THE AMINO ACID SEQUENCE OF *SCENEDESMUS* FERREDOXIN*

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The amino acid sequences of certain ferredoxins isolated from bacterial species (Tanaka et al, 1964, 1966; Benson et al, 1966, 1967; Tsunoda et al, 1967) and higher plants, such as spinach (Matsubara et al, 1967, 1968) and alfalfa (Keresztes-Nagy et al, 1968) have been established. Comparisons of the amino acid sequences of ferredoxins from different species are of interest from the standpoint of structural, functional, and evolutionary relationships.

We have studied the primary structure of ferredoxin from a green alga, *Scenedesmus*. In earlier report (Matsubara, 1968), the molecular weight of *Scenedesmus* ferredoxin was determined to be 11,500 and its absorption spectrum was found to be very similar to those of the typical plant ferredoxins (Arnon, 1965; San Pietro and Black, 1965; Keresztes-Nagy and Margoliash, 1966). Two atoms of iron and two moles of inorganic sulfide were found per mole of *Scenedesmus* ferredoxin, as in all other well-characterized chloroplast-type ferredoxins (Arnon, 1965; San Pietro and Black, 1965; Keresztes-Nagy and Margoliash, 1966).

The ferredoxins of spinach, Swiss chard, and *Scenedesmus* were found to have nearly identical activities when assayed by the photoreduction of TPN with illuminated spinach chloroplasts. The total number of amino acid residues was 96, which is one less than those of higher plant ferredoxins. The amino- and

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carboxyl-terminal sequences were Ala-Thr-Tyr-Lys-Val-Thr-Leu-Lys-Thr-Pro and Leu-Phe, respectively. This article reports the complete amino acid sequence of Scenedesmus ferredoxin.

MATERIALS AND METHODS

Scenedesmus ferredoxin was prepared as described elsewhere (Matsubara, 1968). These preparations had A_{420}/A_{276} = 0.62 to 0.65.

Preparation of S-carboxymethylcysteinylferredoxin (CMCFd) and oxidized ferredoxin (OFd), hydrolysis of the protein by trypsin and chymotrypsin, purification of the peptide fragments, and determination of the seuquences were carried out as reported previously (Matsubara et al, 1968). About 13 μ moles of CMCFd were hydrolyzed with trypsin, and about 10 μ moles of CMCFd with chymotrypsin. The peptide fragments were purified by ion exchange chromatography, and when necessary by paper chromatography or paper electrophoresis. Some of the peptides were further hydrolyzed with thermolysin or subtilisin. Sequence determinations were carried out on these peptides. Cyanogen bromide cleavage of OFd was performed in formic acid essentially as described by Steers, Craven and Anfinsen (1965). About 2 μ moles of OFd were cleaved with cyanogen bromide, and the peptides were separated by paper chromatography.

RESULTS AND DISCUSSION

Two large peptides, one from residue 1 to 69 and the other from residue 70 to 96, were obtained by cyanogen bromide cleavage. These overlapping peptides and the sequence data derived from tryptic and chymotryptic peptides revealed the complete amino acid sequence of Scenedesmus ferredoxin as shown in Figure 1. The distribution of amide residues previously reported (Matsubara and Sugeno, 1968) is revised in this figure.

The amino acid composition deduced from the sequence is as follows: $Lys_4 His_1 Arg_1 Asp_{12} Thr_{10} Ser_8 Glu_6 Gln_4 Pro_4 Gly_7 Ala_{10} Cys_6 Val_5 Met_1 Ile_3 Leu_7 Tyr_4 Phe_3$. This value is in good agreement with that of direct amino acid analysis of intact Scenedesmus ferredoxin (Matsubara, 1968), except for the number of amide groups. There are four glutamine residues and no asparagine in the

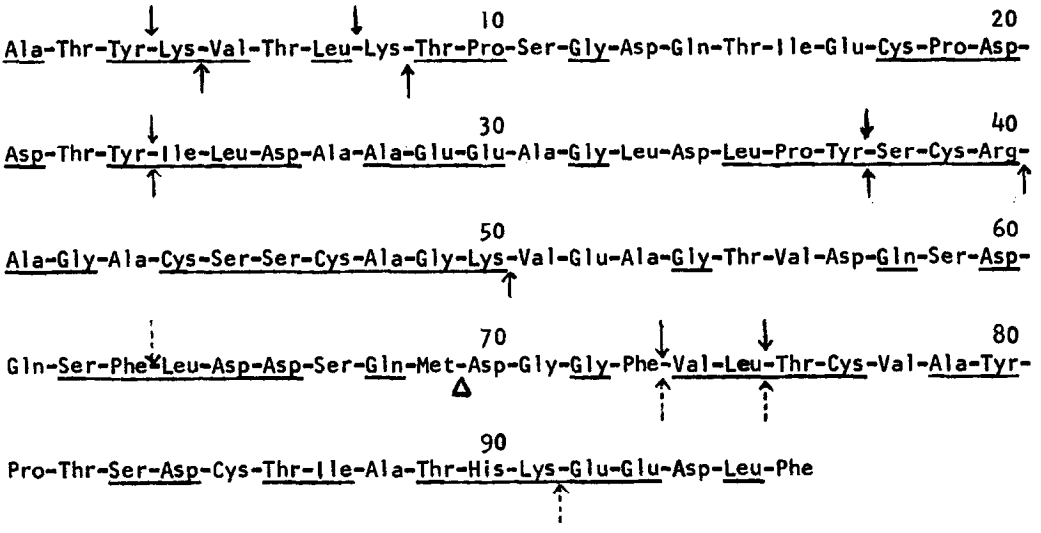

Figure 1. The amino acid sequence of Scenedesmus ferredoxin.

The peptide bonds cleaved by trypsin in S-carboxymethylcysteinyl-ferredoxin are shown by upward arrows, and those by chymotrypsin by downward arrows. Dotted-line arrows represent the positions relatively less susceptible to hydrolysis. The peptide bond cleaved by cyanogen bromide in oxidized ferredoxin is shown by a triangle. The residues common to Scenedesmus, spinach and alfalfa ferredoxins are underlined.

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sequence. The total of 96 amino acids corresponds to a molecular weight of 10,172, not including iron and inorganic sulfide.

The sequence differs in 29 positions from that of spinach ferredoxin, 19 of which can be explained by one base change in their respective codons. The average minimum base difference per codon between the two ferredoxins is 0.41, between ferredoxins of Scenedesmus and alfalfa is 0.43, and between those of spinach and alfalfa is 0.27. The similarity between the ferredoxins of Scenedesmus and those of two higher plants is quite striking and they are clearly homologous to each other, but Scenedesmus ferredoxin differs more from the other two than they do from each other, as might be expected from taxonomical considerations. Spinach and alfalfa, both of which are dicotyledonous angiosperms in the order Tracheophyta, are in the Chenopodiaceae and Leguminosae families, respectively, while Scenedesmus is in the green algae,

Chlorophyta. Comparisons of the amino acid sequences of bacterial and chloroplast-type ferredoxins, and evolutionary aspects have recently been described in detail by Matsubara, Jukes and Cantor (1968).

Scenedesmus ferredoxin is the first chloroplast-type ferredoxin found lacking in tryptophan and asparagine, and containing methionine (Keresztes-Nagy and Margoliash, 1966; Sasaki and Matsubara, 1967; Matsubara et al, 1967, 1968). Evidently neither tryptophan nor methionine can be responsible for electron-transfer activity.

In the three chloroplast-type ferredoxins, the positions of one histidine (residue 90), one arginine (residue 40), three of four lysines (residues 4, 50 and 91), three of four prolines (residues 10, 19 and 36), five of six cysteines (residues 18, 39, 44, 47 and 77), six of seven glycines (residues 12, 32,42,49,54 and 72), six of seven leucines (residues 7, 25, 35, 64, 75 and 95), all of four tyrosines (residues 3, 23, 37, 80), two Asp-Asp groups (residues 20-21 and 65-66), and two Glu-Glu groups (residues 29-30 and 92-93) are invariant. This fact seems to suggest the importance of these residues for the maintenance of the protein molecule for its function.

It is of interest that some of the invariant residues are located in approximately equidistant positions. For example, the cysteines at positions 18, 47, and 77, the glycines at positions 12, 42 and 72 and the leucines at positions 7, 35, 64 and 95 are approximately equidistant. There is an apparently repetitive structure in clostridial ferredoxins (Tanaka et al, 1964, 1966; Benson et al, 1966, 1967). It was first proposed (Tanaka et al, 1964, 1966) that the molecule was partially repeated for 26 consecutive residues, but recently a revised homology was proposed (Benson et al, 1967; Matsubara, Jukes and Cantor, 1968) in which the repetition was 29 sites in length. Similarities in certain regions of chloroplast-type and clostridial ferredoxins were observed (Matsubara et al, 1967, 1968) and repetitive sequences were also found in spinach ferredoxin. The equidistant distribution of some of the invariant residues in chloroplast-type ferredoxins may possibly be related to

the repetitive structure of the molecule. The status of the cysteine residue at position 85, is under investigation.

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REFERENCES

Arnon, D. I., Science, 149, 1460 (1965).

Benson, A. M., Mower, H. F. and Yasunobu, K. T., Proc. Natl. Acad. Sci., 55, 1532 (1966); Arch. Biochem. Biophys., 121, 563 (1967).

Keresztes-Nagy, S. and Margoliash, E., J. Biol. Chem., 241, 5955 (1966).

Keresztes-Nagy, S., Perini, F. and Margoliash, E., personal communication (1968).

Matsubara, H., J. Biol. Chem., 243, 370 (1968).

Matsubara, H., Jukes, T. H. and Cantor, C. R., Brookhaven Symposia in Biology, Structure, Function and Evolution in Proteins, No. 21 (1968), in press.

Matsubara, H., Sasaki, R. M. and Chain, R. K., Proc. Natl. Acad. Sci., 57, 439 (1967); J. Biol. Chem., 243, 1725 (1968); Matsubara, H. and Sasaki, R. M., J. Biol. Chem., 243, 1732 (1968).

Matsubara, H. and Sugeno, K., Fed. Proc., 27, 392 (1968).

San Pietro, A. and Black, C. C., Annu. Rev. Plant Physiol., 16, 155 (1965).

Sasaki, R. M. and Matsubara, H., Biochem. Biophys. Res. Commun., 28, 467 (1967).

Steers, E., Jr., Craven, G. R. and Anfinsen, C. B., J. Biol. Chem., 240, 2478 (1965).

Tanaka, M., Nakashima, T., Benson, A., Mower, H. F. and Yasunobu, K. T., Biochem. Biophys. Res. Commun., 16, 422 (1964); Biochemistry, 5, 1666 (1966).

Tsunoda, J., Whiteley, H. and Yasunobu, K. T., Abstract of Pacific Slope Biochemical Conference (1967) p. 103; personal communication (1967).