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Ferredoxin from fern and amaranthus: Two diverse plants with similar ferredoxins

Ferredoxins are a group of iron–sulfur proteins which serve as electron carriers in plants, photosynthetic bacteria, and certain nonphotosynthetic bacteria. There is a growing body of evidence to support the view that ferredoxin is native to all green plants (including algae), and, through its role in NADP reduction and photophosphorylation, is required ubiquitously for the conversion of sunlight into chemical energy by oxygen-evolving cells[1–4].

If ferredoxin has a general role in photosynthesis, it must be present in all major groups of plants. Ferredoxin has indeed been isolated from a variety of algae and higher plants[4] but not, so far, from members of two groups of plants which merit special attention: ferns (lower plants which due to their inability to flower are considered relatively primitive taxonomically) and amaranths (higher plants similar to spinach taxonomically but distinguished by differences in CO_2 metabolism[5]). We report here the isolation and certain characteristics of ferredoxins from the fern *Polystichum munitum* (commonly called swordfern) and *Amaranthus edulis* (commonly called pigweed).

Fig. 1 shows the absorption spectra of ferredoxins from fern and amaranthus

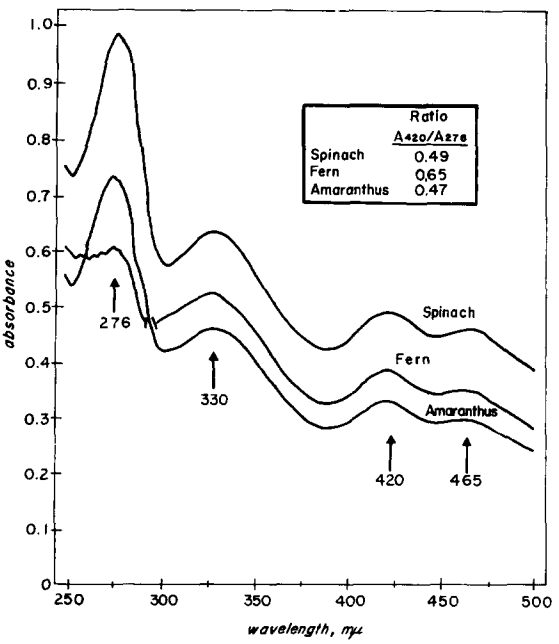

Fig. 1. Absorption spectra of plant ferredoxins. Spectra were measured in 1.0-cm cuvettes with a Cary 14 M recording spectrophotometer. The cuvettes contained the following amounts in 0.3 M Tris–HCl buffer, pH 7.3, containing 0.54 M NaCl: spinach ferredoxin, 0.65 mg/ml; fern ferredoxin, 0.51 mg/ml; and amaranthus ferredoxin, 0.45 mg/ml. Ferredoxin concentration was determined spectrally at 420 mμ, using, in all cases, the extinction coefficient of 0.835 $(mg/ml)^{-1} \times cm^{-1}$ found for spinach ferredoxin[8].

compared with that from spinach. The three ferredoxins show nearly identical absorption spectra in the visible and near-ultraviolet regions (peaks at 465, 420, and 330 mμ), but certain differences are apparent in the ultraviolet. Spinach and amaranthus ferredoxins both show a single peak at 276 mμ (with similar 420/276 mμ absorbance ratios, of 0.49 and 0.47, respectively). Fern ferredoxin also shows an absorption maximum at 276 mμ, but the peak is less pronounced and the total ultraviolet absorption is lower than with the other ferredoxins (420/276 mμ ratio for fern

TABLE I

IRON–SULFIDE CONTENT OF FERN, AMARANTHUS AND SPINACH FERREDOXINS

The three ferredoxins were isolated by the procedure of TAGAWA AND ARNON[8]. Amaranthus plants were grown from seed in a greenhouse on nutrient solutions; fern fronds were collected from the north shore of Alpine Lake, Marin County, California; spinach leaves were purchased from a local market. Ferredoxin concentrations were determined as described in Fig. 1.

Ferredoxin	*Iron (μatoms/μmole ferredoxin)*	*Labile sulfide (μmoles/μmole ferredoxin)*
Fern	2.1	1.8
Amaranthus	1.6	1.6
Spinach	2.0	1.9

TABLE II

AMINO ACID COMPOSITION OF FERN, AMARANTHUS AND SPINACH FERREDOXINS

Amino acid analyses were carried out with a Beckman Model 120C amino acid analyzer by using 22- and 72-h acid hydrolysates (6 M HCl, 100°) of native ferredoxin and of the oxidized and carboxymethylcysteinyl ferredoxin derivatives (prepared as described by MATSUBARA *et al.*[9]). Cysteine was estimated both as carboxymethylcysteine after carboxymethylation and as cysteic acid after oxidation of the native ferredoxins.

Amino acid	*Fern*	*Amaranthus*	*Spinach (ref. 9)*
Lysine	4	4	4
Histidine	2	1	1
Arginine	1	1	1
Tryptophan	0	1	1
Aspartic acid	14	12	13
Threonine	6	8	8
Serine	7–8	8	7
Glutamic acid	9	14	13
Proline	4–5	4–5	4
Glycine	9	6	6
Alanine	7	10	9
Half-cystine	5	5	5
Valine	5	5	7
Methionine	2	1	0
Isoleucine	6	6	4
Leucine	7	6	8
Tyrosine	3	4	4
Phenylalanine	4	1	2
Total	95–97	97–98	97

ferredoxin = 0.65). The weak ultraviolet absorption displayed by fern ferredoxin is also characteristic of ferredoxins from certain types of algae[1,6].

Despite certain spectral differences, fern and amaranthus ferredoxins resemble to a remarkable degree, spinach ferredoxin as well as other plant ferredoxins. Table I shows that each ferredoxin examined contains two iron and labile sulfide groups per mole (based on a molecular weight of 12000). When assayed for activity, the three ferredoxins were equally effective in photoreduction of NADP by isolated spinach chloroplasts[10].

Fern, amaranthus, and spinach ferredoxins are also similar in their amino acid compositions (Table II). Each ferredoxin contains a total of 95 to 98 amino acids with a distribution remarkably similar to that of the other two ferredoxins (and to other plant ferredoxins which have been examined[4]). A noteworthy difference is the absence of tryptophan in fern ferredoxin, a difference which explains its low ultraviolet absorption.

In sum, the isolation of ferredoxin from fern and amaranthus is consistent with the key role assigned to ferredoxin in plant photosynthesis[1]. Fern and amaranthus ferredoxins are remarkably similar to other plant ferredoxins in visible absorption characteristics, iron–sulfide content, and amino acid composition. Fern ferredoxin (like certain algal ferredoxins) is distinguished from higher plant ferredoxins by a low absorbance in the ultraviolet and by the absence of tryptophan.

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