AMINO ACID SEQUENCE OF THE PEPTOSTREPTOCOCCUS ELSDENII FLAVODOXIN

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SUMMARY - The amino acid sequence of the Peptostreptococcus elsdenii flavodoxin has been determined except for a few residues. The protein contains 137 amino acid residues and the NH_2- and COOH-terminal residues are methionine and alanine respectively. The protein contains two cysteine residues in positions 54 and 127. However, the cysteine residue in position 127 appears to be involved in binding FMN and therefore the active site is located near the COOH-terminal position of the protein. There are no aromatic amino acid residues in this region of the protein.

Flavodoxin is found in certain anaerobic bacteria where it is capable of replacing ferredoxin in certain reactions such as the phosphoroclastic cleavage of pyruvate (1). When the bacteria are grown on an iron deficient media, the flavodoxin is produced instead of ferredoxin (1).

The protein has now been isolated from Clostridium pasteurianum (2), Peptostreptococcus elsdenii (3), Desulfovibrio gigas (4) and Desulfovibrio vulgaris (4). The partial amino acid sequence of the P. elsdenii flavodoxin has been reported as well (6). Due to the demand of the knowledge of the primary structure of flavodoxins by the X-ray diffractionists (7), we now wish to present the total sequence of the P. elsdenii flavodoxin except for the assignment of amino acids to positions 94, 95 and 96.

MATERIALS AND METHODS

The P. elsdenii flavodoxin was obtained in a crystalline form as described in a previous report (3). FMN was removed as reported earlier (3) and the carboxymethylcysteine-derivative was prepared by the method of Crestfield, Moore and Stein (8).

The details of the primary structure determination will be published elsewhere. However, the procedures for sequence determination have been described in a previous report from our laboratory (9). In general, the Cys(Cm)-flavodoxin was digested with trypsin to obtain the tryptic peptides and with chymotrypsin to liberate the chymotryptic peptides. The first 41 residues from the NH_2-terminal end of the protein were determined by the use of the Beckman protein sequencer (10). The phenylthiohyandantoin-derivatives of the amino acids were identified as previously reported (6). In addition, the sequence of the peptides were determined manually by the subtractive Edman degradation as described by Konigsberg and Hill (11).

RESULTS

The sequence of residues 1-41 and 136-137. The amino acid sequence of residues 1-41 of flavodoxin was determined by the use of the protein sequencer and has already been reported (6). In addition, the carboxyl and penultimate amino acids have been shown to be alanine and lysine (6).

The sequence of residues 42-135. The tryptic peptides shown in Table I were obtained by chromatography on Dowex 50-X2 followed by paper chromatography. In order to determine the sequence of some of the tryptic peptides, it was necessary to fragment the peptides with thermolysin, chymotrypsin or by the use of CNBr. Furthermore, sequence studies were not performed on peptides T-1 and T-2 since the sequence had already been determined using the Beckman protein sequencer (6).

The isolation of cyanogen bromide fragments of Cys(Cm)-flavodoxin. In order to place the tryptic peptides, peptide fragments were obtained by the action of CNBr on the Cys(Cm)-derivative of flavodoxin. The fragments which were obtained are summarized in Table II. In order to obtain the purified fragments, the products of cyanogen bromide cleavage were chromatographed on a column of Sephadex G-50 (1.5 x 90 cm) in 0.1 M NH_4OH. The main fractions were pooled and further purified by paper chromatography in the solvent system 1-butanol:pyridine:acetic acid:water (60:40:12:48).

TABLE I

Tryptic peptides from Cys(Cm)-flavodoxin from P. elsdenii

Peptide number	Sequence
T-1	Met-Val-Glu-Ile-Val-Tyr-Trp-Ser-Gly-Thr-Gly-Asn-Thr-Glu-Ala-Met-Ala-Asn-Glu-Ile-Glu-Ala-Ala-Val-Lys
T-2	Ala-Ala-Gly-Ala-Asp-Val-Glu-Ser-Val-Arg
T-3	Phe-Glu-Asp-Thr-Asn-Val-Asp-Asn-Val-Ala-Ser-Lys
T-4	Asp-Val-Ile-Leu-Leu-Gly-Cys-Pro-Ala-Met-Gly-Ser-Glu-Glu-Leu-Glu-Asp-Ser-Val-Val-Glu-Pro-Phe-Phe-Thr-Asp-Leu-Ala-Pro-Lys
T-5	Gly-Lys-Lys
T-6	Leu-Lys
T-7	Val-Gly-Leu-Phe-Gly-Ser-Tyr-Gly-Trp-Ser-Trp(Gly,Gly,Glu)-Met-Asp-Ala-Trp-Lys
T-8	Gln-Arg
T-9	Thr-Glu-Asp-Thr-Gly-Ala-Thr-Val-Ile-Gly-Thr-Ala-Ile-Val-Asn-Glu-Met-Pro-Asp-Asn-Ala-Pro-Glu-Cys-Lys
T-10	Glu-Leu-Gly-Glu-Ala-Ala-Ala-Lys
T-11	Ala

The complete sequence. As mentioned previously, the protein sequencer was used to establish the sequence of the first 41 residues from the NH_2-terminal end of the protein. Thus, peptide T-1 is the NH_2-terminal peptide and is followed by peptide T-2 and then peptide T-3. Cyanogen bromide fragments F-1 and F-2 are components of peptide T-1 as well as the first 9 residues of CNBr-F-3. The last 20 residues of T-4, T-5, T-6 and the first 15 residues of T-7 are components of CNBr-F-4. CNBr-F-5 contains the last 4 residues of peptide T-7 and T-8 and the first 17 residues of peptide T-9. CNBr-F-6 contained the last 8 residues of peptide T-9 as well as peptide T-10 and T-11. We have already reported that the COOH-terminal sequence was -Lys-Ala-COOH (6).

TABLE II

Cyanogen bromide fragments from Cys(Cm)-flavodoxin from P. elsdenii

CNBr fragment number	Sequence
CNBr F-1	Hsr
CNBr F-2	Val-Glu-Ile-Val-Tyr-Trp-Ser-Gly-Thr-Gly-Asn-Thr-Glu-Ala-Hsr
CNBr F-3	Ala-Asn-Glu-Ile-Glu-Ala-Ala-Val-Lys-Ala-Ala-Gly-Ala-Asp-Val-Glu-Ser-Val-Arg-Phe-Glu-Asp-Thr-Asn-Val-Asp-Asn-Val-Ala-Ser-Lys-Asp-Val-Ile-Leu-Leu-Gly-Cys-Pro-Ala-Hsr
CNBr F-4	Gly-Ser-Glu-Glu-Leu-Glu-Asp-Ser-Val-Val-Glu-Pro-Phe-Phe-Thr-Asp-Leu-Ala-Pro-Lys-Gly-Lys-Lys-Leu-Lys-Val-Gly-Leu-Phe-Gly-Ser-Tyr-Gly-Trp-Ser-Trp(Gly,Gly,Glu)Hsr
CNBr F-5	Asp-Ala-Trp-Lys-Gln-Arg-Thr-Glu-Asp-Thr-Gly-Ala-Thr-Val-Ile-Gly-Thr-Ala-Ile-Val-Asn-Glu-Hsr
CNBr F-6	Pro-Asp-Asn-Ala-Pro-Glu-Cys-Lys-Glu-Leu-Gly-Glu-Ala-Ala-Ala-Lys-Ala

Thus by a combination of the cyanogen bromide fragments and the tryptic peptides, it was possible to obtain overlaps and to reconstruct the sequence as shown in Figure 1.

A comparison of the P.elsdenii and C. pasteurianum flavodoxins. The sequences of the two flavodoxins are compared in Figure 2. From the sequence determined to date, about 33 out of 74 residues are identical or there is about a 45% homology when the flavodoxins from P. elsdenii and C. pasteurianum are compared with one another. As in the case of other proteins isolated from different species, most of the glycine residues in the two flavodoxins are constant.

DISCUSSION

Several laboratories are currently determining the amino acid sequence

Amino acid sequences of the P. elsdenii flavodoxin

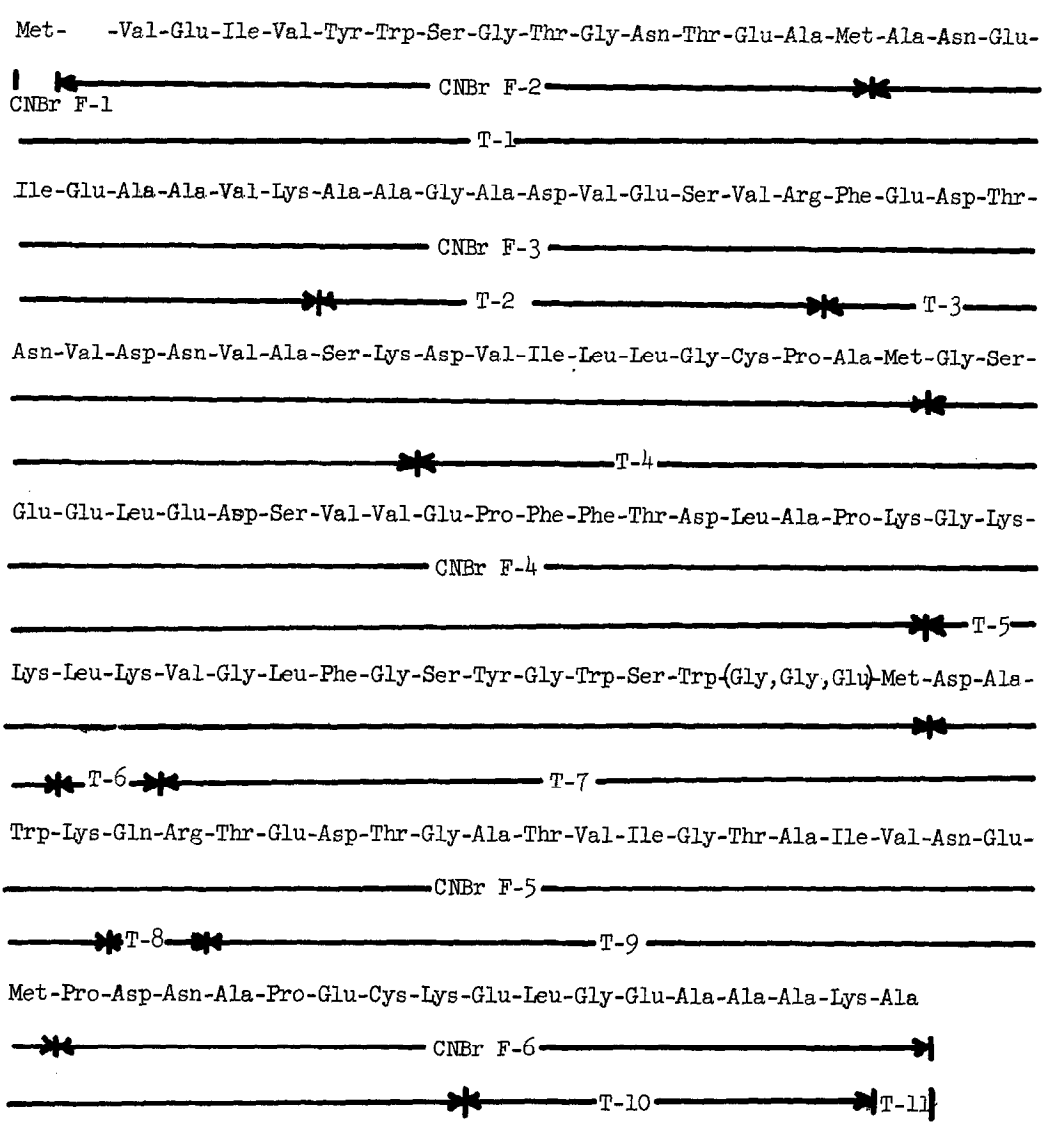

Fig. 1

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of flavodoxin, the first flavoprotein whose primary structure is being completely determined. Fox and Brown (5) have determined about 80 of the residues of the C. pasteurianum flavodoxin. Our laboratory has determined the first 41 residues of the P. elsdenii and the first 51 residues of the C. pasteurianum flavodoxin (6).

In the present report, the complete sequence of the P. elsdenii flavodoxin

Amino acid sequences of the P. elsdenii and C. pasteurianum flavodoxins

P.E. Met- -Val-Glu-Ile-Val-Tyr-Trp-Ser-Gly-Thr-Gly-Asn-Thr-Glu-Ala-Met-Ala-

C.P. Met-Lys-Val-Asn-Ile-Ile-Tyr-Trp-Ser-Gly-Thr-Gly-Asn-Thr-Glu-Ala-Met-Ala-

P.E. Asn-Glu-Ile-Glu-Ala-Ala-Val-Lys-Ala-Ala-Gly-Ala-Asp-Val-Glu-Ser-Val-Arg-

C.P. Lys-Leu-Ile-Ala-Glu-Gly-Ala-Gln-Glu-Lys-Gly-Ala-Glu-Val-Lys-Leu-Leu-Asn-

P.E. Phe-Glu-Asp-Thr-Asn-Val-Asp-Asn-Val-Ala-Ser-Lys-Asp-Val-Ile-Leu-Leu-Gly-

C.P. Val-Ser(Asp,Ala)Lys-Glu-Asp-Asp-Val-Lys-Glu-Ala-Asp-Val-Val-Ala-Phe-Gly-

P.E. Cys-Pro-Ala-Met-Gly-Ser-Glu-Glu-Leu-Glu-Asp-Ser-Val-Val-Glu-Pro-Phe-Phe-

C.P. Ser-Pro-Ser-Met-Gly-Ser-Glu-Val(Ser,Gln,Glu,Glu,Pro,Met)Phe-Leu-Asp-Val-

P.E. Thr-Asp-Leu-Ala-Pro-Lys-Gly-Lys-Lys-Leu-Lys-Val-Gly-Leu-Phe-Gly-Ser-Tyr-

C.P. Val-Ser-Ser-Ile-Val-Thr-Gly-Lys-Lys- - - - - - - - - -

P.E. Gly-Trp-Ser-Trp(Gly,Gly,Glu)Met-Asp-Ala-Trp-Lys-Gln-Arg-Thr-Glu-Asp-Thr-

P.E. Gly-Ala-Thr-Val-Ile-Gly-Thr-Ala-Ile-Val-Asn-Glu-Met-Pro-Asp-Asn-Ala-Pro-

P.E. Glu-Cus-Lys-Glu-Leu-Gly-Glu-Ala-Ala-Ala-Lys-Ala-COOH

Fig. 2

is presented, with the exception of the assignment of residues 94, 95 and 96 which are occupied by two glycine and a glutamic acid residues. Knight and Hardy (2) have reported the possibility that a cysteine residue is involved in the binding of FMN to the protein. This possibility was based on the fact that the cysteine residue could not be titrated in the native C. pasteurianum flavodoxin but was titrated in the apoprotein. In addition, the sulfhydryl reagent, sodium mersalyl, displaced the FMN from the native protein. Although

the P. elsdenii flavodoxin has two cysteine residues, one in position 54 and the other in position 127 it is the latter which is probably involved in the binding of FMN. Thus, it is likely that the active center of the protein which binds the FMN is located in the COOH-terminal position of the flavodoxin molecule. It is of interest that no aromatic residues are located in the vicinity of cysteine residue 127 which can form π-bonds with the isoalloxazine ring of the sole FMN present in the protein.

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