The complete amino acid sequence of a bacteriochlorophyll *a* binding polypeptide isolated from the cytoplasmic membrane of the green photosynthetic bacterium *Chloroflexus aurantiacus*

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A polypeptide soluble in organic solvents was isolated from whole membrane fractions of the green thermophilic bacterium *Chloroflexus aurantiacus* by chromatography on Sephadex LH-60, Whatman DE-32 and Bio Gel P-10. The complete amino acid sequence of this 4.9 kDa polypeptide (44 amino acid residues) was determined. The polypeptide shows a 3-domain structure, similar to the domain structure of the antenna BChl polypeptides of purple photosynthetic bacteria, and sequence homologies (27–39%) to the light-harvesting α-polypeptides of the B870 (890) antenna complexes from purple bacteria. Therefore, the 4.9 kDa polypeptide is designated B(808-866)-α. The typical His residue (conserved His residue identified in all antenna polypeptides of purple bacteria as possible BChl binding site) is found within the hydrophobic domain, which extends from Asn 10 to Leu 30.

Chloroflexus aurantiacus *Green photosynthetic bacteria* *B808-866 antenna complex* *Light-harvesting polypeptide* *Amino acid sequence*

1. INTRODUCTION

The photosynthetic unit of the gliding filamentous green bacterium *Chloroflexus aurantiacus* [1] consists of the photochemical reaction centre and 3 pigment protein antenna complexes [2]. The BChl *a* B808-866 antenna complex is located within the cytoplasmic membrane in association with the reaction centre. The B740 antenna has as its major light-harvesting pigment BChl *c* which is organized in the extramembrane antenna system of the chlorosome. Besides the B740 complex a B790 pigment protein containing BChl *a* has been identified as a component of purified chlorosomes and bridges energy transfer from B740 to the B808-866 complex and reaction centre in the cytoplasmic membrane [3]. All known BChl antenna complexes are composed of specific antenna polypeptides binding BChl [2]. In B808-866, B740 and B790 from *Chloroflexus* only a single antenna polypeptide for each complex was found. These have apparent M_r values of 5300, 5500 (formerly determined M_r 3700 [2]) and 5800, respectively. The primary structure of the BChl *c* binding polypeptide B740 has been determined [4]. This protein revealed new structural features with respect to the pigment protein structure and BChl *c* binding sites.

Since the reaction centre-antenna complex of *Chloroflexus* has physical and kinetic properties similar to those of *Rhodopseudomonas sphaeroi-*

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

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des [5] it is reasonable to assume that the B808-866 complex may be similar to those of the purple photosynthetic bacteria [11–17]. Primary structure analyses of the BChl *a* antenna polypeptides from Rhodospirillaceae indicated that these polypeptides have a 3-domain structure corresponding to a transmembrane orientation of these polypeptides via a hydrophobic, central (α-helical) domain [11–17]. Here, we report on the isolation, purification and sequence analysis of the antenna BChl *a* binding polypeptide of the green bacterium *C. aurantiacus*. The polypeptide is structurally related to the intramembrane antenna polypeptides of purple photosynthetic bacteria. These data further demonstrate a phylogenetic relationship between the synthetic apparatus of green and purple photosynthetic bacteria.

2. MATERIALS AND METHODS

Cells of *C. aurantiacus* were grown anaerobically as in [6]. The polypeptides soluble in organic solvents were extracted from whole membrane fractions (obtained by differential centrifugation) with C/M/NH_4OAc. The 4.9 kDa polypeptide (and other small polypeptides) were separated from large polypeptides (>18 kDa) and from pigments by gel filtration on Sephadex LH-60 (Pharmacia, 3 × 150 cm column). Further purification was achieved by consecutive anion-exchange chromatography on DE-32 cellulose (Whatman, 1.1 × 23 cm column) in C/M/NH_4OAc and by gel filtration on Bio Gel P-10 (-400 mesh, Biorad, 2 × 100 cm) in 50% formic acid.

For amino acid analysis polypeptide samples were hydrolysed in constant boiling 6 N HCl (110°C) in vacuo and analysed on a Biotronic LC 6000 E analyser. The manual Edman degradation procedure as described in [4] was used for identification of amino-terminal amino acid residues and of polypeptide constituents of different fractions. To determine the carboxy-terminal amino acid residue hydrazinolysis was carried out [7]. The amino acid sequence was determined by the automated Edman degradation procedure in a Beckman 890 C sequencer. PTH amino acid derivatives were identified by the HPLC procedure in [8]. PTH-Arg and PTH-His were identified on an isocratic HPLC system on Partisil 5 PAC (Whatman) [9]. Cleavage of tryptophan was carried out by a modified BNPS-skatol method [10]: ~1 mg pure polypeptide was dissolved in 700 μl of 80% propionic acid/4 M guanidine hydrochloride (containing 2 mg/ml resorcine and 0.5 mg/ml tyrosine) and incubated for 1 h. 20 mg BNPS-skatol (Pierce) was added to the incubation mixture and the solution incubated for 16 h at 40°C after protection with Freon (CCl_2F_2, Du Pont). After addition of 700 μl of 50% formic acid the skatol precipitated was removed by centrifugation. The supernatant was applied to a Bio Gel P-4 column (200-400 mesh, 1.6 × 60 cm) in 50% formic acid.

3. RESULTS

The whole membrane fraction of *C. aurantiacus* was extracted with C/M/NH_4OAc and the dark-green extract applied to a Sephadex LH-60 column (elution diagram, fig.1A). SDS-PAGE of peak II showed 4 single bands with apparent molecular masses of 4, 6, 8 and 11 kDa. The 4 kDa polypeptide was the previously described BChl *c* binding polypeptide from chlorosomes as was revealed by Edman degradation [4]. The 6, 8 and 11 kDa polypeptides are most probably related to the polypeptides of 5.3, 5.8, 11 kDa reported by Feick and Fuller [2]. The fractions of peak II were applied to a DE-32 column. The polypeptides were eluted by a stepwise gradient (0, 3 and 20% acetic acid in C/M/NH_4OAc, respectively). The elution diagram is shown in fig.1B. Fractions of peaks I, II, IV–VI contained the 11 and 4 kDa polypeptides and small amounts of the 6 and 8 kDa polypeptides as shown by manual Edman degradation, whereas the fractions of peak III contained a mixture of 6 and 8 kDa polypeptides. For further purification the fractions corresponding to peak III were dialysed against distilled water and lyophilised. The sample was redissolved in 50% formic acid applied to a Bio Gel P-10 column and eluted with 50% formic acid (elution diagram, fig.1C). Fractions of peak I contained the aggregated pure 6 kDa polypeptide, whereas a mixture of the 6 and 8 kDa polypeptides was found in peak II. The lyophilised 6 kDa polypeptide from peak I was subjected to amino acid and sequence analysis. The amino acid composition of the 6 kDa polypeptide is depicted in table 1. No lysine and

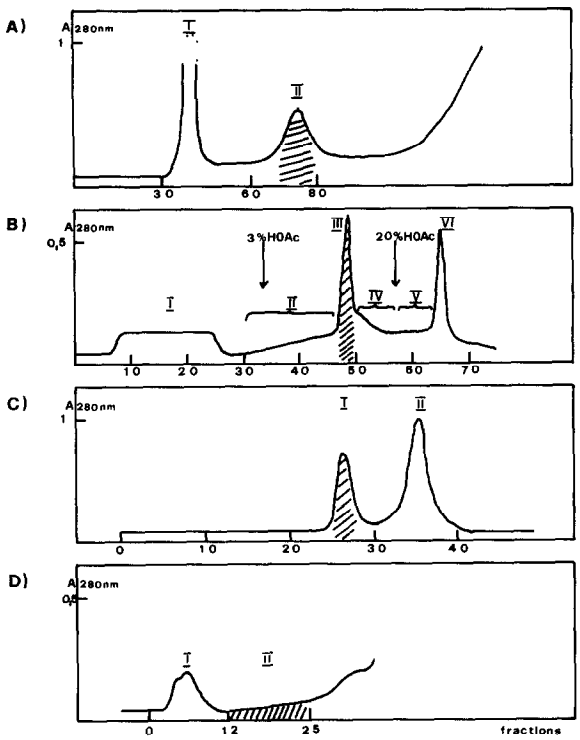

Fig.1. (A) Gel filtration of the polypeptide extract solubilised with C/M/NH_4OAc from whole membrane fractions on Sephadex LH-60 in C/M/NH_4OAc; 7 ml fractions. (B) Anion exchange chromatography of pooled peak II fractions (from LH-60 gel filtration) on Whatman DE-32 in C/M/NH_4OAc; 8 ml fractions. (C) Gel filtration of lyophilised fractions from peak III (DE-32) on Bio Gel P-10 in 50% formic acid; 2.5 ml fractions. (D) Gel filtration of BNPS-skatol fragments from peak I (Bio Gel P-10) on Bio Gel P-4 in 50% formic acid; 3.2 ml fractions.

cysteine and a single methionine, histidine and tyrosine were found.

For sequence analysis deblocking of the N-terminus as described in [11] was necessary. This indicates that the N-terminal Met is most probably formylated. Hydrazinolysis showed Gly as the carboxy-terminal residue. Automated Edman degradation established the amino acid sequence of 41 residues. In addition, to determine its carboxy-terminal amino acid sequence, the 6 kDa polypeptide was cleaved by BNPS-skatol at Trp 37. The fragments were separated by gel filtration on Bio Gel P-4 in 50% formic acid (elution diagram, fig.1D). Fractions 12–25 contained the

Table 1

Amino acid composition of the 4.9 kDa polypeptide of *C. aurantiacus*

Amino acid	Number of residues[a]			Amino acid	Number of residues[a]		
	A[b]	B	C		A[b]	B	C
Asx	2.9	3	3	Met	0.9	1	1
Thr	1.8	2	2	Ile	4.3	5	5
Ser	3.6	4	4	Leu	4.8	5	5
Glx	3.5	3	3	Tyr	0.9	1	1
Pro	2.9	3	3	Phe	3.0	3	3
Gly	3.1	3	3	His	0.8	1	1
Ala	1.9	2	2	Arg[a]	2.0	2	2
Val	4.9	5	5	Trp	n.d.	n.d.	1

[a] Data based on 2 Arg residues per polypeptide chain
[b] Results expressed in mol residue/mol polypeptide

(A) Average of 24, 48, 87 and 96 h hydrolyses, except for Thr and Ser (uncorrected values after 24 h hydrolysis) and Val, Ile, Phe and His (average of 87 and 96 h hydrolyses). (B) Nearest integer, (C) number of residues as derived from amino acid sequence

small carboxy-terminal peptide (7 residues), as determined by amino acid analysis (table 2). Edman degradation of this fragment revealed the sequence Leu-Ser-Asn-Ala-Glu-Gly-Gly-COOH (overlapping sequence: -Leu-Ser-Asn-Ala-). From these data the complete amino acid sequence could be derived (fig.2). Based on the amino acid se-

Table 2

Amino acid composition of the overlapping, C-terminal BNPS fragment of the 4.9 kDa polypeptide

Amino acid	Number of residues[a]		
	A[b]	B	C
Asx	1.2	1	1
Ser	0.8	1	1
Glx[a]	1.0	1	1
Gly	2.1	2	2
Ala	0.8	1	1
Leu	0.9	1	1

[a] Data based on 1 Glx residue per polypeptide chain
[b] Results expressed in mol residue/mol polypeptide

(A) 24 h hydrolysis, (B) nearest integer, (C) number of residues as derived from amino acid sequence

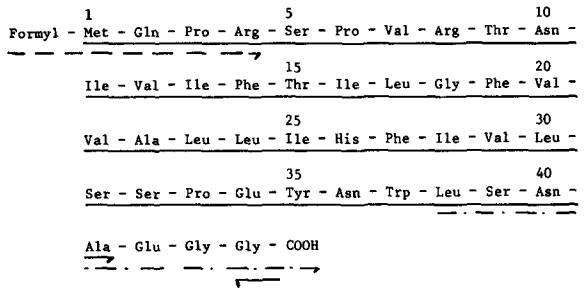

Fig.2. Amino acid sequence of the 4.9 kDa polypeptide from *C. aurantiacus*. (——→) Automated Edman degradation, (– – –) manual Edman degradation, (–·–·→) automated Edman degradation of the BNPS-skatol fragment, (⟝——) C-terminal amino acid residue as determined by hydrazinolysis.

quence, this 6 kDa polypeptide (44 residues) has a true molecular mass of 4929 Da (including the N-terminal formyl group) and is designated the 4.9 kDa polypeptide.

4. DISCUSSION

The number of amino acid residues (44) of the 4.9 kDa polypeptide from the green bacterium *C. aurantiacus* (fig.2) is similar to that of the light-harvesting polypeptides (α, β) from purple photosynthetic bacteria (48–58 amino acid residues, fig.3) [11–17]. The 4.9 kDa polypeptide also has the typical 3-domain structure found in the antenna polypeptides of purple bacteria (fig.3) [11–17], identifying the 4.9 kDa polypeptide with its hydrophobic domain (21 residues in position 10–30) as an intramembrane light-harvesting polypeptide (most probably of the B808-865 complex). The N-terminal polar domain consists of only 10 amino acid residues, reducing its size by 3–13 amino acid residues compared to the one of the antenna polypeptides from purple bacteria (fig.3) [11–17]. The N-terminal domain is located near or at the membrane surface. Differences in this region are probably related to the different environmental (structural, functional) conditions at the membrane surface of the cytoplasmic (green bacteria) and intracytoplasmic (purple bacteria) membrane. The antenna polypeptide nature of the 4.9 kDa polypeptide is further confirmed by the existence of one His residue in position 26 (fig.3, conserved His found in all purple bacteria antenna polypep-

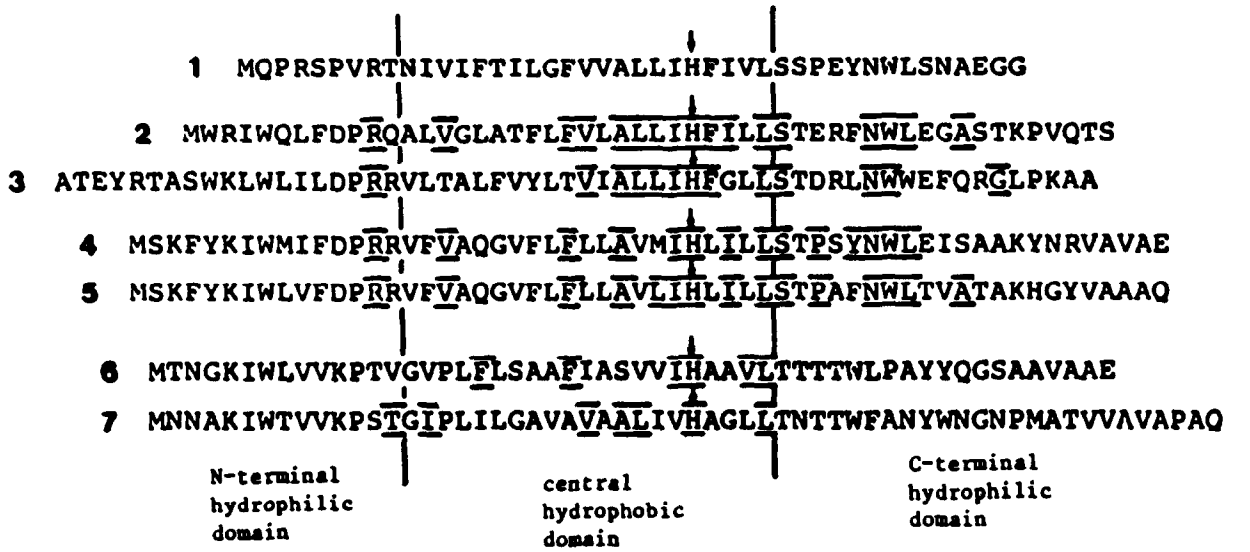

Fig.3. Sequence homology between the 4.9 kDa polypeptide (B808-865-α apoprotein) of the green bacterium *C. aurantiacus* [1] and the antenna α-polypeptides of purple photosynthetic bacteria (2–7). Aligned amino acid sequences: (1) B808-865-α, *C. aurantiacus*; (2) B875-α, *Rhodospirillum rubrum*; (3) B1015-α, *Rps. viridis*; (4) B870-α, *Rps. sphaeroides*; (5) B870-α, *Rps. capsulata*; (6) B800-850-α, *Rps. sphaeroides*; (7) B800-850-α, *Rps. capsulata*. Homologous amino acid residues are boxed (underlined). The arrow indicates the conserved His residue within the hydrophobic domain. Polypeptides 1, 2, 4 and 5 are *N*-formylated.

tides [11–17]). It is most probably also the main binding site for BChl *a* (via an Mg atom) in the *Chloroflexus* antenna polypeptide. The amino acid sequence of the 4.9 kDa polypeptide, particularly in the neighbourhood of His 26 (about 10 residues), is homologous to the sequences of the antenna polypeptides from purple bacteria (fig.3, active site region for BChl *a* binding). The sequence homology is particularly pronounced between the 4.9 kDa polypeptide and the light-harvesting α-polypeptides of the B870 (890) complex of purple bacteria (27–39% [11–18], indicating that the 4.9 kDa polypeptide is most probably the light-harvesting α-polypeptide (B808-866)-α apoprotein) of *C. aurantiacus*. Therefore the 4.9 kDa polypeptide should be part of the antenna complex B808-866 close to the reaction centre (similar to the B870 (890) complex). The sequence homology between the 4.9 kDa polypeptide and α-polypeptides of B800-850 from purple bacteria is lower: 14–23% (fig.3, as was found between α-polypeptides of different antenna complexes from purple bacteria, 13–18%) [18]. Its sequence homology to the β-polypeptides of purple bacteria is very weak: 9–14% (as is the case between α- and β-polypeptides of purple bacteria, 7–13%) [18]. On the basis of these homologies, it can be postulated that the antenna polypeptide B808-866-α of the green bacterium *C. aurantiacus* is phylogenetically related to the B870 (890)-α apoproteins of purple bacteria. The phylogenetic relationship seems to be as close as between B870 (890)-α of *Rps. viridis* and *Rps. sphaeroides, Rps. capsulata* or *Rps. gelatinosa*.

The main difference between the BChl *a* antenna complex of the purple bacteria and that of *Chloroflexus* is the fact that a second (β)-antenna polypeptide has not been found [2]. In addition, the isolation procedure described here and data of Feick and Fuller using HPLC analysis (unpublished) indicate that the 808-866 antenna complex contains no β-polypeptide, or that it has so far defied isolation or identification. To explain the existence of a single light-harvesting α-polypeptide we can speculate that the evolutionary separation of the green bacterium *C. aurantiacus* and purple bacteria was before the gene duplication of α- and β-polypeptide genes of B870 (B890), and before the gene duplication to form α- and β-polypeptides of B800-850 complex in purple bacteria [18,19].

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