THE AMINO ACID SEQUENCE OF MICROCOCCUS AEROGENES RUBREDOXIN*

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In the past few years, several non-heme iron proteins have been isolated and characterized (San Pietro, 1965). Among others, the ferredoxins have come under extensive investigation. The amino acid sequences of Clostridium pasteurianum and C. butyricum ferredoxins have been reported from this laboratory (Tanaka, et al., 1964; Benson, et al., 1966). Another non-heme iron protein, rubredoxin, was first obtained in the crystalline state from C. pasteurianum and was shown to replace ferredoxin in certain enzymatic reactions (Lovenberg and Sobel, 1965). The protein was reported to contain one gram atom of non-heme iron per mole of protein and to have a molecular weight around 6,000.

The present report is concerned with the determination of the complete amino acid sequence of M. aerogenes rubredoxin.

EXPERIMENTAL PROCEDURES

The methods used for growing the bacteria and for the isolation of rubredoxin have been described previously (Bachmayer, et al., 1967). Apo-

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* Abbreviations used: AEC-, S-β-aminoethylcysteinyl-; TLCK, L-(tosylamido-2-lysyl) ethylchloromethylketone; TPCK, L-(tosylamido-2-phenyl) ethyl chloromethylketone.

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rubredoxin was converted to S-β-aminoethylcysteinyl-derivative (AEC-derivative) for digestion with TLCK-chymotrypsin and TPCK-trypsin in separate experiments (Bachmayer, et al., 1967). Peptide fractions were obtained by column chromatography on Dowex AG 1-X2 and Dowex 50-X2, Sephadex G-25, paper chromatography, and paper electrophoresis. In the case of peptide C-8a, 1 μmole of the peptide was digested with 2% (w/w) of the B. subtilis neutral protease (McConn, et al., 1964) in 2 ml of 0.01 M ammonium acetate buffer, pH 7.0. The reaction was allowed to proceed for 18 hr at 38° and then terminated by boiling the reaction mixture. The sample was taken to dryness in vacuo. The peptide mixture was separated by paper chromatography using Whatman 3MM paper and the solvent system butanol:pyridine:acetic acid:water (15:10:3:12,v/v). The individual peptides were localized by spraying with 0.05% ninhydrin in acetone and the ninhydrin-positive bands were eluted from the paper with 20% acetic acid. The sequence determinations of all the purified peptides were carried out as described elsewhere (Tanaka, et al., 1966).

RESULTS

Amino acid sequence determination of the tryptic peptides. The amino acid sequences of the tryptic peptides are shown in Table I. The peptides are listed in the order of their elution from the Dowex AG 1-X2 column. The complete amino acid sequence of peptide T-5 was not determined since the sequence of this section of the rubredoxin molecule was established using the chymotryptic peptides.

Amino acid sequences of the chymotryptic peptides. Table II summarizes the amino acid sequence data for the chymotryptic peptides. Again, the peptides are listed in order of emergence from the Dowex AG 1-X2 column. The complete amino acid sequence was not determined for all of the peptides, but only where it was necessary to establish overlapping sequences and the complete sequence from either the chymotryptic or tryptic peptides. The sequence of peptide C-8a was constructed from peptide fragments obtained by digestion with the B. subtilis neutral protease. The sequence studies performed on these fragments

TABLE I

AMINO ACID SEQUENCE OF TRYPTIC PEPTIDES*

Peptide	Position in the peptide chain	Amino acid sequence
T-1	42 - 45	Gly-Ala-Gly-Lys
T-2	1 - 3	Met-Gln-Lys
T-3	7 - 9	Thr-Leu-AEC
T-4	4 - 6	Phe-Glu-AEC
T-5	10 - 41	Gly-Tyr-Ile-(Tyr, Asp, Pro, Ala, Leu, Val, Gly, Pro, Asp, Thr, Pro, Asp, Glu, Asp, Gly, Ala, Phe, Glu, Asp, Val, Ser, Glu, Asn, Trp, Val, AEC, Pro, Leu)-AEC
T-6	46 - 53	Glu-Asp-Phe-Glu-Val-Tyr-Glu-Asp

*Arrows to the right show sequences determined by the Edman procedure, those to the left sequences determined by the use of carboxypeptidase. Dashed arrows to the right indicate sequences determined by the use of leucine aminopeptidase and dashed arrows to the left sequences determined by hydrazinolysis.

(C-8a-P1, C-8a-P2, C-8a-P3a and C-8a-P3b) are also summarized in Table II.

Overlapping and complete amino acid sequence. Both the tryptic and chymotryptic peptides account for all of the amino acid residues in the original protein. From the structures of these peptides, the complete amino acid sequence of the M. aerogenes rubredoxin can be deduced as shown in Fig. 1. Since the sole methionine residue of the protein was determined to be in the NH_2-terminal position of the protein by the action of leucine aminopeptidase, peptides T-2 and C-2 are the NH_2-terminal peptides. From this point on, there

TABLE II

AMINO ACID SEQUENCE OF CHYMOTRYPTIC PEPTIDES*

Peptide	Position in the peptide chain	Amino acid sequence
C-1	37 - 40	Val-AEC-Pro-Leu
C-2	1 - 4	(Met, Glu, Lys)-Phe
C-3a	5 - 8	Glu-AEC-Thr-Leu
C-3b	9 - 11	AEC-Gly-Tyr
C-4	41 - 51	AEC-Gly-Ala-Gly-Lys-Glu-(Asp, Phe, Glu)-Val-Tyr
C-5	41 - 53	AEC-Gly-Ala-Gly-(Lys, Glu, Asp, Phe, Glu, Val, Tyr, Glu)-Asp
C-6	30 - 40	Glu-(Asp, Val, Ser, Glu, Asn, Trp, Val, AEC, Pro)-Leu
C-7	52 - 53	Glu-Asp
C-8a	12 - 29	Ile-Tyr-Asp-Pro-Ala-Leu-(Val, Gly, Pro, Asp, Thr, Pro, Asp) Gln-Asp-Gly-Ala-Phe
C-8a-P1	12 - 16	Ile-(Tyr, Asp, Pro)-Ala
C-8a-P2	18 - 27	Val-Gly-Pro-Asp-Thr-Pro-Asp-Gln-Asp-Gly
C-8a-P3a	28 - 29	(Ala, Phe)
C-8a-P3b	17	Leu
C-8b	30 - 36	Glu-Asp-Val-Ser-Glu-Asn-Trp

*See footnote of Table I for legends.

are overlapping peptides which make it possible to arrange the various peptide fragments in their proper positions. Hydrazinolysis demonstrated that

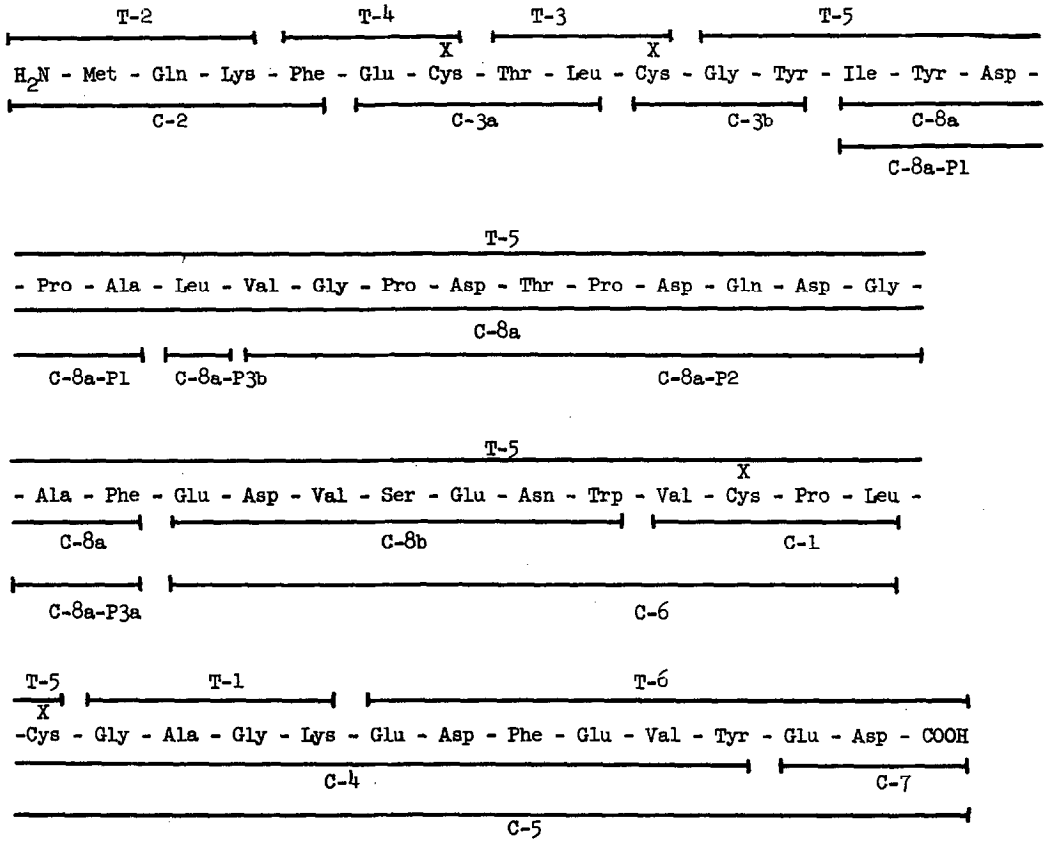

Figure 1: The amino acid sequence of Micrococcus aerogenes rubredoxin The one Fe is attached to the 4 Cysteine residues in positions 6, 9, 38 and 41 (X)

aspartic acid is the COOH-terminal amino acid and therefore, T-6, C-5 and C-7 represent COOH-terminal peptides.

The chelate structure. Lovenberg and Sobel (1965), Mayhew and Peel (1966) and Bachmayer, et al. (1967) all have reported that rubredoxin from several species of bacteria contains one gram atom of iron per mole of protein. For M. aerogenes rubredoxin, Bachmayer, et al. (1967) have demonstrated that the iron is attached to the cysteine residues in positions 6, 9, 38 and 41. Studies to determine the nature of the two other ligands are in progress in our laboratory.

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