The Complete Amino Acid Sequences of the B800–850 Antenna Polypeptides from *Rhodopseudomonas acidophila* strain 7750

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Spectrally pure B800–850 light harvesting complexes of *Rhodopseudomonas acidophila* 7750 were prepared by chromatography of LDAO-solubilised photosynthetic membranes on Whatmann DE-52 ion exchange resin. Two low molecular mass polypeptides (α, β) have been isolated by organic solvent extraction of the lyophilised B800–850 light harvesting complexes. Their primary structures were determined by liquid phase sequencer runs, by the sequence analyses of C-terminal *o*-iodosobenzoic acid fragments, by hydrazinolysis and by carboxypeptidase degradation.

B800–850-α consists of 53 amino acids and is 45.3% and 50.9% homologous to the B800–850-α antenna polypeptides of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*, respectively. The second very short polypeptide (B800–850-β, 41 amino acids) is 61.0% and 56.1% homologous to the corresponding polypeptides of *Rb. sphaeroides* and *Rb. capsulatus*. The molar ratio of the two polypeptides is about 1:1. Both polypeptides show a hydrophilic N-terminal domain, a very hydrophobic central domain and a short C-terminal domain. In both polypeptides the typical His residues, identified in all antenna polypeptides of purple nonsulphur bacteria as possible bacteriochlorophyll binding sites, were found.

Introduction

Purple nonsulphur bacteria may contain one to three different light harvesting complexes (LHC). Whereas *Rhodospirillum rubrum* and *Rhodopseudomonas viridis* form only one LHC with an absorption maximum at 880 nm and 1015 nm, respectively, *Rb. sphaeroides, Rb. capsulatus* and *Rp. acidophila* strain 10050 contain two LHC's: B800–850 with absorption maxima at 800 nm and 850 nm, and B890 (next to the reaction center) with an absorption maximum at 890 nm. The photosynthetic membranes of *Chromatium vinosum* and *Rp. acidophila* strain 7050 and strain 7750 form three distinct light harvesting antenna complexes (B800–820, B800–850 and B890), depending on the cell growth conditions.

In this paper the preparation of spectrally pure B800–850 antenna complex, the isolation and the sequence determination of the two major light harvesting polypeptides of B800–850 antenna complex from *Rp. acidophila* strain 7750 are described. Most of the methods used were developed during the elucidation of the primary structures of the antenna polypeptides from *Rs. rubrum* [2 and 3], *Rb. capsulatus, Rb. sphaeroides* [5 and 6], *Rp. viridis* [4] and *Chloroflexus aurantiacus* [7, 8 and 14] or for the spectral analyses of spectrally pure antenna complexes [1].

Abbreviations: PTH, phenylthiohydantoin; C/M/NH_4OAc = 1/1/0.1 M, chloroform/methanol 1:1 (v/v) containing 0.1 M ammonium acetate; B800–850, antenna complex with absorption maxima at 800 nm and 850 nm; B800–850-α(β), α(β)-polypeptide of the B800–850 antenna complex; Cp, carboxypeptidase; LHC, light-harvesting complex.

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Materials and Methods

Cell growth

Cells of *Rp. acidophila* strain 7750 (DSM 141) (kindly provided by Karin Schmidt, Göttingen (Germany)) were grown anaerobically in 10 l flasks with Malik's medium [9] at a light intensity of 2000 lux and a temperature of 35 °C. Cells were harvested in the late phase of exponential growth by centrifugation, washed twice with 20 mM Tris-HCl pH 8.0 and stored at −20 °C.

Preparation of spectrally pure antenna complexes

Spectrally pure antenna complexes were prepared as described by Cogdell *et al.* [13]. The B800–850 antenna complex was dialysed against deionized water und lyophilized.

Isolation of B800–850-α and B800–850-β antenna polypeptides

About 150 mg of freeze-dried B800–850 antenna complex was extracted 5 times with 4 ml C/M/NH_4OAc = 1/1/0.1 M at room temperature in dim light. The extracts were applied on a Sephadex LH-60 column (3.5 cm × 150 cm) equilibrated in the same solvent. Fractions were collected and their absorption at 280 nm recorded by a LKB Uvicord system. Fractions containing B800–850-α were purified on a Schleicher and Schüll-DEAE column (3.2 cm × 35 cm) in C/M/NH_4OAc = 1/1/0.1 M. The purified polypeptides were dialysed against deionized water and lyophilized.

Primary structure analysis

Partial cleavage of B800–850-α with *o*-iodosobenzoic acid was done according to Mahoney and Hermodson [10]. The resulting peptides were separated on Biogel P-4(−400 mesh) and then subjected to sequence analysis. Automated and manual N-terminal amino acid sequence analysis was performed as described earlier by Brunisholz *et al.* [4]. PTH-arginine and PTH-histidine were identified by HPLC on a Partisil-5 PAC 5 μm column (isocratic solvent system; 10% methanol, 2.5% 1,4-dioxan, 30 mM ammonium acetate in dichloromethane). The C-terminal amino acid sequence determination was done by carboxypeptidase A/B digestion according to Ambler [15, 16], by carboxypeptidase P digestion and by hydrazinolysis as described by Akabori and Narita [11]. The amino acids were identified on a Biotronic LC-6000E amino acid analyser.

Results

Isolation of spectrally pure antenna complexes

After DE-52 ion exchange chromatography of LDAO-extracted photosynthetic membranes of *Rp. acidophila* strain 7750 grown at high temperature, two spectrally pure antenna complexes were isolated: B800–850 (Fig. 1) and B890. Upon manual Edman degradation of B800–850 three distinct PTH-amino acids (Val, Thr, Asn) were found in the second cycle. The comparison with the sequences of the peptides of the B800–820 antenna complex [paper in preparation] showed that the B800–850 LHC-preparation contained a variable amount of peptides typical for B800–820 LHC (usually less than 20%).

Isolation of B800–850-α and B800–850-β

The extraction of freeze-dried B800–850 antenna complex with C/M/NH_4OAc = 1/1/0.1 M and subsequent size exclusion chromatography on Sephadex LH-60 resulted in a baseline separation of B800–850-α and -β (Fig. 2a). The void volume fraction contains unidentified polypeptides in small amounts. Peak II contains B800–850-α and peak III B800–850-β. Fractions containing B800–850-α were purified on a DEAE ion exchange column in C/M/NH_4OAc = 1/1/0.1 M from the B890-β-polypeptides [Fig. 2b]. B800–850-α was not adsorbed to the column whereas B890-β eluted with 8% acetic acid in the solvent mixture.

Amino acid sequence determination

The strategy for elucidation of the complete primary structure was as described in earlier papers [2–8]. The N-terminal amino acid residues were determined by a long sequencer run. The C-termini were verified by Edman degradation of C-terminal *o*-IBA fragments, by carboxypeptidase digestions and by hydrazinolysis. In contrast to the light harvesting polypeptides B880-α from *Rs. rubrum* [2], B890-α from *Rb. sphaeroides* [6] and B806–866-α of *Chloroflexus aurantiacus* [7 and 14], the N-termini of B800–850-α and -β are not blocked by a formyl group.

B800–850-α: Automated Edman degradation in a liquid phase sequencer revealed the first 50 (out of 53) amino acid residues without gaps (Fig. 3a). The determination of the remaining residues was not possible as a result of an excessive PTH-amino acid background. B800–850-α contains 3 Trp residues located in positions 7, 40 and 45. By partial cleavage of

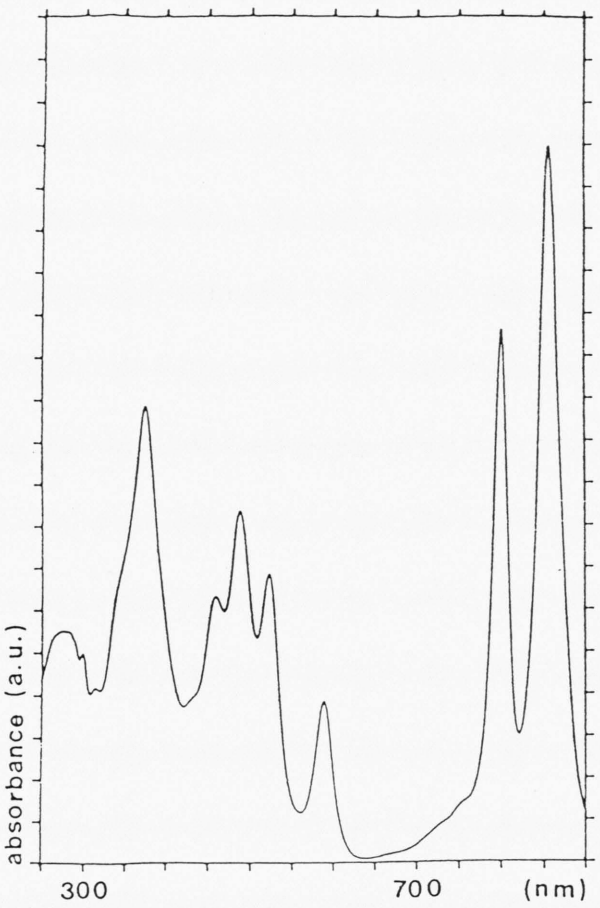

Fig. 1. Absorption spectrum (250–900 nm) of the B800–850-light harvesting complex from *Rhodopseudomonas acidophila* 7750.

B800–850-α with *o*-iodosobenzoic acid and chromatography on Biogel P-4 a C-terminal polypeptide Phe^{41}–Ala^{53} was isolated and sequenced. The short C-terminal peptide Gln^{46}–Ala^{53} was isolated as well but manual Edman degradation failed, probably as a result of pyroglutamate formation in position 46. *Hydrazinolysis of B800–850-α:* Amino acid analysis of the hydrazinolysis of B800–850-α revealed Ala as the C-terminal amino acid residue. *Carboxypeptidase A/B digestion:* Digestion of B800–850-α with carboxypeptidase A cleaved only Ala and small amounts of Lys. Addition of carboxypeptidase B liberated Lys and Val with a final apparent ratio of Ala/Lys/Val = 2/2/1. Together with the results described above the C-terminus Val^{49}–Lys^{50}–Lys^{51}–Ala^{52}–Ala^{53}–OH was confirmed.

B800–850-β: N-terminal sequence analysis by automated Edman degradation in a liquid phase sequencer established all 41 amino acids (Fig. 3b). Pro in position 38 caused merging of that PTH-amino acid with the PTH-amino acid background, but Trp^{39}, Leu^{40} and His^{41} could clearly be identified. After position 41 the PTH-amino acid background decreased and no amino acids were detectable suggesting His^{41} as the C-terminal amino acid. *Hydrazinolysis of B800–850-β:* Hydrazinolysis and subsequent amino acid analysis established His as the C-terminal residue. *Carboxypeptidase P digestion:* Digestion of B800–850-β with carboxypeptidase P released only His and Leu in this order and in a ratio of 1/1. Thus, the C-terminus was stated to be Leu^{40}–His^{41}–OH.

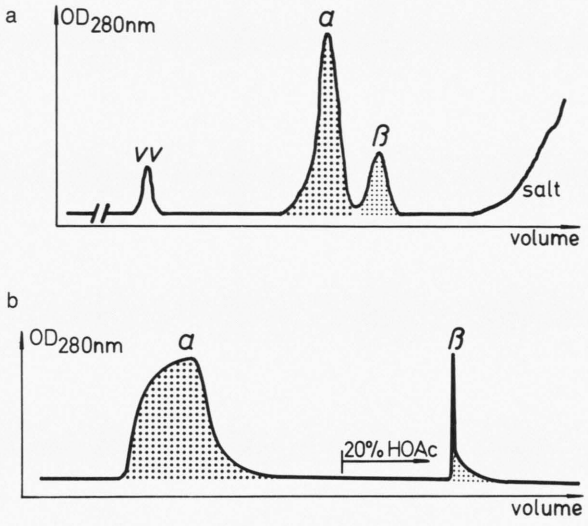

Fig. 2. a. Gel-filtration of the B800−850-antenna complex extracted with organic solvent. Separation was performed on a LH-60 column in chloroform/methanol = 1/1 (v/v) containing 0.1 M ammonium acetate.
v.v.: void volume,
α: B800−850-α with minor amounts of B890-α and B890-β,
β: pure B800−850-β.

b. Ion exchange chromatography on DE-32 of B800−850-α (Fig. 2a) in chloroform/methanol = 1/1 (v/v) containing 0.1 M ammonium acetate.
α: B800−850-α with some B890-α,
β: B890-β.

a

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                                1   2   3   4   5   6   7   8   9  10  11
H-Met-Asn-Gln-Gly-Lys-Ile-Trp-Thr-Val-Val-Asn-

 12  13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28
Pro-Ala-Ile-Gly-Ile-Pro-Ala-Leu-Leu-Gly-Ser-Val-Thr-Val-Ile-Ala-Ile-

 29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
Leu-Val-His-Leu-Ala-Ile-Leu-Ser-His-Thr-Thr-Trp-Phe-Pro-Ala-Tyr-Trp-
                                                ••••••••••••••••••••••

 46  47  48  49  50  51  52  53
Gln-Gly-Gly-Val-Lys-Lys-Ala-Ala-OH
••••••••••••••••••••••••••••••••••
                 ++++++++++++++++++++++++++
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b

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   1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17
H-Ala-Thr-Leu-Thr-Ala-Glu-Gln-Ser-Glu-Glu-Leu-His-Lys-Tyr-Val-Ile-Asp-

 18  19  20  21  22  23  24  25  26  27  28  29  30  31  32  33  34
Gly-Thr-Arg-Val-Phe-Leu-Gly-Leu-Ala-Leu-Val-Ala-His-Phe-Leu-Ala-Phe-

 35  36  37  38  39  40  41
Ser-Ala-Thr-Pro-Trp-Leu-His-OH
                  ••••••••••
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Fig. 3. a. Amino acid sequence of B800−850-α from *Rhodopseudomonas acidophila* 7750 determined by:
(----------) automated Edman degradation of $Met^{1}-Lys^{50}$,
(••••••••••) automated Edman degradation of the C-terminal partial Trp-fragment ($Phe^{41}-Ala^{53}$),
(++++++++) amino acids released by CpA/B-digestion,
(·····) C-terminal amino acid residue as determined by hydrazinolysis.

b. Amino acid sequence of B800−850-β from *Rhodopseudomonas acidophila* 7750 determined by:
(----------) automated Edman degradation of $Ala^{1}-His^{41}$,
(••••••••••) amino acids released by CpP-digestion,
(·····) C-terminal amino acid residue as determined by hydrazinolysis.

Discussion

The isolated B800–850 antenna complex of *Rp. acidophila* strain 7750 consists of two polypeptides soluble in organic solvents: B800–850-α with one and B800–850-β with two conserved His residues and sequence homologies to the corresponding light harvesting polypeptides of *Rb. sphaeroides* and *Rb. capsulatus*. A nonpigmented polypeptide described earlier [13] could not be found under the experimental conditions described. On the basis of amino acid sequence analysis B800–850-α consists of 53 amino acids having a molecular weight of 5653.9 Dalton, a polarity of 28.3% as defined by Capaldi and Vanderkooi [12] and is 45.3% and 50.9% homologous to the corresponding polypeptides of *Rb. sphaeroides* and *Rb. capsulatus*. The comparison with the B800–850-α's of *Rb. sphaeroides* and *Rb. capsulatus* (Fig. 4) shows in particular high sequence homology for the N-terminus (residues 1–17: 52.9% of all positions identical), the C-terminus (residues 38–47: 50% identical) and the environment of His^{31}, the postulated fifth ligand of the Mg^{2+} of bacteriochlorophyll a. At positions 7 and 40 (9^{th} residue downstream of His^{31}) two conserved Trp are found. Together with some aromatic residues at the C-terminus they probably interact with bacteriochlorophyll a, influencing its spectral properties. The hydrophobic core of the three polypeptides (residues 18–37: 15% identical) is much less conserved but all exchanged amino acids are structurally related. It is postulated that the hydrophobic core regions forms an α-helix beginning at position 18 (α-helix breaking Pro residues are found

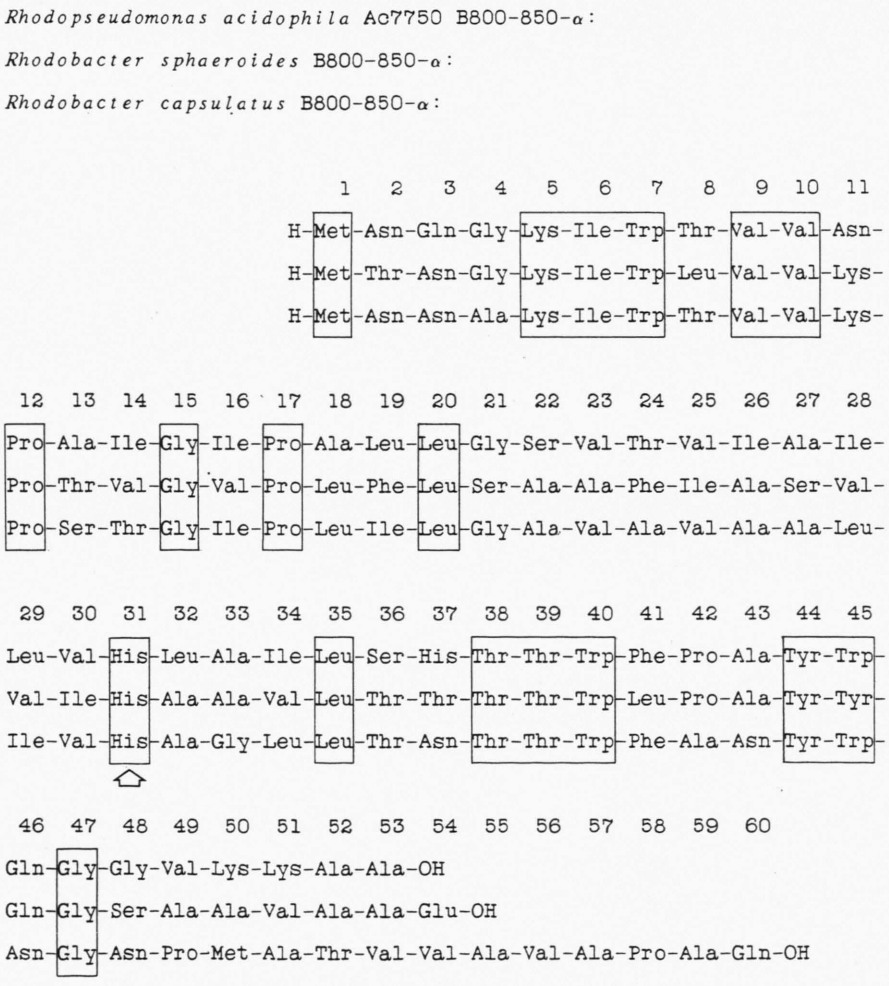
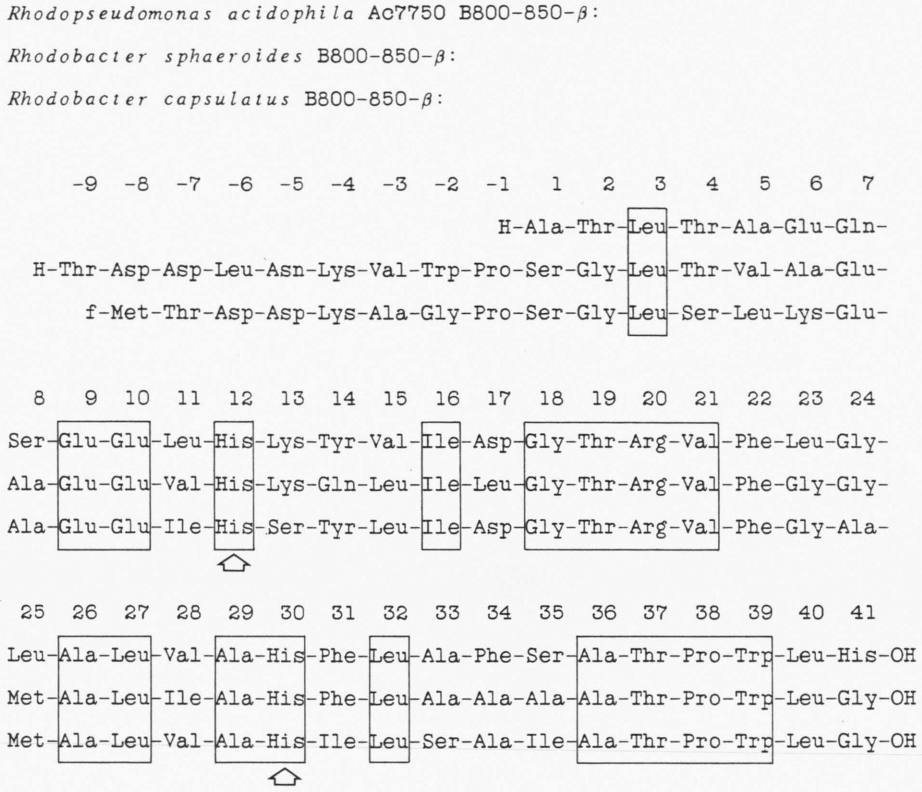


Fig. 4. Sequence homology between B800–850-antenna polypeptides from *Rhodopseudomonas acidophila* 7750, *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*.
Homologous amino acid residues are boxed. The arrows indicate the conserved His residues.



at positions 12 and 17) and ending at position 37 (residues 38–41, very hydrophilic cluster of polar residues) and spans the apolar core region of the photosynthetic membrane. The hydrophobicity plot (not shown) indicates a three domain structure with hydrophilic N- and C-terminal regions and a central hydrophobic core as has been found with other antenna polypeptides from purple bacteria. B800–850-β consists of 41 amino acids with a molecular weight of 4554.2 Dalton, 39.0% polarity and 61.0% and 56.1% homology to B800–850-β of *Rb. sphaeroides* and *Rb. capsulatus,* respectively (Fig. 4). In contrast to the β-polypeptides of the latter two bacteria the β-polypeptide of *Rp. acidophila* strain 7750 has a much shorter N-terminus. In the three β-polypeptides compared in Fig. 4, the residues in regions 9–22 (64.3% of all residues identical) and 26–41 (62.5% identical) are conserved. The residues in the neighborhood of His^{12} and His^{30} are particularly conserved. As in B800–850-α, Trp^{39} downstream of His^{30} influences the spectral properties of bacteriochlorophyll a. The hydrophobicity plot of B800–850-β (not shown) of *Rp. acidophila* clearly shows its three domain structure with a hydrophilic N-terminal and C-terminal and a hydrophobic central part.

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