AMINO ACID SEQUENCE OF THE SPIRULINA MAXIMA FERREDOXIN, A FERREDOXIN FROM A PROCARYOTE

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SUMMARY The amino acid sequence of the Spirulina maxima ferredoxin has been determined. Spirulina maxima is a blue green algae and is a procaryote. The ferredoxins of the plant-algal type sequenced to date have all been isolated from eucaryotes. The S. maxima ferredoxin was composed of 98 amino acids arranged in a single polypeptide chain. The sequences of the various procaryote-eucaryote ferredoxins are compared and the differences discussed.

The ferredoxins are classified into three types, i.e. the bacterial type, the photosynthetic bacterial type and the plant-algal type (1,2). Ferredoxins of the plant-algal type sequenced to date are from spinach (3), alfalfa (4), Leucaena glauca (5), taro (1) and Scenedesmus (6). These ferredoxins contain 96-97 amino acids and have molecular weights of about 10,000-11,000 and contain 2 g atoms of iron and 2 moles of sulfide per mole of protein. Four cysteine residues are thought to be chelated to the iron and these cysteine residues are present at positions 39,44,47 and 77. For the first time, the amino acid sequence of procaryote ferredoxin from a blue green algae has been determined and the sequence and a brief account of the research which led to the determination of the structure is presented here.

EXPERIMENTAL PROCEDURES

Spirulina maxima ferredoxin- The methods used for culturing the algae and the isolation of the ferredoxin have been reported previously

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(7). The Cys(Cm)-ferredoxin was prepared by the method reported by Crestfield et al (8).

Tryptic digestion- About 2.5 μmoles of the Cys(Cm)-ferredoxin were hydrolyzed with 5% trypsin for 22 hours at 28°.

Isolation of the tryptic peptides- The tryptic digest mentioned above was fractionated on a Sephadex G-50 column (1.9 x 55 cm). The peak fractions were collected, pooled and concentrated. When necessary, the peptides were further purified by paper chromatography.

End group analyses- NH_2-terminal end group analysis of the protein and peptides were determined by the manual Edman degradation procedure (9). The carboxyl terminal amino acid analyses were performed by hydrazinolysis (10) or by carboxypeptidase (11).

Sequence determinations- The manual Edman degradation or the automated Edman degradation (12) were used to sequence the protein and peptides (100 to 350 nmoles). The Beckman Model 890 Protein Sequencer under the control of the protein double cleavage program was used for the latter procedure.

Amino acid analyses- The Beckman Model 120C automatic amino acid analyzer was used to determine the amino acid composition of the protein and peptides (13).

RESULTS

Purity and amino acid composition of the S. maxima ferredoxin- The purity of the Cys(Cm)-ferredoxin was ascertained by NH_2- and COOH-terminal analyses. The Edman degradation yielded phenylthiohydantoin of alanine in 100% yield. No other end groups were detected. COOH-terminal analysis of the derivative by carboxypeptidase A showed that tyrosine was the carboxyl-terminal amino acid (100% yield). With the assurance that the ferredoxin was pure, the amino acid composition of the ferredoxin was determined and the data is shown in Table I.

Protein Sequencer run- About 225 nmoles of the Cys(Cm)-ferredoxin

TABLE I

Amino Acid Composition of Spirulina max Ferredoxin

Amino Acid	From Acid Hydrolysates[a]	From the Sequence
Lysine	2.00 (2)	2
Histidine	0.94 (1)	1
Arginine	1.03 (1)	1
Carboxymethylcysteine	5.83 (6)	6
Aspartic Acid	12.98 (13)	13[b]
Threonine	9.70 (10)	10
Serine	7.56 (8)	8
Glutamic Acid	13.07 (13)	13[c]
Proline	1.94 (2)	2
Glycine	6.97 (7)	7
Alanine	10.07 (10)	10
Valine	2.98 (3)	3
Isoleucine	7.80 (8)	8
Leucine	7.03 (7)	7
Tyrosine	5.83 (6)	6
Phenylalanine	1.05 (1)	1
Total Residues	98	98

[a] Acid hydrolyses were carried out on carboxymethyl-ferredoxin for 24,48 and 72 hours at 110° with 6 N HCl. The amino acid residues were calculated on the basis of a lysine content of 2.00 mol/mol of protein. The values of threonine and serine were obtained by extrapolating to zero time. The values of valine, isoleucine and leucine were the maximun values (72 hours). Numbers in parenthes es indicate values rounded off to nearest whole number.

[b] Sum of twelve aspartic acids and one asparagine.

[c] Sum of eight glutamic acids and five glutamines.

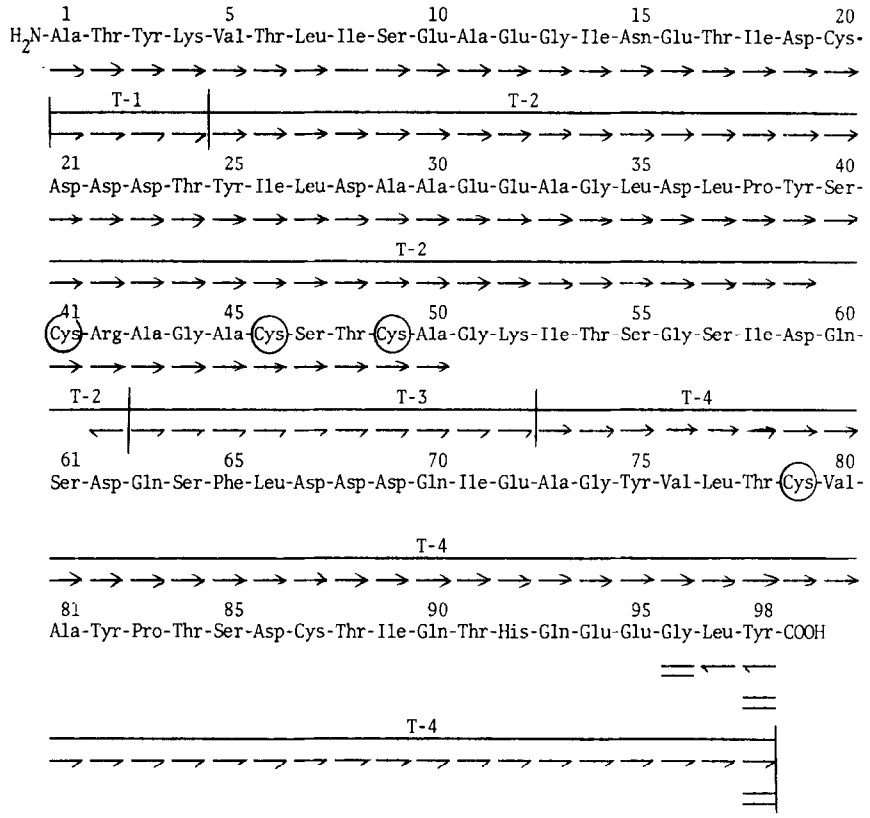

Figure 1. Reconstruction of the complete amino acid sequence of Spirulina maxima ferredoxin and sequence data of peptide fragments. In the figure, the symbols →, ⟶, ←, ⟸ represent sequences determined by use of the Beckman Sequencer, direct manual Edman degradation, carboxypeptidase A or B, and hydrazinolysis experiments, respectively.

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was analyzed in the Protein Sequencer. As shown in Fig. 1, it was possible to determine the first 50 amino acid residues. The average repetitive yield of Pth-alanine was 95% and the recovery of Pth-alanine at the fiftieth step was about 10% and therefore, all the amino acids were clearly identifiable.

Tryptic peptides- The amino acid composition of the various tryptic peptides isolated and summarized in Table II as well as their properties and methods used to purify the peptides. The sequence of these peptides were determined as shown in Fig. 1.

TABLE II

Amino Acid Composition[a] and Properties of Tryptic Peptides of Carboxymethyl-ferredoxin.

Amino Acid	T-1	T-2	T-3	T-4	Total Residues
Lysine	0.95 (1)		0.96 (1)		2
Histidine				0.86 (1)	1
Arginine		0.85 (1)			1
Carboxymethylcysteine		1.97 (2)	1.84 (2)	1.88 (2)	6
Aspartic Acid		7.04 (7)		5.99 (6)	13
Threonine	0.88 (1)	2.84 (3)	0.92 (1)	4.92 (5)	10
Serine		1.77 (2)	0.86 (1)	4.75 (5)	8
Glutamic Acid		5.06 (5)		8.10 (8)	13
Proline		0.97 (1)		0.90 (1)	2
Glycine		1.99 (2)	1.94 (2)	2.93 (3)	7
Alanine	1.00 (1)	4.00 (4)	3.00 (3)	2.00 (2)	10
Valine		1.05 (1)		1.98 (2)	3
Isoleucine		4.06 (4)		4.09 (4)	8
Leucine		4.04 (4)		3.10 (3)	7
Tyrosine	0.94 (1)	1.98 (2)		2.87 (3)	6
Phenylalanine				0.99 (1)	1
Total Residues	4	38	10	46	98
Recovery (%)	74	71	86	71	
R_F in BPAW[b]	0.45	0.00	0.20	0.41-0.69	
R_F in PIN[b]	0.63	0.23	0.41	0.33	
Color Reaction with Ninhydrin	Purple	Grayish Violet	Violet	Purple	
Pauly Reaction	Orange Brown	Brown		Reddish Brown	
Purification Method[b]	BPAW	BPAW PIN	BPAW	BPAW PIN	

[a]Results from 6 N HCl hydrolyses for 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: BPAW, paper chromatography in the solvent system, 1-butanol/pyridine/acetic acid/water (60:40:12:48, v/v); and PIN, paper chromatography with pyridine/isoamyl alcohol/0.1 N ammonium hydroxide (60:30:50,v/v).

Reconstruction of the amino acid sequence- The Protein Sequencer run plus the tryptic peptide data provided overlapping sequences as shown in Fig. 1. The Protein Sequencer run showed that peptide T-1 was the NH_2-terminal peptide and was followed by peptide T-2. Peptide T-3 was next in order. Since peptide T-4 contained all of the residues from this point to the C-terminal amino acid, the sequence of the S. maxima ferredoxin was clearly established.

DISCUSSION

The S. maxima ferredoxin contained 98 amino acids as compared to the 96-97 amino acids found in the other ferredoxins of the plant-algal type. The S-β-carboxymethylcysteine derivative turned out to be especially suitable for sequence determination in the automated instrument and 50 of the 98 amino acid sequences were determined in this way. In general, one is struck more by the similarities in sequence of the various plant-algal ferredoxins but some important differences were noted. All of the plant ferredoxins contain a tryptophan residue (residue in Fig. 2) but neither of the two algal ferredoxins, i.e. the S. maxima and Scenedesmus ferredoxin, contained tryptophan. When the sequences are aligned as shown in Fig. 2, the possibility of an insertion of a glutamate at position 12 and an isoleucine residue at position 14 in the S. maxima ferredoxin or a deletion of these residues in the other ferredoxins was vital. A deletion of an alanine at the C-terminal of the S. maxima and Scenedesmus ferredoxins or an insertion of an alanine residue in the plant ferredoxins was evident. A cysteine residue is present at residue 87 in the two algal ferredoxins (Fig. 2) and is absent in the other ferredoxins. The iron binding cysteine residues are constant in all of the plant and algal ferredoxins and are thought to be the cysteine residues 41, 46, 49 and 79 (Fig. 2).

The blue green algae (Cyanophyta) are very primitive and are thought to have evolved in the Archeozac Era some 3 billion years ago. Scenedesmus

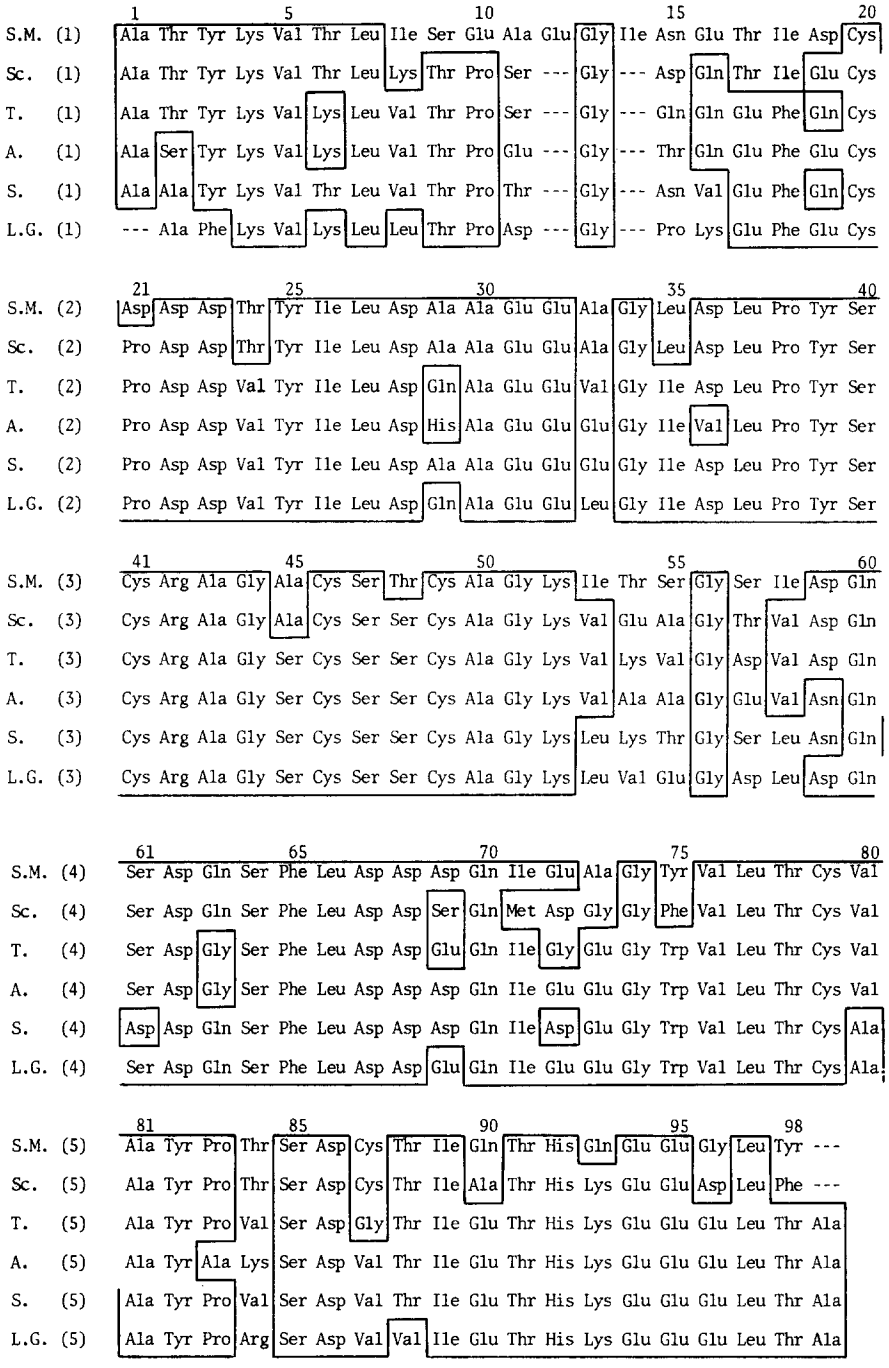

Figure 2. Comparison of the amino acid sequences of various plant and algal ferredoxins. The abbreviations used are: S.M., Spirulina maxima; Sc., Scenedesmus; T., Taro; A., Alfalfa; S., Spinach; and L.C., Leucaena glauca.

		1 5 10 15 20
Spirulina maxima Fd	(1)	Ala-Thr-Tyr-Lys-Val-Thr-Leu-Ile-Ser-Glu-Ala-Glu-Gly-Ile-Asn-Glu-Thr-Ile-Asp-Cys-Asp-Asp-
Aphanothece Fd	(1)	Ala-Xxx-Tyr-Lys-Val-Thr-Leu-Xxx-Thr-Pro-Asp- -Gly- -Asp-Gln-Val-Ile(Thr)Val-Xxx-Asp-Asp-
Scenedesmus Fd	(1)	Ala-Thr-Tyr-Lys-Val-Thr-Leu-Lys-Thr-Pro-Ser- -Gly- -Asp-Gln-Thr-Ile-Glu-Cys-Pro-Asp-Asp-

		25 30 35 39 97 98
Spirulina maxima Fd	(2)	Thr-Tyr-Ile-Leu-Asp-Ala-Ala-Glu-Glu-Ala-Gly-Leu-Asp-Leu-Pro-Tyr----------Leu-Tyr
Aphanothece Fd	(2)	Xxx-Tyr-Ile-Leu-Asp-Xxx-Xxx-Glu-Glu-Glu-Xxx-Leu-Xxx-L/I-Xxx(Tyr)----------Leu-Tyr
Scenedesmus Fd	(2)	Thr-Tyr-Ile-Leu-Asp-Ala-Ala-Gln-Glu-Ala-Gly-Leu-Asp-Leu-Pro-Tyr----------Leu-Phe

Figure 3. NH_2-terminal sequences of the various algal ferredoxins which have been sequenced.

(Chlorophyta) is thought to have evolved in the Proterozoic Era some 2 billion years ago. According to Tilden (14), the procaryotes and the eucaryotes were in existence in the Paleozoic Era. According to Craig (15), the blue green algi underwent endosymbiotic relationship with the primitive plant cell precursor and the former became the chloroplast in the present day cell. A comparison of the sequence differences between the S. maxima and Scenedesmus show 25 sequence differences. Taking 2 billion years ago as the time that the Scenedesmus evolved, it can be calculated that one amino acid replacement took 80 million years. This value for the hemoglobin family was calculated to be about 7.3 million years (16) and the value for protamine was 75 million years ago (17). The value for cytochrome c is intermediate (17). These values are very rough estimates because such factors as back mutation, fatal mutations, etc. are not taken into account. However, the rough values show that the rate of amino acid replacements is quite slow.

The partial amino acid sequence of the Aphanothece sacrum ferredoxin has been reported by Wada et al (18) and is shown in Fig. 3. The Spirulina maxima ferredoxin sequence is more like that of the Scenesdesmus ferredoxin than the A. sacrun ferredoxin although the latter organism is also a blue-green algi. Either the ferredoxins are not suitable evolutionary markers because of rapid mutation rates or as discussed

previously, because of the multiple ferredoxin genes shown to be present in some plants (19). It is possible that different ferredoxin genes are being compared in the algae and plants. Additional ferredoxins of the plant-algi class must be sequenced to clarify the situation.

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