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Large differences in amino acid sequences among ferredoxins from several species of genus *Solanum*☆

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Abstract

The complete amino acid sequences of [2Fe-2S] ferredoxins from four species of genus *Solanum* (*S. nigrum*, *S. lyratum*, *S. indicum*, and *S. abutiloides*) were determined by automated Edman degradation of the entire S-carboxymethylcysteinyl proteins and of the peptides obtained by enzymatic digestion. The amino acid sequences of these four ferredoxins differed from each other by 12–19, whereas 0–4 differences have been observed among ferredoxins from plants in the same genus and 14–40 differences were seen between different families. This suggests that these *Solanum* plants are distantly related to each other taxonomically.
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Keywords: *Solanum nigrum*; *Solanum lyratum*; *Solanum indicum*; *Solanum abutiloides*; Solanaceae; Amino acid sequence; Protein chemo-taxonomy; Ferredoxin

1. Introduction

There have been many examples of the use of protein characteristics in chemotaxonomy since the seminal paper by Zuckerkandl and Paulings (1965). Recently, nucleotide sequences of a chloroplast gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) were used to produce phylogenetic trees of many higher plants (Chase et al., 1993). Although comprehensive phylogenetic studies of *rbcL* sequences have also been reported, only limited data are available concerning solanaceous plants, which are of great importance to humans. While the Solanaceae family is one of the best-studied groups of plants, there are still many unsolved questions concerning the relations among its members.

The present study is one of a series designed to provide sequence information for solanaceous ferredoxins (Fds) with the aim of determining the effectiveness of "protein chemotaxonomy", i.e., molecular taxonomy based on the primary structures of common plant proteins. Fd, an iron-sulphur electron transfer protein, was chosen for this study because it is easy to isolate and has an appropriate molecular weight for determining the primary structure. We have previously reported the primary structures of Fds from seven *Datura* plants (Mino et al., 1993; Mino, 1994a,b, 1995), *Physalis alkekengi* var. *francheti* (Mino and Yasuda, 1998), *Nicotiana tabacum* (Mino and Iwao, 1999a), *Capsicum annuum* (Mino and Iwao, 1999b), *Lycium chinense* (Mino, 2002), and *Scopolia japonica* (Mino, 2002). These results were consistent with the taxonomic relationship of these solanaceous plants, except that Fd from *D. arborea* was more similar to those of *L. chinense* and *S. japonica* than to those of other *Datura* plants (Mino, 2002).

In this study, we determined the amino acid sequences of Fds from four species of genus *Solanum* (*S. nigrum*, *S. lyratum*, *S. indicum*, and *S. abutiloides*), and compared their primary structures with those of other higher plants as well as those of solanaceous plants. Their phylogenetic relationships based on the amino acid sequences of Fds are also discussed.

☆ Part 9 in the series "Protein Chemotaxonomy of the Solanaceae". For Part 8, see Mino (2002).

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2. Results and discussion

2.1. Homogeneity and properties of ferredoxins

Four *Solanum*-Fds, *S. nigrum* (*So.n*)-, *S. lyratum* (*So.l*)-, *S. indicum* (*So.i*)-, and *S. abutiloides* (*So.a*)-Fds, were isolated in a high degree of purity with maximum absorption indices A_{max}/A_{275} of 0.85 (at 330 nm), 0.65 (at 420 nm), and 0.60 (at 465 nm), respectively. The spectra were characteristic of [2Fe-2S] Fd of higher plants (Buchanan and Arnon, 1971; Palmer, 1973). The homogeneity of the proteins was confirmed by isoelectric focusing. Hydrophobic column chromatography also showed that there were no isoforms of Fd in these *Solanum* plants.

2.2. Sequence determination

The complete amino acid sequences of the four *Solanum*-Fds are shown in Fig. 1, together with details of the overlapping sites and fragments which aided in deducing the sequences. The amino acid compositions of S-carboxymethylcysteinyl (Cm) Fds and the peptides obtained by enzymatic digestion showed good agreement with those of the final sequences. In the case of *So.n*-Fd, automated Edman degradation of the Cm-Fd yielded the amino-terminal sequence up to the 42nd cycle, except for several slightly doubtful amino acid residues. Lysyl endopeptidase digestion gave three short peptides [L-1 (1–4), L-2 (5–6), and L-5,6 (83–97)] and two long peptides [L-3 (7–50) and L-4 (51-82)], which is in accordance with the one less number of peptides expected from the five Lys residues determined by amino acid analysis of the protein. We assumed the presence of a Lys residue resistant to the enzyme. These peptides were isolated by reversed-phase HPLC, and only L-2 was not obtained. Edman degradation of L-3–T-2, obtained by tryptic digestion of L-3 (7–50), confirmed the sequence of 41–50. L-4 and L-5 covered the sequences of 50–82 and 83–97 (C-terminal), respectively. The N-terminal sequence was confirmed by isolating L-1 (Ala-Thr-Tyr-Lys). The C-terminal sequence of the Cm-Fd was found to be-Leu-Thr-Gly-COOH after digestion with carboxypeptidase Y for different periods of time. This result was in good agreement with the C-terminal sequence determined by Edman degradation of the peptide, L-5,6 (83–97).

The amino acid sequences of the other *Solanum*-Fds were determined in basically the similar manner. In the case of *So.a*-Fd, Lys-82 in the other *Solanum*-Fds was replaced by Gln-82. For the C-terminal sequence of *So.a*-Fd, L-4,5 (51–97), obtained by digestion with lysyl endopeptidase, was subjected to digestion with Asp-N to give L-4,5–D-6 (84–97). Sequencing of this peptide clarified the somewhat doubtful C-terminal sequence. Finally, the primary structures of the four *Solanum*-Fds were determined, as shown in Fig. 1. Among them, 25 amino acid substitutions (boxed) were found.

The amino acid sequences of *Solanum*-Fds are compared to those of other solanaceous Fds in Fig. 2. Compared to other solanaceous Fds (*Datura*-, *Pa*-, *Nt*-, *Ca*-, *Lc*-, and *Sj*-Fds), differences were observed at Ala-43, Asn-61, and Ser-62 in *So.l*- and *So.i*-Fds, at Ser-55 in

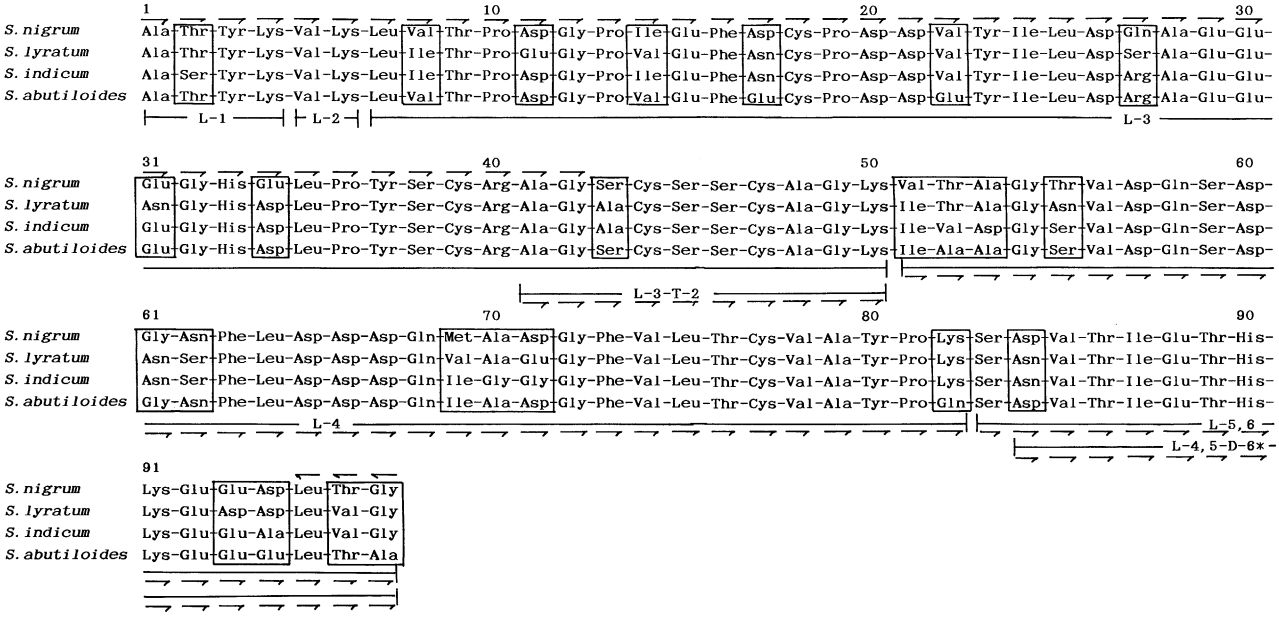

Fig. 1. Amino acid sequences of *Solanum nigrum, S. lyratum, S. indicum, and S. abutiloides* ferredoxins. Arrows (→) and (←) represent residues determined by automated Edman degradation and carboxypeptidase Y digestion, respectively. L(1-5), T(1-2), and D(1-6) represent peptides obtained from digestion with lysyl endopeptidase, trypsin, and endoproteinase Asp-N, respectively. L-4,5–D-6* was necessary only for the case of *S. abutiloides*, which lacks a Lys residue at position 82 in the amino acid sequence of its ferredoxin.

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                                1         2         3         4         5         6         7         8         9
                                0         0         0         0         0         0         0         0         0
(1) Solanum nigrum              ATYKVKLVTPDGPIEFDCPDDVYILDQAEEEGHELPYSCRAGSCSSCAGKVTAGTVDQSDGNFLDDDQMADGFVLTCVAYPKSDVTIETHKEEDLTG
(2) Solanum lyratum             ATYKVKLITPEGPVEFNCPDDVYILDSAEENGHDLPYSCRAGACSSCAGKITAGNVDQSDNSFLDDDQVAEGFVLTCVAYPKSNVTIETHKEDDLVG
(3) Solanum indicum             ASYKVKLITPDGPIEFNCPDDVYILDRAEEEGHDLPYSCRAGACSSCAGKIVDGSVDQSDNSFLDDDQIGGGFVLTCVAYPKSNVTIETHKEEALVG
(4) Solanum abutiloides         ATYKVKLVTPDGPVEFECPDDEYILDRAEEEGHDLPYSCRAGSCSSCAGKIAAGSVDQSDGNFLDDDQIADGFVLTCVAYPQSDVTIETHKEEELTA
(5) Scopolia japonica           ATYKVKLVTPDGPVEFDCPDDVYILDQAEEEGHELPYSCRAGSCSSCAGKVSAGTVDQSDGNFLDDDQMADGFVLTCVAYPQSDVIIETHKEEELTG
(6) Lycium chinense             ATYKVKLVTPDGPVEFDCPDDVYILDQAEEEGHELPYSCRAGSCSSCAGKVSAGTVDQSDGNFLDDDQIADGFVLTCVAYPQSDVTIETHKEEALTG
(7) Capsicum annuum             ASYKVKLITPDGPIEFDCPDDVYILDQAEEAGHDLPYSCRAGSCSSCAGKIAGGAVDQTDGNFLDDDQLEEGWVLTCVAYPQSDVTIETHKEAELVG
(8) Nicotiana tabacum           ASYKVKLITPEGAVEFDCPDDVYILDQAEEMGHDLPYSCRAGSCSSCAGKVTAGNVDQSDGNFLDDDQMADGFVLTCVAYPQSDVTIETHKEEELTA
(9) Physalis alkekengi*         ATYKVKLITPDGPVVFDCPDNEYILDAAEEQGHDLPYSCRAGSCSSCAGKVTAGTVDQSDGNFLDDDQVADGFVLTCVAYPQSDVTIETHKEEELTA
(10) Datura stramonium†         ATYKVKLVTPDGPVEFNCPDDVYILDQAEEEGHDLPYSCRAGSCSSCAGKVTAGTVDQSDGNYLDDDQMADGFVLTCVAYPQSDVTIETHKEEELTG
(11) Datura metel‡              ATYKVKLVTPDGPVEFDCPDDVYILDRAEEEGHDLPYSCRAGSCSSCAGKVTAGTVDQSDGNFLDDDQMADGFVLTCVAYPQSDVTIETHKEEELTG
(12) Datura arborea             ATYKVKLVTPDGPVEFDCPDDVYILDQAEEEGHELPYSCRAGSCSSCAGKVTAGTVDQSDGNYLDDDQMAEGFVLTCVAYPQSDVTIETHKEEELTG
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Fig. 2. Comparison of sequences of [2Fe-2S] ferredoxins from solanaceous plants. Amino acids are represented by one-letter abbreviations. *: *Physalis alkekengi* var. *francheti*, †: var. *stramonium* and var. *tatula*, and *D. quercifolia*, ‡: *D. metel*, *D. innoxia*, and *D. fastuosa*. References for the sequences are: (5, 6), Mino (2002); (7), Mino and Iwao (1999b); (8), Mino and Iwao (1999a); (9), Mino and Yasuda (1998); (10), Mino et al. (1993); (11), Mino (1994a); (12), Mino (1994b); (1–4), Present work.

So.i- and *So.a*-Fds, at Ser-27 and Asn-31 in *So.l*-Fd, and at Val-52 and Asp-53 in *So.i*-Fd. The residue Ala-43 in the primary structures of *So.l*- and *So.i*-Fds is rare in sequences of other higher plants (Mino and Iwao, 1999b). These residues should be characteristic for each *Solanum*-Fd. Although the residues His-33, Asn-62, and Gln-82 seemed to be characteristic of other solanaceous Fds reported previously, in this work only His-33 was conserved as a characteristic residue of solanaceous Fds. Based on a comparison with about 40 other sequences, the occurrence of a histidine residue at position 33 appears to be very unusual in both higher plants and cyanobacteria (Matsubara and Hase, 1983). This histidine residue might be indispensable for solanaceous Fds to act as biological electron carriers. In Fds, the sequences 35–50 and 74–77, including the cysteine ligands for the iron atoms (–C39–C44–C47– and –C77–), and a later region, 83–93, are almost completely conserved. This was also true in the case of the *Solanum*-Fd except for Asp-93 in *So.l*-Fd and Ala-43 in *So.l*- and *So.i*-Fds.

2.3. Taxonomic considerations

Many primary structures have been reported for chloroplast [2Fe-2S] Fds (Matsubara and Hase, 1983; Kamo et al., 1989; Takruri, 1991; Mino et al., 1993; Mino, 1994a,b, 1995, 2002; Mino and Yasuda, 1998; Mino and Iwao, 1999a,b). There are 14–40 differences in the amino acid residues of the Fds from different families and 0–4 differences in Fds from plants in the same genus (Mino, 2002). Although a few sequences for plants in the same family are available, the sequences of several solanaceous Fds have recently been determined (Mino et al., 1993; Mino, 1994a, b, 1995, 2002; Mino and Yasuda, 1998; Mino and Iwao, 1999a, b). Table 1 shows a matrix of the amino acid differences in solanaceous Fds that have been determined so far. Two to nineteen amino acid differences were observed among different genera, *Datura*, *Physalis*, *Nicotiana*, *Capsicum*, *Scopolia*, and *Lycium*. On the other hand, there were only 0–4 differences in the Fds from seven *Datura* plants. In the present study, large differences (12–19) in the amino acid sequence were observed among the four

Table 1
Amino acid difference matrix for the sequences of [2Fe-2S] ferredoxin from higher plants[a]

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1) *Solanum nigrum*	0	17	18	12	5	5	17	11	12	6	7	3
(2) *Solanum lyratum*	17	0	13	19	20	19	20	17	19	17	17	18
(3) *Solanum indicum*	18	13	0	17	21	18	18	21	23	19	19	20
(4) *Solanum abutiloides*	12	19	17	0	10	9	17	12	10	9	9	9
(5) *Scopolia japonica*	5	20	21	10	0	3	17	10	11	5	6	2
(6) *Lycium chinense*	5	19	18	9	3	0	17	11	11	6	7	3
(7) *Capsicum annuum*	17	20	18	17	17	17	0	16	19	17	16	16
(8) *Nicotiana tabacum*	11	17	21	12	10	11	16	0	10	9	10	8
(9) *Physalis alkekengi**	12	19	23	10	11	11	19	10	0	10	10	9
(10) *Datura stramonium*†	6	17	19	9	5	6	17	9	10	0	3	3
(11) *Datura metel*‡	7	17	19	9	6	7	16	10	10	3	0	4
(12) *Datura arborea*	3	18	20	9	2	3	16	8	9	3	4	0
(13) *Brassica napus*	25	23	24	23	25	24	25	22	24	24	23	24
(14) *Leucaena glauca*	25	28	25	24	24	24	23	26	28	24	24	24
(15) *Medicago sativa*	19	24	23	17	20	19	21	18	23	20	19	19
(16) *Petroselinum sativum*	27	30	27	27	25	27	29	26	29	25	26	25
(17) *Phytolacca americana*	24	28	30	28	28	26	30	28	26	27	26	26
(18) *Phytolacca esculenta*	23	28	30	27	27	25	30	27	25	26	25	25
(19) *Spinacia oleracea*	26	28	26	21	25	24	26	24	26	24	23	24
(20) *Sambucus nigra*	22	23	19	18	23	22	20	20	22	22	21	22
(21) *Arctium lappa*	20	24	25	19	21	22	24	17	21	19	19	19
(22) *Colocasia esculenta*	20	25	24	19	20	19	22	19	22	19	19	19
(23) *Triticum aestivum*	23	27	27	22	22	24	27	22	27	22	23	22
(24) *Hordeum vulgare*	22	25	26	21	21	23	25	21	26	21	21	21
(25) *Oryza sativa*	23	23	26	26	24	24	27	25	26	24	25	24
(26) *Equisetum telmateia*	38	40	38	38	37	36	38	36	36	38	36	37
(27) *Equisetum arvense*	39	41	39	39	38	40	39	37	37	39	37	38
(28) *Gleichenia japonica*	34	34	33	33	35	35	36	34	34	34	33	34

[a] Data for higher plants other than solanaceous plants are available elsewhere (Mino and Iwao, 1999b), and are omitted here. See the legend to Fig. 2. (1)–(12) Solanaceae; (13) Cruciferae; (14) and (15) Leguminosae; (16) Umbelliferae; (17) and (18) Phytolaccaceae; (19) Chenopodiaceae; (20) Caprifoliaceae; (21) Compositae; (22) Araceae; (23)–(25) Gramineae; (26) and (27) Equisetales; (28) Filicales, respectively.

Solanum-Fds, suggesting that these *Solanum* plants are quite remotely related. The number of differences among *Solanum*-Fds is significantly greater than those (0–4) for Fds for plants in the same genus, and also greater than or comparable to those (3–13) for Fds from plants in different genera: e.g., *Hordeum*, *Triticum*, and *Oryza* of the Gramineae (Mino and Iwao, 1999b). In addition, *So.n*- and *So.a*-Fds were fairly similar to *Datura*-Fds, with only 3–9 differences, but not to the other *Solanum*-Fds. On the other hand, *So.i*- and *So.l*-Fds showed 18–23 and 16–20 differences, respectively, compared to the Fds of solanaceous plants outside of the genus *Solanum*. Thus, *Solanum* plants might be taxonomically distributed over all *Solanaceae*.

Fig. 3 shows a phylogenetic tree based on the amino acid sequences of Fds of higher plants. Twelve solanaceous plants form a cluster that is distinctly separate from other angiospermous plants, a fern, and horsetails by significantly long branch lengths, which increase in that order. In this solanaceous cluster, these four *Solanum*

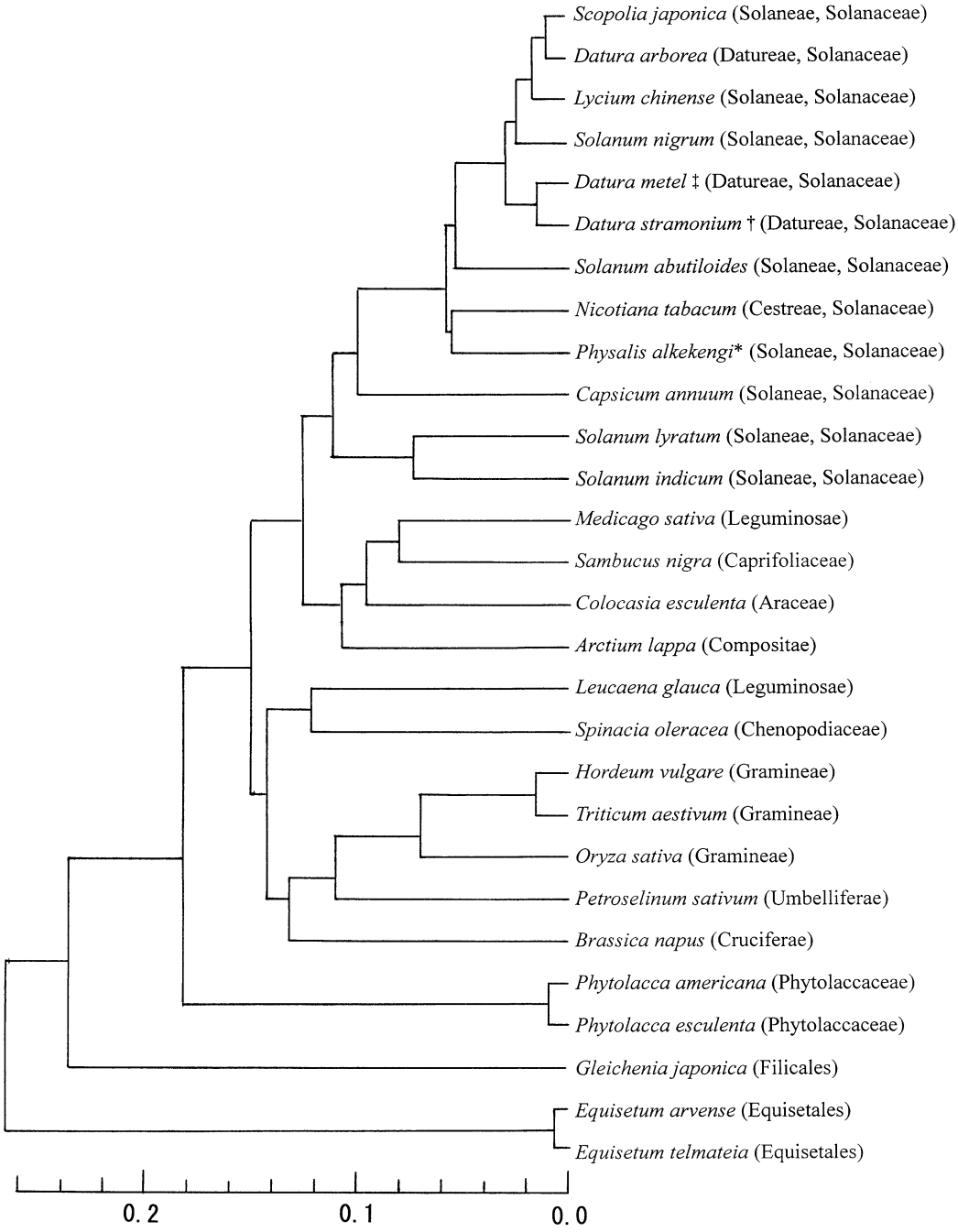

Fig. 3. Phylogenetic tree based on the amino acid sequences of ferredoxins from higher plants. The phylogenetic tree was constructed using the UPGMA method of Nei (GENETYX software) (Nei, 1987). Genetic distances are represented by the proportion of amino acid differences between each taxa (1.0 = 100%).

plants are located among genera, *Datura, Lycium, Scopolia, Physalis, Nicotiana,* and *Capsicum*, indicating a discrepancy between protein chemotaxonomy and classical taxonomy. These results probably suggest that these *Solanum* plants are distantly related to each other taxonomically, like plants belonging to different genera, and that genus *Solanum* includes a very wide variety of plants. This view is consistent with the fact that *Solanum* is a huge genus that contains over 1000 of the total number of solanaceous plants (ca. 3500 species) (D'Arcy, 1986; Hawkes et al., 1991). To explore this issue further, we would need additional information regarding the amino acid sequences of Fds from *Solanum* plants. The amino acid sequences of minor Fds (so-called Fd) (Sakihama et al., 1986; Sakai et al., 1994), if available, as well as major Fds (Fd I) from *Solanum* plants, would be helpful for the taxonomic classification of these plants.

3. Experimental

3.1. Materials

S. lyratum and *S. nigrum* were cultivated in the herb garden at this university. Fresh leaves of *S. indicum* and *S. abutiloides* were kind gifts from Prof. T. Nohara, Kumamoto University.

3.2. Isolation of ferredoxin

The proteins were purified from the leaves (ca. 0.6 kg) of each *Solanum* plant as described previously (Mino et al., 1993; Mino and Yasuda, 1998).

3.3. Sequence determination

The amino acid sequences of the Fds were determined using a gas-phase protein sequencer with the automated Edman degradation of Cm-Fd, and the peptides obtained by digestion with lysyl endopeptidase, trypsin, or endoproteinase Asp-N. C-terminal analysis was performed using carboxypeptidase Y.

3.4. Construction of a phylogenic tree

A phylogenic tree was constructed from the amino acid sequences (97 residues) of higher-plants Fds (28 species) using the unweighted pair-group method with the arithmetical averages (UPGMA) method of Nei (GENETYX software, Software Development, Japan) (Nei, 1987).

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References

Buchanan, B.B., Arnon, D.I., 1971. Ferredoxins from photosynthetic bacteria, algae, and higher plants. Methods Enzymol. 23, 413–440.

Chase, M.W., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hills, H.E., Qiu, Y.-L., Kron, K.A., Rettig, J.H., Conti, E., Palmer, J.D., Manhart, J.R., Sytsma, K.J., Michaels, H.J., Kress, W.J., Karol, K.G., Clark, W.D., Hedren, M., Gaut, B.S., Jansen, R.K., Kim, K.-J., Wimpee, C.F., Smoth, J.F., Furnier, G.R., Strass, S.H., Xiang, Q.-Y., Plunkett, G.M., Soltis, P.S., Swensen, S.M., Williams, S.E., Gadek, P.A., Quinn, C.J., Eguiarte, L.E., Golenberg, E., Learn, G.H., Graham, S.W., Barrett, S.C.H., Dayanandan, S., Albert, V.A., 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. Ann. Missouri Bot. Gard. 80, 528–580.

D'Arcy, W.G., 1986. Taxonomy and biogeography. In: D'Arcy, W.G. (Ed.), Solanaceae: Biology and Systematics. Columbia University Press, New York, pp. 1–4.

Hawkes, J.G., Lester, R.N., Nee, M., Estrada, N., 1991. Introduction. In: Hawkes, J.G., Lester, R.N., Nee, M., Estrada, N. (Eds.), Solanaceae III—Taxonomy, Chemistry, Evolution. The Royal Botanic Gardens Kew, Richmond.

Kamo, M., Kotani, N., Tsugita, A., He, Y.K., Nozu, Y., 1989. Amino acid sequences of ferredoxins from rice cultivars, japonica and indica. Protein Seq. Data Anal. 2, 289–293.

Matsubara, H., Hase, T., 1983. Phylogenetic consideration of ferredoxin sequences in plants, particularly algae. In: Jansen, U., Fairbrothers, D.E. (Eds.), Proteins and Nucleic Acids in Plant Systematics. Springer-Verlag, Berlin, pp. 168–181.

Mino, Y., Usami, H., Inoue, S., Ikeda, K., Ota, N., 1993. Protein chemotaxonomy of genus *Datura*: identical amino acid sequence of ferredoxin from two varieties of *Datura stramonium*. Phytochemistry 33, 601–605.

Mino, Y., 1994a. Identical amino acid sequence of ferredoxin from *Datura metel* and *D. innoxia*. Phytochemistry 35, 385–387.

Mino, Y., 1994b. Amino acid sequence of ferredoxin from *Datura arborea*. Phytochemistry 37, 429–431.

Mino, Y., 1995. Protein chemotaxonomy of genus *Datura*. IV. Amino acid sequence of *Datura* ferredoxin depends not on the species but the section of *Datura* plants from which it comes. Chem. Pharm. Bull. 43, 1186–1189.

Mino, Y., Yasuda, K., 1998. Amino acid sequence of ferredoxin from *Physalis alkekengi* var. *francheti*. Phytochemistry 49, 1631–1636.

Mino, Y., Iwao, M., 1999a. Protein chemotaxonomy of the Solanaceae. VI. Amino acid sequence of ferredoxin from *Nicotiana tabacum*. Biol. Pharm. Bull. 22, 96–99.

Mino, Y., Iwao, M., 1999b. Protein chemotaxonomy of the Solanaceae. VII. Amino acid sequence of ferredoxin from *Capsicum annuum*. Natural Med. 53, 37–41.

Mino, Y., 2002. Amino acid sequences of ferredoxins from *Scopolia japonica* and *Lycium chinense*: their similarities to that of *Datura arborea*. Biol. Pharm. Bull. 25, 1367–1369.

Nei, M., 1987. Phylogenic trees. In: Nei, M. (Ed.), Molecular Evolutionary Genetics. Columbia University Press, New York, pp. 287–326.

Palmer, G., 1973. Current insights into the active center of spinach ferredoxin and other iron-sulfur proteins. In: Lovenberg, W.

(Ed.), Iron-Sulfur Proteins II. Academic Press, New York, pp. 285–325.
Sakai, H., Kamide, K., Morigasaki, S., Sanada, Y., Wada, K., Ihara, M., 1994. Amino acid sequences of ferredoxin isoproteins from Japanese taro (*Colocasia esculenta* Schott). J. Plant Res. 107, 299–305.
Sakihama, N., Shin, M., Toda, H., 1986. Separation and identification of two native forms of spinach ferredoxin by hydrophobic chromatography. J. Biochem. 100, 43–47.
Takruri, I.A.H., 1991. The amino acid sequence of ferredoxin from *Hordeum vulgare*. Phytochemistry 30, 415–418.
Zuckerkandl, E., Pauling, L., 1965. Molecules as documents of evolutionary history. J. Theor. Biol. 8, 357–366.