The complete amino-acid sequence of the small bacteriochlorophyll-binding polypeptide B800–850β from light-harvesting complex B800–850 of *Rhodopseudomonas capsulata*

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The small bacteriochlorophyll-binding polypeptide (β) of the light-harvesting complex B800–850 from the phototrophic bacterium *Rhodopseudomonas capsulata* was purified from the complex and the membrane, respectively. The complete amino-acid sequence has been determined. The M_r of the polypeptide is 4597. The protein consists of 49 amino acids, 64% of which are hydrophobic. Homology with other bacteriochlorophyll-binding polypeptides is discussed.

Bacteriochlorophyll-binding polypeptide *Primary structure* Rhodopseudomonas capsulata *B800–850 antenna pigment complex*

1. INTRODUCTION

Rhodopseudomonas capsulata is an anoxygenic, facultative, phototrophic bacterium, which produces ATP by cyclic phosphorylation when grown anaerobically in the presence of light [1]. The photosynthetically active pigments bacteriochlorophyll *a* and carotenoids are non-covalently bound to proteins in 3 pigment-protein complexes. These are the photochemical reaction centre and the two light-harvesting complexes B870 and B800-850 [2,3]. In both light-harvesting complexes the pigments are bound in stoichiometric amounts to two small polypeptides of $M_r < 10000$. While the B870 complex is synthesized in a constant ratio to the reaction centre and surrounds the reaction centre, the B800-850 complex is a variable component of the photosynthetic apparatus and is synthesized in large amounts under conditions of low light intensity [4].

The light-harvesting complex B800-850 has been isolated and characterized [3,5,6]. The complete amino-acid sequence of the large bacteriochlorophyll-binding polypeptide B800-850α has been published [7]. Here we describe the complete sequence of the small pigment-binding polypeptide B800-850β.

2. MATERIALS AND METHODS

2.1. *Isolation of membranes*

The isolation of membranes containing the light-harvesting complex B800-850 from *R. capsulata* strain Y5 and the purification of the B800-850α polypeptide have been described [6]. Polypeptides present in the light-harvesting complex were characterised by the Laemmli SDS-polyacrylamide gel electrophoresis procedure [5,6].

2.2. *Amino acid analysis*

The proteins were hydrolyzed [6] and the amino-acid composition determined using an automatic amino-acid analyser (Durrum D-500). Tryptophan and cysteine were determined by established methods [8,9].

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2.3. *Sequence analysis*

Automated Edman degradation was performed in a self-constructed gas-liquid phase microsequencer [10]. The amino PTH-derivatives were identified by HPLC as in [11]. For this purpose the sequencer was equipped with an on-line HPLC system. Manual Edman degradation was performed as described in [6].

2.4. *Cyanogen bromide cleavage of the polypeptide and fractionation of the fragments*

1.2 mg (approx. 261 nmol) protein was cleaved with CNBr (2.8 mg) in 0.8 ml of 70% formic acid for 24 h at room temperature. After cleavage, the mixture was concentrated to 0.4 ml and applied to a column (1.2 × 110 cm) of Bio-Gel P6 (200–400 mesh) which was equilibrated with 50% formic acid. Elution was carried out with 50% formic acid at a flow rate of 2.6 ml/h; 300 fractions of 2 ml each were collected.

2.5. *Digestion with carboxypeptidase A+B (Boehringer, Mannheim)*

To determine the carboxy-terminal sequences, carboxypeptidase A+B was used at pH 8.0 as described in [12].

2.6. *Nomenclature of peptides*

The cyanogen bromide peptides are designated with the prefix B. The peptides are numbered in the order of their elution from the Bio-Gel P6 columns.

3. RESULTS

3.1. *Polypeptide isolation and purification*

The light-harvesting polypeptides were isolated from the freeze-dried light-harvesting complex B800-850 or from freeze-dried membranes by chromatography on Sephadex LH60 (Pharmacia) in organic solvents as described [6]. The peak fraction III (fig.2 in [6]) showed one band in SDS-polyacrylamide gel electrophoresis, but 3 N-terminal amino acids were found by manual Edman degradation. The peak fraction III was dialyzed against distilled water and centrifuged (30 min, 48000 × *g*). The pellet was lyophilized and dissolved (10 mg in 1 ml chloroform/methanol, 3:1, v/v). The volume was reduced to about 0.4 ml by vacuum evaporation and a mixture of 0.8 ml chloroform/methanol/0.1 M Tris buffer (pH 7.4, final concentration 20 mM), 2:6:2, by vol., was added. The solubilized protein was applied to a DEAE-cellulose column (1.2 × 30 cm) equilibrated with chloroform/methanol/Tris buffer, pH 7.4 (2:6:2), and eluted with the same solvent (flow rate 10 ml/h). After fraction 50 a linear elution gradient up to 0.5 M NaCl was applied. Two protein peaks were obtained. The peak containing B800-850β proteins eluted at 0.35 M NaCl. The amino-acid composition of that fraction is presented in table 1.

3.2. *Sequence determination*

Automated Edman degradation of this polypep-

Table 1

Amino acid composition of the bacteriochlorophyll *a*-binding polypeptide B800-850β of the light-harvesting complex B800-850 and of the C-terminal fragment B-2 after CNBr cleavage of the polypeptide

Amino acid	B800-850 polypeptide			Peptide B-2	
	Molar ratio*		Integer	Molar ratio	Integer
	24 h	72 h		24 h	
Asp	2.90	2.97	3 (3)	+	0 (0)
Thr	2.98	3.19	3 (3)	0.89	1 (1)
Ser	4.83	4.33	5 (4)	1.21	1 (1)
Glu	2.81	2.86	3 (3)	0	0 (0)
Pro	1.74	1.68	2 (2)	0.98	1 (1)
Gly	5.14	4.60	5 (5)	0.12	1 (1)
Ala	7.00	7.00	7 (7)	4.03	4 (4)
Cys	n.d.	n.d.	n.d. (0)	0	0 (0)
Val	1.40	2.06	2 (2)	1.02	1 (1)
Met	1.35	0.87	1 (2)	0	0 (0)
Ile	2.99	3.33	3 (4)	1.87	2 (2)
Lac	5.64	5.75	6 (6)	2.83	3 (3)
Tyr	0.84	0.71	1 (1)	0	0 (0)
Phe	0.97	0.99	1 (1)	0	0 (0)
His	1.61	1.80	2 (2)	1.00	1 (1)
Lys	1.71	1.81	2 (2)	0	0 (0)
Arg	0.90	0.96	1 (1)	+	0 (0)
Trp	n.d.	n.d.	n.d. (1)	0.89	1 (1)
Polarity			36%		

Values in parentheses are the number of residues found by sequencing; +, amounts less than 0.1 nmol. * Data published in [6]; polarity is calculated as defined in [13]

tide revealed methionine as the N-terminal residue. However, only 21% of the expected amount of methionine was obtained. Therefore the polypeptide was incubated in 5% concentrated HCl in chloroform/methanol (1:1, v/v) for 24 h at room temperature [6] to deblock the N-terminus. After this treatment the yield of methionine from the N-terminus increased to 60% of that expected. It appears that this polypeptide exists in two states, one that has a free N-terminus and one that is blocked, probably with a formyl group. Attempts to improve the deblocking of the N-terminus by increasing the HCl concentration to 10% led to partial release of the N-terminal residue.

The automated Edman degradation of 6.9 μg (approx. 1.5 nmol) of deblocked polypeptide (5% concentrated HCl in chloroform/methanol, 1:1, v/v, for 10 h at room temperature) resulted in the sequence of 37 N-terminal residues (fig.1).

3.3. *The cyanogen bromide fragments of the polypeptide*

After cyanogen bromide cleavage (described in section 2) the fragments were isolated by chromatography on Bio-Gel P6 (200–400 mesh) (fig.2). Elution patterns, amino-acid composition (table 1) and manual Edman degradation indicated that peak B-1 (fig.2) consisted mainly of the N-terminal fragment with residues 2–33. The C-terminal fragment (residues 34–49) was recovered in peak B-2; it had the sequence Ala-Val-Ala-His-Ile-Leu-Ser-Ala-Ile-Ala-Thr-Pro-Trp-Leu-Gly-COOH (fig.1). C-terminal analysis of fragment

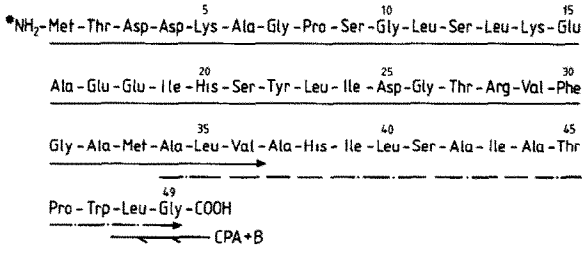

Fig.1. Amino acid sequence of the bacteriochlorophyll-binding polypeptide B800-850β of the light-harvesting complex B800-850 from *R. capsulata*, strain Y5. ——→, N-terminal sequence of B800-850β in the sequencer; ----, sequence determination of the CNBr-C-terminal fragment in the sequencer; ←——, sequence determination of B800-850β polypeptide and CNBr–C-terminal peptide.

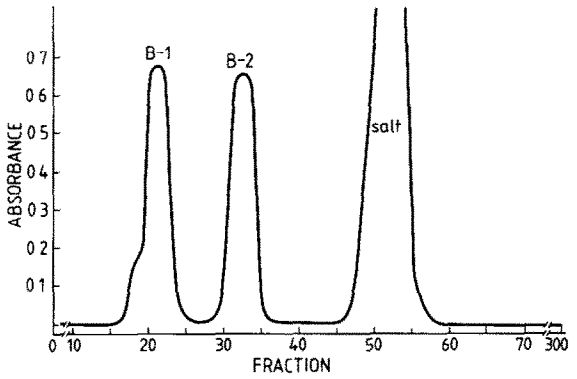

Fig.2. Gel filtration on Bio-Gel P6 (200–400 mesh) of the CNBr peptide fragments of the B800-850β polypeptide (see section 2).

B-2 (fig.2) and of the whole polypeptide by carboxypeptidase A + B resulted in the same degradation pattern revealing Trp-Leu-Gly in the ratio of 0.9:1:1.1.

4. DISCUSSION

The small bacteriochlorophyll-binding polypeptide of the light-harvesting complex B800-850 has an apparent M_r of 8000 by SDS-polyacrylamide gel electrophoresis. On the basis of the amino-acid sequence analysis the polypeptide consists of 49 amino-acid residues having an M_r of 4597. This protein contains 64% hydrophobic amino acids as defined in [13], which is characteristic of an integral membrane protein.

The amino-acid sequence (fig.1) shows a hydrophilic N-terminal region (residues 1–17). The remaining polypeptide including the C-terminus is hydrophobic. Comparison of the amino-acid sequence of B800-850β from *R. capsulata* (fig.1) with B800-850β from *R. sphaeroides* [14] shows strong homology. The homology is not restricted to the hydrophobic transmembrane region but is extended to the C- and N-terminal sequences, which demonstrates that these regions are also of importance for the organisation of the complex. There is, however, no homology between the α and β polypeptides of the B800-850 complex from *R. capsulata*. The N-terminal region of the α polypeptide [7] has a net positive charge whereas the β (fig.1) subunit has a net negative charge, which can be of importance for the interactions between both

polypeptides in the complex. There is some homology between the primary structures of B870β [12] and B800-850α. Thus, functionally and structurally the β polypeptides of different species [4,7,12,14–16] from both complexes are related. From the amino-acid sequence of the B870α polypeptide from *Rhodospirillum rubrum* [15] a tripartite structure has been reported: a polar N-terminal region is separated from a polar C-terminal region by a segment of about 21 predominantly hydrophobic amino acids. This tripartite structure described in terms of polarity and hydrophobicity in [13] does not fit the primary structure of the light-harvesting polypeptide of *R. capsulata* [7,12]. All pigment binding polypeptides which have been analysed contain one (α) or two (β) histidine residues. Histidine is proposed to function as a ligand for the Mg^{2+} of the bacteriochlorophyll ring.

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REFERENCES

[1] Drews, G. and Oelze, J. (1981) Adv. Microbiol. Physiol. 22, 1–92.
[2] Peters, J. and Drews, G. (1983) FEMS Microbiol. Lett. 17, 235–237.
[3] Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499–513.
[4] Drews, G. (1985) Mikrobiol. Rev., in press.
[5] Shiozawa, J.A., Welte, W., Hodapp, N. and Drews, G. (1982) Arch. Biochem. Biophys. 213, 473–485.
[6] Tadros, M.H., Zuber, H. and Drews, G. (1982) Eur. J. Biochem. 127, 315–318.
[7] Tadros, M.H., Suter, F., Drews, G. and Zuber, H. (1983) Eur. J. Biochem. 129, 533–536.
[8] Hirs, C.H.W. (1967) Methods Enzymol. 11, 197–199.
[9] Lin, T.Y. and Chany, Y.H. (1971) J. Biol. Chem. 246, 2942–2948.
[10] Frank, R. and Trosin, M. (1984) in: Modern Methods in Protein Chemistry (Tschesche, H. ed.) De Gruyter, Berlin, in press.
[11] Lottspeich, F. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 1829–1834.
[12] Tadros, M.H., Suter, F., Seydewitz, H.H., Witt, I., Zuber, H. and Drews, G. (1984) Eur. J. Biochem. 138, 209–212.
[13] Capaldi, R.A. and Vanderkooi, G. (1972) Proc. Natl. Acad. Sci. USA 69, 930–932.
[14] Theiler, R., Suter, F., Wiemken, V. and Zuber, H. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 703–719.
[15] Brunisholz, R.A., Cuendet, P.A., Theiler, R. and Zuber, H. (1981) FEBS Lett. 129, 150–154.
[16] Brunisholz, R.A., Suter, F. and Zuber, H. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 675–688.