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Four-Iron (Sulfide) Ferredoxin from *Bacillus polymyxa*

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Ferredoxin from *Bacillus polymyxa* contains (per mole) four non-heme iron residues, four acid-labile sulfide residues, and four cysteine residues. Its molecular weight is approximately 8,800, and it has an oxidation-reduction potential (E_m) of −390 mv. It is active as an electron carrier in several ferredoxin-linked enzyme systems.

Ferredoxins are a major class of electron carriers whose functions and chemical properties have received a great deal of attention in recent years. Except for a previous report of a four-iron (sulfide) ferredoxin from *Desulfovibrio gigas* (4), ferredoxins are of either the bacterial [eight-iron (sulfide)] or plant [two-iron (sulfide)] type (2). We now report *B. polymyxa* ferredoxin [previously isolated by Shethna et al. (7)] to be an intermediate form of ferredoxin containing a four-iron (sulfide) prosthetic group. The amino acid composition, oxidation-reduction potential (E_m), and biological activity of this ferredoxin are also reported.

Extracts of *B. polymyxa*, grown in medium (3) containing $(NH_4)_2SO_4$ as the nitrogen source, were prepared with a Hughes press (nitrogen-fixing extracts were prepared from cells grown on N_2). Ferredoxin was isolated from crude extracts by the acetone extraction method of Mortenson (6). *B. polymyxa* ferredoxin chromatographed on diethylaminoethyl (DEAE) cellulose as a major and a minor band (ratio 5:1), the minor band eluting prior to the major band. The two ferredoxin fractions had equal biological activity when compared on the basis of their absorption at 390 nm. After further purification of the combined major and minor ferredoxin components, only a single ferredoxin peak was observed on both DEAE cellulose and Sephadex G-50 chromatography. There is no readily apparent explanation for a second ferredoxin band, unless a small amount of the ferredoxin was complexed with another protein, thus changing its electrical charge and resulting in a different rate of migration on DEAE cellulose. Non-heme iron and acid-labile sulfide were estimated by the method of Lovenberg et al. (5). The E_m of *B. polymyxa* ferredoxin was determined by measuring its percent reduction when at equilibrium with the H_2-hydrogenase system (8). *B. polymyxa* ferredoxin was assayed for biological activity in the photochemical reduction of nicotinamide adenine dinucleotide phosphate (NADP) by spinach chloroplasts (1).

The amino acid composition and iron-sulfide content of *B. polymyxa* ferredoxin are presented in Table 1. The protein contains 77 to 79 amino acid residues (minimal molecular weight, including iron and sulfide, estimated to be 8,800) and is devoid of histidine, methionine, and possibly tyrosine. The minimal molecular weight (8,800) of *B. polymyxa* ferredoxin is in good agreement with the value of 9,000 reported by Shethna et al. (7) from Sephadex G-100 chromatography data. Furthermore, we have observed that *B. polymyxa* ferredoxin elutes after horse heart cytochrome *c* (molecular weight = 12,400) on Sephadex G-50, indicating a molecular weight less than that of cytochrome *c*. Thus from both amino acid analysis and Sephadex elution patterns the molecular weight of *B. polymyxa* ferredoxin appears to be approximately 9,000.

The number of iron and sulfide groups appears to be four per molecule based on a molecular weight of 8,800. This unusual number of irons and sulfides (one-half that of clostridial ferredoxin) is confirmed by the finding of four cysteine residues in the ferredoxin apoprotein; all bacterial ferredoxins examined to data have equal numbers of iron, sulfide, and cysteine (2).

The midpoint E_m (Fig. 1), measured at different *p*H values and a constant partial pressure of H_2, was −390 mv. The corresponding value of n, the number of electrons transferred, was 1.0. Thus the four-iron (sulfide) ferredoxin from *B. polymyxa* has the same potential and

TABLE 1. *Amino acid composition of Bacillus polymyxa ferredoxin*[a]

Amino acid	Amino acid residues per molecule	
	From analysis	Nearest integer
Lysine	3.25	3
Histidine	0	0
Arginine	1.25	1
Tryptophan	ND	ND
Aspartic acid	16.00	16
Threonine	5.74	6
Serine	3.10	3
Glutamic acid	8.00	8
Proline	4.12	4
Glycine	6.60	7
Alanine	10.90	11
Half-cystine[b]	4.10	4
Valine	1.95	2
Methionine	0	0
Isoleucine	7.19	7
Leucine	3.48	3-4
Tyrosine	0.57	0-1
Phenylalanine	2.09	2
Total residues		77-79
Non-heme iron[c]		4
Acid-labile sulfide[c]		4

[a] Amino acid analyses were carried out using general methods described by Yasunobu and co-workers (9) with 40 hr of hydrolysis (6 N HCl) at 110 C. Abbreviation: ND, not determined.

[b] Cysteine was determined as cysteic acid after performate oxidation of the protein sample.

[c] Atoms per molecule.

number of electrons transferred as does the eight-iron (sulfide) ferredoxin from *Clostridium* (8).

B. polymyxa ferredoxin has biological activity common to both two- and eight-iron (sulfide) ferredoxins. *B. polymyxa* ferredoxin was required for nitrogenase activity in extracts of this organism with reducing power supplied by either pyruvate (7) or illuminated chloroplasts (*unpublished data*). *B. polymyxa* ferredoxin also replaced spinach ferredoxin [a two-iron (sulfur) protein] in mediating the photochemical reduction of NADP by chloroplasts (Fig. 2). *B. polymyxa* ferredoxin was approximately 80% as effective as spinach ferredoxin (on a molar basis) in mediating this electron transfer reaction.

Bacterial ferredoxins with similar biological activities have now been isolated which contain either two, four, six (6*a*), or eight irons (sulfides) per molecule. Although intermediate to the plant and bacterial-type ferredoxins in

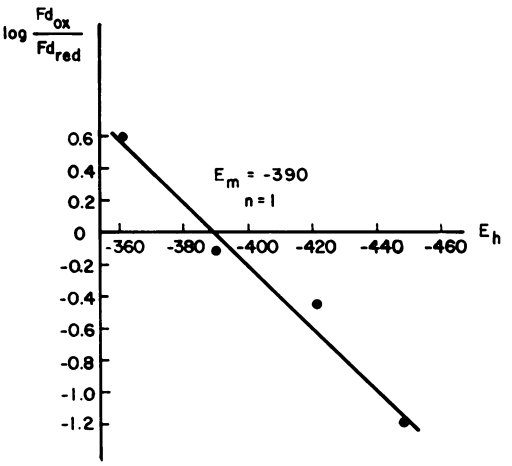

FIG. 1. *Oxidation-reduction potential (E_m) of B. polymyxa ferredoxin determined by varying the pH at a constant partial pressure of H_2. Reaction mixtures contained, in a volume of 2.0 ml: ferredoxin, absorbance at 390 nm = 0.5; HEPES buffer, pH values from 6.0 to 7.5, 500 μmoles; and clostridial hydrogenase, 0.02 ml [prepared as previously described (8)].*

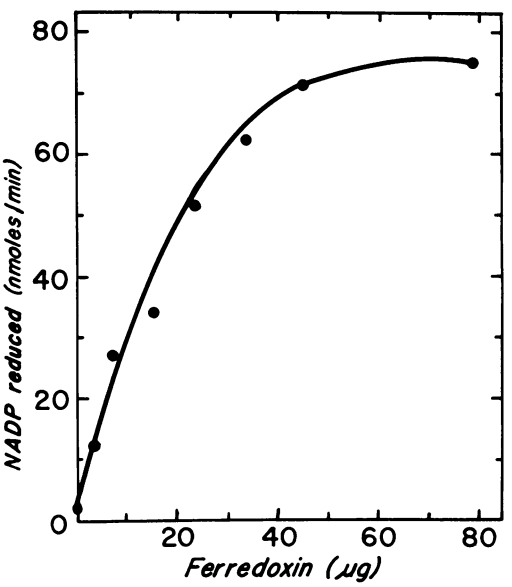

FIG. 2. *Activity of B. polymyxa ferredoxin in the photochemical reduction of NADP by spinach chloroplasts. The complete reaction mixture contained, in 1.0 ml, spinach chloroplasts (100 μg of chlorophyll) and the following in μmoles: tricine buffer, pH 8.2, 50; ascorbate, 7.5; 2,6-dichlorophenol indophenol, 0.035; $MgCl_2$, 7.5; NADP, 2; and B. polymyxa ferredoxin as indicated.*

its iron-sulfide content, *B. polymyxa* ferredoxin more closely resembles the eight-iron bacterial ferredoxins in its spectrum (7), E_m, and amino acid profile.

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