THE AMINO ACID SEQUENCE OF CLOSTRIDIUM PASTEURIANUM FERREDOXIN*

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Ferredoxin, a nonheme iron-containing protein was isolated from C. pasteurianum by Mortenson, Valentine and Carnahan (1-3). The electron transpor ing protein has also been isolated (3-5) in crystalline form (6,7) from numerou anaerobic bacteria. The latest reports on the physicochemical properties of C. pasteurianum ferredoxin indicate that the protein has a molecular weight of about 6,000,with about 50 amino acid residues, and that it may contain 7 sulfide and 7 iron atoms per mole (7,8). The amino-terminal residue has been shown to be alanine (7,9).

Due to the scarcity in the protein of peptide bonds which are hydrolyzed b trypsin and chymotrypsin and the high content of cysteine residues, this protei posed certain problems. Therefore, it was necessary to prepare S-β-aminoethyl-cysteinyl-ferredoxin (AECFd)[1], cysteicyl-ferredoxin (CFd) and S-carboxamidometh cysteinyl-ferredoxin (CAMCFd) using the procedures described by Lindley (10), as modified by Raftery and Cole (11), Moore (12) and Crestfield et al. (13), respectively. The method for the preparation of the bacteria, the method for the preparation of the iron-free ferredoxin and a few of the amino- and carboxy terminal sequences have already been published (9).

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1. The abbreviations used are: AECFd, S-β-aminoethylcysteinyl-ferredoxin; CFd, cysteicyl-ferredoxin; CAMCFd, S-carboxamidomethylcysteinyl-ferredoxin; and PTH-amino acids, phenylthiohydantoin-amino acids.

For the liberation of peptides of convenient size, AECFd was particularly useful. AECFd (58 μM) was incubated with 2% trypsin in 15 ml of water. The hydrolysis was allowed to proceed for 55 hr @ 28° during which time the solution was maintained at pH 8.0 by the manual addition of 10% trimethylamine. The reaction mixture was then lyophilized and dissolved in 16.6 ml of pyridine-acetic acid buffer, pH 3.1. The solution was applied to a Dowex 50-X2 column (2 x 108 cm) and the peptides were eluted with pyridine-acetic acid buffers as described by Margoliash (14). The peptides were purified using rechromatography on Dowex 50-X2 as described by Konigsberg and Hill (15), paper electrophoresis using pyridine-acetic acid buffer, pH 6.5, or paper chromatography as described by Margoliash (14).

In a separate experiment, 50 μM of CFd were hydrolyzed for 12 hr at 28° by pepsin (10%). The reaction mixture was lyophilized and dissolved in 15 ml of pH 7.8 pyridine-acetic acid buffer. The solution was applied to a column of Dowex AG1-X2 (2 x 100 cm) and the elution carried out as described by Schroeder et al. (16). The peptide fractions were pooled and purified as described for AECFd.

Also, 35 μM of CAMCFd were hydrolyzed for 32 hr by chymotrypsin (6%) during which time the solution was maintained at pH 8.0 by the manual addition of base. The reaction mixture was lyophilized and dissolved in 15 ml of pyridine-acetic acid buffer, pH 3.1. The digest was applied to a column of Dowex 50-X2 (2 x 100 cm) and the peptides eluted using pyridine-acetic acid buffer, pH 3.1. The peptides were further purified as described above for AECFd.

The various peptides which were isolated are summarized in Table I. The amino acid composition of the various peptides was determined by the usual procedure (17) after hydrolysis with 6N HCl for 22-48 hr. The amino acid sequence of the peptides was determined by the substractive Edman procedure (18) as well as by direct identification of the PTH-amino acids by paper chromatography (19). In certain cases, carboxypeptidase A (15) or carboxypeptidase B (11) was used to determine the carboxyl-terminal amino acids.

TABLE I

The amino acid composition and sequence of peptides from various derivatives of C. pasteurianum ferredoxin

Sequence	Peptide No.	Amino Acid Composition	Substrate
1	P-2	Alanine	CFd
2	P-3	Tyrosine	CFd
1-2	C-2	Ala-Tyr	CFd
1-2	C-9	Ala-Tyr	CFd
1-3	T-3	Ala-Tyr-Lys	CFd
1-3	T-9	Ala-Tyr-Lys	AECFd
3-30	C-II	Lys-(Ileu,Ala,Asp,Ser,CyS,Val,Ser, CyS,Gly,Ala,CyS,Ala,Ser,Glu,CyS,Pro, Val,$AspNH_2$,Ala,Ileu,Ser,$GluNH_2$,Gly, Asp_2,Ser,Ileu,Phe)	CAMCFd
3-11	C-8	Lys-(Ileu,Ala,Asp,Ser,CyS,Val,Ser,CyS)	CAMCFd
3-10	C-3-b	Lys-Ileu-Ala-(Asp,Ser,CyS,Val,Ser)	CAMCFd
4-8	T-5	Ileu-Ala-Asp-Ser-CyS	AECFd
9-11	T-6-a	Val-Ser-CyS	AECFd
12-14	T-6-b	Gly-Ala-CyS	AECFd
15-22	T-4-c	Ala-Ser-Glu-(CyS,Pro,Val)-$AspNH_2$-Ala	AECFd
15-30	T-4-p	Ala-Ser-Glu-CyS-Pro-Val-$AspNH_2$-(Ala, $Ileu_2$,Ser_2,Glu,Gly,Asp,Phe)	AECFd
23-30	T-4-a	Ileu-(Ser,Glu,Gly,Asp,Ser)-Ileu-Phe	AECFd
22-26	P-4	Ala-Ileu-Ser-$GluNH_2$-Gly	CFd

The majority of the peptides produced are as expected from the known specifi city of the proteases. Two peptides, T-4-a and T-4-p, appear to have been formed as a result of chymotryptic contamination of the trypsin preparation. It has

(TABLE I continued)

Sequence	Peptide No.	Amino Acid Composition	Substrate
23-29	P-15	Ileu-Ser-GluNH$_2$-Gly-Asp-(Ser,Ileu)	CFd
23-26	P-6	Ileu-Ser-GluNH$_2$-Gly	CFd
27-29	P-16	Asp-Ser-Ileu	CFd
27-30	P-23-II	Asp-Ser-Ileu-Phe	CFd
31-37	T-3'	Val-Ileu-Asp-Ala-Asp-Thr-CyS	AECFd
31-55	C-III	Val-(Ileu,Asp,Ala,Asp,Thr,CyS,Ileu, Asp,CyS,Gly,Asp,CyS,Ala,AspNH$_2$,Val, CyS,Pro,Val,Gly,Ala,Pro,Val,Glu,Glu)	CAMCFd
38-43	T-8	Ileu-Asp-CyS-Gly-AspNH$_2$-CyS	AECFd
44-51	T-2-P-2	Ala-AspNH$_2$-Val-(CyS,Pro,Val)-Gly-Ala	AECFd
44-55	T-2	Ala-AspNH$_2$-Val-CyS-Pro-Val-Gly-Ala-Pro-Val-(Glu,Glu)	AECFd
52-55	P-11	Pro-Val-Glu-Glu	CFd
53-55	P-18	Val-Glu-Glu	CFd
52-55	T-2-P-1	Pro-Val-GluNH$_2$-Glu	AECFd

The following symbols were adopted: P, C, and T represent peptides produced by pepsin, chymotrypsin and trypsin, respectively. Arrows to the right represent sequences determined by the Edman method (27). Arrows to the left represent sequences determined by carboxypeptidase A or B. Dashed arrows pointing to the right indicate sequences determined by dinitrophenylation.

been reported that trypsin fails to cleave the lysyl-proline peptide linkage in β-corticotrophin (20) and oxidized ribonuclease (21). The AEC-cysteinyl-proline bond in ferredoxin, which are found in peptides T-4-p and T-2-P-2, are also resistant to tryptic hydrolysis. Furthermore, peptide T-8 appeared anomalous in that the AEC-cysteinyl-glycine peptide linkage was not hydrolyzed by trypsin. However, the Edman procedure clearly gave the results presented.

From the structures of the various peptides isolated, it becomes possible to propose the amino acid sequence of C. pasteurianum ferredoxin which is shown in Fig. 1. The amino-terminal analysis of ferredoxin indicated that alanine was at the amino terminus (9). By considering peptides T-3, C-II and C-III, the fifty-five amino acids are accounted for. Since there is only one lysine residue in the molecule, C-II must be adjacent to T-3. Therefore, C-III must represent the carboxy-terminal peptide. Also, since there is only one phenylalanine residue per molecule of protein, C-II and C-III must be adjacent to one another. The problem now consisted of obtaining fragments from C-II and C-III with the desired overlaps. As far as C-II is concerned, peptides C-8, C-3-b, T-5, T-6-a, T-6-b, T-4-c, T-4-p, P-4, P-15, P-6, P-16 and P-23-II provide the necessary sequence and overlaps. Peptide T-6-b occurs only once in the molecule and it must, therefore, be located between peptides C-8 and T-4-p in order to satisfy the amino acid composition of C-II. In the case of peptide C-III, fragments T-3', T-8, T-2-P-2, T-2, P-11 and P-18 provides the necessary sequence and overlaps. The penultimate amino acid in the carboxyl-terminal portion is glutamine. It was erroneously reported to be serine (9) due to the fact that the positions of gluta-

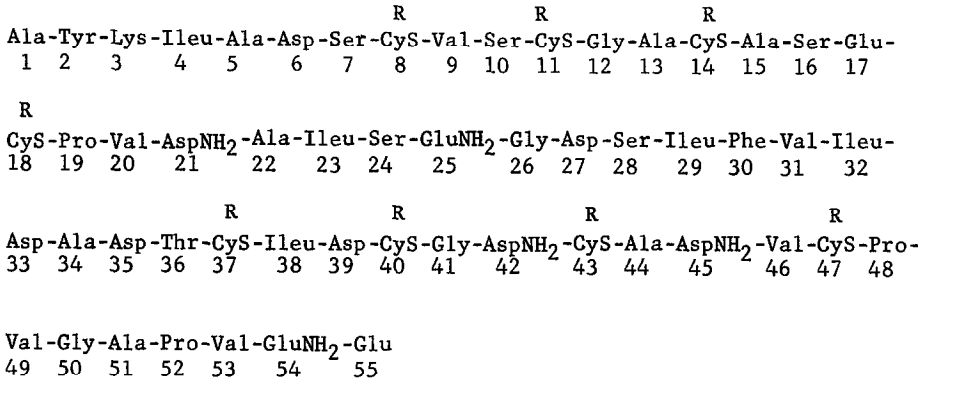

Figure 1 The amino acid sequence of Clostridium pasteurianum ferredoxin. The R radicals represent sulfide and ferric ions which are attached in an unknown manner to the cysteine residues.

mine and serine are identical on the chromatogram obtained with the automatic amino acid analyzer. In peptides P-11 and P-18, the glutamine residue was deaminated but was intact in the case of peptide T-2-P-1.

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