *E. halochloris*. Important residues or domains are boxed. Arrows indicate the conserved histidines.

Chromatiaceae [10]. It was therefore of interest to investigate the primary structures of the antenna polypeptides of such a species and to compare them with the known sequences of antenna polypeptides of members of the Rhodospirillaceae. A further point of interest was the extreme bathochromic shift of the antenna complex from *E. halochloris* (structure-function relationship). The 7.4 kDa polypeptide shows the same three domain structures as the intramembrane antenna polypeptides of other purple bacteria [13–19] (fig.4). As compared to the sequences of purple non-sulfur bacteria the 7.4 kDa polypeptide (65 amino acid residues) exhibits an extended C-terminal domain. The first cysteine found in a bacterial antenna polypeptide is located in position 12, four residues upstream of the β-typical conserved histidine in the N-terminal domain. The most interesting mutation is the exchange of histidine 34 to asparagine. From its conservation it was postulated that this specific histidine acts as the fifth ligand to the Mg atom of bacteriochlorophyll. Thus, it is possible that in the 7.4 kDa polypeptide asparagine as a nucleophile offers bacteriochlorophyll a binding site, as proposed in model systems by Cotton et al. [21]. In the case of the bacteriochlorophyll *c*-binding protein, which is located in the chlorosomes of the green bacterium *Chloroflexus aurantiacus*, asparagine as a possible binding site for bacteriochlorophyll has been suggested recently by Wechsler et al. [20]. Comparative structure analyses of antenna β-polypeptides from purple non-sulfur bacteria revealed exclusively alanine in the hydrophobic stretch in position His-4 (4 amino acids upstream of the conserved His) (fig.4). A small amino acid in this position with respect to the vicinal bound Bchl seems to be essential for sterical reasons [25]. In *E. halochloris* alanine is replaced by glycine. The 7.4 kDa polypeptide shows the typical elements that are characteristic for β-chains: four Glx within residues 10–14, a second conserved His in position 16, aromatic residues in positions 26 and 43 (fig.4). However, there are some distinct differences, which can probably be ascribed partially to the extreme growth conditions of the cells. Interestingly, the β-chain of *R. viridis*, another Bchl *b* containing bacterium with only one antenna complex (B1020), shows (except *Chloroflexus aurantiacus*) the least homology (23.1%). Therefore, the appearance of Bchl *b* does not seem to be the decisive factor for the differences described above (e.g. His-34, Cys-12, extended C-terminus). Taking into account the fact, that in *E. halochloris* at least five polypeptides in the molecular mass range of antenna polypeptides are synthesized, it seems reasonable to propose, that this photosynthetic bacterium contains two or maybe even more antenna complexes: e.g. a core antenna B1020 and a peripheral antenna B800–830. From its primary structure the possible relationship of the 7.4 kDa polypeptide however is not yet clear. The overall homology to the core antenna polypeptides of purple non-sulfur bacteria ranges from 23.1% to 36.4% and to the peripheral antenna polypeptides from 24.5% to 33.3%. Only a single element typical of core antenna [26] WRPF (residues 40–43) has been found. Thus homologies and typical elements can give at best evidence for a probable assignment of the 7.4 kDa polypeptide. The extended C-terminus and the existence of an additional histidine in this region (position 55) as well as in the hydrophobic stretch (position 24) (fig.4) raise the question as to whether there are considerable differences regarding number and organization of the antenna Bchl-molecules as compared to the antenna complexes of other purple bacteria.

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