THE PRIMARY STRUCTURE OF *CLOSTRIDIUM PERFRINGENS* FERREDOXIN

YASUhide SEKI,* SACHIKO SEKI, AND MAKOTO ISHIMOTO†

*Department of Chemical Microbiology, Faculty of Pharmaceutical Sciences,
Hokkaido University, Kita-ku, Sapporo, Hokkaido 060, Japan*

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The complete amino acid sequence of ferredoxin from *Clostridium perfringens* was established by a combination of Edman degradation and protease digestion. The sequence was: AYKILDTCVSCGACAAECPV-DAISQGDTQFVIDADTCIDCGNCANVCPVGAPVQE. It shows a high degree of similarity to *Clostridium pasteurianum* ferredoxin (89% homology), and its residual conversions are located at the non-conservative sites of the clostridial-type ferredoxins: at 6 sites, discrete except for the last two, all along the amino(N)-terminal half of the chain, while none at the carboxyl(C)-terminal half (residue numbers from 30 to 55). The occurrence of the 8 cysteine residues at the conservative sites strongly suggested the presence of the conventional two [4Fe-4S] clusters in the molecule.

Bacterial ferredoxins are iron-sulfur proteins with low molecular weights of 6,000 to 15,000 (6). Of these, the first and best characterized is that from an anaerobic bacterium *Clostridium pasteurianum* (5). The clostridial-type ferredoxin consists of 55 amino acid residues, 8 iron atoms, and 8 acid-labile sulfurs per molecule of the protein. It contains 8 cysteine residues and has two [4Fe-4S] clusters.

Ferredoxin acts as an electron donor for nitrate reductase in *Clostridium perfringens* (9). In a previous paper (10), we tentatively postulated that a ferredoxin isolated from the bacterium contained a single [4Fe-4S] cluster instead of the two, based on the evaluations of four atoms of iron, four atoms of acid-labile sulfur, and six half-cystine residues per molecule of the protein. In this paper we report primary structure of *C. perfringens* ferredoxin and propose the presence of two [4Fe-4S] clusters in the molecule.

* Address reprint requests to: Dr. Yasuhide Seki, Department of Chemical Microbiology, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060, Japan.

† Present address: Sengoku 3-37-10, Bunkyo-ku, Tokyo 112, Japan.

Abbreviations: PE-Fd, pyridylethyl-apoferredoxin; N-, amino; and C-, carboxyl.

MATERIALS AND METHODS

Materials. *Clostridium perfringens* was harvested from a GAM culture supplemented with nitrate (11), and ferredoxin was isolated from the cells as previously described (10). Ferredoxin was further purified by HPLC (CCPD system, Tosoh) on a TSK-Phenyl-5PW RP column (0.46×7.5 cm) with a linear concentration gradient of from 10 to 90% (v/v) acetonitrile containing 0.05% trifluoroacetic acid, monitored by the absorbance at 220 nm. It was finally purified by reverse-phase HPLC on a TSK-ODS-120T column (0.46×25 cm) in 0.05% trifluoroacetic acid with a linear concentration gradient from 10 to 36% acetonitrile.

Staphylococcal V8 protease [EC 3.4.21.19] was purchased from Boehringer Mannheim GmbH.

Fragmentation of ferredoxin. The ferredoxin was pyridylethylated with 4-vinylpyridine (4) and the pyridylethyl-apoferredoxin (PE-Fd) was isolated by HPLC with a Phenyl-5PW column and purified on an ODS-120T column. To the purified PE-Fd (about 5 nmol) dried under reduced pressure was added 96 μ l of 50 mM potassium phosphate buffer, pH 7.8, and 4 μ l of aqueous staphylococcal V8 protease solution (50 ng/ μ l), and the mixture was incubated for 12 h at 37°C. After further addition of 10 μ l of the V8 protease solution and another 9 h of incubation, the digest was added with 0.9 ml of the solution composed of 0.05% trifluoroacetic acid and 10% acetonitrile. The resulting peptides were separated on an ODS-120T column by a gradient of acetonitrile from 10 to 45%.

Amino acid analysis. The amino acid compositions of PE-Fd and derived peptides were determined with a Hitachi-835 autoanalyzer system after 14–16 h hydrolysis with 6 N HCl. The *o*-phthalaldehyde method (3) was employed when the amounts of the subjected samples were present only in an order of pmol.

The N-terminal sequences of the proteins and derivative peptides were determined by an automated protein sequencer (Applied Biosystems 477A), amino acids being determined as the phenylthiohydantoin-derivatives.

RESULTS AND DISCUSSION

The complete amino acid sequence of the ferredoxin from *C. perfringens* is shown in Fig. 1. The amino acid composition calculated from the sequence agreed

Fig. 1. The amino acid sequence of *C. perfringens* ferredoxin.

V- refers to peptides derived by V8 protease digestion and the additional numbering indicates the order of elution by HPLC (see Fig. 2). The arrows (→) represent the results of automated Edman degradation.

Fig. 2. The elution pattern of the V8 protease fragments of PE-Fd. The chromatographic conditions are described in MATERIALS AND METHODS.

Table 1. Amino acid compositions of peptides obtained by V8 protease digestion.

	V-2	V-7
Cys	(3)	(2)
Asp	2.1(2)	4.5(5)
Thr		1.4(2)
Ser		1.0(1)
Glu	1.9(2)	1.8(2)
Gly	2.2(2)	1.4(1)
Ala	1.8(2)	1.6(2)
Val	2.7(3)	1.5(2)
Ile		2.3(3)
Phe		1.2(1)
Pro	1.3(2)	0.7(1)
Position	40–55	18–39

Values in parentheses are deduced from sequences.

fairly well with that determined previously (10). Since the close resemblance of the composition with that of *C. pasteurianum* ferredoxin suggested also a very close resemblance in their primary structure, the construction of the final sequence relied largely on the alignments of the peptides to fit the corresponding sequence of *C. pasteurianum* ferredoxin.

The amino acid sequence of the N-terminal region of *C. perfringens* ferredoxin as isolated could be determined up to the 34th residue by automated Edman degradation except those corresponding to cysteine residues in *C. pasteurianum* ferredoxin. Partial digestion of PE-Fd with V8 protease at pH 7.8 for 21 h gave more than 12 fractions on HPLC (Fig. 2). Seven major fractions were analyzed for

Cpa, *Cb*, *Cau*, *Cl*, *Cth*, CME, and *Pa* are abbreviations for *C. pasteurianum*, *C. butyricum*, *C. aciduri*, *C. tartarivorum*, *C. thermosaccharolyticum*, *Clostridium* species ME, and *Peptococcus aerogenes*, respectively. The residues that were identical (including the gaps) within all eight sequences are boxed, and the residues identical in *C. perfringens* and *C. pasteurianum* ferredoxins are boxed with bold lines.

activity as electron donor for nitrate reductase with *C. perfringens* ferredoxin than those with *C. pasteurianum* ferredoxin (10).

Of the seven ferredoxins compared here, *C. perfringens* ferredoxin is most closely related to *C. pasteurianum* ferredoxin. Between these two strains, 6 residues out of the 55 varied only in the N-terminal halves of the ferredoxin molecules. The latter C-terminal halves (residues 30–55) were identical. This is the solitary exception among *Clostridia* with leucine residue at position 5 (and Thr at 7) because even though *C. butyricum* ferredoxin has a C-half almost identical to the *C. pasteurianum* ferredoxin, there exists one replacement at position 53 (Asn in *C. butyricum* ferredoxin instead of Val in *C. pasteurianum* ferredoxin). It is interesting that the “connector” (residues at position 27–29 in this case), which plays an important role other than just providing an arm for the connection of the first (residues 1–26) and the second (30–55) units in the functional ferredoxin(7), has a sequence of Asp-Thr-Gln (27–29) in the *C. perfringens* ferredoxin just as in *C. butyricum* ferredoxin. On the other hand it is Asp-Ser-Ile (27–29) in *C. pasteurianum* ferredoxin. These are the only successive variations observed between *C. perfringens* ferredoxin and *C. pasteurianum* ferredoxin (the other 4 variations occur not successively but dispersively).

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