THE AMINO ACID SEQUENCE OF FERREDOXIN FROM THE SULFATE REDUCING BACTERIUM, *DESULFOVIBRIO GIGAS*[1]

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Summary: The primary structure of a ferredoxin from the sulfate reducing bacterium, *Desulfovibrio gigas* has been determined by conventional procedures. The molecule is unique among bacterial ferredoxins in that it contains only six cysteine residues, four in the first half and two in the second half of the sequence. Preliminary structural considerations suggest that it may be derived from a prototype ferredoxin intermediate between green plants and bacteria.

Some chemical and physical properties of a ferredoxin isolated from the sulfate reducing bacterium, *Desulfovibrio gigas* have been previously reported (1,2). This ferredoxin has a molecular weight and spectrum typical of that shown by ferredoxins isolated from other non-photosynthetic anaerobes but atypically contains two methionine residues, four atoms each of iron and sulfide and six residues of cysteine per molecule. Because of this unusual composition and the unique physiology of the sulfate reducing bacteria (3), the primary structure of this ferredoxin was of intense interest from both evolutionary and biochemical aspects. In this communication we wish to briefly describe our procedures and present the complete amino acid sequence of this ferredoxin. Details of our methods will be reported separately.

Materials and Methods

Desulfovibrio gigas was grown and the ferredoxin purified by previously described methods (2,4). Oxidized ferredoxin was prepared by precipitation of the apo-protein with 10% trichloroacetic acid (5) followed by oxidation with performic acid (6). Tryptic peptides were obtained by digestion of the oxidized

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protein with TPCK-treated porcine trypsin. Cyanogen bromide fragmentation was performed by treatment of the apo-protein with CNBr (7) followed by performic acid oxidation.

Tryptic and cyanogen bromide fragments could not be easily separated by ion-exchange chromatography because of their strongly acidic nature. Therefore, in all cases, peptides from individual digests were purified by high voltage paper electrophoresis at pH 3.7 (8), followed by elution of the separated components with 1% pyridine. In order to unambiguously determine the sequence of individual peptide components and to provide overlap with other peptides, chymotryptic, peptic, subtilisin, and acetic acid hydrolysis was sometimes required. Individual peptides were sequenced by a combination of techniques including Edman degradations, aminopeptidase and carboxypeptidase A and B digestion, and hydrazinolysis.

Results and Discussion

Amino Acid Composition: The initial fragmentation of the ferredoxin from *D. gigas* was accomplished by either cyanogen bromide cleavage of apo-ferredoxin or tryptic digestion of the oxidized protein. Chymotryptic digestion was not utilized as a primary step because of its inability to cleave the one theoretically susceptible peptide bond provided by the single leucine residue. The composition of each of these peptides together with the composition obtained by complete hydrolysis of the native enzyme is given in Table 1. This composition is slightly different from that reported previously (2) and results from a small contaminating peptide which frequently co-chromatographed with the ferredoxin during its isolation.

Sequences of Tryptic and CNBr Peptides: The sequences of the six major peptides (Table 1) obtained by tryptic digestion and cyanogen bromide cleavage which were used in determining the structure of this ferredoxin are shown in Tables 2 and 3. Sequences of smaller peptides whose structures were required to complete the structure of each of the larger fragments are also included in these tables. It was found that CN-1 and CN-2 provided the best starting mate-

Table 1

The Amino Acid Composition of *D. gigas* Ferredoxin and its Constitutive Peptides

	F_d	T_1	T_2	T_3	C_1	C_2	C_3
LYS	1	1	0	0	0	0	1
HIS	0	0	0	0	0	0	0
ARG	1	0	1	0	0	0	1
ASP	11	6	5	0	2	2	7
THR	0	0	0	0	0	0	0
SER	3	0	2	1	0	0	3
GLU	9	6	3	0	1	3	5
PRO	4	2	2	0	1	1	2
GLY	1	1	0	0	0	0	1
ALA	6	2	4	0	0	2	4
VAL	5	3	2	0	1	2	2
CYS (As Cysteic Acid)	6	2	4	0	1	3	2
MET or MET SO_2	2	2	0	0	0	0	0
ILEU	5	2	3	0	1	1	3
LEU	1	1	0	0	0	0	1
TYR	0	0	0	0	0	0	0
PHE	1	1	0	0	0	1	0
HOMOSER or HOMOSER Lactone	0	0	0	0	1	1	0
TOTAL	56	29	26	1	8	16	32

Fd; oxidized protein: T_{1-3}; tryptic peptides: C_{1-3}; cyanogen bromide peptides.

rials for elucidating the structure of the first half of the ferredoxin molecule because of their smaller size relative to T-1. Similarly, T-2 provided most of the data for the structure of the remainder of the molecule since CN-3 was larger in size. Finally, digestion of CN-3 with trypsin yielded both T-2 and a frag-

Table 2

Sequences of Tryptic Peptides Utilized in Deriving the Structure of Ferredoxin from *D. gigas*

T-1	PRO-ILE-GLN-VAL-ASP-ASN-CYS-MET-ALA-CYS-GLN-ALA-CYS-ILE-ASN-GLU-CYS-PRO-VAL-ASP-VAL-PHE-GLN-MET-ASP-GLU-GLN-GLY-ASP-LYS
T-1,C-1	PRO-ILE-GLN-VAL-ASP-ASN-CYS-MET-ALA-CYS-GLN-ALA-CYS-ILE-ASN-GLU-CYS-PRO-VAL-ASP-VAL-PHE
T-1,C-2	GLN-MET-ASP-GLU-GLN-GLY-ASP-LYS
T-2	ALA-VAL-ASN-ILE-PRO-ASN-SER-ASN-LEU-ASP-ASP-GLN-CYS-VAL-GLU-ALA-ILE-GLN-SER-CYS-PRO-ALA-ALA-ILE-ARG
T-2,P-1	ASP-GLN-CYS-VAL-GLU
T-2,P-2	ASP-GLN-CYS-VAL-GLU-ALA
T-2,P-3	ILEU-GLN-SER-CYS-PRO-ALA
T-2,P-4	ALA-ILE-GLN-SER-CYS-PRO-ALA
T-2,P-5	ALA-ILE-GLN-SER-CYS-PRO-ALA-ALA
T-2,P-6	ALA-VAL-ASN-ILE-PRO-ASN-SER-ASN-LEU
T-2,P-7	ALA-ILE-ARG
T-2,S-1	SER-ASN-LEU-ASP-ASP-GLN-CYS-VAL
T-2,S-2	SER-CYS-PRO-ALA

T; trypsin: C; chymotrypsin: P; pepsin: S; subtilisin.

ment, CN-3-T-1, which contained the residues not provided for by CN-1, CN-2, and T-2. The remainder of the large fragments were used mainly to provide over lapping sequences.

Primary Structure of the Ferredoxin from *D. gigas*: Dinitrophenylation and Edman degradation of oxidized ferredoxin yielded proline as the amino terminus of this protein, while hydrazinolysis yielded only free serine as the carboxyl terminal. Based on these results as well as those from overlapping sequences, the order of tryptic and cyanogen bromide peptides were easily placed to give

Table 3

Sequences of Cyanogen Bromide Peptides Utilized in Deriving the Structure of Ferredoxin from *D. gigas*

CN-1	PRO-ILE-GLN-VAL-ASP-ASN-CYS-HSER
CN-1, S-1	ASP-ASN-CYS-HSER
CN-2	ALA-CYS-GLN-ALA-CYS-ILE-ASN-GLU-CYS-PRO-VAL-ASP-VAL-PHE-GLN H-SER
CN-2, C-1	ALA-CYS-GLN-ALA-CYS-ILE-ASN-GLU-CYS-PRO-VAL-ASP-VAL-PHE
CN-2, C-2	GLN-HSER
CN-2, S-1	ALA-CYS-GLN
CN-2, S-2	ALA-CYS-ILE-ASN-GLU-CYS-PRO-VAL-ASP-VAL-PHE-GLN-HSER
CN-2, S-2, A-5	VAL-PHE-GLN-HSER
CN-2, S-2, A-3	ALA-CYS-ILE-ASN-GLU-CYS-PRO-VAL-ASP-VAL
CN-2, S-2, A-1	GLU-CYS-PRO-VAL-ASP
CN-3	ASP-GLU-GLN-GLY-ASP-LYS-ALA-VAL-ASN-ILE-PRO-ASN-SER-ASN-LEU-ASP-ASP-GLN-CYS-VAL-GLU-ALA-ILE-GLU-SER-CYS-PRO-ALA-ALA-ILE ARG-SER
CN-3, T-1	ASP-GLU-GLN-GLY-ASP-LYS

CN; Cyanogen bromide: S; subtilisin: C; chymotrypsin: A; acetic acid.

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the structure of the ferredoxin from *D. gigas* shown in Table 4.

Analysis of the primary structures of bacterial ferredoxins has resulted in the conclusions that this group of non-heme iron proteins is evolutionarily related and that gene duplication was a major event in the evolution of known sequences (9,10). Comparison of the primary structure of the ferredoxin from *D. gigas* with the sequences of other bacterial ferredoxins (5,11,12,13,14,15) indicates that a strong degree of homology exists from residues 1-29, the first half of the molecule. The second half of the sequence is unique in containing only two cysteine residues and homology with either its own first half or the

Table 4

Primary Structure of Ferredoxin from *D. gigas*

NH_2-PRO-ILE-GLN-VAL-ASP-ASN-CYS-MET-ALA-CYS-GLN-ALA-CYS-ILE-ASN-GLU-CYS-

PRO-VAL-ASP-VAL-PHE-GLN-MET-ASP-GLU-GLN-GLY-ASP-LYS-ALA-VAL-ASN-ILE-PRO-

ASN-SER-ASN-LEU-ASP-ASP-GLN-CYS-VAL-GLU-ALA-ILE-GLN-SER-CYS-PRO-ALA-ALA-

ILE-ARG-SER-COOH

second halves of other bacterial ferredoxins is not as high as that usually observed; however, the ferredoxin does appear to have been evolved after gene duplication occurred. Curiously, portions of this new sequence exhibit an unusually high degree of homology with the ferredoxins from green plants and a detailed analysis of the structure will be reported in a future communication.

Finally, ferredoxins (10,16) have been classified into groups I, II and III based on both physical and chemical data. In the light of the properties of the ferredoxin from *D. gigas* it would seem that this classification should be re-evaluated in order to account for the low iron, sulfur, and unusual amino acid sequence of this ferredoxin.

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