

The primary structure of two chlorosome proteins from *Chloroflexus aurantiacus*

Georg Niedermeier, Judith A. Shiozawa, Friedrich Lottspeich, Reiner G. Feick*

Max-Planck-Institut für Biochemie, D 82152 Martinsried, Germany

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Abstract

The complete nucleotide sequence of two chlorosome proteins with apparent molecular weights of M_r 18,000 and M_r 11,000 from *Chloroflexus aurantiacus* have been determined. The two polypeptides were 145 and 97 amino acids long and possessed true molecular masses of 15,545 and 10,820 Da, respectively. Protein chemical sequencing was done in parallel to confirm the primary structure deduced from nucleotide sequencing. By Northern blot analysis of RNA isolated from phototrophically grown cells a transcript of 0.95 kb was detected which is the expected length for a mRNA encoding both genes.

Key words: Green bacteria; Light-harvesting; Chlorosome; Primary structure; *Chloroflexus aurantiacus*

1. Introduction

Conversion of light into chemical energy requires, firstly, the absorption of photons by antenna or light-harvesting pigments and, secondly, the primary charge separation in the photochemically active reaction center pigment–protein complexes. Characteristic of green sulfur and green filamentous bacteria and, hence, for *Chloroflexus aurantiacus* is the presence of chlorosomes and two types of bacteriochlorophyll (Bchl), Bchl *a* and Bchl *c*, *d*, or *e* [1,2]. Chlorosomes are oblong bodies of $100\text{--}200 \times 30 \times 12 \text{ nm}^3$ and are firmly attached to the inner side of the cytoplasmic membrane. The chlorosome is surrounded by a 2–5 nm thick envelope [3,4].

Three different light-harvesting pigment complexes are present in *C. aurantiacus*: the major antenna pigment Bchl *c* is located solely in chlorosomes and absorbs at 740 nm (B 740) [5]. An additional chlorosome constituent is a Bchl *a*-containing complex which absorbs at 790 nm (B 790). This pigment is thought to be part of a baseplate which functionally and physically attaches the chlorosome to the cytoplasmic membrane [3,6]. The cytoplasmic membrane is the location of the third antenna component, a Bchl *a*-containing complex with absorption bands at 806 and 866 nm (B 806–866) and of the photochemical reaction center [5,7].

Purified chlorosomes of *C. aurantiacus* isolated according to Feick [5] are comprised of three major polypeptides with apparent M_r of 18,000, 11,000, 3,700 as

determined by SDS-PAGE; a fourth polypeptide (M_r 5,800) is present in small amounts. Topographical studies showed that the two larger polypeptides are constituents of the chlorosome envelope [7,8]. The primary structure of the M_r 3700 protein has been determined [9,10]; it has a true molecular mass of 5.7 kDa. Its location and function and, in particular, its role in Bchl *c* binding is controversial. While some groups believe that protein is essential for Bchl *c* organization [7,9,11,12] others favour Bchl *c* oligomers as the organizational principle in chlorosomes [13–16]. While the first idea is based on the sensitivity of the *in vivo* absorbance to proteolysis, the latter one is based on the tendency of Bchl *c* molecules to form aggregates in aqueous suspensions with spectroscopic properties similar to those of intact chlorosomes.

Compared to other antenna pigment systems there is a lack of detailed information on the structure of the chlorosome as a whole and the pigment organization within. With regard to this shortcoming we have isolated the two largest chlorosome polypeptides and present here the primary structure of the two largest structural polypeptides of chlorosomes from *C. aurantiacus*.

2. Materials and methods

2.1. Biochemicals and reagents

Restriction endonucleases and other enzymes were purchased from either Amersham Buchler (Braunschweig, Germany), Biozym Diagnostik (Hameln, Germany), Boehringer Mannheim (Mannheim, Germany), New England Biolabs (Schwalbach, Germany), Pharmacia Biotech (Freiburg Germany) or US Biochemicals Corporation (Bad Homburg, Germany). Digoxigenin-11-dUTP and detection reagents were from Boehringer Mannheim (Mannheim, Germany).

*Corresponding author. Fax: (49) (89) 8578 3557.

[³⁵S]dATP[αS] was purchased from Amersham Buchler (Braunschweig, Germany). Qiagen plasmid kits used for plasmid DNA isolation and nylon membrane for nucleic acid transfer and analysis were from Diagen. Plasmid pGEM-3Zf(+) was obtained from Promega (Madison, USA). All other chemicals were either reagent or HPLC grade.

2.2. Growth of bacteria

Chloroflexus aurantiacus, strain J10-fl (Deutsche Sammlung von Mikroorganismen, Göttingen) were grown photoheterotrophically in 15-l fermentors as described in [5].

Escherichia coli strain DH 5α (F[−], recA1, endA1, gyrA96, thi-1, hsdR17[r_k[−], m_k⁺], supE44, relA1, φ80d/lac ZΔM15, Δ(lac ZYA-argF)U169, λ[−]; Gibco-BRL, Eggenstein, Germany) were grown in LB medium at 37°C. Plasmid containing *E. coli* were grown in medium containing 100 mg/l ampicillin.

2.3. Isolation of chlorosome proteins

Chlorosomes were isolated by repeated sucrose density gradient centrifugation in the absence of detergent according to [5]. Isolation of chlorosome polypeptides was achieved by preparative SDS/PAGE and subsequent elution employing FAPH-Superose-chromatography as previously reported in [12,17].

2.4. Chemical and enzymatic cleavage of chlorosome proteins

CNBr cleavage of chlorosome proteins was performed as described in [17]. Proteolytic digestion with trypsin, chymotrypsin or endoproteinase Glu-C was carried out as described by the manufacturer. Peptide fragments were separated by reversed phase HPLC on a C₄ or a C₁₈ column (Partisil 5 ODS 3, Kontron; ET 250/8/4 Nucleosil 1,000–7 C₄, Macherey-Nagel). Elution of peptide fragments was accomplished by applying a linear gradient from eluant A to 60% eluant B within 60 min at a flow rate of 1 ml/min. Eluant A contained 0.1% (v/v) CF₃COOH in water and eluant B consisted of 0.1% (v/v) CF₃COOH in acetonitrile/isopropanol (2:1, v/v) (B). Some polypeptide fragments could be directly subjected to amino acid sequence analysis; a few had to be rechromatographed on a C₁₈ column using a gradient from A (50 mM CH₃COONH₃ in H₂O) to 60% B (CH₃CN).

2.5. Amino acid sequence analysis

Automated Edman degradation of isolated chlorosome peptides or undigested chlorosome proteins was performed on a gas-phase sequencer (Applied Biosystems 477A or 470A), see [18].

2.6. Isolation of DNA

C. aurantiacus chromosomal DNA was isolated as described in [18]. Small-scale plasmid preparations were made by the alkaline lysis method [19] omitting the phenol/chloroform extraction. For large-scale plasmid preparations Qiagen columns were used.

2.7. Cloning of the 11 kDa and 18 kDa chlorosomal genes

Based upon the peptide sequences of the M_r 18,000 protein, three pools of degenerate oligonucleotides (Fig. 1) were synthesized (DNA synthesizer 380 B, Applied Biosystems), two of them, N-Term 1 and anti sense 18Try12 were used as primers for the amplification of the genes in *C. aurantiacus* chromosomal DNA with a thermostable DNA polymerase.

The PCR was performed in a reaction mixture (100 μl) containing 100 ng chromosomal DNA. Thirty cycles of denaturation at 94°C for 1 min, primer annealing at 44°C for 1 min and extension at 72°C for

3 min. Ten percent of the reaction volume was taken as template DNA for a second amplification using the same conditions as above.

The reaction products were analyzed on a 1.5% agarose gel, transferred to nylon membrane and hybridized with Dig-11-dUTP-labelled oligonucleotide N-Term 2 (Fig. 1) [20]. Hybridization was carried out for 20 h in 5 × SSPE [19], 5 × Denhardt's [19] and 20 μg/ml denatured calf-thymus DNA at 42°C. Nylon membranes were washed and the hybridized DNA was detected using alkaline phosphatase conjugated anti-digoxigenin antibody and AMPPD as substrate according to the manufacturer's protocol. Fragments which hybridized to N-Term 2 and which had the expected size were recovered from agarose gel and ligated into pGEM3Zf(+). *E. coli* transformants were screened by colony hybridization using one of the oligonucleotides N-Term 2. After identifying the two genes encoding the 11 kDa and 18 kDa proteins by nucleotide sequence analysis, a new oligonucleotide was synthesized (F1-UNI) and used for the identification and cloning of the genes from genomic DNA.

2.8. DNA sequence analysis

Nucleotide sequencing of both strands of the cloned genomic DNA fragment was accomplished by generating subclones and using standard and specifically synthesized oligonucleotides as primers. Sequencing of DNA was performed either as described previously [18] or using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit with an automated sequencer (Applied Biosystems model 373A, version 1.1.1). For computer analysis of DNA and derived protein sequence data the UWGCG software package [21] on a VAX computer and DNA-Strider (Commissariat à l'Energie Atomique, France) on an Apple-Macintosh computer were used.

2.9. RNA isolation and Northern analysis

C. aurantiacus total RNA was isolated according to [22]; a DNase treatment after the precipitation step was included [23]. Denatured total RNA was size fractionated in a formaldehyde-containing 1.2% agarose gel [24] and transferred to nylon membrane. Hybridization, washing procedures and detection were performed according to [25]. Hybridization probes were generated by amplification with a thermostable DNA polymerase, using the appropriate primers and plasmid bearing the template DNA. Digoxigenin-11-dUTP at a final concentration of 10 μM was added to the reaction mixture.

3. Results

3.1. Polypeptide isolation and sequencing

Using protein chemical methods we were able to determine 52% and 78% of the amino acid sequence of the M_r 18,000 and M_r 11,000 chlorosome proteins (Fig. 2). The sequence of the first 19 amino acids from the N-terminus of the M_r 18,000 chlorosome protein has been previously published [11]. Our sequencing data corroborated their results with the exception that the first amino acid after the initial methionine was found to be a serine instead of an alanine. The M_r 11,000 polypeptide was blocked at its N-terminus.

N-Term 1:	N-Term 2:
Met Ser Asn Glu Thr Thr Asn Glu Arg Asp Gly Leu Phe Glu Met Ala Ala Gly Phe	TTY GAR ATG GCN GCN GG
AAY GAR ACV ACV AAY GA	
18Try12:	F1-UNI:
Lys Val Asp Glu Trp Val Glu Thr Pro	Gly Gly Ile Val Arg Leu Gly
GAY GAR TGG GTW GAR AC	GGT GGG ATT GTT CGG CTC GG

Fig. 1. Oligonucleotides used for detection and isolation of the genes *csmM* and *N*. The upper amino acid sequence represents the N-terminus of the 18 kDa protein; 18Try12 is the oligonucleotide derived from the same polypeptide. F1-UNI is a part of the *csmM* gene, which encodes the 11 kDa protein.

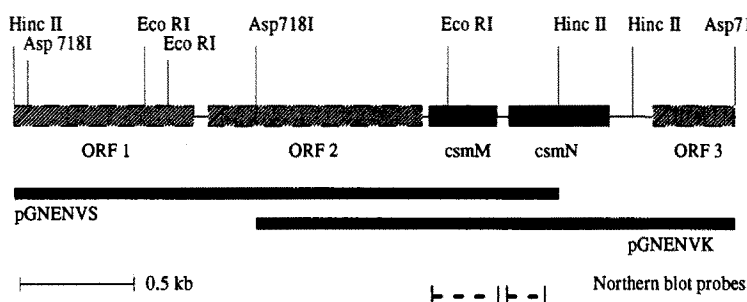


Fig. 2. Restriction map of the *C. aurantiacus* *csmM* and *N* genomic region including three open reading frames (ORF) transcribed in the same direction. Only restriction sites used for subcloning are shown. Additionally, the two isolated *Asp718I* and *HincII* chromosomal fragments are shown. Northern blot probes are indicated in dotted lines.

3.2. Molecular cloning of the genes encoding the 11 kDa and 18 kDa chlorosomal proteins

Two genomic regions, a 2.3 kb *HincII* and a 2.0 kb *Asp718I* fragment, carried the genes *csmM* and *csmN*. By Southern blot analysis only one hybridization signal per digest could be detected (data not shown). The fragments were isolated and ligated into the appropriate sites in the polycloning region of pGEM3Zf(+). The genetic and restriction map of the 3.1 kb genomic region encompassing the two overlapping fragments is shown in Fig. 3. Both genes, which were separated by 39 bp, were preceded by a putative Shine-Dalgarno sequence GGAG [26]. The two polypeptides encoded by the genes named *csmM* and *csmN* were 145 and 97 amino acids long and possessed a true molecular mass of 15545 and 10820 Da, respectively, which is in reasonable agreement with the previously determined M_r of 18,000 and M_r 11,000 [7]. Nucleotide sequences resembling the consensus sequence for the σ^{70} subunit of RNA polymerase were found 168–

143 bp and 200–171 bp upstream of the *csmM* start codon [27]. In the same transcriptional direction seven additional open reading frames (ORFs) longer than 25 amino acids were observed. Twelve ORFs in the opposite direction were found. The three longest ORFs are shown in Fig. 3. A computer search of the combined protein sequence data bank (Pir, SwissProt, MIPS) did not reveal any sequences with significant similarity to the *csm* gene products and only the putative polypeptide of ORF 2 exhibited significant similarity to any known protein. ORF 2 was 31.5% identical and 59.8% similar to a bacteriochlorophyll synthase 33 kDa chain of *Rhodobacter capsulatus* [28].

3.3. Northern analysis

Digoxigenin-labeled DNA probes specific for either *csmM* and *csmN* both hybridized to RNA migrating at about 0.95 kb. This is the expected size of RNA encoding both genes. No additional transcripts were detected (Fig. 4).

ACCCTGATCG	TTGCTACGT	CACCATTAAC	AGCTTCGAGG	CAATGCTGAT	GATCCTGGCG	TTGATTGGG	GACAGTACTG	GGTAGCGGCA	90
TTTATGTTTC	TGGCCCTGGT	GGCACCAGTC	TACAATCAGA	TCAAACCTCTA	CCAGGAACCA	ACCCAGCAAA	ACTATGTTTCG	CTATCTACTG	180
								<i>csmM</i>	
GCCTCAAATC	CATTCTGTCG	GTTGATTACG	ATTATTTTCG	GTTTCTCTCG	AGGCGGCTAT	TTTGGCTGAC	CGTATCTAAG	GAGGTGCGAT	270
								M	
GATGACAGAG	AGTGAAGGCG	AGGTGCGGGT	ACGCAGCGTA	CCGGTGGGAC	GCAACGACAG	CTTCGTTGAG	TCGGCGATGG	AATTCGGAGG	360
M T E S E G E V R V	R S V P V R R	N D S F V E S A M E F G G							
TGGGATTGTT	CGSCTCGGTT	TCTCGATCTT	TACCTCTCCC	CTGGCTCTGC	TGCGGCCGGA	GTCGCGCCAG	CACATGCACA	ATGCTACCAA	450
G I V R L G F S I F T L P L A L L L P P E S R Q H M H N A T T K									
AGAGTTGATG	TACGCTTTTG	CTTCACTACC	ACGCGATTTC	GCCGAGATTG	CGGGTAAGAG	CATCGAGAAA	TGGGCGGAAG	AGGGTGAAGA	540
E L M Y A F A S L P R D F A E I A G K S I E K W A E E G E E									
								<i>csmN</i>	
GCCAAAAGGA	GAGGCGAAGT	AGGGCCATTT	GCTCTCTTCA	GAGCAGAGGA	GGAGTGTAGT	ATGAGCAATG	AGACAACAAA	CGAGCGCGAT	630
P K G E A K *						M S N E T T N E R D			
GGGCTGTTTG	AGATGGCAGC	CGGCTTCGTC	GGTGGTGCTA	CACGGATTGG	ATTGACGGTA	GCATCCGTAC	CGCTGGTCTT	GCTGCCGCGC	720
G L F E M A A A G F V G G A T R I G L T V A S V P L V L L P R									
AACTCGCGCC	GCCGGGTACG	ACGGGCGATG	GCTGAAGTGG	CAATGGCCGT	TGTAGCTTTT	CCCAAAGAGC	TGGCCAACGT	CTCTGAACGG	810
N S R R R V R R A M A E V A M A V V A F P K E L A N V S E R									
GTGGTTGACG	ACATCTTTTC	CGCCGATCCA	CCCCAGATCA	ACCTGCCCAG	CCCGCAGCGT	GTCGGCGAAC	AGGTGCGTTC	GTTTACCGAG	900
V V D D I F A A D P P O I N L P S P Q R V G E Q V R S F T E									
CGCCTGSCCC	GTGCGGCAGA	AGAGTTGGGC	ACCAGCTTTA	GCCGGGCTGC	CGGTCGTGCC	GCTGATGCCG	TCGAACAGGG	TGCGGCGAAG	990
R L A R A A E E L G T S F S R A A G R A A D A V E O G A A K									
GTTGATGAGT	GGGTCGAAAC	ACCACCAAG	ACACCACCGG	CGCCGTGATC	GTTTACCGCT	TGCTGAGTGA	CGCAGAGGTG	TAGGATGGGC	1080
V D E W V E T P P P A P *									
AATTGAACAT	CCTGGCCTCT	GCGTTTGCCA	CTCTGAGAGG	AATGTCGCTG	TGTTATAAGG	TTGACCGGCA	ACCAGACCGT	GGTATGCTGT	1140

Fig. 3. Nucleotide sequence and deduced amino acid sequence of *C. aurantiacus* *csmM* and *N*. Double underlined nucleotide sequences indicate putative σ^{70} promoter sequences, dotted lines show Shine-Dalgarno sites. Underlined peptides were identified by amino acid sequencing.

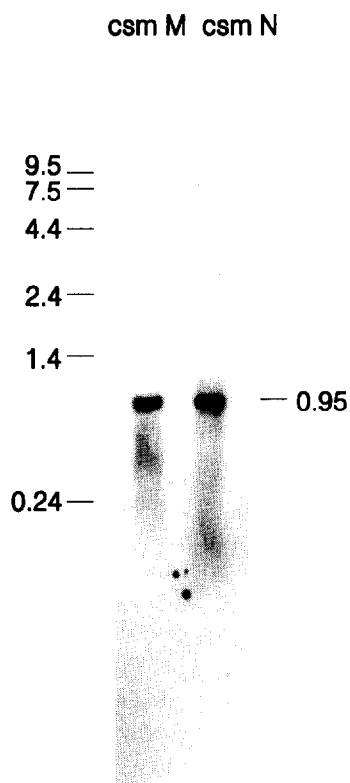


Fig. 4. Northern blot analysis of phototrophically grown *C. aurantiacus* cells using the probes shown in Fig. 2. Only one transcript of about 0.95 kb could be detected with each of the DNA probes.

4. Discussion

The two genes, *csmM* and *csmN*, coding for the M_r 11,000 and M_r 18,000 chlorosome proteins of *C. aurantiacus*, respectively, have been cloned and sequenced. The *csm* locus designations have been chosen because both gene products are chlorosome components [29]. So far it is not known how far apart *csmA*, the gene coding for the 5.7 kDa chlorosome polypeptide [10], is located from *csmM* and *csmN*. Both genes are co-transcribed into a 0.95 kb long mRNA. ORF2 has significant sequence identity to the 33 kDa subunit of a Bchl synthase of *Rhodobacter capsulatus* [28]. The proximity of the gene encoding a polypeptide involved in Bchl synthesis to *csmM* and *N* could indicate that genes required for chlorosome assembly are grouped together in a manner similar to that of purple nonsulfur bacteria [30–32]. In the *puf1* and *puf2* operons of *C. aurantiacus*, putative promoter sequences similar to the σ^{70} consensus sequence preceding the two chlorosomal genes have been found [18,33]. On the other hand, the *csmA* gene coding for the 5.7 kDa chlorosome protein is preceded by a putative σ^{54} consensus sequence [10].

With respect to secondary structure prediction, the amino acid composition of the two chlorosome polypeptides revealed some interesting features, notably the rela-

tive high content of Ala (9 and 16%), Glu (14 and 9%) and Pro (6 and 8%) in the M_r 11,000 and 18,000 proteins, respectively. The former two amino acids are frequently found in α -helical regions while Pro is known as a secondary structure breaking amino acid residue (see Fig. 5).

From earlier topographic studies it was surmised that the two polypeptides are located at or within the chlorosome envelope membrane [7,12]. Therefore, a hydropathy plot [33] was chosen for secondary structure prediction. The overall shape and polarity of the hydropathy profiles are indicative of the presence of one and perhaps two putative transmembrane regions in the M_r 11,000 and M_r 18,000 proteins, respectively. The hydropathic profiles of the M_r 11,000 protein between amino acids 20–60 and that of the M_r 18,000 protein between residues 10–50 are very similar. This is not too surprising since the two proteins are 40% identical and 67% similar in these two regions. In summary, all these features corroborate the earlier topographic findings that the two polypeptides should be located within or proximal to the chlorosome envelope membrane [7,12] and, furthermore, could possess the same N-terminal orientation. The high content of Arg and Lys in both proteins – there is a cluster of 6 Arg between residues 40–49 in the M_r 18,000 protein – explains the high yield of homo- and heterodimer formation in chemical cross-linking experiments [7]. Considering that there are 13 Glu residues in each polypeptide (5 Glu at the C-terminal end of the 11,000-protein) it is tempting to speculate that this amino acid might be of functional relevance for the chlorosome structure as a whole or for the envelope and/or the pigment organization within the chlorosome.

The functional role of the M_r 11,000 and M_r 18,000 polypeptides seems to be in stabilizing the characteristic

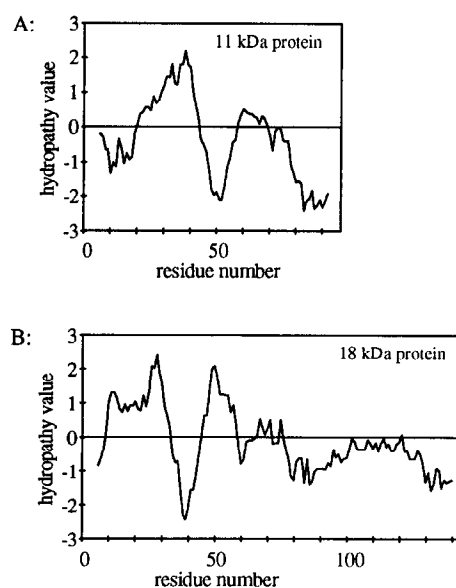


Fig. 5. Hydropathy plots of the *C. aurantiacus* *csmM* and *N* using a window size of 11 amino acid residues.

ellipsoid form of chlorosomes. This idea is substantiated by electron micrographs of protease-treated chlorosomes, where in addition to an absorption maximum shift, a drastic change in shape – from ellipsoid to spherical – has been observed [12].

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