THE COMPLETE AMINO ACID SEQUENCE OF THE SPIRULINA PLATENSIS FERREDOXIN

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SUMMARY: The complete amino acid sequence of ferredoxin from the Spirulina platensis, a blue green non-filamentous algae has been determined. The amino acid sequence differs from the reported sequence of Wada et al. (1) at residue 9. Our results indicate that the residue is Asn while Wada et al. have reported it to be Asp. In addition, residues 86 and 87 have been determined in our study while Wada et al. have assumed these residues by homology to other ferredoxins which have been sequenced. Modifications of the automated sequence procedure previously described (2) were again found to be useful for peptide sequencing.

Eucaryotic ferredoxins of the plant type which have been sequenced to date (3) include the proteins from spinach (4), Leucaena glauca (5), alfalfa (6), taro (7), and Scenedesmus (8). Only one procaryotic ferredoxin has been completely sequenced, namely the Spirulina maxima ferredoxin (9). Nearly the complete sequence of the Spirulina platensis ferredoxin, another procaryotic ferredoxin has just been reported by Wada et al. (1) in a preliminary note. Our laboratory has independently just completed the amino acid sequence of the S. platensis ferredoxin by an independent procedure from Wada et al. (1) and the sequence results from the two laboratories are compared in this report.

EXPERIMENTAL PROCEDURES

Spirulina platensis ferredoxin. Ferredoxin was extracted and purified from the dried Spirulina platensis cells by the procedure described previously for the Spirulina maxima (10). The preparation of the carboxymethylferredoxin has been described previously (9).

Trypsin digestion of carboxymethylferredoxin and gel filtration of

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tryptic digest. About 25 mg of carboxymethylferredoxin were digested with 4% TPCK-trypsin (11) in pH 8 at 28° for 24 hours. The dried digest was applied to a Sephadex G-50 column (1.9 x 57 cm). The solvent used was 0.1 N ammonium hydroxide.

Isoelectric focusing of the mixture of peptides, T2 and T3, and further purification of the isolated peptides. The lyophilized mixture (5 mg) of peptide T2 and T3 obtained from the first peak during gel filtration of the tryptic digest of carboxymethylferredoxin was separated into two peptides, T2 and T3, by electrofocusing techniques (12) using ampholine (pH range of 3.5 - 10). The applied voltage was 300 volts and a column of 148 ml capacity was used. After 11.5 hours, prior to the attainment of an equilibrium state, fractions of 3.5 ml were collected and the pH of the various samples were determined. Each peak fraction was again rechromatographed on a Sephadex G-50 column.

Sequence determinations and amino acid analyses. Amino-terminal end group analyses of the protein and peptides (155 to 300 nmoles) were carried out by the manual Edman degradation (13) or by the automated Edman degradation procedure (14) in the Beckman-Spinco Model 890 Protein/Peptide Sequencer. All of the automated runs utilized the protein double cleavage program and 0.2 M Quadrol as the coupling buffer. Prior to the Sequencer analysis, peptide T3 was reacted with 4-sulfophenylisothiocyanate (15). The phenylthiohydantoins of the amino acids were determined by gas chromatography (16) or by thin layer chromatography (14), or by amino acid analyses of the 6N HCl hydrolysates of the amino acid phenylthiohydantoins (17).

COOH-terminal end groups of the protein and peptides were determined by the action of carboxypeptidase B and/ or A digestion (18), and/ or by hydrazinolysis experiments (19).

The amino acid compositions of the protein and peptides were determined on 6N HCl hydrolysates in a Beckman-Spinco Model 120C automatic amino acid analyzer (20).

RESULTS

Amino acid composition and end group analyses of Spirulina platensis ferredoxin. The amino acid composition of the Spirulina platensis ferredoxin was determined on 24-, 48-, and 72-hours hydrolysates of carboxymethylferredoxin and the protein was shown to consist of 98 residues. The NH_2-terminal amino acid of the S. platensis ferredoxin was shown to be alanine (100% yield) by Protein Sequencer analysis. Hydrazinolysis of the carboxymethylferredoxin yielded tyrosine in 86% yield. Carboxypeptidase A digestion (6 hours) followed by hydrazinolysis of the carboxymethylferredoxin yielded glycine (79%), leucine (100%), and tyrosine (100%). Thus, the COOH-terminal sequence of the S. platensis ferredoxin was shown to be -Gly-Leu-Tyr-COOH. The end group analyses indicated that the ferredoxin was pure.

Separation and purification of tryptic peptides. Tryptic peptides of carboxymethylferredoxin were separated by Sephadex G-50 column chromatography. Three peaks were observed. The first peak contained two long peptides, T2 and T3, together with a small amount of undegraded protein. The isoelectrofocusing technique was used to separate peptides T2 and T3. The second and third peaks contained peptides T4 and T1, respectively. The method for the further purification of the peptide fractions, their amino acid compositions and properties are summarized in Table I.

Sequencer results of carboxymethylferredoxin (Residues 1-45). Sequence analysis of the Spirulina platensis carboxymethylferredoxin (300 nmole) was performed twice in the Beckman-Spinco Protein/Peptide Sequencer. It was possible to determine the first 45 residues from the amino-terminal end of the protein. In a typical experiment, the average repetitive yield of Pth-alanine[1] was 94% and the recovery of Pth-alanine at the forty-fifth step was 7%.

Sequencer analyses and COOH-terminal analysis of peptide T3 (Residues 43-90). The sequence analyses of the peptide T3 was carried out on both the 4-sulfophenylthiocarbamyl derivative of the peptide (300 nmole) and the underivatized peptide (300 nmole). In the Sequencer run of the 4-sulfophenylthiocarbamyl derivative, the residual peptide after step 24 was removed from the cup. Subsequent Edman degradation steps were performed manually. By this modification (2), it was possible to sequence an additional 23 residues of the peptide. The recovery of Pth-isoleucine at the forty-seventh step was 10%.

The COOH-terminal analyses of the peptide T3 utilized the carboxypeptidase B procedure and the carboxypeptidase B-A procedure. In the latter type of experiment, after 6 hours of digestion with carboxypeptidase A,

[1] The abbreviation used is: Pth, phenylthiohydantoin.

TABLE I: Amino Acid Composition[a] and Properties of Tryptic Peptides of the Carboxymethyl-ferredoxin from S. platensis.

Amino Acid	T1	T2	T3	T4	Total Residues
Carboxymethylcyetine		1.88 (2)	3.86 (4)		6
Aspartic acid		8.05 (8)	6.07 (6)		14
Threonine	0.93 (1)	2.97 (3)	6.89 (7)	0.95 (1)	12
Serine		0.88 (1)	4.76 (5)		6
Glutamic acid		4.97 (5)	4.03 (4)	2.98 (3)	12
Proline		0.87 (1)	0.94 (1)		2
Glycine		1.99 (2)	3.97 (4)	0.99 (1)	7
Alanine	1.00 (1)	4.00 (4)	5.00 (5)		10
Valine		1.09 (1)	1.98 (2)		3
Isoleucine		3.96 (4)	3.86 (4)		8
Leucine		3.95 (4)	2.02 (2)	1.00 (1)	7
Tyrosine	0.97 (1)	1.98 (2)	2.01 (2)	0.95 (1)	6
Phenylalanine			0.98 (1)		1
Lysine	0.98 (1)		0.95 (1)		2
Histidine				0.91 (1)	1
Arginine		0.87 (1)			1
Total residues	4	38	48	8	98
Recovery (%)	78	77	77	84	
Color reaction with ninhydrin	Purple	Purple	Purple	Yellow to Purple	
Purification method[b]	PIN Rf= 0.67	Electro-focusing	Electro-focusing	BPAW Rf= 0.49	

[a] Results from 6N HCl hydrolyzates (24 and 48 hours). The numbers in parentheses refer to the assumed stoichiometric number of residues per molecule of pure peptide.

[b] The abbreviations used are: PIN, paper chromatography in the solvent system, pyridine/isoamyl alcohol/0.1 N ammonium hydroxide (60 : 30 : 50, v/v); and BPAW, paper chromatography with 1-butanol/pyridine/acetic acid/water (60 : 40 : 12 : 48, v/v).

yield in addition to threonine (54%), isoleucine (100%), and lysine (100%). The sequence data of peptide T3 are summarized in Figure 1.

Sequence determination of peptide T4 (Residues 91-98). Seven steps of the manual Edman degradation established the sequence of this peptide (200 nmole). After the seventh step of Edman degradation, free tyrosine

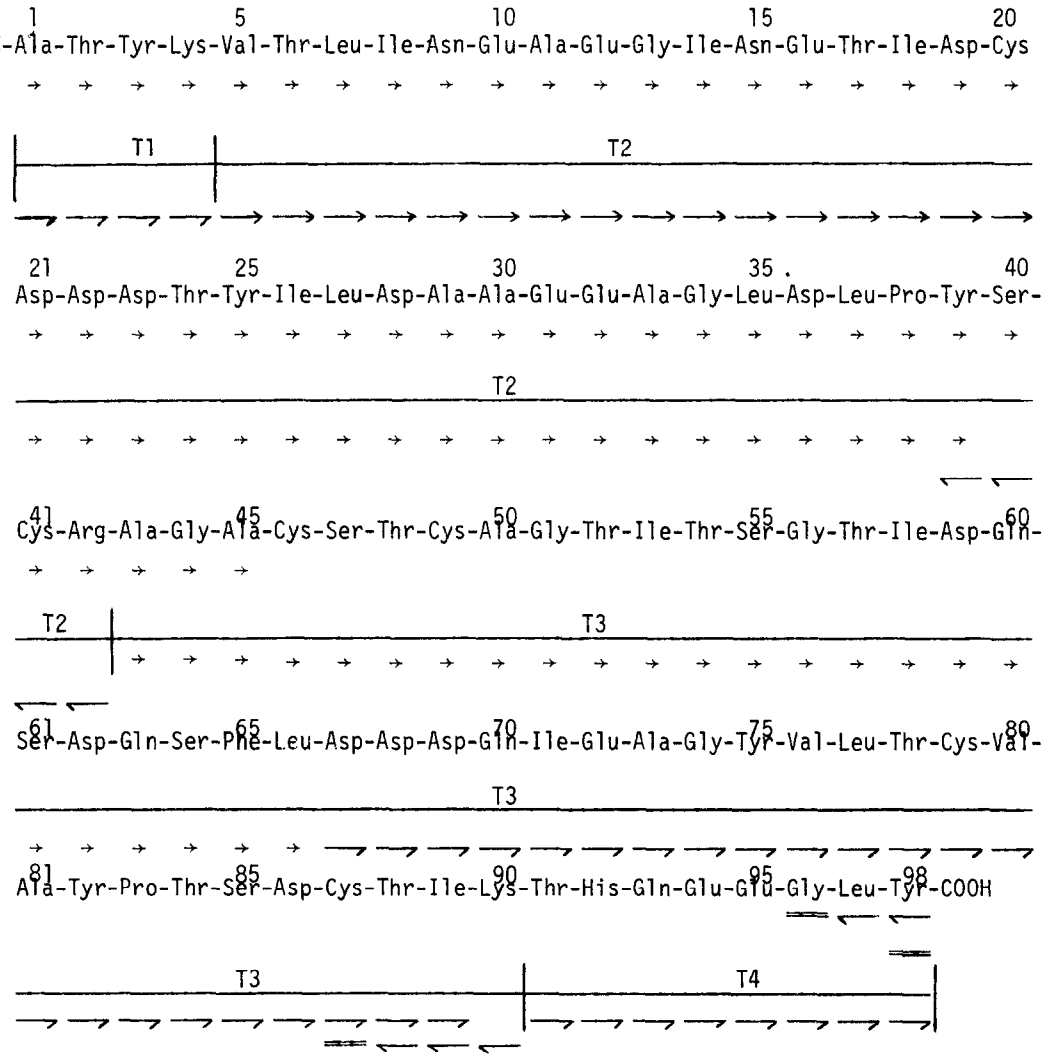

Figure 1: Reconstructrion of the complete amino acid sequence of Spirulina platensis ferredoxin and sequence data of peptide fragments. In the figure, the symbols → , ⟶, ⟵ , and ⟸ represent sequences determined by use of the Beckman-Spinco Model 890 Protein/Peptide Sequencer, direct manual Edman degradation, carboxypeptidase B and/or A digestion, and hydrazinolysis experiments, respectively.

hydrazinolysis of the digest yielded S-β-carboxymethylcysteine in 27% was quantitatively determined in the sample by direct amino acid analysis and the recovery of tyrosine was 49%. The results obtained are summarized in Figure 1.

Complete sequence. The Protein Sequencer run of the Spirulina platensis ferredoxin showed that peptide T1 was the NH_2-terminal peptide and that it was followed by peptide T2 and then by peptide T3, in this order. Peptide T4 is the COOH-terminal peptide from the carboxyl-terminal analysis of the protein. All the sequence data used to establish the complete amino acid sequence are summarized in Figure 1.

DISCUSSION

Sequence investigations of the ferredoxins are in progress in several laboratories with the goal of obtaining structure-function and evolutionary data since these Fe-S proteins are thought to be ancient relics. In the case of the S. platensis ferredoxin, two different approaches for the amino acid sequence determination were simultaneously and independently carried out by both Wada et al. (1) and our laboratory, and both the groups arrived at nearly the same sequence. However, there was one difference noted in the sequence. Our sequence study showed that amino acid residue 9 is Asn rather than Asp. Our thin layer chromatographic analysis of residue 9 clearly showed that it is Asn. In addition, Wada et al. did not determine the sequence of residues 86 and 87 but assumed these residues were constant in the various algal-plant ferredoxins. This assumption was shown to be correct by our complete sequence investigations of the S. platensis ferredoxin[2].

In a previous report from our laboratory (2), a modification of the automated sequence determination was described. This technique again was used in the sequence determination of the S. platensis ferredoxin and was found to be a suitable modification for the sequence determination of peptide T3.

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[2] The studies reported here were completed in September 1975. At a meeting in Dec. 1975, Matsubara indicated that residue 9 has been corrected to Asn. Thus, the sequence determination by the two groups is now in complete agreement. However, it is important to have published verification of the sequence, especially when different experimental approaches have been used.

The amino acid sequence of the S. platensis ferredoxin shows considerable homology with the S. maxima ferredoxin. Only four differences were noted in positions 9, 52, 57, and 90. The sequences of the other plant and algal ferredoxins have been compared and discussed in a previous report from our laboratory (9) and need not be discussed here.

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