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The BChlc/e-binding polypeptides from chlorosomes of green photosynthetic bacteria

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A 6.3 kDa polypeptide has been isolated from chlorosomes of the green photosynthetic bacterium *Pelodictyon luteolum*, and its complete amino acid sequence has been determined. It exhibits an overall homology of 30% to the BChlc-binding protein of *Chloroflexus aurantiacus*. Preliminary results from the N-terminal sequence analyses of the analogous polypeptides isolated from *Chlorobium limicola*, *Prosthecochloris aestuarii* and *Chlorobium phaeovibrioides* revealed a highly conserved sequence. This protein is suggested to be the BChlc/e-binding polypeptide in the family of the Chlorobiaceae.

Chlorosome; BChlc/e-binding polypeptide;
(Pelodictyon luteolum, Chlorobium limicola, Prosthecochloris aestuarii, Chlorobium phaeovibrioides)

1. INTRODUCTION

Green photosynthetic bacteria, the sub-order of the Chlorobiineae, consist of two families: the Chloroflexaceae and the Chlorobiaceae. The best characterized species of the Chloroflexaceae is *Chloroflexus aurantiacus*. Its photosynthetic apparatus contains two different light harvesting complexes: an intramembranously located BChla-binding complex B 806–866 consisting of an α/β pair of polypeptides [1,2], and an additional antenna containing BChlc absorbing at 740 nm. The latter is organized in the so-called chlorosomes, surface-located oblong vesicles [3–6]. Freeze fracture electron microscopy of chlorosomes by Staehelin et al. [7] showed rod-like three dimensional structures. SDS-polyacrylamide gel electrophoresis of chlorosomes revealed three major polypeptides (18, 11 and 3.7 kDa) and a fourth polypeptide (5.8 kDa) in minor amounts [8]. Crosslinking as well as proteolytic digestion experiments led to the conclusion that the BChlc is associated with the 3.7 kDa polypeptide. This polypeptide was isolated from purified chlorosomes and its amino acid sequence was determined [9], yielding a true molecular mass of 5.6 kDa. Seven Asn/Gln specifically arranged on one side of the presumptive α-helix were suggested to bind the BChlc [9].

In the family of the Chlorobiaceae far less is known about the photosynthetic apparatus. There are some distinct differences between the Chloroflexaceae and the Chlorobiaceae. So far, no intracytoplasmatic core antenna complex has been found in cells of Chlorobiaceae. However, an additional water-soluble BChla-binding protein, located between the chlorosome and the reaction center, has been isolated and crystallized [10]. The existence of a further BChla-containing complex absorbing at 794 nm located in the chlorosome membrane of *Chlorobium limicola* has been reported by Gerola et al. [11]. From chlorosomes of this bacterium a 74 amino acid residues polypeptide has been isolated and sequenced [12,13]. Although the homology between this polypeptide and the BChlc-binding protein of *C.*

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Abbreviations: PTH, phenylthiohydantoin; TFA, trifluoroacetic acid

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aurantiacus is only 17.6%, it has been designated as BChl-binding protein. At the same time [14] we reported on a polypeptide isolated from *Pelodictyon luteolum* which is 30% homologous to the c-binding protein of *C. aurantiacus*. We postulated that it most probably represents the BChlc-binding protein [14]. Interestingly no significant sequence homology was found between the proposed BChlc-binding proteins of *C. limicola* [12,13] and *P. luteolum* [14].

Here we report on the isolation and primary structure analysis of the 6.3 kDa polypeptide, the probable BChlc-binding protein, isolated from the BChlc-containing bacterium *P. luteolum*. In addition, analogous proteins were isolated from the BChlc-containing bacteria *C. limicola* and *Prosthecochloris aestuarii* and from the brown-green BChle-containing bacterium *Chlorobium phaeovibrioides*. Partial N-terminal sequences have been determined from these polypeptides.

2. MATERIALS AND METHODS

Cells of *P. luteolum* strain 2530, *C. limicola* strain 6230, *P. aestuarii* strain SK 413 and *C. phaeovibrioides* strain 2631 (kindly provided as a gift by Professor Norbert Pfennig, Konstanz) were grown according to Pfennig and Trüper [15] with trace element solution SL 10B [16] containing 300 mg H_3BO_3 in 10 l batch cultures.

Chlorosomes were prepared as described [8] with the following modifications: the chlorosomes were purified only once over a sucrose-gradient (1.2/0.9/0.6 M) and were washed by dilution with 10 mM Tris, pH 8.0, and consecutive sedimentation instead of dialysis.

Cells or chlorosomes were extracted with an organic solvent mixture of methylenechloride/methanol/ammonium acetate (1:1, v/v, 0.1 M), with the addition of 20% acetic acid in some cases. Gel filtration of this extract on Sephadex LH-60 yielded the 6.3 kDa and the 74 amino acid polypeptide described by Gerola et al. [12,13]. The 6.3 kDa polypeptide was further purified by reversed phase chromatography on a self-packed Diphenyl Si 300 (Serva) column by HPLC with a linear water/acetonitrile gradient (containing 0.1% TFA).

Cleavage at lysine was carried out as described [17]. Fragments were separated by reversed phase chromatography on a PepRP HR 5/5 (Pharmacia) column by fast protein liquid chromatography (FPLC).

Determination of the carboxy-terminal sequence was achieved by hydrazinolysis as described [18] and by combined digestion with carboxypeptidase A and B (Boehringer) as described [19].

For amino acid analysis polypeptide samples were hydrolyzed in constantly boiling 6 N HCl for 24 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 Biosystems 470 A protein sequencer. The PTH amino acids were identified as described [20].

3. RESULTS

The organic solvent extract of lyophilized cells or chlorosomes of *P. luteolum* was chromatographed on a Sephadex LH-60 column. The peak containing the 6.3 kDa polypeptide, completely separated from larger proteins as well as from pigments, was dialyzed and lyophilized and then used for Edman degradation without any further purification. Treatment with a solution of 5% HCl in methanol was necessary to deblock the N-terminus. The automated Edman degradation liberated 51 of 60 amino acids (fig.1). In order to get a C-terminal fragment the 6.3 kDa polypeptide was cleaved with endoproteinase Lys-C at Lys 38 and the 22 amino acid residues C-terminal fragment was purified by reversed phase chromatography on a PepRP HR 5/5 column by FPLC. Primary structure analysis of this fragment provided the amino acid sequence of the C-terminal 22

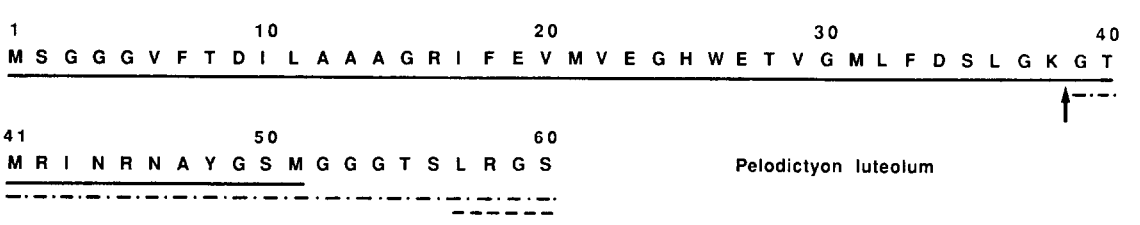

Fig.1. Amino acid sequence of the 6.3 kDa polypeptide of *P. luteolum*. (——) N-terminal sequence. (-·-·-) C-terminal fragment after cleavage at lysine 38 (arrow). (- - -) C-terminal amino acids confirmed by carboxypeptidase digestion. (···) C-terminal amino acid determined by hydrazinolysis. The N-terminus is blocked, probably by a formyl group.

amino acids. The C-terminus was confirmed by carboxypeptidase digestion and hydrazinolysis (fig.1). For amino acid analysis the 6.3 kDa fraction from LH-60 was further purified by reversed phase chromatography on a Diphenyl Si 300 column by HPLC. Based on the amino acid sequence the 6.3 kDa polypeptide has a true molecular mass of 6255 Da.

The analogous proteins of *C. limicola, P. aestuarii* and *C. phaeovibrioides* were isolated and purified in a similar manner from cells, in the case of *C. limicola* also from chlorosomes. N-terminal sequence analyses were performed as described for the 6.3 kDa polypeptide of *P. luteolum*. Protein sequences were established for *C. limicola* and for *P. aestuarii* up to position 48 and for *C. phaeovibrioides* up to position 51 (fig.2). All four polypeptides depicted in fig.2 show an overall homology of 30% to the BChlc-binding polypeptide of *C. aurantiacus*. The homologous residues are clustered mainly in two regions between residue 21 and 29 and between 43 and 48. The homology within the members of the Chlorobiaceae is extremely high: there are no substitutions between *P. luteolum* and *C. limicola* up to position 48. Only one mutation is present between *P. aestuarii* and *P. luteolum*, and there are 8 differences between *P. luteolum* and *C. phaeovibrioides*.

From cells of *C. limicola* and *P. luteolum* an additional polypeptide was isolated from LH-60. It

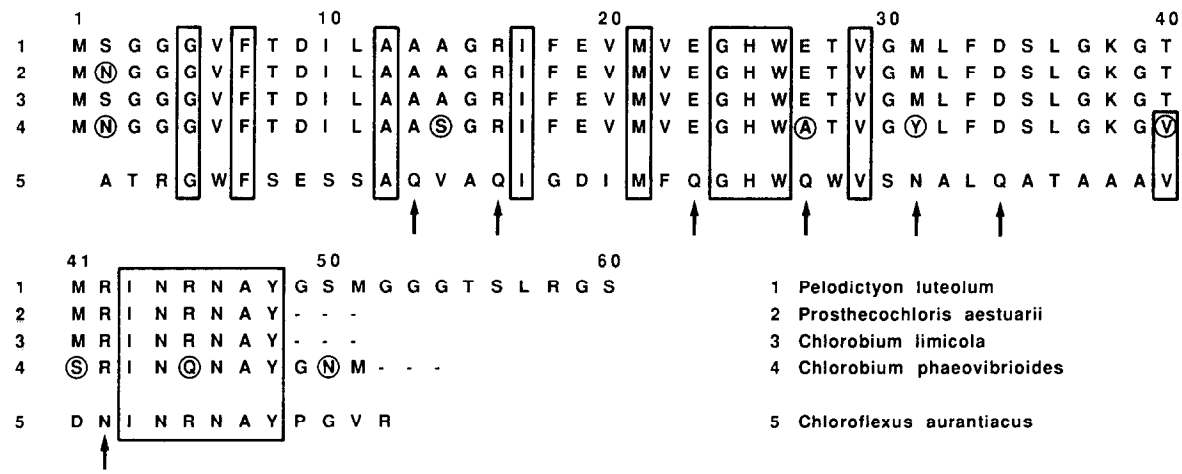

Fig.2. BChlc/e-binding polypeptides from chlorosomes of green photosynthetic bacteria. Homologous amino acids between the BChlc-binding polypeptide of *C. aurantiacus* and the BChlc/e-binding polypeptides of the Chlorobiaceae are boxed. Differences between the proteins of the Chlorobiaceae are marked with circles. The possible binding sites for bacteriochlorophyll *c* in *C. aurantiacus* suggested by Wechsler et al. [9] are indicated by arrows.

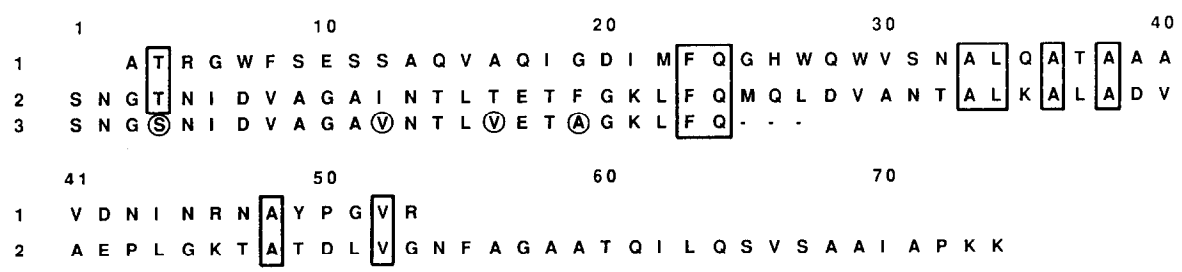

Fig.3. BChlc-binding polypeptide of *C. aurantiacus* (1). 74 amino acid polypeptide (see text [12,13]) of *C. limicola* (2) and *P. luteolum* (3) with a relatively low homology to 1 (17.6% for *C. limicola*). Homologous positions are boxed. Amino acids substitutions between 2 and 3 are indicated by circles.

eluted in front of the 6.3 kDa polypeptide according to its higher molecular weight. These polypeptides were dialyzed and lyophilized and then subjected to the automated Edman degradation procedure. The primary structure analysis of the polypeptide from *C. limicola* yielded the already established sequence published by Gerola et al. [12,13] (fig.3). An analogous polypeptide has been isolated from cells of *P. luteolum* (fig.3). However, it is remarkable that it was not possible to isolate this polypeptide from purified chlorosomes of *P. luteolum* under exactly the same conditions.

4. DISCUSSION

The 6.3 kDa polypeptide from *P. luteolum*, as well as the equivalent polypeptides isolated from *C. limicola*, *P. aestuarii* and *C. phaeovibrioides*, show a homology of 30% to the BChlc-binding polypeptide of *C. aurantiacus*. This is rather high considering that there are significant differences between the Chlorobiaceae and Chloroflexaceae with respect to the photosynthetic apparatus. The Gln and Asn residues which have been suggested to coordinate to the central Mg-atom of the bacteriochlorophyll *c* in *C. aurantiacus* [9] are replaced by other amino acids in the 6.3 kDa polypeptide of the Chlorobiaceae. However, in three cases (fig.2, positions 23, 27, 34) the amide group is substituted by a carboxyl group which possibly can bind BChlc similarly via the oxygen atom. Remarkable is the highly conserved region around the His (position 25), which also is a possible ligand to the Mg-atom. Another strictly homologous region is found from residue 43 to 48.

The homology of the 6.3 kDa polypeptide within the members of the Chlorobiaceae is extremely high (fig.2). Besides the close relationship, there must be an additional reason for this fact. Most probably this 6.3 kDa polypeptide has a very important functional role that allows practically no substitutions in the amino acid sequence. It is notable that only the polypeptide of *C. phaeovibrioides* exhibits some structural differences to the three other polypeptides, even in the homologous clusters (fig.2, positions 27, 45). This may be explained by the fact that this bacterium belongs to the brown-green Chlorobiaceae species containing BChle in the chlorosome antenna.

The 74 amino acid polypeptide of *C. limicola* described by Gerola et al. [12,13] as BChlc-binding polypeptide (fig.3, line 2) exhibits only 17.6% homology to the BChlc-binding protein of *C. aurantiacus* (fig.3). Most of the homologous amino acids are alanines and other residues which seem to be of less functional importance. The point that we could only isolate the analogous protein from cells of *P. luteolum* but not from chlorosomes is possibly due to different preparation procedures for the chlorosomes. The miranol treatment probably induced a release of some proteins including the 74 amino acid polypeptide which are not released by the method of Gerola et al. [11]. Thus the 74 amino acid polypeptide is presumably not part of the internal B 740 antenna complex of the chlorosome. It could be bound to chlorosome surface or membrane surface. On the other hand it is reasonable to assume that the 6.3 kDa polypeptide from *P. luteolum*, as well as the equivalent proteins from *C. limicola*, *P. aestuarii* and *C. phaeoverioides* are the BChlc/e-binding polypeptides in the Chlorobiaceae.

This is suggested by the following observations: (1) the 6.3 kDa polypeptide is found in isolated chlorosomes and seems to be located within the chlorosome; (2) the sequence homology of the 6.3 kDa polypeptides of several Chlorobiaceae is 30% to the BChlc-binding polypeptide of *C. aurantiacus*; (3) the 74 amino acid polypeptide is also missing in the chlorosome of *C. aurantiacus*.

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