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The Amino Acid Sequence of Ferredoxin II from *Chlorobium limicola,* a Photosynthetic Green Bacterium†

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ABSTRACT: The amino acid sequence of ferredoxin II from the photosynthetic green sulfur-reducing bacterium, *Chlorobium limicola,* was deduced to be: Ala-His-Arg-Ile-Thr-Glu-Glu-Cys-Thr-Tyr-Cys-Ala-Ala-Cys-Glu-Pro-Glu-Cys-Pro-Val-Asn-Ala-Ile-Ser-Ala-Gly-Asp-Glu-Ile-Tyr-Ile-Val-Asp-Glu-Ser-Val-Cys-Thr-Asp-Cys-Glu-Gly-Tyr-Tyr-Asp-Glu-Pro-Ala-Cys-Val-Ala-Val-Cys-Pro-Val-Asp-Cys-Ile-Ile-Lys-Val. The ferredoxin was shown to consist of 61 amino acids in a single polypeptide chain. The presence of 8 g-atoms of Fe and 8 mol of sulfide led to a calculated molecular weight of 7289. In contrast to the ferredoxin I from *C. limicola,* ferredoxin II contains basic amino acids in positions 2 and 3 and 60 from the NH_2-terminal end of the protein. The sequences of all the various ferredoxins from photosynthetic bacteria reported to date are compared with one another.

The amino acid sequence of ferredoxin I, one of the two types of ferredoxin present in the photosynthetic green sulfur-reducing bacterium, *Chlorobium limicola,* has been determined (Tanaka et al., 1974). The amino acid sequence of ferredoxin from a photosynthetic, but purple sulfur-reducing bacterium, *Chromatium,* has also been reported by Matsubara et al. (1970). From the comparison of the amino acid sequences of the *Chlorobium limicola* ferredoxin I and the *Chromatium* ferredoxin, it was pointed out that both ferredoxins show great sequence homology although the latter contains 21 additional amino acids at the C-terminal end of the protein.

Both of these ferredoxins are of the 8 Fe, 8 S^{2-}-type in which the iron is probably chelated to 8 cysteine residues to form two iron–sulfur clusters which contain 4 Fe and 4 S^{2-} each (Adman et al., 1973).

Now the amino acid sequence of ferredoxin II, the other ferredoxin in the photosynthetic green bacterium, *Chlorobium limicola,* has been determined. The present report gives the details of the research which has led to the elucidation of the primary structure of the *Chlorobium limicola* ferredoxin II.

Materials and Methods

Chlorobium limicola Ferredoxin II. The preparation of ferredoxin from a pure culture of *C. limicola* and the procedures for the isolation and purification of the CM[1] derivative of *C. limicola* ferredoxin II have already been described (Tanaka et al., 1974). The *C. limicola* CM-ferredoxin II was then further purified by column rechromatography on Dowex 1-X2 and was used in the present sequence studies.

Chymotrypsin was obtained from the Worthington Biochemical Corp. as a three-times crystallized preparation. L-(1-Tosylamido-2-lysyl)ethyl chloromethyl ketone was purchased from Cyclo Chemical.

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[1] Abbreviations used are: CM, carboxymethyl; PTH, phenylthiohydantoin.

Sequanal grade protein sequenator reagents and solvents (including 5% phenyl isothiocyanate in heptane, 1.0 *M* Quadrol–trifluoroacetic acid buffer (pH 9.0), anhydrous *n*-heptafluorobutyric acid, benzene, ethyl acetate, and 1-chlorobutane) were purchased from the Pierce Chemical Co.

The sources of the other reagents used in the present investigation have been described in previous reports from our laboratory (Tanaka et al., 1973).

Amino Acid Analyses. Purified samples of the protein and peptides were hydrolyzed in 6 *N* HCl at 110° for 24, 48, or 72 hr in sealed, evacuated Pyrex glass tubes. The amino acid composition of the protein and peptides were determined on their 6 *N* HCl hydrolysates in a Beckman-Spinco Model 120 C automatic amino acid analyzer as described by Spackman et al. (1958). The instrument was equipped with high sensitivity cuvets and a 4–5 mV full scale range card.

Amino Acid Sequence Determinations. The NH_2-terminal sequences of the CM-ferredoxin were determined by the Beckman-Spinco Model 890 protein/peptide sequencer utilizing the protein double cleavage program. Prior to the sequencer analysis, peptide CT-5 was reacted with 4-sulfophenyl isothiocyanate (Braunitzer et al., 1970). The sequencer run was carried out on both the 4-sulfophenylthiocarbamyl derivative of the peptide CT-5 and the underivatized peptide CT-5. The NH_2-terminal sequences of all the other peptides were obtained by the usual manual Edman degradation method (Edman and Sjoquist, 1956). Amounts of material used in sequence studies for both the sequencer run and the manual Edman reaction varied from 170 to 250 nmol. The phenylthiohydantoins of the amino acids were identified by gas chromatography in a Beckman GC-45 gas chromatograph as described by Pisano and Bronzert (1969), or by thin-layer chromatography as described by Edman and Begg (1967), or by amino acid analyses of the 6 *N* HCl hydrolysates of the amino acid phenylthiohydantoins (Van Orden and Carpenter, 1964). In the present sequence studies, the quantitative determination and identification methods for PTH-amino acids were selected as follows. (1) A combination of gas chromatography and amino acid analysis of the 6 *N* HCl hydrolysates was used for the identification of PTH-alanine, PTH-valine, PTH-proline, PTH-isoleucine, and PTH-leucine. (2) A combination of gas chromatography and thin-layer chromatography was carried out for the identification of PTH-carboxymethylcysteine, PTH-serine, and PTH-threonine. (3) Both amino acid analyses of 6 *N* HCl hydrolysates and thin-layer chromatography were used for the identification of PTH-aspartic acid, PTH-asparagine, PTH-glutamic acid, and PTH-glutamine. (4) Only amino acid analysis of acid hydrolysates was used for the identification of PTH-lysine, PTH-histidine, and PTH-arginine. (5) All three methods were used for identifying PTH-glycine and PTH-tyrosine. Prior to the sequence studies, amounts of the protein and peptides to be sequenced were quantitatively determined by amino acid analyses of their 6 *N* HCl hydrolysates and theoretical yields were calculated in order to measure the recovery of PTH-amino acids in both the sequencer run and the manual Edman reaction. The average repetitive yields in the Edman reaction are shown in each experimental table. In the present investigation, carryover of PTH-amino acids was below 5% at each step. No problems of assignment were observed throughout the sequence determinations. The COOH-terminal amino acids of the protein and peptides were determined by hydrazinolysis (Bradbury, 1958).

Table I: Amino Acid Compositions of *Chlorobium limicola* Ferredoxins, I and II.

Amino Acid	Ferredoxin II: From Analysis Data[a]	Ferredoxin II: From Sequence Studies	Ferredoxin I[b]
Lysine	1.01 (1)	1	0
Histidine	0.95 (1)	1	0
Arginine	0.94 (1)	1	0
Aspartic acid	6.02 (6)	6[c]	6[d]
Threonine	3.02 (3)	3	4
Serine	1.76 (2)	2	1
Glutamic acid	8.05 (8)	8[e]	9[f]
Proline	3.92 (4)	4	3
Glycine	2.08 (2)	2	4
Alanine	6.97 (7)	7	9
Cysteine[g]	8.82 (9)	9	9
Valine	7.02 (7)	7	5
Isoleucine	5.84 (6)	6	5
Leucine	(0)	0	2
Tyrosine	4.00 (4)	4	3
Total residues	61	61	60
Molecular weight[h]		7289	6919

[a] Acid hydrolyses were performed on CM-ferredoxin for 24, 48, and 72 hr at 110° with 6 *N* HCl. The amino acid residues were calculated on the basis of a tyrosine content of 4.00 mol/mol of protein. Extrapolations were made for threonine and serine. Values for valine, isoleucine, and leucine were taken from 72-hr hydrolysates. Values in parentheses indicate values rounded off to nearest whole number. [b] Taken from reference of Tanaka et al. (1974). [c] Sum of five aspartic acid and one asparagine. [d] Sum of four aspartic acid and two asparagine. [e] No glutamine exists. [f] Sum of seven glutamic acid and two glutamine. [g] Determined as CM-cysteine. [h] Intact protein including 8 iron and 8 sulfide.

Chymotrypsin Digestion, Chromatography of the Digest, and Further Purification of the Peptides. About 2.5 μmol of CM-ferredoxin II was digested with chymotrypsin (enzyme to substrate was 1:30, w/w) at pH 8.0 in a total volume of 1.0 ml. Chymotrypsin used in this study was obtained from the Worthington Biochemical Corp. as a three times crystallized preparation. Prior to the use, chymotrypsin was treated with L-(1-tosylamido-2-lysyl)ethyl chloromethyl ketone (Mares-Guia and Shaw, 1963). Additional chymotrypsin was added at 6 hr and the digestion was carried out at 28° for 20 hr. The N_2-dried chymotryptic digest of CM-ferredoxin was applied to a Dowex 1-X2 column (1.0 × 20 cm). The digestion mixture on the column was eluted by a linear gradient elution technique by mixing 200 ml of water in the mixing chamber and 200 ml of 6.0 *M* acetic acid in the reservoir. The flow rate was 60 ml/hr. Fractions of 4.5 ml were collected and an aliquot of each fraction was assayed by alkaline ninhydrin procedure (Crestfield et al., 1963). The peptides, after column chromatography, were further purified by paper electrophoresis in a pyridine–acetic acid–water buffer (the pH of 10% pyridine solution was adjusted to 6.50 with glacial acetic acid) or by paper chromatography (1-butanol–pyridine–acetic acid–water; 60:40:12:48, v/v), or by rechromatography on Dowex 1-X2.

Peptide Nomenclature. Peptides obtained from the chymotryptic hydrolysis of the CM-ferredoxin II are designated by the symbol CT.

Results

Amino Acid Composition and End Group Analysis. The

Table II: Amino Acid Composition[a] and Properties of Chymotryptic Peptides of CM-ferredoxin.

Amino Acid	CT-1[c]	CT-2	CT-3	CT-4	CT-5[d]	CT-6	CT-7	CT-8[e]	Total Residues[b]
Cysteine[f]	0.92 (1)		0.85 (1)	2.86 (3)	4.80 (5)	1.82 (2)	2.84 (3)	1.86 (2)	9
Aspartic acid				2.08 (2)	4.05 (4)	1.83 (2)	2.06 (2)	0.97 (1)	6
Threonine	1.89 (2)		1.92 (2)		0.90 (1)	0.90 (1)			3
Serine				0.83 (1)	0.85 (1)	0.81 (1)			2
Glutamic acid	2.01 (2)		2.09 (2)	2.96 (3)	3.03 (3)	1.88 (2)	1.03 (1)		8
Proline				2.01 (2)	1.92 (2)		1.86 (2)	0.84 (1)	4
Glycine				1.00 (1)	1.00 (1)	1.00 (1)			2
Alanine	1.00 (1)	1.00 (1)		3.87 (4)	1.95 (2)		2.00 (2)		7
Valine				0.93 (1)	5.85 (6)	1.86 (2)	3.88 (4)	2.84 (3)	7
Isoleucine	0.96 (1)		0.97 (1)	1.94 (2)	2.85 (3)	0.97 (1)	1.86 (2)	2.01 (2)	6
Tyrosine	0.95 (1)		0.98 (1)	1.00 (1)	2.00 (2)	1.00 (1)	1.00 (1)		4
Lysine					0.96 (1)		0.92 (1)	1.00 (1)	1
Histidine	0.91 (1)	0.86 (1)							1
Arginine	1.01 (1)		1.00 (1)						1
Total residues	10	2	8	20	31	13	18	10	61
Recovery (%)	32	27	29	66	44	35	29	10	
R_f[g]	0.31	0.26	0.41	0.45	0.55	0.31	0.45	0.52	
Color reaction with ninhydrin	Violet	Violet	Violet	Yellow to Violet	Violet	Violet	Violet	Violet	
Purification method[h]	PAW and BPAW	PAW and BPAW	BPAW	Dowex 1 and BPAW	Dowex 1 and BPAW	BPAW	BPAW	BPAW	

[a] Results from samples hydrolyzed for 24 and 48 hr in 5.7 *N* HCl. The numbers in parentheses refer to the assumed stoichiometric number of residues per molecule of pure peptide. [b] Sum of the peptides, CT-1, CT-4, and CT-5. [c] Sum of the peptides, CT-2 and CT-3. [d] Sum of the peptides, CT-6 and CT-7. [e] Unusual chymotryptic peptide which was liberated by the cleavage of an Ala-Val linkage. [f] Determined as CM-cysteine. [g] Paper chromatography with 1-butanol–pyridine–acetic acid–water (60:40:12:48, v/v). [h] The abbreviations used are: PAW, paper electrophoresis in the electrolyte system, pyridine–acetic acid–water (pH of a 10% pyridine solution was adjusted to 6.50 with glacial acetic acid); BPAW, paper chromatography in the solvent system, 1-butanol–pyridine–acetic acid–water (60:40:12:48, v/v); and Dowex 1, rechromatography on a Dowex 1-X2 column.

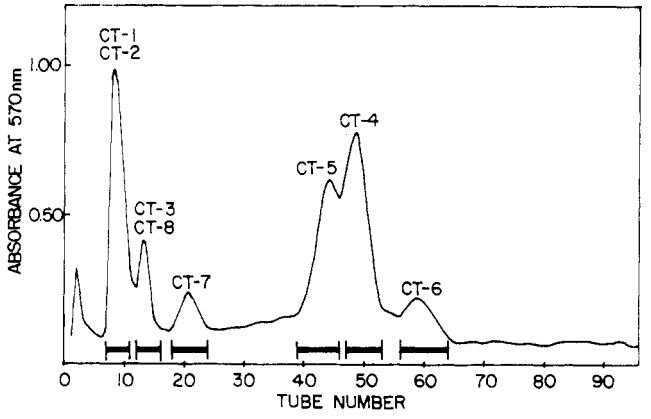

FIGURE 1: Dowex 1-X2 column chromatography of the chymotryptic peptides of CM-ferredoxin II. See Experimental Section for details of experimental conditions used. Fractions under each peak which were pooled are indicated by solid bars.

amino acid composition of the *C. limicola* ferredoxin II was obtained from 24-, 48- and 72-hr hydrolysates of the CM-ferredoxin. The results are summarized in Table I along with the amino acid composition data of *C. limicola* ferredoxin I previously reported by us (Tanaka et al., 1974). The NH_2-terminal amino acid of the *C. limicola* ferredoxin II was shown to be alanine from the sequencer analysis. The COOH-terminal amino acid of the ferredoxin II was determined to be a valine (75% yield) by hydrazinolysis.

Isolation and Purification of Chymotryptic Peptides. The chymotryptic digest of CM-ferredoxin II was chromatographed on a column of Dowex 1-X2 as shown in Figure 1. A total of six main peaks were detected by the ninhydrin assay method after alkaline hydrolysis. The first peak contained two peptides, CT-1 and CT-2. Paper electrophoresis of peak 1 peptides in pyridine–acetic acid–water (pH 6.50) separated the acidic peptide CT-1 and the basic peptide CT-2. Both peptides were further purified by paper chromatography in 1-butanol–pyridine–acetic acid–water. The second peak also contained two peptides, CT-3 and CT-8, and both of the peptides were purified by paper chromatography in 1-butanol–pyridine–acetic acid–water. The peptides present in the fourth (CT-5) and fifth (CT-4) peaks were first purified by rechromatography on columns of Dowex 1-X2 and were then further purified by paper chromatography. The peptides in the third (CT-7) and sixth (CT-6) peaks also were purified by paper chromatography. The amino acid compositions and properties of these peptides are summarized in Table II.

Sequencer Results of CM-ferredoxin II. The sequence analyses of the *C. limicola* CM-ferredoxin II (250 nmol) were determined twice in the Beckman-Spinco protein sequencer (protein double cleavage program was used). It was possible to determine the first 39 residues from the amino-terminal end of the protein. A typical result, which was obtained in one of the two separate sequencer runs of the CM-ferredoxin II, is summarized in Table III.

Sequencer Analyses of Peptide CT-5 (Residues 31–61). The sequence analyses of the peptide CT-5 were carried out on both the 4-sulfophenylthiocarbamyl derivative of the peptide (200 nmol) and the underivatized peptide (200 nmol). In both cases, the conditions of the sequencer run were same as described above for the CM-ferredoxin except for use of 0.25 *M* Quadrol in place of 1.0 *M* Quadrol (Braunitzer et al., 1972). In the case of the sequencer run of the 4-sulfophenylthiocarbamyl derivative of peptide, the 4-sulfophenylthiohydantoin from the NH_2-terminal residue was not quantitatively extracted by the organic solvents because of its strong acidic property and, therefore, the yield

Table III: Amino-Terminal Amino Acid Sequence Analysis of the *Chlorobium limicola* Ferredoxin II.

Step No.	Sequence[a]	Yield[b] (%)	Identification Method[c]		
1	Alanine	98	GC	HYD	
2	Histidine	88	HYD		
3	Arginine	30	HYD		
4	Isoleucine	70	GC	HYD	
5	Threonine	52	GC	TLC	
6	Glutamic acid	50	HYD	TLC	
7	Glutamic acid	46	HYD	TLC	
8	Cysteine	48	GC	TLC	
9	Threonine	40	GC	TLC	
10	Tyrosine	36	GC	HYD	TLC
11	Cysteine	37	GC	TLC	
12	Alanine	32	GC	HYD	
13	Alanine	28	GC	HYD	
14	Cysteine	29	GC	TLC	
15	Glutamic acid	25	HYD	TLC	
16	Proline	23	GC	HYD	
17	Glutamic acid	22	HYD	TLC	
18	Cysteine	25	GC	TLC	
19	Proline	21	GC	HYD	
20	Valine	19	GC	HYD	
21	Asparagine	20	HYD	TLC	
22	Alanine	21	GC	HYD	
23	Isoleucine	19	GC	HYD	
24	Serine	14	GC	TLC	
25	Alanine	18	GC	HYD	
26	Glycine	16	GC	HYD	TLC
27	Aspartic acid	17	HYD	TLC	
28	Glutamic acid	15	HYD	TLC	
29	Isoleucine	15	GC	HYD	
30	Tyrosine	13	GC	HYD	TLC
31	Isoleucine	15	GC	HYD	
32	Valine	14	GC	HYD	
33	Aspartic acid	14	HYD	TLC	
34	Glutamic acid	13	HYD	TLC	
35	Serine	10	GC	TLC	
36	Valine	12	GC	HYD	
37	Cysteine	11	GC	TLC	
38	Threonine	10	GC	TLC	
39	Asx[d]	7	HYD		

[a] CM-ferredoxin was sequenced in the protein sequencer. See Methods for experimental details. [b] Theoretical yield determined by gas chromatography of PTH-amino acid except for PTH-histidine, -arginine, -glutamic acid, -asparagine, and -aspartic acid which were determined by amino acid analyses. The average repetitive yield was 99.3%. [c] The abbreviations used are: GC, gas chromatography of PTH-amino acid; TLC, thin-layer chromatography of PTH-amino acid; and HYD, 6 *N* HCl hydrolysis of the PTH-amino acid to the free amino acid and subsequent amino acid analyses. [d] "Asx" means aspartic acid or asparagine. From the sequence analyses of the peptide CT-5, this position was determined to be aspartic acid.

of this amino acid was quantitated on the underivatized peptide (Tanaka et al., 1974). Sixteen residues from the NH_2-terminal end of the peptide were determined from the combined sequencer runs. The results of these analyses are summarized in Table IV.

Manual Edman Degradation Results of Peptide CT-7 (Residues 44–61). Twelve steps of the manual Edman degradation were carried out on this peptide (180 nmol). The results obtained are summarized in Table V.

Sequence Determination of Peptide CT-8 (Residues 52–61). Nine steps of the manual Edman degradation established the sequence of the peptide (170 nmol). The results of the sequence analyses are summarized in Table VI.

Complete Sequence. The sequencer analysis of the *C. limicola* ferredoxin II showed that peptide CT-1 was NH_2-terminal and was followed by peptide CT-4 and then by

Table IV: Automatic Sequenator Results of Peptide CT-5.

Step No.	Sequence[a]	Yield (%)[b] Run 1[c]	Run 2[d]	Identification Method[e]		
1	Isoleucine	100	54[f]	GC	HYD	
2	Valine	80	87	GC	HYD	
3	Aspartic acid	64	73	HYD	TLC	
4	Glutamic acid	55	65	HYD	TLC	
5	Serine	43	55	GC	TLC	
6	Valine	40	55	GC	HYD	
7	Cysteine	32	49	GC	TLC	
8	Threonine	22	33	GC	TLC	
9	Aspartic acid	18	30	HYD	TLC	
10	Cysteine	12	25	GC	TLC	
11	Glutamic acid	9	24	HYD	TLC	
12	Glycine	6	17	GC	HYD	TLC
13	Tyrosine		15	GC	HYD	TLC
14	Tyrosine		13	GC	HYD	TLC
15	Asx		9	HYD		
16	Glx		8	HYD		

[a] Step No. 15 and 16 in the sequence were determined to be aspartic acid and glutamic acid, respectively, from the sequence analyses of the peptide CT-7. [b] See footnote *b* in Table III. The average repetitive yields were calculated to be 77.4% for run 1 and 84.5% for run 2. [c] Results obtained with underivatized peptide CT-5. [d] Results obtained with 4-sulfophenylthiocarbamyl derivative of peptide CT-5. [e] See footnote *c* in Table III. [f] Could not be quantitatively extracted by the organic solvent because of its strongly acidic property.

Table V: Manual Edman Degradation Results of Peptide CT-7.

Step No.	Sequence	Yield[a] (%)	Identification Method[b]		
1	Tyrosine	90	GC	HYD	TLC
2	Aspartic acid	88	HYD	TLC	
3	Glutamic acid	84	HYD	TLC	
4	Proline	70	GC	HYD	
5	Alanine	66	GC	HYD	
6	Cysteine	56	GC	TLC	
7	Valine	45	GC	HYD	
8	Alanine	43	GC	HYD	
9	Valine	37	GC	HYD	
10	Cysteine	21	GC	TLC	
11	Proline	16	GC	HYD	
12	Valine	14	GC	HYD	

[a] See footnote *b* in Table III. [b] See footnote *c* in Table III.

Table VI: Sequence Determination[a] of Peptide CT-8.

Sequence No.	Amino Acid	Yield[b] (%)	Identification Method[c]	
1	Valine	100	GC	HYD
2	Cysteine	85	GC	TLC
3	Proline	72	GC	HYD
4	Valine	60	GC	HYD
5	Aspartic acid	44	HYD	TLC
6	Cysteine	36	GC	TLC
7	Isoleucine	31	GC	HYD
8	Isoleucine	25	GC	HYD
9	Lysine	20	HYD	
10	Valine	12	Direct analysis[d]	

[a] Manual Edman degradation established the sequence of this peptide. [b] See footnote *b* in Table III. [c] See footnote *c* in Table III. [d] After the ninth step of Edman degradation, free valine was determined in the sample by the direct amino acid analysis without acid hydrolysis.

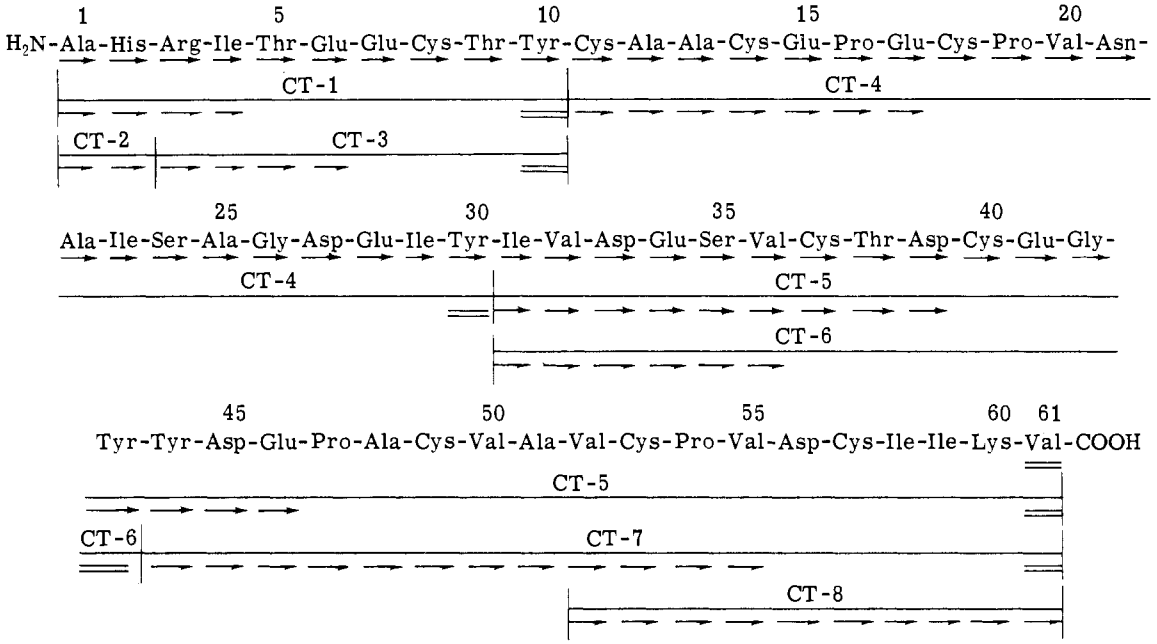

FIGURE 2: Reconstruction of the complete sequence of *Chlorobium limicola* ferredoxin II and sequence data of peptide fragments. In the figure, the symbols →, ⇀, and = represent sequences determined by use of the sequencer, the manual Edman degradation, and hydrazinolysis experiments, respectively.

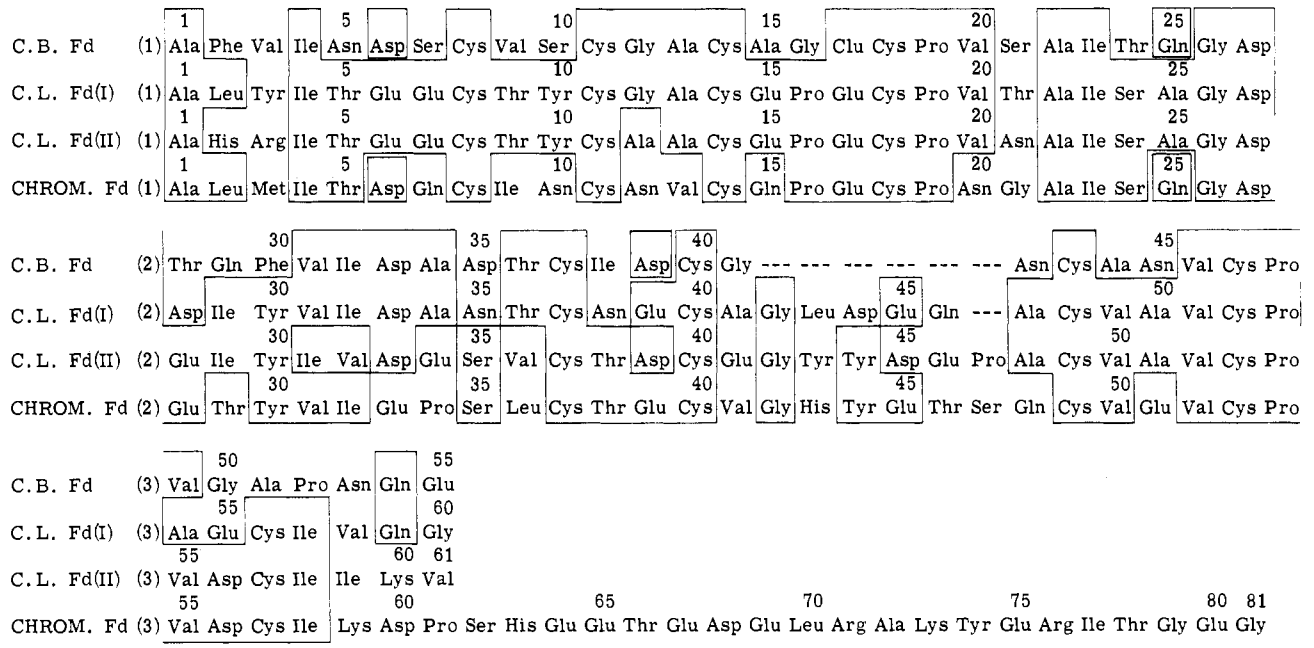

FIGURE 3: Comparison of the amino acid sequences of *Clostridium butyricum* ferredoxin and three photosynthetic bacterial ferredoxins. The abbreviations used are: C.B. Fd, *Clostridium butyricum* ferredoxin; C.L. Fd (I), *Chlorobium limicola* ferredoxin I; C.L. Fd (II), *Chlorobium limicola* ferredoxin II; and CHROM. Fd, *Chromatium* ferredoxin. In the figure, the altered sequence of the *Chromatium* ferredoxin was used to obtain better homology among the four ferredoxins (Tanaka et al., 1974).

peptide CT-5 in that order. Peptide CT-5 is the COOH-terminal peptide based on the COOH-terminal analysis of the protein. The sequence data necessary to establish the total sequence which have been covered in the previous section and also additional sequence studies which were determined are summarized in Figure 2.

Discussion

In a previous report (Tanaka et al., 1974), our aims and goals for a sequence study of ferredoxins from various photosynthetic bacteria were given. Thus far, the ferredoxins from *Chromatium* (Matsubara and Sugeno, 1969) and from *Chlorobium limicola* have been sequenced (Tanaka et al., 1974). The former bacterium is a photosynthetic purple bacterium while the latter is a photosynthetic green bacterium. In the case of *C. limicola,* two different types of ferredoxin which have been designated as ferredoxins I and II have been found. The amino acid sequence of ferredoxin I has been reported previously (Tanaka et al., 1974) and, in the present report, the details of the investigation which

Table VII: Comparison between the Amino Acid Sequences of Three Photosynthetic Bacterial Ferredoxins and *Clostridium butyricum* Ferredoxin.[a]

	C.L. Fd (I)	C.L. Fd (II)	Chrom. Fd.	C.B. Fd.
C.L. Fd (I)		38	31	27
C.L. Fd (II)	(62.2)		32	23
Chrom. Fd	(50.9)	(52.5)		23
C.B. Fd	(44.3)	(37.7)	(37.7)	
Between three photosynthetic ferredoxins		26 (42.6)		
Between all four ferredoxins		18 (29.5)		

[a] In the table, numbers not in parentheses show number of identical amino acids and values in parentheses show percentage of identical amino acids in the total of 61 residues used for comparison.

lead to the sequence determination of the *C. limicola* ferredoxin II are presented.

The amino acid analyses of ferredoxin II disclosed a number of differences from the reported amino acid analyses of ferredoxin I. Ferredoxin II contained the basic amino acid residues histidine, arginine, and lysine and none of these amino acids were present in ferredoxin I. Also, ferredoxin I contained 2 leucine residues while ferredoxin II contained no leucine residues. However, rather than pointing out the differences between ferredoxins I and II, one should note the great similarity in the sequence of these two ferredoxins. Ferredoxin II contains one more amino acid residue than ferredoxin I and in our alignment, we have assumed that there has been a deletion of a residue after residue 46 in ferredoxin I. Both proteins contain 60–61 amino acids, 9 cysteine residues located at positions 8, 11, 14, 18, 37, 40, 49, 53, and 57, and most of these latter residues are involved in iron chelation and the two sequences show 62% homology.

When a comparison of the sequences of the various ferredoxins from photosynthetic bacteria is made, one notes certain constant features. (1) They appear thus far to contain 9 cysteine residues. (2) When their sequences are compared with clostridial ferredoxins, one notes an insertion of 5–6 residues between residues 41 and 42 in the *C. butyricum* ferredoxin (see Figure 3). In the *Chromatium* ferredoxin, an insertion of additional residues at the C-terminal end of the protein has occurred and this section of the *Chromatium* ferredoxin was not included in the sequence comparisons. (3) The sequences of the *C. limicola* ferredoxins are more similar to the *Chromatium* ferredoxin than the nonphotosynthetic bacterial ferredoxin such as from *C. butyricum* as shown in Table VII.

Sequence determination of the *C. limicola* ferredoxin II was done partly in the automated instrument as well as by manual Edman degradation on the isolated chymotryptic peptides. The automated instrument established the sequence of the first 39 residues from the NH_2-terminal end of the protein. In addition, peptide CT-5 was also analyzed in the protein sequencer using the conditions described by Braunitzer et al. (1970, 1972), *i.e.*, the protein program with 0.25 *M* Quadrol in place of 1.0 *M* Quadrol. The remainder of the peptides were sequenced by the manual Edman degradation. Provided that the yields of the phenylthiohydantoin of the amino acids were 10% or greater, it was possible to identify each amino acid during the Edman degradation. Yields of 10% made it possible to use at least two methods, e.g., amino acid analysis of the acid hydrolyzates of the PTH-amino acids plus either gas or thin-layer chromatography. Each of the three methods mentioned above require about 5 pmol of the PTH-amino acids. The only unusual observation made during the isolation and characterization of the chymotryptic peptides was the cleavage of an Ala–Val bond (residues 51–52) by chymotrypsin. Although it was not reported in an earlier paper (Tanaka et al., 1974) this same peptide bond was cleaved in the *C. limicola* ferredoxin I.

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