CHEMICAL COMPOSITION OF A FERREDOXIN ISOLATED FROM COTTON

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Summary. This communication describes the isolation, purification and the amino acid, iron and sulfur composition of a type I ferredoxin from cotton.

Within the last two years, the primary structures of four type I ferredoxins have been reported (Spinach, Matsubara, *et al.*, 1968; Alfalfa, Keresztes-Nagy, *et al.*, 1969; *Leucaena glauca*, Benson and Yasunobu, 1969; and *Scenedesmus*, Sugeno and Matsubara, 1969). In addition, the chemical composition of a fifth ferredoxin, that from *Anacystis nidulans*, has recently appeared (Yamanaka, *et al.*, 1969). We would like to report the properties of a type I ferredoxin isolated from cotyledons of germinating cotton seed (*Gossypium hirsutum*).

Methods

Green cotyledons from 6 days germinated cotton plantlets, germinated on agar, were frozen in liquid nitrogen, lyophilized and exhaustively extracted with carbon tetrachloride-hexane (2:1; v/v). The solvent-extracted residue was vacuum dried and then vigorously extracted with cold 0.1 M sodium phosphate buffer, pH 6.6. The buffer-soluble protein was fractionated in cold with solid ammonium sulfate and the 50-80% precipitate collected. This fraction was redissolved in 0.1 M buffer, dialyzed against a lower buffer concentration (0.01 M, pH 6.6) overnight in the cold and the resulting precipitate discarded. The soluble fraction was then applied to a DEAE-cellulose column (Whatman DE 23, equilibrated with 0.01 M buffer, pH 6.6).

and a gradient of NaCl (0 to 0.4 M in 0.01 M buffer) passed through the column. A reddish-brown band of ferredoxin formed near the top of the column during this gradient, and was eluted with 0.6 M NaCl in 0.01 M buffer. The eluate was again fractionated with ammonium sulfate and the 80-100% precipitate collected. This fraction was better than 99% pure ferredoxin and appeared homogeneous on disc electrophoresis performed at pH 8.6. However, it was slightly contaminated with a yellowish pigment that absorbed strongly in the U.V. at wave lengths lower than 270 mμ. From 50 g. of solvent-extracted dry cotyledons, 10 mgs. of ferredoxin of this purity were obtained. Based on the total buffer-soluble protein from the dry cotyledons, this constituted a 2000 fold purification.

Results

This cotton ferredoxin has the usual type I spectrum; the extinction coefficients at 460 mμ, 419 mμ, and 325 mμ are 6.55×10^3, 7.58×10^3 and 10.82×10^3 respectively. These were calculated on the basis of amino acid content of the protein, and are very similar to those reported for the Alfalfa protein (Keresztes-Nagy, *et al.*, 1969). Because of the pigment absorption it was not possible to compute a 280 mμ extinction. The methods described by Lovenberg, *et al.* (1963), were used to determine the iron and labile sulfide content and, on a molar basis, these were 2 Fe : 1.85 S : 1 Ferredoxin, which ratio is characteristic of the other type I ferredoxins, except for the *Anacystis nidulans* protein which is reported to contain only 1 labile sulfur (Yamanaka, *et al.*, 1969). The E.P.R. spectrum at 80°K of the protein when reduced with dithionite was similar in all respects to those published for type I ferredoxins (Palmer and Sands, 1966), and the oxidized protein was active in the photoreduction of $NADP^+$ performed with spinach chloroplasts (San Pietro, 1963).

Amino-acid analysis, computed from data obtained by 22 and 48 hour hydrolysis of both the native and performic acid oxidized apo-protein, is

TABLE I

Amino acid compositions of type I Ferredoxins

	Spinach	Alfalfa	*L.glauca*	*Scenedesmus*	*A.nidulans*	Cotton
Lys	4	5	5	4	3	3
His	1	2	1	1	1	1
Arg	1	1	2	1	1	2
Asp	13	9	10+	12	15	16
Thr	8	6	4	10	12	4
Ser	7	8	7	8	7	6
Glu	13	16	16+	10	11	18
Pro	4	3	4+	4	3	4
Gly	6	7	6+	7	6	8
Ala	9	9	7	10	12	8
1/2 Cys	5	5	5	6	6	4[a]
Val	7	9	6+	5	9	8
Met	0	0	0	1	0	1
Ile	4	4	4+	3	5	4
Leu	8	6	9+	7	7	6
Tyr	4	4	3	4	5	2
Phe	2	2	3	3	2	3
Trp	1	1	1	0	0	1[b]
N-terminal	Ala	Ala	Ala	Ala	?	Ala[c]
Total	97	97	96	96	105	99

a. as cysteic acid

b. by the method of Opienska-Blauth, *et al.* (1963)

c. by Mass spectroscopy (Lovins and Travis, in preparation)

shown in Table I, along with the five published analyses of type I ferredoxins for comparison.

The protein contains 99 amino acids, giving a computed molecular weight, including the iron and sulfur, of 10,950. In comparison with the composition of the other five type I ferredoxins, the following points are noteworthy. Cotton ferredoxin has the highest percentage of putatively acidic residues,

is the only plant ferredoxin to contain methionine and has only 4 residues of cysteine per mole of protein.

The N-terminal amino acid was determined by mass spectrographic analysis of the DNP derivative, a technique developed by Lovins and Travis (in preparation), and, as in the other type I ferredoxins, appeared to be alanine.

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