THE COMPLETE AMINO ACID SEQUENCE OF THE SINGLE LIGHT HARVESTING PROTEIN FROM CHROMATOPHORES OF *RHODOSPIRILLUM RUBRUM* G-9$^+$

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1. Introduction

Pigment-free preparations of the single light-harvesting protein from the purple photosynthetic bacterium *Rhodospirillum rubrum* have been reported both from the antenna bacteriochlorophyll–protein complex and directly from chromatophores by chloroform/methanol extraction [1,2]. Molecular mass determinations yielded values of 9000–19 000 [1–4]. Preliminary sequence information was obtained from a few very small fragments of limited acid-treated LHP from wild-type *R. rubrum* [5]. N-Terminal amino acid sequence data have been published for LHP from the carotenoidless mutant *R. rubrum* G-9$^+$, which was found to be blocked, presumably by a formyl group [6]. Aside from the elucidation of the initial N-terminal sequences of the 10 000 M_r and the 8000 M_r polypeptides from the light-harvesting B800–850 complex from *Rhodopseudomonas capsulata* strain Y5 [7], this was the first report on extensive N-terminal sequence data of a LHP from a purple photosynthetic bacterium.

Here we present the complete amino acid sequence of LHP from *R. rubrum* G-9$^+$, thus opening the way for progress in localizing it within the chromatophore membrane and for studying its in vivo aggregational state in the BChl–protein complex. On the basis of the amino acid sequence, LHP consists of 52 amino acid residues yielding 6106 M_r. Of particular interest is a hydrophobic segment from residue 13–33, carrying in position 29 the single His residue of the polypeptide.

Abbreviations: LHP, light harvesting protein; BChl, bacteriochlorophyll; RC, reaction center; PTH, phenylthiohydantoin; *o*-IBA, *o*-iodosobenzoic acid; C/M/NH_4OAc, chloroform/methanol (1:1, v/v) containing 0.1 M ammonium acetate

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2. Material and methods

LHP from *R. rubrum* G-9$^+$ was extracted with a mixture of C/M (1:1, v/v) from freeze-dried RC-depleted chromatophores as in [1]. The extracted protein was liberated from pigment and phospholipids by gel filtration on Sephadex LH-60 (Pharmacia) in C/M/NH_4OAc [7]. Fractions containing LHP were dialyzed extensively against water and lyophilized. To obtain accurate amino acid analyses LHP was further purified by ion-exchange chromatography on CM-cellulose (Whatman, CM-52) essentially pretreated as in [8]. LHP was applied onto the CM-cellulose column in C/M/NH_4OAc and eluted with this solvent containing 0.5% (v/v) acetic acid. A contaminant containing tyrosine was eluted when the concentration of acetic acid was increased to 20% (v/v).

Excellent deblocking conditions for the N-terminal blocked LHP were achieved with 5% HCl in C/M (1:1, v/v) during 24 h at room temperature. For automated amino-terminal sequence analyses this protein solution was directly applied into the spinning cup of the sequencer.

Fragmentation of LHP with *o*-IBA was performed in 80% acetic acid, 4 M guanidinium–HCl according to [9]. LHP lacking in tyrosine was not preincubated with *p*-cresole prior to fragmentation, as in [10]. After fragmentation the protein solution was desalted on a P-2 (–400 mesh, Biorad) column (2.5 × 25 cm) in 80% acetic acid, while separation of the fragments was done on a P-6 (–400 mesh, Biorad) column (2.5 ×

70 cm) in 80% acetic acid. Each gel filtration run was recorded at 280 nm on the Uvicord system from LKB. In addition, in some runs fractions were checked for amino acid composition. Protein and peptide samples of ~30–50 nmol (added up to 0.1–0.2 ml of constant boiling HCl) were used for amino acid analyses. The samples were hydrolyzed in vacuo at 110°C, usually for 24, 48 and 72 h. The hydrolysis time for LHP was even extended up to 96, 192 and 240 h. After drying the samples were analyzed on a Biotronik LC 6000 E analyzer. Tryptophan determination was done as in [11] using 4 N methanesulfonic acid from Pierce. Special comments on the calculation of the amino acid composition in section 3.

Amino acid sequences were determined by Edman degradation either in an automated sequencer (Beckman 890 C) using the Quadrol (0.25 M) program together with Polybrene (Sigma) as an additive [12], or by a slightly modified manual procedure [13]. PTH-amino acid derivatives were identified by the HPLC procedure in [14]. PTH-Arg and PTH-His were identified on an amino acid analyzer after back hydrolysis [15]. The carboxyl terminal analyses were performed enzymatically with carboxypeptidase Y (Boehringer) at pH 5.5 [16]. Aliquots were removed after 0, 3, 9, 15, 30, 60, 150 and 360 min; lyophilized and subjected to an amino acid analysis. In order to detect any sugar moiety, *o*-glycosidically attached LHP was treated with 0.1 N NaOH in the presence of 1 M sodium borohydride at 37°C for 50 h [17]. After carboxypeptidase Y digestion any release of α-aminobutyric acid or alanine was checked on the amino acid analyzer.

3. Results and discussion

3.1. *Purification of LHP*

Separation of LHP from the pigment/phospholipid fraction on a Sephadex LH-60 column in C/M/NH_4OAc was better than the methods in [1,2]. Although such preparations were slightly contaminated (indicated by ~0.1 M tyrosine/mol LHP) LHP was pure enough to determine its complete amino acid sequence. LHP designated for accurate amino acid analyses was further purified by column chromatography on CM-cellulose (elution diagram not shown).

Table 1
Amino acid composition of LHP (LH-60 and CM-52) and the *o*-IBA dodecapeptide. The results are expressed as residue/molecule

Amino acid	LHP[a] LH-60		LHP[a] CM-52		LHP[g]	*o*-IBA[b] dodecaptide	
Asp	1.8	(2)	1.8	(2)	2	0.2	(0)
Thr	2.8[c]	(3)	2.5[c]	(3)	4	1.7	(2)
Ser	1.6[c]	(2)	1.5[c]	(2)	3	1.5	(2)
Glu	5.7	(6)	5.1	(5)	5	1.9	(2)
Pro	3.2	(3)	2.4	(2)	2	0.9	(1)
Gly	3.0	(3)	2.0	(2)	2	[f]	(1)
Ala	4.8	(5)	4.0	(4)	4	1.0	(1)
Val	3.8	(4)	3.1	(3)	3	0.9	(1)
Met	1.3	(1)	1.0	(1)	1	0.0	(0)
Ile	3.4	(3)	2.9	(3)	3	0.1	(0)
Leu	10.1	(10)	9.4	(9)	10	1.1	(1)
Tyr	0.1	(0)	0.0	(0)	0	0.0	(0)
Phe	5.2	(5)	4.8	(5)	5	0.2	(0)
His	[e]	(1)	[e]	(1)	1	0.0	(0)
Lys	1.4	(1)	0.9	(1)	1	0.9	(1)
Arg	3.0	(3)	2.9	(3)	3	0.2	(0)
Trp[d]	2.6	(3)	n.d.		3	n.d.	

[a] 240 h hydrolysis; [b] 24 h hydrolysis, represents uncorrected data
[c] uncorrected; [d] methanesulfonic acid hydrolysis according to [9]
[e] data based on 1 His/LHP molecule; [f] data based on 1 Gly/LHP molecule
[g] according to amino acid sequence

The values in parentheses are the most probable numbers of residues

3.2. *Amino acid analyses*

The amino acid compositions of LHP after the LH-60 and the CM-cellulose purification step (both from 240 h hydrolysis) are compiled in table 1. The amino acid analysis of the latter is in good accordance with the composition established by the amino acid sequence. Calculations of earlier reported amino acid analyses of LHP [1,2] based on 1 His residue/polypeptide assumed that His was totally hydrolyzed after 24, 48 and 72 h. However, new investigations with LHP purified on Sephadex LH-60 showed that His is apparently not completely hydrolyzed even after 96 h incubation in 6 N HCl. This is compatible with the localization of His within a hydrophobic amino acid segment (fig.3). On the basis of 1 His residue, the total number of amino acid residues of 73 decreases after 24 h to 53 residues after 240 h hydrolysis (fig.1). This indicates a relative increase of His compared to other amino acid residues. The numbers of Ser and Thr are not extrapolated to zero time, nor is Trp compiled in this graph. Leu shows a decreasing tendancy over 96 h, then increases continously to the 240 h experiment. This implies that Leu was not completely hydrolyzed until after the 240 h experiment, at the earliest. Based on the amino acid analysis after 240 h hydrolysis, LHP has M_r ~6000. The much lower number of 73 amino acid residues/1 His residue after 24 h (fig.1) compared to [1,2] is apparently the result of the improved chromatographic purification of LHP on Sephadex LH-60 in C/M/NH_4OAc.

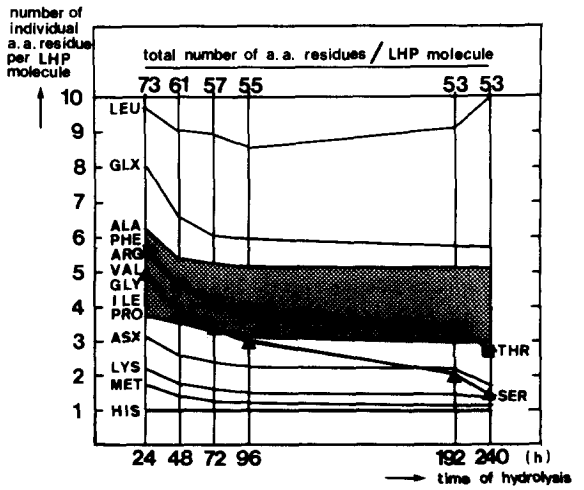

Fig.1. Amino acid composition of LHP (purified on LH-60), depending on time of hydrolysis of LHP. The calculation is based on 1 His residue/LHP molecule: (▲) Ser; (■) Thr; Trp is not shown.

3.3. *Deblocking and amino acid sequence analyses of LHP*

Deblocking conditions for LHP were improved by taking 5% HCl in C/M (1:1, v/v) instead of 1 N aqueous HCl [6]. As judged by the yield of PTH amino acids, ≥90% of LHP were deblocked. The automated Edman degradation of 3 mg LHP (500 nmol) resulted in the determination of 49 amino terminal residues (fig.3). Enzymatic digestion of LHP with carboxypeptidase Y revealed Ser as the C-terminal amino acid residue.

3.4. *Elucidation of the complete amino acid sequence by o-IBA fragmentation*

After fragmentation of LHP by *o*-IBA, a specific tryptophanyl peptide bond splitting reagent, the peptides generated were desalted on P-2 (fig.2A). Manual Edman degradation and amino acid analyses indicated that peak IIa consists mainly of two small fragments originating from the N-terminal region of LHP (fig.3). Rechromatography of peak Ia on P-6 (fig.2B) yielded, beside the core fraction Ib (mainly the fragment with residue number 6–40), a peptide IIb showing the sequence Leu–Glu–Gly–Ala–Ser–Thr–Lys–Pro–Val–Gln–Thr–Ser-COOH. Thus this fragment comprises the adjacent segment from residue 40 (Trp).

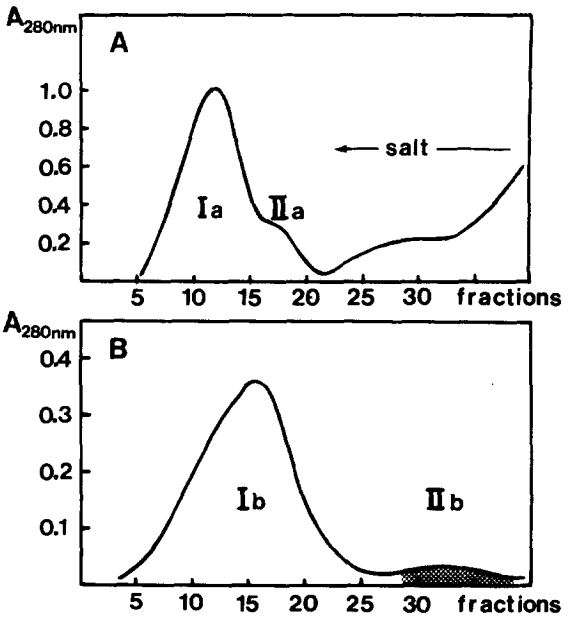

Fig.2. (A) Gel filtration of the *o*-IBA fragments of LHP (20 mg) on Biogel P-2 (–400 mesh), 4 ml/fraction. (B) Gel filtration of peak Ia (from P-2) on Biogel P-6 (–400 mesh). 5 ml/fraction.

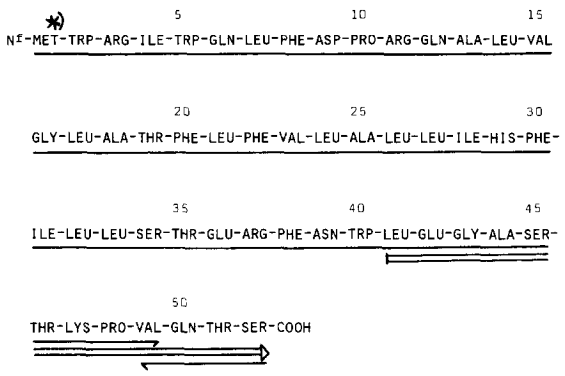

Fig.3. Amino acid sequence of LHP from *R. rubrum* G-9$^+$: (—▸) sequence determination of deblocked LHP in the sequencer; (⇒) sequence determination of the *o*-IBA dodecapeptide in the sequencer; (——) sequence determination of LHP and the *o*-IBA dodecapeptide by carboxypeptidase Y' (*) The amino group of Met is presumably blocked by a formyl group [6]. The N-terminal Met was also detected from the *o*-IBA fragment IIa (fig.2A, from untreated blocked LHP) by amino acid analysis as Met-sulfone.

The complete amino acid sequence (fig.3) of this dodecapeptide is compatible with the amino acid analysis (table 1). C-terminal analysis of this *o*-IBA fragment by carboxypeptidase Y resulted in the same degradation pattern as obtained for LHP revealing Val:Thr:Ser in the ratio of ~1:1:4. Thus, this dodecapeptide is apparently the C-terminal *o*-IBA fragment. From the carboxypeptidase Y experiment the question arises as to whether there is >1 Ser (ratio: Thr = 4:1) at the C-terminal end (see also [6]). Since both amino acid sequence analysis and amino acid analysis confirmed 1 Ser residue at the C-terminal end, either a blocked Thr (residue 51) or the kinetics of carboxypeptidase Y degradation might be the cause of the increased amount of Ser. Carboxypeptidase Y degradation of alkali-treated LHP (under reducing conditions) showed that neither Ser nor Thr are involved in carbohydrate binding. Therefore, the increased amount of Ser liberated by carboxypeptidase Y is probably due to a much slower degradation of Thr than Ser in this specific amino acid sequence constellation. In addition it must be taken into account that Gln runs at the same position as Ser on the amino acid analyzer. According to the definition in [18], LHP of *R. rubrum* G-9$^+$ shows a polarity of 37%. The peculiar feature of its amino acid sequence is a hydrophobic stretch from residue 13–33 (fig.3). Surrounded with an almost symmetrical arrangement of hydrophobic residues (–Leu–Leu–Ile–His–Phe–Ile–Leu–Leu–), the single His in position 29 might be involved in the pigment–protein interaction. At least in the case of the green photosynthetic bacterium *Prosthecochloris aestuarii,* 5 or 7 BChl were reported to interact with His sidechains [19]. Furthermore, it is interesting to note that the 10 000 M_r protein from the B800–850 light-harvesting complex of *Rhodopseudomonas capsulata* exhibits a His residue in a hydrophobic segment at the same position (unpublished).

The complete amino acid sequence now offers a helpful tool for determining arrangement and localization of LHP within the chromatophore membrane. Experiments in this field are currently in progress in our laboratory.

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