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Short Communication

Isolation and Partial Characterization of Ferredoxin from *Zea mays*[1]

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Ferredoxins have been isolated and purified from several varieties of higher plants. These proteins appear to have similar molecular weights (10–12,000), contain 2 g-atoms of iron per mole, and support photoreduction of NADP with chloroplasts. Recently, great interest has been focused on some plant species that have a high photosynthetic capacity for CO_2 assimilation. These include many of the tropical grasses such as corn, sugar cane, and sorghum. With these species, CO_2 appears to be fixed by a pathway in which C_4-dicarboxylic acids (oxalacetate, malate, and aspartate) are the initial primary products (2, 3). However, most of the ferredoxins which have been studied are confined to those plants having a lower photosynthetic capacity where 3-phosphoglycerate is the initial product of CO_2 fixation (C_3 pathway). Since the carbon products of photosynthesis differed, we thought it possible that the ferredoxins involved in photoreduction of NADP from the two plant types might differ. A technique for isolation and purification of ferredoxin in good yields from leaves of *Zea mays* has been developed and the amino acid composition, molecular weight, and biological activity of the protein have been determined.

MATERIALS AND METHODS

Hybrid sweet corn, "Golden Gross Bantam T-51", was grown in a greenhouse and used 20 to 35 days after planting. For isolation of corn ferredoxin, 200 g of leaves were ground 4 min in a blender with 400 ml of 50% acetone-water containing 0.84 g of tris base, precooled to −15 C. The tris was needed to maintain a pH greater than 7.

The homogenate was filtered through four layers of cheesecloth and centrifuged 10 min at 20,000*g* at −15 C. The following steps were done at 4 C and all solutions were adjusted to pH 7.3 and contained 1 mM β-mercaptoethanol. The supernatant solution from 400 g of leaves was passed through a 4 × 4 cm DEAE-cellulose[2] bed, previously equilibrated with 50% acetone-water containing 2 mM tris-HCl, in a 60-ml fritted disc funnel. The bed was washed with 0.01 M tris-HCl until the effluent was clear, and the bound protein was eluted with 0.1 M tris-HCl, 0.8 M NaCl. The eluate was diluted 1:4 with water and readsorbed on a 2.5- × 45-cm DEAE-cellulose column equilibrated with 0.1 M tris-HCl, 0.2 M NaCl. The column was washed with the equilibration solution (1.5 liters) until a diffuse red band containing the ferredoxin separated from the dark material retained at the top. The red band was then eluted with 0.1 M tris-HCl, 0.3 M NaCl.

The fractions containing ferredoxin were pooled, diluted 1:3, and concentrated by readsorption on a 1- × 3-cm DEAE-cellulose column and elution with 0.1 M tris-HCl, 0.8 M NaCl. One and one-half milliliters of this concentrated solution was put on a 1.5- × 100-cm column of Sephadex G-75 and eluted with 0.01 M tris-HCl, 0.2 M NaCl. The purified ferredoxin separated from a slower moving fluorescent band and was collected and concentrated on a 1- × 3-cm DEAE-cellulose column. This procedure yielded about 15 mg of ferredoxin from 400 g of leaves.

Amino acid content was determined with a Beckman Model 121 Amino Acid Analyzer on samples which had been hydroyzed in 6 N HCl for 24 hr and 72 hr at 110 C. Cysteine was determined as cysteic acid after oxidation of the protein by performic acid (5). Sedimentation equilibrium centrifugation runs were made in an analytical ultracentrifuge with a Yphantis cell (13), with patterns taken at 24 hr and 28 hr. The runs were made at 4 C and 20,000 rpm. The iron content was determined by the method of Cameron (1) by formation of the ferrous-*o*-phenanthroline complex.

The activity of corn ferredoxin in mediating photoreduction of NADP was assayed with corn and spinach chloroplasts and compared to the activity using spinach ferredoxin. The plastids were isolated in 0.25 M tris-HCl, pH 7.5, 0.5 M sorbitol, 5 mM β-mercaptoethanol. The leaves were chopped in a blender for 30 sec and filtered to remove material greater than 25 μ. The chloroplasts were collected by centrifugation at 2000*g* at 4 C. Light-dependent reduction of NADP was determined in a cuvette containing 0.1 mg of chlorophyll, 0.1 mg of ferredoxin (corn or spinach), and 2 μmoles of NADP in 3.0 ml of the above sorbitol buffer. The cuvette was illuminated at 4000 ft-c and 20 C and absorbance change was monitored at 340 nm. Spinach ferredoxin was isolated by essentially the same technique used with corn.

RESULTS AND DISCUSSION

The initial steps of isolation as reported for spinach (11) or alfalfa (6) ferredoxin were not satisfactory for isolation of corn ferredoxin. If corn leaves were ground in aqueous buffer followed by DEAE chromatography, much of the ferredoxin activity was lost. If, however, the corn leaves were ground in 50% acetone, buffered with tris at 7.3 or higher, the solids removed, and the solution adsorbed to DEAE, a high yield of ferredoxin was obtained.

The complete isolation procedure was accomplished in 36 hr with no loss of activity when the solutions contained β-

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[2] Abbreviation: DEAE: diethylaminoethyl.

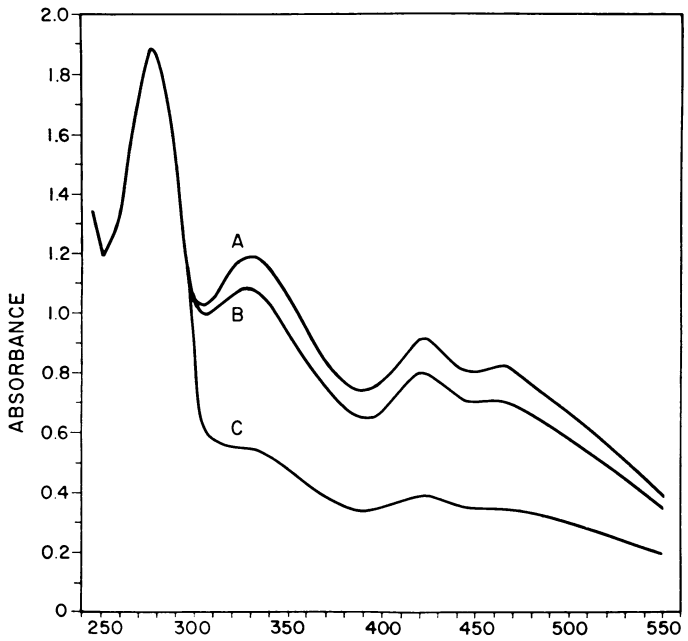

FIG. 1. Absorption spectrum of ferredoxin from *Zea mays* and its change during denaturation at 23 C. The ferredoxin was desalted on a G-25 Sephadex column (2.5 × 15 cm) and the spectrum of the colored eluate taken directly.

Table I. *Amino Acid Composition of Ferredoxin from Zea mays and Comparison to Other Higher Plant Ferredoxins*

Amino Acid	Residues per Molecule					
	Values Obtained after Hydrolysis (Leucine = 8)		Nearest Integer	Spinach (9)	Alfalfa (7)	Cotton (10)
	24 hr.	72 hr.				
Lysine	3.35	3.32	3	4	5	3
Histidine	1.84	1.79	2	1	2	1
Arginine	1.07	1.04	1	1	1	2
Aspartic Acid	12.95	12.94	13	13	9	16
Threonine	5.79	5.36	5	8	6	4
Serine	8.67	7.20	8	7	8	6
Glutamic Acid	14.20	14.27	14	13	16	18
Proline	4.35	4.38	4	4	3	4
Glycine	7.99	8.02	8	6	7	8
Alanine	8.11	8.06	8	9	9	8
Cysteine	3.77[1]	...	4	5	5	4
Valine	9.90	9.99	10	7	9	8
Methionine	0.20	0.18	0	0	0	1
Isoleucine	5.21	5.20	5	4	4	4
Leucine	8.00	8.00	8	8	6	6
Tyrosine	5.12	4.98	5	4	4	2
Phenylalanine	1.11	0.80	1	2	2	3
Tryptophan	...	...	1[2]	1	1	1
Total			100	97	97	99

[1] Determined as cysteic acid (5).

[2] Based on spectrophotometric analysis (9).

mercaptoethanol and the salt concentration and pH were controlled. The protein appeared homogeneous in the ultracentrifuge. It has been stored frozen under nitogen in 0.05 M tris-HCl, pH 7.3, 0.15 M NaCl for long periods with little change in the absorption spectrum. The protein denatures in ion-low water, as shown by the change in the absorption spectrum with curves B and C in Figure 1.

Curve A in Figure 1 is the absorption spectrum of purified corn ferredoxin. The absorption maxima are found at 277, 330, 423, and 463 nm with absorbance ratios at 330, 423, and 463 nm to that at 277 nm of 0.60, 0.48, and 0.43, respectively. The molecular extinction coefficients calculated from a molecular weight of 11,000 were 21.3×10^3 M^{-1} cm^{-1} at 277 nm, 13.3×10^3 M^{-1} cm^{-1} at 330 nm, 10.0×10^3 M^{-1} cm^{-1} at 423 nm, and 8.9×10^3 M^{-1} cm^{-1} at 463 nm. Similar absorption peaks and ratios have been found for other plant ferredoxins (7, 10, 12).

The amino acid analysis was computed from samples hydrolyzed for 24 hr and 72 hr. The values were calculated with leucine normalized to eight residue per molecule, and results from a typical experiment are shown in Table I. Corn ferredoxin, like other ferredoxins, has a high number of acidic amino acids, a low content of basic and aromatic residues, and lacks methionine.

Tryptophan was not determined chemically, but it is assumed to be one per molecule based on spectrophotometric analysis (4, 9). The absorbance ratios of corn ferredoxin are very similar to spinach and alfalfa ferredoxin, and a different number of tryptophan residues could be expected to alter these ratios (compare *Scenedesmus* ferredoxin [8] which lacks tryptophan).

Iron determinations on three samples gave values of 1.73, 1.65, and 2.21 g-atoms Fe per mole, indicating 2 g-atoms Fe per mole, which is typical of other plant ferredoxins.

Labile sulfur content was not determined. Performic acid oxidation of the protein yielded 3.77 residues of cysteic acid (assuming leu = 8) indicating four cysteine residues (5). Values for the other amino acids of the performic acid oxidized protein were in good agreement with those of Table I. The total number of amino acid residues is 100, giving a calculated molecular weight, including 2 iron and 2 sulfur atoms, of 10,950.

The molecular weight was also calculated from sedimentation equilibrium centrifugation experiments. Plots of the logarithm of the fringe displacement with respect to r^2 were virtually linear, indicating a high degree of homogeneity. At protein concentrations of 2 mg/ml and 4 mg/ml, the calculated molecular weight was 10,530 and 11,490, respectively, giving an average molecular weight of 11,000.

Both corn and spinach ferredoxins were found to operate interchangeably with either corn or spinach chloroplast preparations in mediating noncyclic electron flow to NADP. With corn chloroplasts both ferredoxins sustained an initial reduction of NADP of 40 μmoles per mg chlorophyll per hr. With spinach chloroplasts, both proteins gave initial rates of 100 μmoles per mg chlorophyll per hr.

Ferredoxin from corn, a C_4 pathway plant, appears quite similar to the ferredoxins obtained from C_3 pathway plants in amino acid composition, iron content, molecular weight, and biological activity.

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