The Primary Structure of the Presumable BChl *d*-Binding Polypeptide of *Chlorobium vibrioforme f. thiosulfatophilum*

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In addition to the previous isolated and sequenced polypeptides from green photosynthetic sulfur bacteria, which are presumably involved in binding BChl *c* and *e*, an analogous polypeptide has been purified from the BChl *d*-containing bacterium *Chlorobium vibrioforme f. thiosulfatophilum*. The primary structure of this 6.15 kDa polypeptide was determined. It shows an extremely high homology (98.3%) to the corresponding polypeptide from *Pelodictyon luteolum,* indicative of an important functional role.

Introduction

The photosynthetic apparatus of green photosynthetic bacteria is characterized by the appearance of an extramembrane organized, additional light-harvesting system, the chlorosome. These vesicles, oblong bodies, directly attached to the cytoplasmic membrane [1, 2] contain BChl *c, d* or *e* as photosynthetic pigment, absorbing light between 720 and 750 nm [3]. In the case of *Chloroflexus aurantiacus* energy is transferred from the chlorosome *via* a crystalline base-plate (792 nm) to the B806–866 intramembrane located core complex [4, 5] and finally to the reaction center (P865) [6]. In members of the Chlorobiaceae no intracytoplasmic core complex has been found so far. However, an additional water-soluble BChl *a*-binding protein (807 nm) located between the reaction center and the chlorosome [7] could serve as an energy transmitter from a chlorosomal BChl *a* complex, absorbing light at 795 nm [8] to the reaction center (P840).

Freeze fracture electron micrographs of chlorosomes revealed rod-like three-dimensional structures [1, 2]. Protein-chemical investigations of chlorosomes from *Chloroflexus aurantiacus* (proteolytic digestion, cross-linking experiments) by Feick and Fuller [9] have shown the existence of three major polypeptides (3.7, 11 and 18 kDa) and a fourth one (5.6 kDa) appearing only in minor amounts. The 3.7 kDa polypeptide located most probably in the chlorosome has been sequenced. Asn and Gln residues have been proposed to be involved in binding seven BChl *c* pigment molecules per polypeptide and thus building-up the rod-like elements [10]. An analogous polypeptide was isolated from chlorosomes of four members of the Chlorobiaceae exhibiting an overall homology of about 30% to the BChl *c*-binding polypeptide of *Chloroflexus aurantiacus* [11]. The seven proposed binding sites in the polypeptide of *Chloroflexus* are not conserved in the corresponding polypeptides of the Chlorobiaceae. However, two main homologous amino acid clusters are suggestive of functional domains. A 7.5 kDa polypeptide, originally proposed to be the BChl *c*-binding polypeptide of *Chlorobium limicola* [12–14], was shown to be much less homologous (16.7%) to the *c*-protein from *Chloroflexus aurantiacus*.

The homology of the presumable BChl *c*-binding polypeptides between the members of the Chlorobiaceae is extremely high (95.9–100%), except for the corresponding polypeptide of the brown coloured BChl *e*-containing bacterium *C. phaeovibrioides* which shows more substitutions, resulting in a homology of 84.3%. For these reasons a further bacterium, *C. vibrioforme forma thiosulfatophilum* containing BChl *d* as abundant photosynthetic pigment in the chlorosome was

Abbreviations: PTH, phenylthiohydantoin; TFA, trifluoroacetic acid; BChl, bacteriochlorophyll; HPLC, high performance liquid chromatography; *C, Chlorobium*.

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subject of our investigations to find out the influence of the chlorophyll type on the protein structure.

Materials and Methods

Chlorobium vibrioforme f. thiosulfatophilum (DSM 263) was grown photolithoautotrophically at 30 °C and 1000 lux in 1 l screw cap bottles in the medium described by Steinmetz and Fischer [15]. To get a better cell yield, the culture was fed once with 50 ml of a solution containing 0.75 g $Na_2S \times 9H_2O$ and 0.5 g Na_2CO_3, neutralized by the addition of 2 ml of a sterilized 2 M H_2SO_4 solution. Cells were harvested by continuous flow centrifugation at 10,000 rpm in a Christ Labofuge 15,000, washed once in a 0.9% NaCl solution prior to lyophilization and stored at −20 °C until used.

Lyophilized cells were extracted with a mixture consisting of methylenchloride/methanol/ammonium acetate (1:1 (v/v), 0.1 M). The resulting extract was chromatographed by means of gel filtration (Sephadex LH-60, Pharmacia, 150 × 4.4 cm). The fraction corresponding to an approximate molecular weight of 6 kDa contained the 6.15 kDa polypeptide as a major component. For further purification, this material was applied on a butyl (C4, Shandon) reversed phase column. High performance liquid chromatography with a linear water/acetonitrile (both containing 0.1% TFA) gradient from 20 to 100% yielded the pure 6.15 kDa polypeptide.

Cleavage at lysine was carried out as described in [16]. Fragments were separated by reversed phase chromatography (HPLC) on a PepRP HR 5/5 (Pharmacia) column with a linear water/acetonitrile (both containing 0.1% TFA) gradient from 0 to 100%.

For amino acid analysis polypeptide samples were hydrolyzed in constantly boiling ~6 N HCl for 24 or 72 h at 110 °C *in vacuo* and analyzed on a Biotronic LC 6000 E analyzer.

The amino acid sequence was determined by the automated Edman degradation procedure on an Applied Biosystem 470 A protein sequencer. The PTH amino acids were identified as described in [17].

Results

The 6.15 kDa polypeptide was separated from larger polypeptides as well as from smaller molecules (pigments, lipids) by gel filtration of the organic extract on a Sephadex LH-60 column. For further purification the fraction corresponding to a molecular weight of 6 kDa was chromatographed on a butyl reversed phase column. The pure 6.15 kDa polypeptide was detected in peak 2 (Fig. 1). The polypeptide was incubated for 2 h with 5% HCl in methanol to cleave off the N-terminal formyl group and was then subjected to the automated Edman degradation procedure. Primary structure analysis of the polypeptide revealed 51 amino acids (Fig. 3). Endoproteinase Lys-C was applied in order to cleave the polypeptide at position Lys_{38}. The resulting fragments were separated on a C18 PepHR 5/5 reversed phase column with a linear water/acetonitrile gradient (Fig. 2). By amino acid analyses of the materials from the different peaks, it was shown that the C-terminal fragment was contained in peak No. 3. Residues 39–59 of the polypeptide were identified by Edman degradation (Fig. 3) of the fragment from peak No. 3. The determination of the C-terminus of the uncleaved polypeptide was neither possible by carboxypeptidase digestion nor

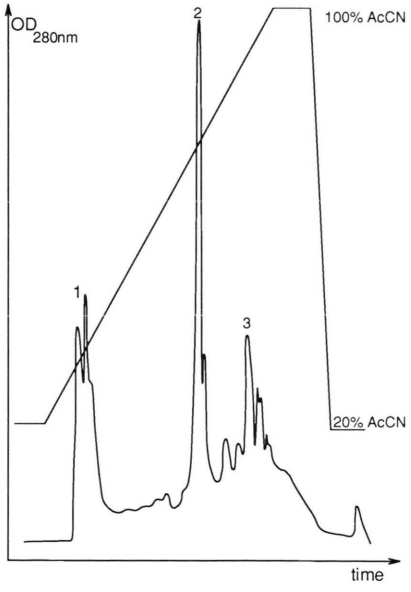

Fig. 1. HPLC chromatography of the extracted *Chlorobium vibrioforme f. thiosulfatophilum* material (see Materials and Methods) from Sephadex LH-60 corresponding to an approx. molecular weight of 6 kDa on a butyl (Shandon) reversed phase column. Peak 2 contains the pure 6.15 kDa polypeptide.

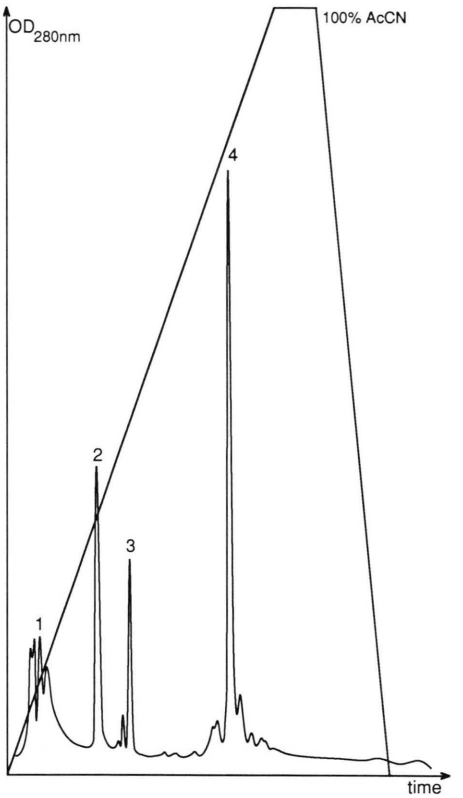

Fig. 2. HPLC chromatography of the polypeptide fragments of the hypothetical BChl *d*-binding polypeptide after digestion with endoproteinase Lys-C on a PepRP HR 5/5 (Pharmacia) reversed phase column. The C-terminal peptide was found in peak 3.

Table I. Amino acid composition (mol/mol) of the uncleaved presumable BChl *d*-binding polypeptide (A) and the C-terminal fragment (D) isolated from *Chlorobium vibrioforme f. thiosulfatophilum*. B, E nearest integer, C, F number of residues derived from the amino acid sequence.

AS	A	B	C	D	E	F
Asx	4.85	5	4	2.47	2	2
Thr	2.88	3	3	1.09	1	1
Ser	4.35	4	5	2.87	3	3
Glx	3.42	3	3	0	0	0
Pro	0	0	0	0	0	0
Gly	11.69	12	13	6.86	7	6
Ala	4.24	4	4	1.09	1	1
Cys	0	0	0	0	0	0
Val	4.03	4	4	0	0	0
Met	3.61	4	5	1.19	1	2
Ile	2.99	3	3	1.00	1	1
Leu	4.01	4	4	0.98	1	1
Tyr	0.84	1	1	0.88	1	1
Phe	2.95	3	3	0	0	0
His	0.98	1	1	0	0	0
Lys	1.03	1	1	0	0	0
Arg	4.00	4	4	3.00	3	3
Trp	n.d.	–	1	n.d.	–	0
Sum			59			21

by hydrazinolysis, probably due to the small amounts of material subjected to these experiments. However, taking into account the amino acid analyses of the whole polypeptide as well as those of the C-terminal fragment it seems very likely that the complete sequence of the BChl *d*-binding polypeptide of *Chlorobium vibrioforme f. thiosulfatophilum* has been established (Table I). Values for serine and methionine are commonly known to be too small. The values for Asx of the hypothetical BChl *d*-binding polypeptide were always too high in our analyses. Although this is the rule for most proteins it cannot be completely excluded, that there is an additional Asx amino acid residue at the end of the polypeptide. But we know for sure that products of methionine (sulfoxide, sulfon) cannot have increased the Asx peak artificially, since their retention times were checked and shown to be identical. Based on the protein sequence know so far the molecular weight of the hypothetical BChl *d*-binding polypeptide of *Chlorobium vibrioforme f. thiosulfatophilum* is 6154.02.

Discussion

The primary structure of the hypothetical BChl *d*-binding polypeptide from *Chlorobium vibrioforme f. thiosulfatophilum* has been deter-

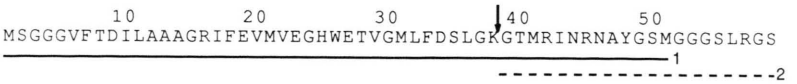

Fig. 3. Amino acid sequence of the hypothetical BChl *d*-binding polypeptide from *Chlorobium vibrioforme f. thiosulfatophilum*. (——) N-terminal sequence, (– – –) sequence of the C-terminal fragment.

mined, although there remains the question of an extra Asx at the C-terminus. This, however, seems to be very improbable with regard to the high homology of the rest of this polypeptide to the corresponding polypeptide from *Pelodictyon luteolum*. The primary structure analysis of the C-terminal fragment has been performed twice and in both experiments there was no hint for the appearance of an Asx following the serine at position 59. A comparison of all known presumable BChl *c*-, *d*- and *e*-binding polypeptides (Fig. 4) shows a high homology between the members of the Chlorobiaceae. With respect to the sequence of the polypeptide from *Pelodictyon luteolum* there is only one change in the polypeptide from *Chlorobium vibrioforme f. thiosulfatophilum*, namely threonine at position 55 is deleted. The homologous regions between positions 21–29 and 43–48 compared to *Chloroflexus aurantiacus* are also conserved just as in the other members of the Chlorobiaceae, thus confirming the presumption that these domains are functionally and structurally important. Above all there is a histidine at position 25, which is a very suitable candidate to bind the bacteriochlorophyll. If these polypeptides are actually involved in binding of BChl pigments, the influence of the structure of the pigment molecules on the primary structure of the polypeptides seems to be quite low. However, there are only very small differences between the structures of BChl *c* and *d* mainly consisting of an additional methyl group of BChl *d* between ring I and IV at position R_2 and partially unequal alkyl rests at ring II and III. These more peripherally located differences do not necessarily require distinct changes in the binding polypeptide, assuming that the BChl molecules are bound *via* the central Mg-atoms. One explanation for this high degree of homology between the members of the Chlorobiaceae might be that the species of this family form a coherent, moderately tight phylogenetic grouping [18]. On the other hand, the degree of homology is lower between the corresponding BChl-binding polypeptides of Chlorobiaceae and *Chloroflexus aurantiacus* (see Fig. 4). This fact can be explained and confirmed by the ribosomal RNA oligonucleotide cataloguing data which have clearly shown that there exists no close relationship between the green photosynthetic bacteria *Chlorobium* and *Chloroflexus* [19]. Beside the possibility of lateral gene transfer the most obvious reason for the high degree of homology seems to be a very important function of these polypeptides, which is in our opinion the association of the BChl pigment molecules in the chlorosome. Otherwise it is hard to believe that the polypeptide structure should have been conserved so strictly.

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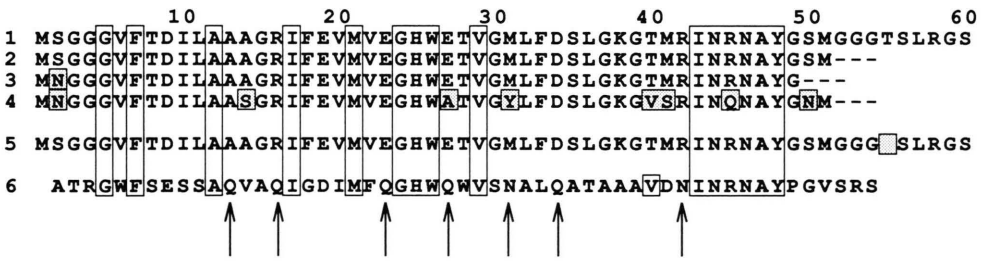

Fig. 4. Primary structure of the proposed BChl *c*-, *d*- and *e*-binding polypeptides from: 1 *Pelodictyon luteolum*[a], 2 *Chlorobium limicola*[a], 3 *Prosthecochloris aestuarii*[a], 4 *Chlorobium phaeovibrioides*[a], 5 *Chlorobium vibrioforme f. thiosulfatophilum*[b], 6 *Chloroflexus aurantiacus*[a]. Homologies between the polypeptides of the members of the Chlorobiaceae and *Chloroflexus aurantiacus* are boxed. Differences between the polypeptides among the members of the Chlorobiaceae are labelled with filled boxes. The originally proposed binding sites for bacteriochlorophyll *c* by Wechsler *et al.* [10] are indicated by arrows. Data taken from: [a] Wagner-Huber *et al.* [11]; [b] these results.

[1] L. A. Staehelin, J. R. Golecki, R. C. Fuller, and G. Drews, Arch. Microbiol. **119,** 269–277 (1978).
[2] L. A. Staehelin, J. R. Golecki, and G. Drews, Biochim. Biophys. Acta **589,** 30–45 (1980).
[3] N. Pfennig, Arch. Microbiol. **63,** 224–226 (1968).
[4] T. Wechsler, R. Brunisholz, F. Suter, R. C. Fuller, and H. Zuber, FEBS Lett. **191** (1), 34–38 (1985).
[5] T. D. Wechsler, R. A. Brunisholz, G. Frank, F. Suter, and H. Zuber, FEBS Lett. **210,** 189–194 (1987).
[6] J. A. Betti, R. E. Blankenship, L. V. Natarajan, L. C. Dickinson, and R. C. Fuller, Biochim. Biophys. Acta **680,** 194–201 (1982).
[7] D. E. Tronrud, M. F. Schmid, and B. W. Matthews, J. Mol. Biol. **188,** 443–454 (1986).
[8] P. D. Gerola and J. M. Olson, Biochim. Biophys. Acta **848,** 69–76 (1986).
[9] R. G. Feick and R. C. Fuller, Biochemistry **25,** 3693–3700 (1984).
[10] T. Wechsler, F. Suter, R. C. Fuller, and H. Zuber, FEBS Lett. **181** (1), 173–178 (1985).
[11] R. Wagner-Huber, R. A. Brunisholz, G. Frank, and H. Zuber, FEBS Lett. **239,** 8–12 (1988).
[12] P. D. Gerola, P. Højrup, and J. M. Olson, in: Photosynthethic Light-Harvesting Systems, pp. 129–139, Walter de Gruyter & Co., Berlin 1988.
[13] J. M. Olson, P. D. Gerola, J. Mikkelsen, P. Højrup, and J. Knudsen, Abstr. Vth Int. Symp. Photosynth. Prokaryotes, 86 (1985).
[14] P. D. Gerola, P. Højrup, J. Knudsen, P. Roepstorff, and J. M. Olson, in Green Photosynthetic Bacteria (J. M. Olson, J. G. Ormerod, J. Amesz, E. Stackebrandt, and H. G. Trüper, eds.), pp. 43–52, Plenum Press, New York 1988.
[15] M. A. Steinmetz and U. Fischer, Arch. Microbiol. **131,** 19–26 (1982).
[16] R. Rümbeli, M. Wirth, F. Suter, and H. Zuber, Biol. Chem. Hoppe-Seyler **368,** 1–9 (1987).
[17] G. Frank, in: Methods in Protein Sequence Analysis, Proceedings of the 7th International Conf. (Brigitte Wittmann-Liebold, ed.), pp. 116–121, Springer Verlag, Berlin 1989.
[18] J. Gibson, W. Ludwig, E. Stackebrandt, and C. R. Woese, System. Appl. Microbiol. **6,** 152–156 (1985).