AMINO ACID SEQUENCE OF SPIRULINA PLATENSIS FERREDOXIN: A FAR DIVERGENCY OF BLUE-GREEN ALGAL FERREDOXINS

Keishiro WADA, Toshiharu HASE, Hiroko TOKUNAGA and Hiroshi MATSUBARA
Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka, Japan

Received 2 May 1975

1. Introduction

Blue-green algae are prokaryotes and occupy an important position in the evolutionary process to eukaryotes. The comparison of ferredoxin structure of blue-green algae with those of photosynthetic bacteria and green plants should give an insight into the evolution of photosynthesis and related metabolic systems. The amino acid sequences of five chloroplast-type ferredoxins have so far been established [1–5], but the sequence of blue-green algal ferredoxin has not been reported except for the study of *Aphanothece sacrum* ferredoxin [6]. The study has strongly suggested the commonness of the blue-green algal ferredoxin and those of green plants including green algae. Several other blue-green algal ferredoxins have been shown to have characteristics of the typical chloroplast-type ferredoxin [7–12] and their amino acid compositions have suggested the common origin of these and green plant ferredoxins.

Recently we have found that *Spirulina platensis* is an excellent source to purify and study ferredoxin, and its properties are those of the chloroplast-type as reported for *Spirulina maxima* ferredoxin [10]. The present paper describes the amino acid sequence of *Spirulina platensis* ferredoxin and it is compared with those of the green plant and *Aphanothece sacrum* ferredoxin.

2. Materials and methods

Spirulina platensis cells were grown as described [13] and the cells were treated with 80% of chilled acetone. The ferredoxin was extracted with a dilute Tris–HCl buffer and purified essentially as described [6,14]. The preparation had an absorbance ratio, A_{422}/A_{275}, of 0.53. The contents of non-heme iron and sulfur atoms were two each per protein molecule.

The major procedure of the sequence study is composed of the use of a sequence analyzer both on the *S*-pyridylethyl (PE) cysteinyl-ferredoxin [15] and on the tryptic digest of succinylated PE-ferredoxin, and the uses of manual Edman degradations, leucine aminopeptidase, and carboxypeptidases on the peptide fragments purified from tryptic, chymotryptic and thermolysin digests of PE-ferredoxin. The detailed procedures for Edman degradations, purification of the peptides on paper and other sequence studies were described in the previous papers [3,6].

3. Results and discussions

The amino acid composition of *Spirulina platensis* ferredoxin was Lys_2, His_1, Arg_1, Asx_{14}, Thr_{12}, Ser_6, Glx_{12}, Pro_2, Gly_7, Ala_{10}, Cys_6, Val_3, Ile_8, Leu_7, Tyr_6, and Phe_1, giving a mol. wt of 10 890 with iron and sulfur atoms. Fig. 1 shows the summary of the sequence studies of this ferredoxin. The sequence analyze established the N-terminal sequence of 40 residues confirmed by the studies on various small peptides. The C-terminal sequence was Leu-Tyr revealed by carboxypeptidase A digestion. Carboxypeptidase B applied on the tryptic digest of succinylated PE-ferredoxin released arginine and PE-cysteine successively. This revealed the C-terminal sequence of N-terminal half fragment ending at residue 42. The sequence analyzer

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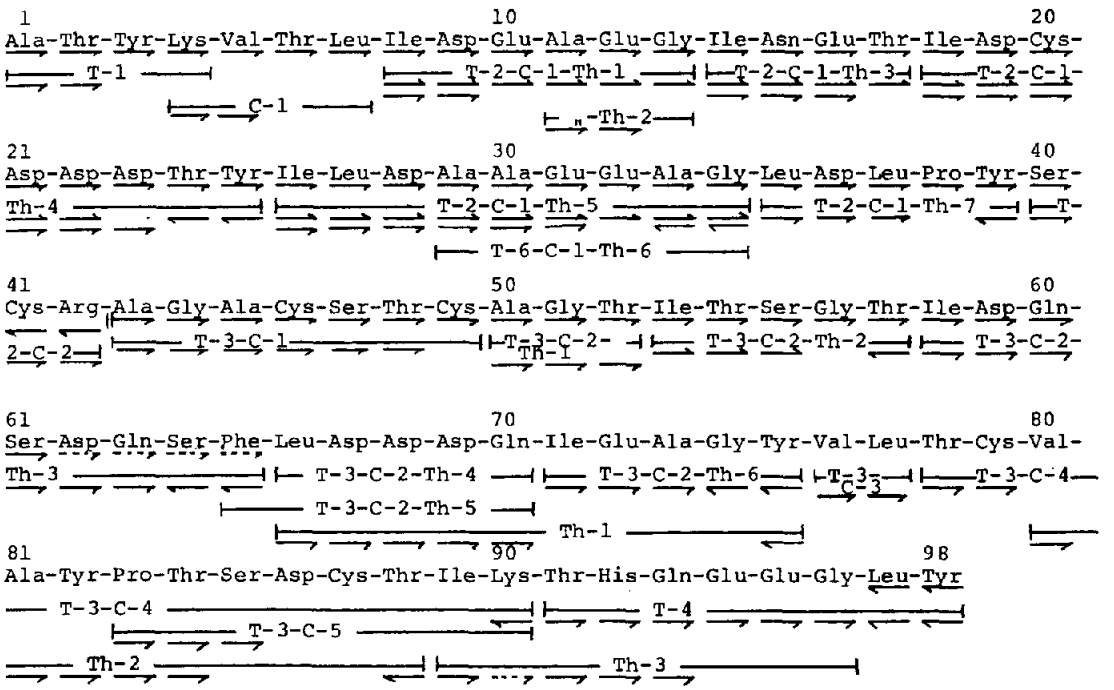

Fig.1. Amino acid sequence of *Spirulina platensis* ferredoxin. Each step of the Edman degradation on PE-ferredoxin by a sequence analyzer is shown by ⟶ just below the sequence. A double vertical line at residue 42 indicates the start of a new Edman degradation on a tryptic digest of succinylated PE-ferredoxin. The dotted arrows indicate uncertain identifications by the sequence analyzer. Peptides derived by trypsin (T), chymotrypsin (C) and thermolysin (Th) digestion are shown below the sequence ⟶ indicates the digestion of peptides by leucine aminopeptidase, ⟶ the manual Edman degradation, and ⟵ the degestion by carboxypeptidase A or B.

applied on this tryptic digest established the N-terminal sequence up to 19 residues of the C-terminal half fragment of ferredoxin. This sequence and that of the rest in the C-terminal half fragment were independently studied by various combinations of the conventional procedures on tryptic and thermolysin peptides. The sequence at residues 86 and 87 could not be completed, but a preliminary study and homologous sequences in other ferredoxins suggest strongly the sequence to be Asp–Cys as shown in fig. 2, in which the sequence of *Aphanothece sacrum* ferredoxin is included (T. Hase, K. Wada and H. Matsubara, unpublished result). The total number of residues was 98 two residues excess

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    1                   10                  20
(A) - A F K V K L L T - P D G - P K E F E C P D D V Y
(B) A A Y K V T L V T - P T G - N V E F Q C P D D V Y
(C) A S Y K V K L V T - P E G - T Q E F E C P D D V Y
(D) A T Y K V K L V T - P S G - Q Q Q F Q C P D D V Y
(E) A T Y K V T L K T - P S G - D Q T I E C P D D T Y
(F) A S Y K V T L K T - P D G - D N V I T V P D D E Y
(G) A T Y K V T L I D E A E G I N E T I D C D D D T Y
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    26          30                  40                  50
(A) I L D Q A E E L G I D L P Y S C R A G S C S S C A
(B) I L D A A E E E G I D L P Y S C R A G S C S S C A
(C) I L D H A E E E G I V L P Y S C R A G S C S S C A
(D) I L D Q A E E V G I D L P Y S C R A G S C S S C A
(E) I L D A A E E A G L D L P Y S C R A G A C S S C A
(F) I L D V A E E E G L D L P Y S C R A G A C S T C A
(G) I L D A A E E A G L D L P Y S C R A G A C S T C A

    51                  60                  70
(A) G K L V E G D L D Q S D Q S F L D D E Q I E E G W
(B) G K L K T G S L N Q D D Q S F L D D D Q I D E G W
(C) G K V A A G E V N Q S D G S F L D D D Q I E E G W
(D) G K V K V G D V D Q S D G S F L D D E Q I G E G W
(E) G K V E A G T V D Q S D Q S F L D D S Q M D G G F
(F) G K L V S G P A P(B,B,Z,Z)S F L D D N Q I Q A G Y
(G) G T I T S G T I D Q S D Q S F L D D D Q I E A G Y

    76          80                  90                  99
(A) V L T C A A Y P R S D V V I E T H K E E E L T G
(B) V L T C A A Y P V S D V T I E T H K E E E L T A
(C) V L T C V A Y A K S D V T I E T H K E E E L T A
(D) V L T C V A Y P V S D G T I E T H K E E E L T A
(E) V L T C V A Y P T S D C T I A T H K E E D L F -
(F) I L T C V A Y P T G D C V I E T H K E E A L Y -
(G) V L T C V A Y P T S D C T I K T H Q E E G L Y -
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Fig.2. Comparison of the amino acid sequences of chloroplast-type ferredoxins. (A) *L. glauca* [1], (B) Spinach [2], (C) Alfalfa [3], (D) Taro [4], (E) *Scenedesmus* [5], (F) *Aphanothece* (Hase, T., Wada, K. and Matsubara, H. unpublished result) and (G) *Spirulina platensis*, present paper.

compared to other algal ferredoxins. Two gaps were introduced in other ferredoxins to make the alignment most probable (fig. 2). Four indispensable cysteine residues are at positions 41, 46, 49 and 79 as in other ferredoxins [6, 16]. It is interesting to note that cysteine-20 is common to the higher plant ferredoxins and cysteine-87 to the algal ferredoxins so far studied. This fact might be an expression of the evolutionary event occurred between primitive plants including blue-green algae and higher plants. A preliminary calculation to construct a phylogenetic tree of ferredoxins suggests that higher plant ferredoxins are highly homologous to one another but not to algal ferredoxins. *Spirulina* ferredoxin is rather similar to *Scenedesmus* ferredoxin than to *Aphanothece* ferredoxin. *Spirulina* ferredoxin is thus far divergent not only from higher plant ferredoxins but from another blue-green algal ferredoxin.

A minor component of ferredoxin has recently been isolated from *Aphanothece sacrum*, and its amino acid composition and terminal sequences are shown to be quite different from those of the major one just as described above [14]. It is clear from these data that the blue-green algal ferredoxins are in a very divergent group and this divergency must be ascribed to the very early appearance of these algae in the evolutionary process of life.

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