THE AMINO ACID SEQUENCE OF TARO FERREDOXIN*

K. Krishna Rao** and Hiroshi Matsubara

Space Sciences Laboratory, University of California, Berkeley, Calif. 94720

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The amino acid sequence of ferredoxin from Colocasia esculenta, commonly known as taro, was determined from sequence studies of chymotryptic and thermolysin peptides. Taro ferredoxin consists of a single polypeptide chain of 97 amino acids. A comparison of structures of five chloroplast ferredoxins shows 61% identity in their sequences.

Ferredoxins are single-chain, non-heme iron proteins found in plants and many anaerobic and photosynthetic bacteria, and are involved in the electron transfer processes in these organisms(Mortenson et al, 1962; Arnon, 1965; Malkin and Rabinowitz, 1967). The amino acid sequence of plant-type ferredoxins isolated from spinach (Matsubara and Sasaki, 1968), alfalfa (Keresztes-Nagy et al, 1969), L. glauca (Benson and Yasunobu, 1969) and Scenedesmus (Sugeno and Matsubara, 1969) were reported. This paper concerns with the amino acid sequence of ferredoxin from the monocot, Hawaiian taro. Purification and some characterizations of taro ferredoxin has recently appeared (Rao, 1969).

MATERIALS AND METHODS

Taro ferredoxin was obtained as described (Rao, 1969). Oxidized ferredoxin (OFd) and S-carboxymethylcysteinylferredoxin (CMCFd) were prepared as described (Matsubara et al, 1968a). About 5 μmoles of CMCFd were digested with 1 mg of chymotrypsin at pH 8.0 and 40° for 16 hr. The reaction was terminated by adding a drop of glacial acetic acid and the solution was lyophilized. About 5 μmoles of OFd were digested with 0.5 mg of thermolysin at pH 8.0 and 40° for 3 hr. The reaction mixture was treated as for the chymotryptic digest. These digests were separately chromatographed on Dowex AG 1-X2 columns (0.9x60 cm) under the similar conditions to those for Scenedesmus ferredoxin (Sugeno and

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**Present address: Botany Department, King's College, University of London, London.

Matsubara, 1969). Further purification of peptides was carried out by paper chromatography (butanol-pyridine-acetic acid-water, 75:50:15:60, v/v) and paper electrophoresis at pH 6.5 and 3.6 as described (Matsubara and Sasaki, 1968). Amino acid analysis of peptides was performed on Beckman/Spinco analysers after hydrolysis with 6 N HCl for 20 to 24 hrs. at 105°. Some peptides containing carboxymethylcysteine (CMC) were hydrolysed in the presence of a trace of purified thioglycolic acid. Sequential degradations were performed by the modified Edman procedures (Light and Greenberg, 1965; Konigsberg and Hill, 1962; Matsubara et al, 1968a). The identification of PTH-amino acids was done by paper chromato-

Table I. Amino Acid Sequence of Chymotryptic Peptides of CMCFd

Peptide	Residue No.	Amino Acid Sequence*	yield %
C-1	1-3	Ala-Thr-Tyr	20
C-2	4-7	Lys-Val-Lys-Leu	12
C-3	4-23	Lys-Val-Lys-Leu-Val(Thr,Pro,Ser,Gly,Glx_3)Phe- C C Gln-CMC-Pro-Asp-Asp-Val-Tyr Th Th	30
C-4	24-37	Ile-Leu-Asp(Glx_2,Ala)Glu-Val-Gly-Ile-Asp-Leu-Pro-Tyr Th Th Th	50
C-5	38-73	Ser(CMC_3,Ser_3,Arg,Ala_2,Gly_2)Lys-Val-Lys-Val(Val,Gly_3, T T T Asx_5,Glx_4,Ser_2,Phe,Leu,Ile)Gly-Trp T	66
C-6	74-75	Val-Leu	35
C-7	74-97	Val-Leu(Thr,CMC)Val(Ala,Tyr,Pro,Val,Ser,Asx,Gly,Thr_2, Th Th T Ile,Glu,His)Lys-Glu-Glu-Glu-Leu-Thr-Ala Th T T	23
C-8	76-97	Thr-CMC[Val,Ala-Tyr-Pro,Val-Ser-Asp-Gly-Thr(Ile,Glx, Th Th Th Thr,His,Lys,Glx_3)] Leu-Thr-Ala Th Th	36

* Arrows above the sequence represent the establishment by the Edman degradation (→) and carboxypeptidases (←) with the parent peptides, respectively, and those below the sequence with the smaller peptides derived from the parent peptides. T,C,Th, and S represent tryptic, chymotryptic, thermolysin , and subtilisin peptides.

graphy (Edman and Sjoquist, 1956), thin-layer chromatography (Brenner et al, 1961) and direct analysis after alkaline hydrolysis (Africa and Carpenter, 1966). The

Table II. Amino Acid Sequence of Thermolysin Peptides of OFd

Peptide	Residue No.	Amino Acid Sequence*	yield %
Th-1	1-2	Ala-Thr	18
Th-2	3-4	Tyr-Lys	30
Th-3	5-6	Val-Lys	62
Th-4	8-23	Val-Thr-Pro-Ser-Gly-Gln(Glx_3,Phe,$CySO_3H$,Pro,Asp_2)Val-Tyr	30
Th-5	8-15	Val-Thr(Pro,Ser,Gly,Glx)Gln-Glu	21
Th-6	16-23	Phe-Gln($CySO_3H$,Pro,Asx_2,Val)Tyr	20
Th-7	24-30	Ile-Leu-Asp-Gln-Ala-Glu-Glu Th ⇐⇐ ⇒⇒ Th	30
Th-8	25-30	Leu-Asp-Gln(Ala,Glx_2)	9
Th-9	28-30	Ala-Glu-Glu	2
Th-10	31-32	Val-Gly	45
Th-11	33-36	Ile-Asp-Leu-Pro	10
Th-12	37-50	Tyr-Ser-$CySO_3H$(Ser_3,Arg,Ala_2,Gly_2)Lys	4
Th-13	33-50	Ile(Asp,Leu,Pro,Tyr,Ser,$CySO_3H$)Arg-Ala-Gly-Ser-$CySO_3H$-Ser-Ser-$CySO_3H$-Ala-Gly-Lys T, T, S_1, S_2, S_3, T, S_2, S_5, S_3, S_4	54
Th-14	53-55	Val-Gly-Asp	12
Th-15	56-63	Val-Asp-Gln(Ser,Asx,Gly)Ser-Phe	40
Th-16	56-62	Val-Asp-Gln-Ser-Asp-Gly-Ser	6
Th-17	64-68	Leu-Asp-Asp-Glu-Gln	25
Th-18	69-73	Ile-Gly-Glu(Gly,Trp**)	15
Th-19	74-77	Val(Leu,Thr,$CySO_3H$)	-
Th-20	75-77	(Leu,Thr,$CySO_3H$)	12
Th-21	78-81	Val(Ala,Tyr,Pro)	60
Th-22	82-94	Val(Ser,Asp,Gly,Thr,Ile,Glx_4,Thr,His,Lys)	2
Th-23	87-94	Ile-Glu(Thr,His,Lys,Glu_3)	20
Th-24	90-94	(His,Lys,Glu_3)	17
Th-25	95-97	(Leu,Thr,Ala)	25

* See the footnote in Table I.
**Ehrlich reaction was positive probably due to partial survival of tryptophan during the performic acid oxidation.

carboxyl-terminal sequence was determined by carboxypeptidase A (Fraenkel-Conrat et al, 1956) and B (Gladner and Folk, 1958). The digestion of the larger peptides with trypsin, thermolysin and subtilisin were performed as described (Matsubara and Sasaki, 1968). The peptides produced were purified on paper and their sequences were determined as mentioned above.

RESULTS AND DISCUSSION

Five steps of Edman degradation of native ferredoxin revealed the amino-terminal sequence to be Ala-Thr-Tyr-Lys-Val. The carboxyl-terminal sequence

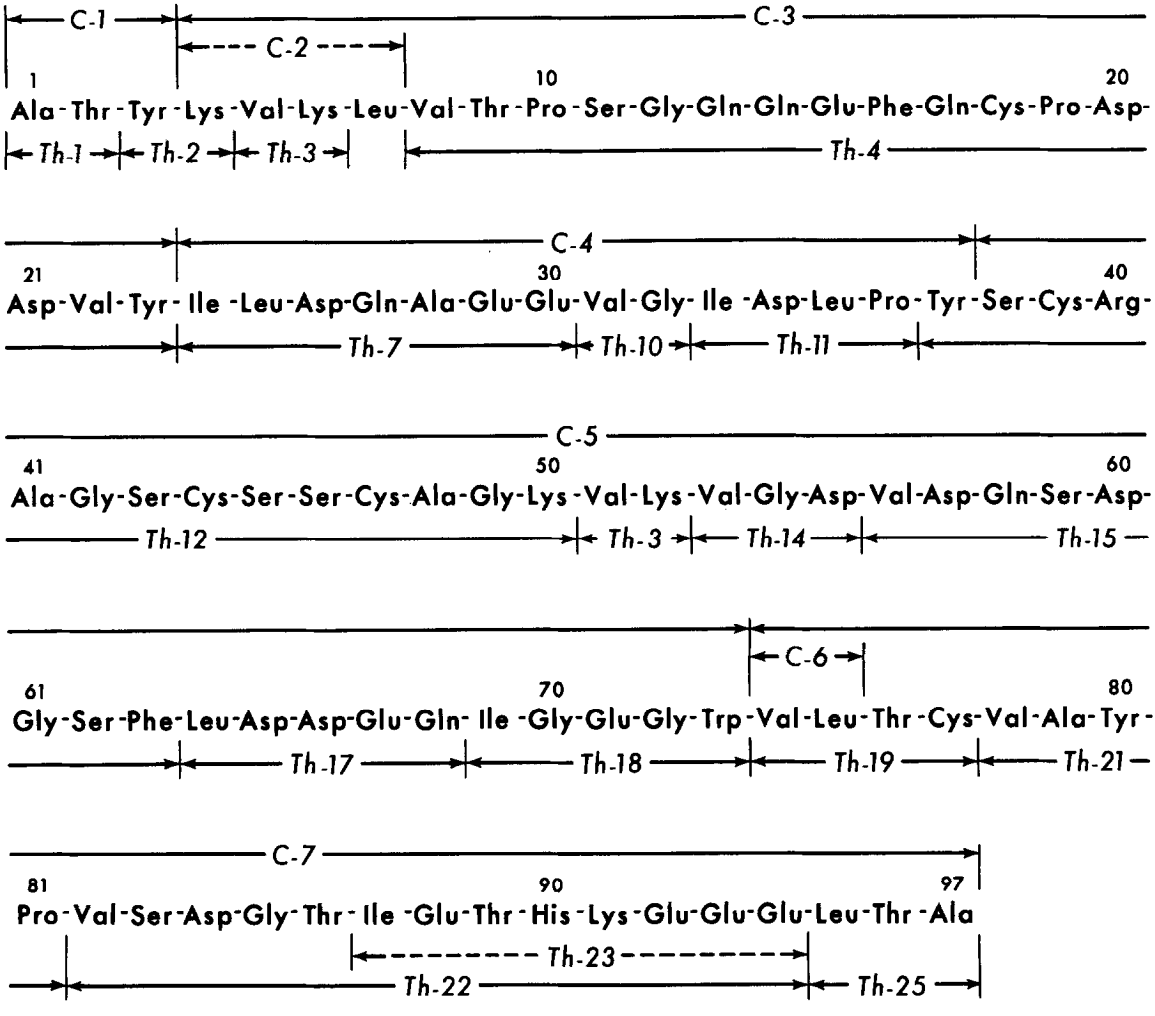


Fig. 1. The amino acid sequence of taro ferredoxin. The chymotryptic and tryptic peptides are arranged in order.

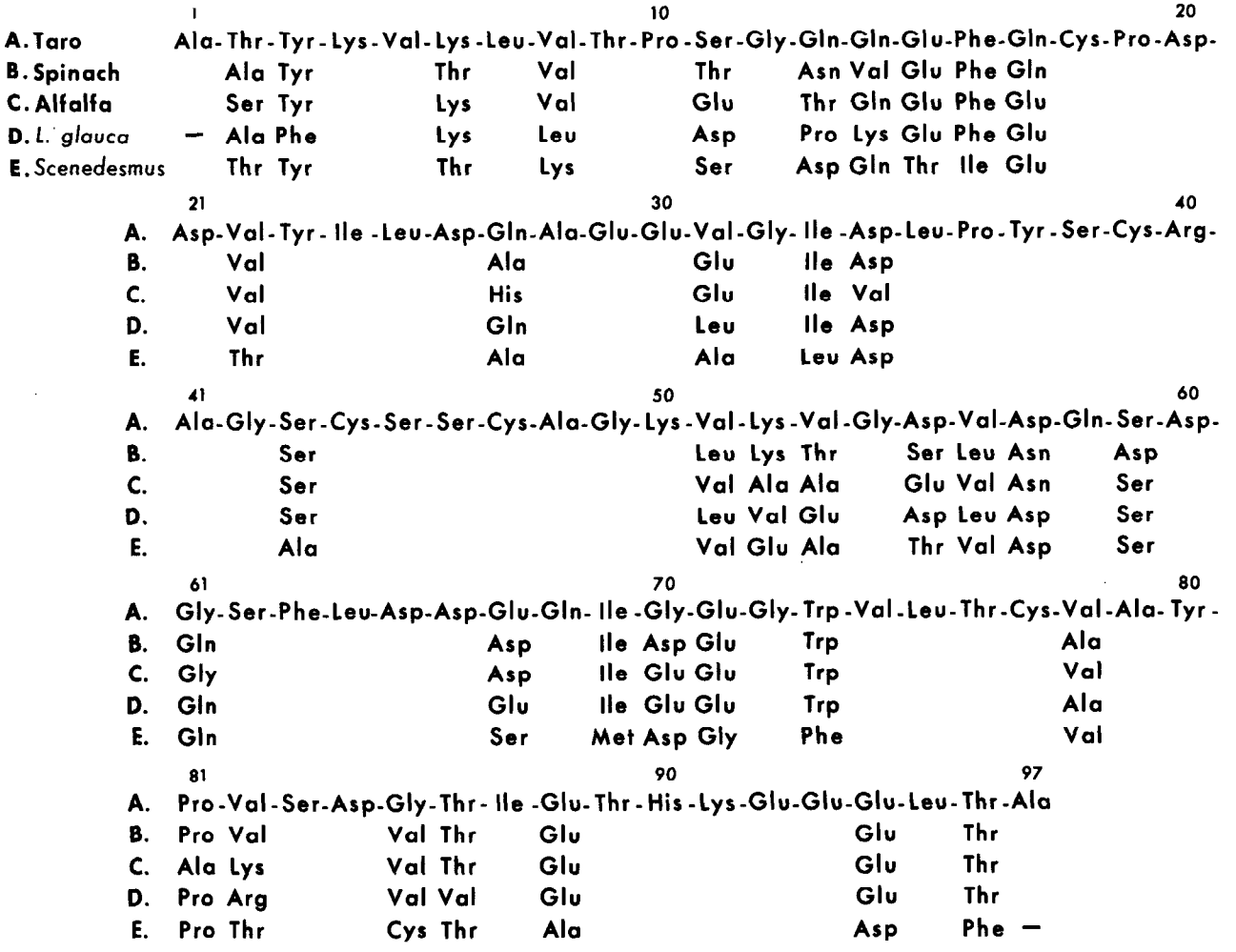
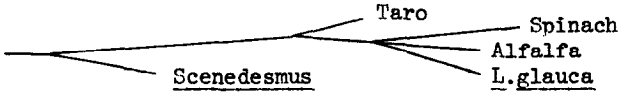


Fig. 2. The amino acid sequence of taro ferredoxin compared with those of spinach, alfalfa, L. glauca and Scenedesmus ferredoxins.

was known to be Leu-Thr-Ala (Rao, 1969). The sequence studies of chymotryptic and thermolysin peptides are summarized in Tables I and II, respectively. From these studies and the comparison with other plant ferredoxin sequences the amino acid sequence of taro ferredoxin is postulated as shown in Fig. 1. This sequence is compared with other four ferredoxin structures in Fig. 2. Taro ferredoxin resembles Scenedesmus ferredoxin in lacking asparagine. However, it resembles the ferredoxins from the dicots in possessing tryptophan and lacking methionine. The positions of all the six glycines (Residues 12, 32, 42, 49, 54, 72), all the five cysteines (Residues 18, 39, 44, 47, 77), three of the four prolines (Residues 10, 19, 36), three of the four tyrosines (Residues 23, 37, 80), three of the four lysines (Residues 4, 50, 91), and the lone residues of arginine (Residue 40) and histidine (Residue 90) found in spinach ferredoxin are invariant in the other four ferredoxins. Residues 35 to 50 are identical in all the five structures except for the alanine at Residue 43 in Scenedesmus ferredoxin. The conservation of this segment, including three cysteines, in all the chloroplast ferredoxins suggests the possiblility of the location of the functional groups in this segment of the molecule. Identical amino acid residues are found in 58 out of 95 residues in the common chain of the five ferredoxins, i.e., there is 61% identity in the structures. If we assume that the genes of plant-type ferredoxins had a common ancestor gene (Matsubara et al, 1968b) then the calculation of the minimum base differences per codon in the five ferredoxins can be fitted into an evolutionary scheme as shown below:



The angiosperms would have originated in the Mesozoic era,roughly 165 million years ago (Hutchinson, 1926; Haupt, 1953; Porter, 1959). The monocots and dicots are believed to have evolved from the Ranales order, one of the earliest fossil angiosperms. Taro belongs to the Arales group of monocotyledons, spinach belongs to the Chenopodiales group of dicots, and alfalfa, and L. glauca belong to the Rosales group of dicots. The taxonomical calssification of these plants agrees well with the evolutionary scheme proposed above.

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REFERENCES

Africa, B. and Carpenter, F. H., Biochem. Biophys. Res. Commun., 24, 113 (1966).

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

Benson, A. M. and Yasunobu, K. T., J.Biol. Chem., 244, 955 (1969).

Brenner, M. Niederwiesser, A. and Pataki, G., Experientia, 17, 145 (1961).

Edman, P. and Sjoquist, J., Acta Chem. Scand., 10, 1507 (1956).

Fraenkel-Conrat, H., Harris, J. I. and Levy, A. L., Method Biochem. Anal., 2, 397 (1956).

Gladner, J. A. and Folk, J. E., J. Biol. Chem., 231, 393 (1958).

Haupt, W. A., in Plant Morphology, McGraw-Hill Book Co. Inc., New York, p. 401 (1953)

Hutchinson, J., in The Families of Flowering Plants, Vols. 1 and 2, MacMillan and Co., London, p. 7 and 8 (1926).

Keresztes-Nagy, S., Perini, F. and Margoliash, E., J. Biol. Chem., 244, 981 (1969).

Konigsberg, W. and Hill, R. L., J. Biol. Chem., 237, 2547 (1962).

Light, A. and Greenberg, J., J. Biol. Chem., 224, 261 (1965).

Malkin, R. and Rabinowitz, J. C., Ann. Rev. Biochem., 36, 113 (1967).

Matsubara, H. and Sasaki, R. M., J. Biol. Chem., 243, 1732 (1968).

Matsubara, H., Sasaki, R. M. and Chain, R. K., J. Biol. Chem., 243, 1725(1968a).

Matsubara, H., Jukes, T. H. and Cantor, C. R., Brookhaven Symp. Biol., 21, 201 (1968b).

Mortenson, L. E., Valentine, R. C. and Carnahan, J. E., Biochem. Biophys. Res. Commun., 7, 448 (1962)

Porter, C. L., in Taxonomy of Flowering Plants, W. H. Freeman and Co., San Francisco, p. 125 (1959).

Rao, K. K., Phytochemistry, 8, 1379 (1969).

Sugeno, K. and Matsubara, H., J. Biol. Chem., 244, 2979 (1969).