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The complete amino acid sequence of the antenna polypeptide B806–866-β from the cytoplasmic membrane of the green bacterium *Chloroflexus aurantiacus*

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The bacteriochlorophyll *a*-binding polypeptide B806–866-β was extracted from membranes of the green thermophilic bacterium *Chloroflexus aurantiacus* with chloroform/methanol/ammonium acetate. Purification of the antenna polypeptide (6.3 kDa) was achieved by chromatography on Sephadex LH-60, Whatman DE-32 and by FPLC. The complete amino acid sequence (53 amino acid residues) was determined. The B806–866-β polypeptide is sequence homologous to the antenna β-polypeptides of purple bacteria (27–40%) and exhibits the characteristic three domain structure of the B870, B800–850 and B800–820 antenna complexes. The two typical His residues, conserved in all antenna β-polypeptides of purple bacteria, were found: His-24 lies within the N-terminal hydrophilic domain and His-42 within the central hydrophobic domain. This polypeptide together with the previously described α-polypeptide form the basic structural unit of the B806–866 antenna complex from *C. aurantiacus*.

Green photosynthetic bacterium; B806–866 antenna complex; Light-harvesting polypeptide; Amino acid sequence; (*Chloroflexus aurantiacus*)

1. INTRODUCTION

Chloroflexus aurantiacus is a green thermophilic photosynthetic bacterium capable of phototrophic growth under anaerobic conditions and chemoheterotrophic growth under aerobic conditions [1]. Its photosynthetic apparatus consists of light harvesting pigment-protein complexes containing bacteriochlorophyll *a* and *c*, and the photochemical reaction center [2]. Three different antenna complexes were found in *Chloroflexus*: the B 740 antenna complex (BChl *c*) of the chlorosome (extramembrane antenna); the B806-866 antenna complex (BChl *a*) situated in the cytoplasmic membrane in association with the reaction center; and the B 790 pigment-protein complex (BChl *a*), most probably located between the B 740 and B806–866 complex and responsible for the energy transfer [3]. The primary structure of the single antenna polypeptide of the B 740 complex was determined recently [4]. Furthermore, the primary structure of the antenna α-polypeptide of the intramembrane B806–866 antenna complex was elucidated [5]. This polypeptide shows the typical features (sequence homologies, three domain structure, conserved His residue for BChl *a* binding) of the α-antenna polypeptides of purple photosynthetic bacteria. In spite of the fact that all antenna complexes of purple photosynthetic bacteria consist of distinct α- and β-polypeptide pairs [6], so far only

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Abbreviations: BChl, bacteriochlorophyll; PTH, phenylthiohydantoin; C/M/NH_4OAc, 1:1 (v/v) chloroform/methanol containing 0.1 M ammonium acetate; TFA, trifluoroacetic acid; Hfo, formic acid; PAGE, polyacrylamide gel electrophoresis

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one single antenna polypeptide was found in the B806-866 antenna complex of *C. aurantiacus* [2,5]. Here we report on the isolation and sequence analysis of a second antenna polypeptide from the B806-866 complex of *Chloroflexus*, showing typical features of the β-antenna polypeptides of purple photosynthetic bacteria [7-11].

2. MATERIALS AND METHODS

Cells of *C. aurantiacus*, strain J-10-fl, were grown anaerobically in 10-l screw cap bottles (Pyrex) at low light intensity. The antenna polypeptides were extracted from lyophilised whole membrane fractions as described in [5]. The β-polypeptide (6.3 kDa) was separated from large polypeptides (> 18 kDa) and from pigments (BChl *a*, BChl *c* and carotenoids) by gel-filtration on Sephadex LH-60 (Pharmacia, 4.5 × 150 cm column) in C/M/NH_4OAc. Further purification was achieved by chromatography on DE-32 cellulose (Whatman, 1.1 × 23 cm column) in C/M /NH_4OAc and by FPLC on ProRPC (Pharmacia, HR 5/10 and HR 10/10) with a linear water/acetonitrile (containing 0.1% TFA) gradient. For amino acid analysis, polypeptide samples were hydrolysed in constantly boiling 6 N HCl (110°C) in vacuo and analysed on a Biotronic LC 6000E analyzer. Amino acid sequencing was carried out by either manual or automated Edman degradation in a Beckman 890C and an Applied Biosystems 470A protein sequencer [4]. PTH-amino acid derivatives were identified by the HPLC procedure described in [16]. PTH-Arg and PTH-His were identified on an isocratic HPLC system on Partisil-5-PAC (Whatman; Suter, F. unpublished). Determination of the carboxy-terminal amino acid residue was achieved by hydrazinolysis as described in [13]. In order to determine the carboxy-terminal sequence, cleavage at aspartic acid [14] and at tryptophan [15] was performed. Cleavage at aspartic acid was carried out by a procedure described in [14]: 3 mg polypeptide was dissolved in 1.5 ml of 25% propionic acid (containing 5 mg/ml tryptophan). The solution was hydrolysed for 15 h at 110°C in vacuo. 10 ml of 50% Hfo was added to the cold solution, which was then carefully evaporated to ~ 2 ml. The solution was applied to a Bio Gel P-10 column (– 400 mesh, 2 × 80 cm) in 50% Hfo. Cleavage at Trp was carried out by a modified BNPS-Skatol procedure [15]: 1 mg polypeptide was dissolved in 1 ml of 80% propionic acid/4 M guanidine hydrochloride (containing 7 mg/ml tyrosine) and incubated for 1 h at room temperature. 40 mg BNPS-Skatol (Pierce) was added and the solution incubated for 24 h at 40°C in the dark after protection with Freon (CCl_2F_2, DuPont). After addition of about 1 ml of 100% Hfo, the precipitated Skatol was removed by centrifugation. The superantant was applied to a Bio Gel P-4 column (200-400 mesh, 1.6 × 60 cm) in 50% Hfo.

3. RESULTS

The organic solvent extract of the membrane fractions was separated on a Sephadex LH-60 column (elution diagram as described in [5], fig. 1A). Peak I contained reaction centers and larger polypeptides. SDS-PAGE (not shown) of peak II

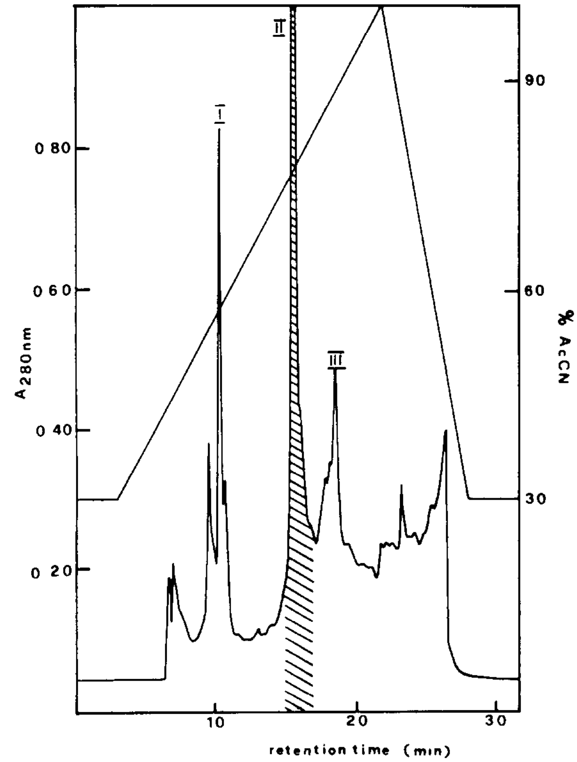


Fig.1. FPLC of pooled peak II fractions (from DE-32 anion-exchange chromatography) on ProRPC: peaks I-III were collected. Peak I, BChl *c*-binding polypeptide; peak II, β-polypeptide; peak III, α-polypeptide.

fractions revealed 4 bands with apparent molecular masses of 4, 6, 8 and 11 kDa (further details are described in [5]). The polypeptides of peak II were fractionated on a DE-32 column by a stepwise gradient system with 0, 3 and 20% acetic acid in C/M/NH_4OAc. The corresponding elution diagram was shown earlier in [5] fig.1B. The fractions of peak III contain largely the α- and β-polypeptides and small amounts of the BChl *c*-binding polypeptide as shown by manual Edman degradation. The polypeptide mixture of peak III was applied to a ProRPC-FPLC column and separated by a linear gradient from 30% acetonitrile in water to 100% acetonitrile (fig.1). Peak I represents the BChl *c*-binding polypeptide, peak II the β-polypeptide and peak III the α-polypeptide, as shown by amino acid analysis and Edman degradation. The amino acid composition of the β-polypeptide is depicted in table 1 (72 h analysis). The data agree well with those derived from the amino acid sequence analysis. No cysteine and threonine were found. For sequence analysis deblocking of the N-terminus as described in [7] was necessary, indicating that the N-terminus is most probably formylated. Hydrazinolysis showed Pro as the carboxy-terminal amino acid residue. 47 amino acid residues were identified by the automated Edman degradation (fig.2). In order to elucidate the carboxy-terminal amino acid sequence, cleavage at aspartic acid residues was performed. The cleavage products were separated on a Bio Gel P-10 column in 50% formic acid (elution diagram in fig.3A). One of the resulting peptides, Ile-26–Pro-53 found in peak b (fig.3A), was subjected to amino acid analysis (table 1).

Automated Edman degradation of this peptide fragment established the sequence Ile-26–Pro-50. This fragment was further cleaved at Trp residues. The resulting peptide fragments were separated on a Bio Gel P-4 column in 50% formic acid (elution

Table 1

Amino acid composition (mol/mol) of (I) the antenna β-polypeptide, (II) the C-terminal Asp fragment Ile-26–Pro-53, (III) the C-terminal Trp fragment Lys-49–Pro-53 and (IV) the C-terminal Trp fragment Leu-52–Pro-53

Amino acid	I			II		III		IV	
	A	B	C	A	C	A	C	A	C
Asx	7.68	8	8						
Thr									
Ser	0.77	1	1	0.9	1				
Glx	1.23	1	1						
Pro	4.53	5	5	1.7	2	2.07	2	0.98	1
Gly	2.31	2	2	2.0	2				
Ala	3.27	3	3	3.0	3				
Val	4.93	5	5	3.0	3				
Met	1.09	1	1						
Ile	2.98	3	3	2.1	3				
Leu	7.93	8	8	3.7	4	1.17	1	1.00	1
Tyr	2.08	2	2	1.5	2				
Phe	3.33	3	3	1.9	2				
His	1.84	2	2	0.9	1				
Lys	2.93	3	3	2.3	2	1.00	1		
Arg	2.00	2	2						
Trp	n.d.	n.d.	4	n.d.	4				

A, 72 h hydrolysis; B, nearest integer; C, number of residues as derived from amino acid sequence; D, 24 h hydrolysis. The data are based on (I) 2 Arg residues per polypeptide chain, (II) 2 Gly/polypeptide, (III) 1 Lys/polypeptide and (IV) 1 Leu/polypeptide

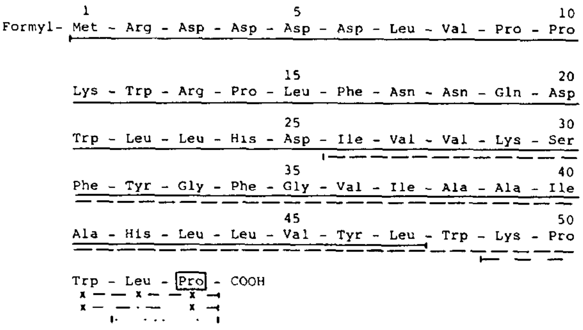
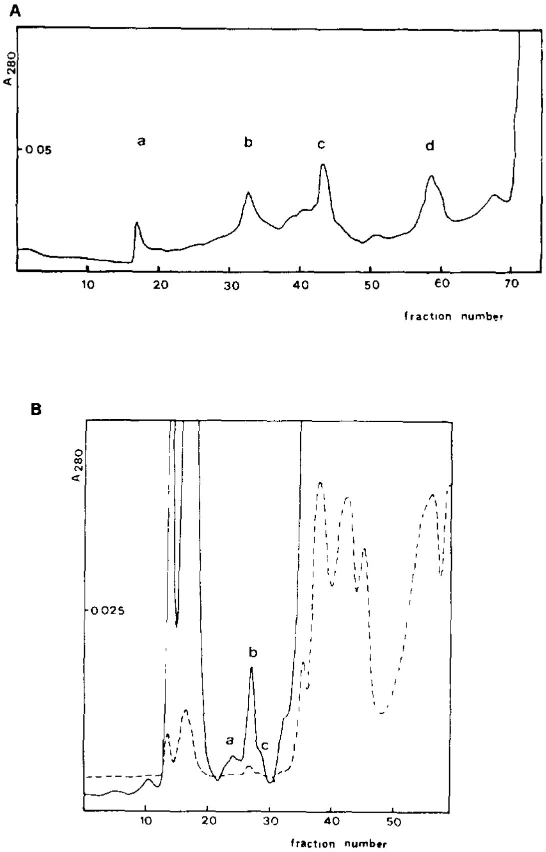


Fig.2. Amino acid sequence of the antenna β-polypeptide from *C. aurantiacus*. Automated Edman degradation of: (——) Met-1-Leu-47, (- - -) C-terminal Asp fragment Ile-26-Pro-50, (-·-·-) Trp fragment Lys-49-Leu-52 (of the Asp fragment), (···) C-terminal Trp fragment Leu-52-Pro-53 (of the peptide Ile-26-Pro-53); x, not detected; □, C-terminal amino acid residue as determined by hydrazinolysis.

→

Fig.3.(A) Gel-filtration on Bio Gel P-10 in 50% formic acid of the fragment from the aspartic acid cleavage of the antenna β-polypeptide: 2.5 ml fractions (explanation of peaks see text). (B) Gel-filtration of the BNPS-Skatol fragments of peptide Ile-26-Pro-50 on Bio Gel P-4 in 50% formic acid: 1.6 ml fractions. Peak a, Lys-Pro-X-Leu-Pro; peak b, Lys-Pro-Trp; peak c, Leu-Pro.



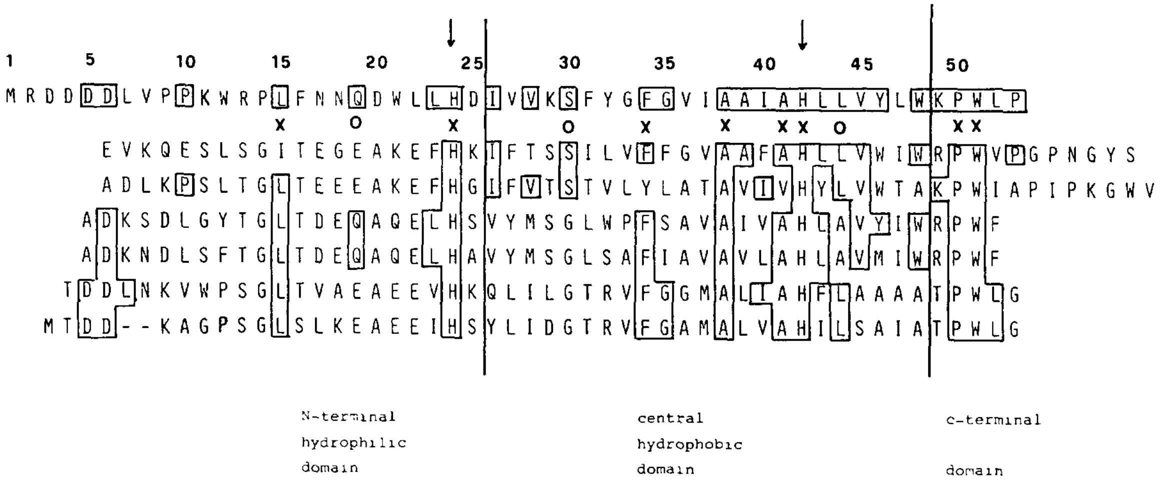


Fig.4. Sequence homology between the B806-866-β-polypeptides of the green photosynthetic bacterium *C. aurantiacus* and the antenna β-polypeptides of the purple photosynthetic bacteria [6-12,17]. Aligned amino acid sequences: (1) B806-866-β *C. aurantiacus;* (2) B890-β *Rs. rubrum*; (3) B1015-β *Rp. viridis*; (4) B890-β *Rb. sphaeroides*; (5) B890-β *Rb. capsulatus*; (6) B800-850-β *Rb. sphaeroides*; (7) B800-850-β *Rb. capsulatus*. Homologous amino acid residues of the antenna polypeptides of *C. aurantiacus* and purple bacteria are boxed. The arrows indicate the conserved His residues. Polypeptides 1, 2, 4 and 5 are formylated. X, amino acid residues conserved; 0, amino acid residues conserved and structurally related in *C. aurantiacus* and in purple bacteria.

diagram in fig.3B). The amino acid composition and the Edman degradation of the peptide from peak a showed 2 Pro, 1 Lys, 1 Leu and the sequence Lys-Pro-X-Leu. Peak c represents the sequence Leu-Pro. On the basis of the overlapping fragments Met-1-Leu-47, Ile-26-Pro-50, Lys-49-Leu-52, Leu-52-Pro-53 and the C-terminal Pro residue (hydrazinolysis) the complete amino acid sequence of B806-866-β was established (fig.2). Based on the amino acid composition and sequence, this polypeptide has the true molecular mass of 6349 Da.

4. DISCUSSION

The 6.3 kDa polypeptide isolated from the cytoplasmic membrane of *C. aurantiacus* shows similar structural features as the intramembrane antenna polypeptides and particularly the β-polypeptides of purple photosynthetic bacteria (fig.4), [6-11]: (i) the number of residues (53) lies within the range of 45-55 residues found in purple bacteria; (ii) the antenna polypeptide has the characteristic three-domain structure [a hydrophilic N-terminal domain (Met-1-Asp-25), the hydrophobic central domain (Ile-26-Trp-48) and the relatively short (compared to the α-polypeptide) C-terminal domain (Lys-49-Pro-53]; (iii) two conserved His residues, possible binding sites for BChl *a*, are present [His-42 in the central hydrophobic domain and His-24 at the C-terminal region of the N-terminal domain]. Similarly to the His residues, other conserved amino acid residues as Leu-15, Phe-34, Ala-38, Ala-41, Pro-50 and Trp-51, demonstrate the structural and also phylogenetic relationship to the β-antenna polypeptides of purple bacteria. In addition, homologous or structurally related amino acid residues are found both in the β-polypeptides of *Chloroflexus* and the purple bacteria in position 19 (Gln, Glu), 30 (Ser, Gly), 44 (Leu, Ala) and 45 (Val, Ala). Futhermore, the amino acid sequence Arg(Lys)-Pro-Trp found in all β-polypeptides of the B870 (B1015) core complex of purple bacteria is also present in the antenna β-polypeptide of B806-866 of *Chloroflexus*. Interestingly, in the primary structure most of these conserved or structurally related residues are 3-5 residues apart, indicating an asymmetrical arrangement of these residues at one side of a hypothetical α-helix formed by the β-polypeptide. These conserved residues probably represent interaction sites between the α- and β-antenna polypeptides (α-, β-heterodimer) within the membrane. The aromatic residues (in position 46, 48, 51) located relatively close on the C-terminal side of the BChl *a*-binding site at His-42 and also found in the β-polypeptides of purple bacteria are most probably of functional importance [2,8]. An unusual feature of the β-polypeptide of *C. aurantiacus*, compared to the β-polypeptide of purple bacteria, is the large N-terminal domain with the cluster of four aspartic acid residues. Together with the α-antenna polypeptides, the β-antenna polypeptide forms the α-β-heterodimer and larger aggregates of this α-β-polypeptide pair constituting the core antenna complex B806-866 in the environment of the reaction center. The core complex B806-866, with its typical two absorption maxima at 806 nm and 866 nm, shows different spectral properties compared to the core complexes B870/890 of purple bacteria, with only one main absorption maximum. These spectral differences should be related to the structural differences between the α- and β-antenna polypeptides of *Chloroflexus* and the purple photosynthetic bacteria.

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