THE AMINO ACID SEQUENCE OF THE AZOTOBACTER VINELANDII FLAVODOXIN

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Received July 17,1975

SUMMARY. The amino acid sequence of a group II flavodoxin, the Azotobacter vinelandii flavodoxin has been determined. The FMN-redox protein was shown to exist as a single polypeptide chain and to contain 179 amino acids. Despite the rather low amino acid sequence homology with the other flavodoxins sequenced, it is concluded that sequences of the group I and group II flavodoxins are homologous. The major differences between the group I and group II flavodoxins appears to be a lengthening in the C-terminal region in the group II flavodoxins.

Flavodoxins are low molecular weight FMN-proteins which are found in bacteria, algae and plants (1,2). A tentative classification of the flavodoxins based on their molecular weights separates them into two types. The molecular weights of the group I flavodoxins are in the vicinity of 15,000 while the molecular weights of the group II flavodoxins are about 22,000. The sequences (3-8) and the 3D structures (9,10) of several groups I flavodoxins have been determined. The NH_2-terminal sequences of several group II flavodoxins have been reported by MacKnight et al. (11). In the present paper, a brief report of the studies which lead to the complete sequence determination of the A. vinelandii flavodoxin (Azotoflavin) is presented.

EXPERIMENTAL PROCEDURES

Methods. A. vinelandii Strain OP (Berkeley) was grown under N_2-fixing conditions in a 200-liter fermentor. The flavodoxin was isolated from a 40% acetone-5% Triton X-100 solution and separated from the ferredoxin fraction as described by Yoch and Arnon (12).

Preparation of Derivatives of Flavodoxin. The procedure of Crestfield et al. (13) was applied to the apoflavodoxin. The succinyl-derivative of the Cys(Cm)-flavodoxin was prepared by reaction with succinic anhydride as described by Klotz (14).

Amino Acid Composition and Sequence Analysis. The procedures used for these determinations have been described in previous reports from our laboratory (6,7). Briefly, amino acid compositions were determined as described by Spackman et al.(15). Sequence determinations were performed manually as described by Edman (16) or in the Beckman Model 890 Protein Sequencer (17). The amino acid phenylthiohydantoins were identified by gas chromatography (18), thin layer chromatography (19) or by amino acid analysis after acid hydrolysis (20). C-terminal amino acid analyses were determined by hydrazinolysis (21) or carboxypeptidase procedures (22).

Enzymatic Hydrolyses. About 1.25 μmoles of Cys(Cm)-flavodoxin in 1.5 ml was adjusted to pH 8 by the addition of N-ethylmorpholine and 1.0 mg of TPCK-trypsin was added. Digestion was allowed to proceed for 24 hours. About 1.4 μmoles of substrate was also treated similarily with 1.0 mg of TLCK-chymotrypsin for 16 hours at 27°. Similarily 1.45 μmole of succinyl-Cys(Cm)-flavodoxin was digested with 1.0 mg of TPCK-trypsin for 22 hours at 26°. Tryptic peptides, tryptic peptides of succinyl-Cys(Cm)-flavodoxin and chymotryptic peptides are designated by the symbols T, ST and CT, respectively.

Purification of Peptides. Peptides were purified by chromatography on Dowex 50-X2 (23), Dowex 1-X2 (24) or by paper chromatography where the solvent system was 1-butanol; pyridine; acetic acid; water (60:40:12:48, v/v) or pyridine: isoamyl alcohol: 0.1 N ammonium hydroxide (60:30:50, v/v).

RESULTS

Amino Acid Composition and End Groups. The amino acid composition of the S-β-carboxymethylcysteine derivative of the azotoflavin was determined and was shown to contain 179 residues. The results of these analyses agreed with the total residues obtained from the sequence data. End group analysis showed that alanine and leucine were the NH_2- and COOH-terminal amino acids, respectively.

Sequence Determination of Purified Peptides and Proteins. Sequence analysis of the Cys(Cm)-flavodoxin in the Protein Sequencer established the order of the first 53 amino acid residues from the NH_2-terminal end of the protein. The majority of Sequence determinations were achieved by manual Edman degradation of the various tryptic and chymotryptic peptides. However, for peptides T-10 and T-12, the Protein Sequencer was used to determine part of the sequences of these peptides. In addition, it was effective to hydrolyze the succinyl-Cys(Cm)-flavodoxin with trypsin in order to obtain overlapping peptides. The methods utilized for the sequence determination of the various peptides are summarized in Table I.

Reconstruction of the Amino Acid Sequence. The Protein Sequencer established the amino acid sequence of the first 53 residues from the NH_2-terminal end of

TABLE I

Summary of Sequence Studies

Residues	Protein of Peptide	Method
1-53		Protein Sequencer run
39-80	T-10	Sequencer run; Thermolytic peptides
78-86	CTa	Manual Edman degradation
81-89	T-11	Manual Edman degradation
87-93	CTb	Manual Edman degradation
90-118	T-12	Sequencer run; Manual Edman degradation
118-128	CTc	Manual Edman degradation
119-120	T-13	Manual Edman degradation
121-123	T-14	Manual Edman degradation
124-145	T-15	Manual Edman degradation
134-146	CTd	Manual Edman degradation
146-159	T-16	Manual Edman degradation
150-168	CTe	Manual Edman degradation
160-163	T-17	Manual Edman degradation
164-179	T-18	Manual Edman degradation

the protein. Sequence determination of peptide T-10 and the thermolytic peptides from this peptide T-10 established the sequence to residue 80. Peptide T-11 established the sequence of azotoflavin up to residue 89 and peptide CTa overlapping peptide T-10 and T-11. Peptide T-12 established the sequence up to residue 118. Peptide CTc established the sequence through residue 128 and contained part of the peptide T-12 and T-15 and peptide T-13 and T-14. Peptide T-15 established the sequence up to 145. Peptide T-16 established the sequence through residue 159 and peptides T-15 and T-16 were overlapped by peptide CTd. Peptides T-17 and T-18 established the remainder of the sequence of azotoflavin and these two peptides were overlapped by peptide CTe as shown in Fig. 1.

DISCUSSION

The FMN-containing flavoprotein from A. vinelandii was discovered by Shethna

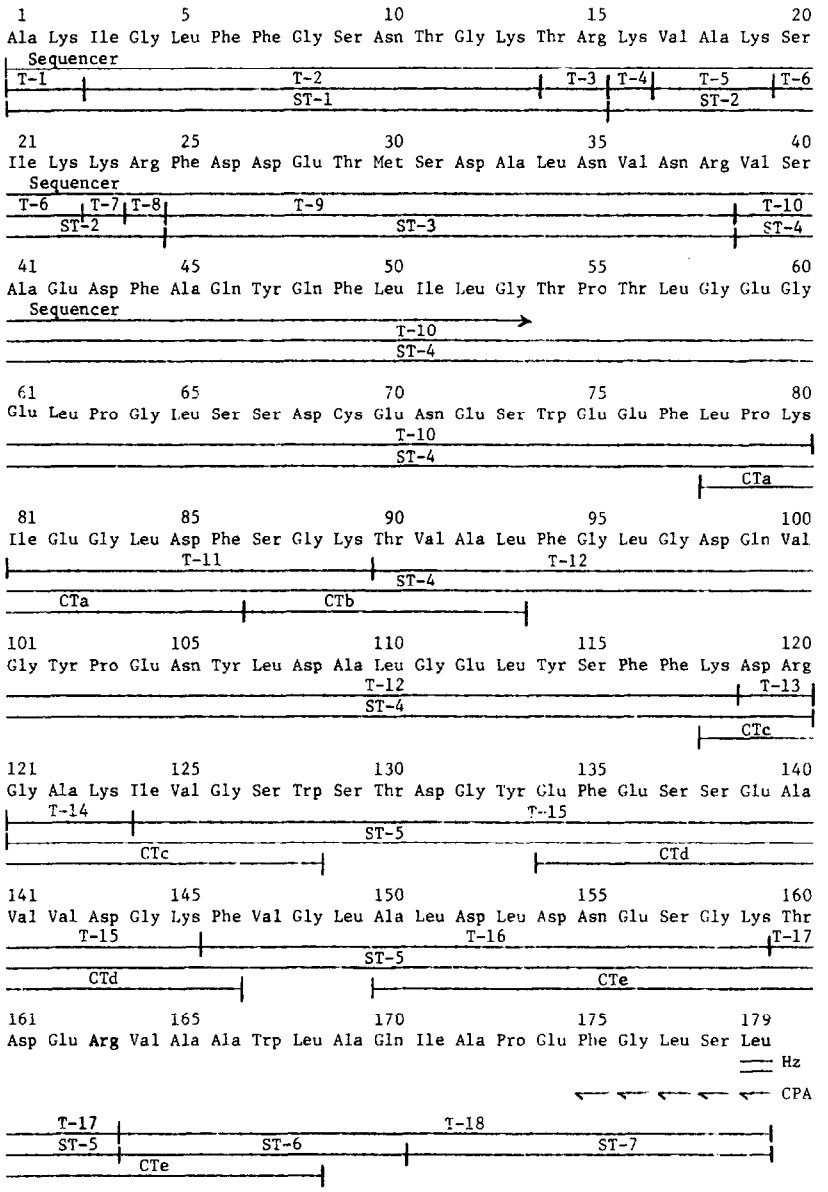

Figure 1. Reconstruction of the amino acid sequence of the A. vinelandii flavodoxin from the Protein Sequencer run and sequence determinations of the tryptic and chymotryptic peptides.

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et al. (2,25), Bulen and coworkers (26,27) and by Benemann et al. (28). Structure-function studies have been initiated by Tollin et al., (29-31). Sequence studies on the amino-terminal region of the A. vinelandii flavodoxin has been reported

by MacKnight et al. (11) and these workers compared it to those of five flavodoxins; Peptostreptococcus elsdenii (6),Clostridium MP (7), Clostridium pasteurianum (3), Desulfovibrio vulgaris (5) and Rhodospirillum rubrum (11). While a significant amino acid homology was shown for the Azotobacter flavodoxin in the region of the FMN-binding site, there was reported to be no correlation between the Azotoflavin and the other flavodoxins outside this region. At the time of the report of MacKnight et al. (11), sequence investigations on the Azotoflavin were in progress in our laboratory. Our present results completely conform to the NH_2-terminal sequence reported by them. In addition, we have completed the sequence determination of the entire protein and have shown it to be a single polypeptide protein consisting of 179 amino acids. The main point we wish to stress is that there is sequence and conformational homologies (32) of the A. vinelandii flavodoxin with the group I flavodoxins. The main difference is that the Azotoflavin is about 31 or more residues longer at the COOH-terminal end. In addition, the two residues flanking the planar FMN ring are probably glutamic acid and tyrosine rather than methionine and tryptophan in the Clostridium MP flavodoxin (10) and tryptophan and tyrosine in the D. vulgaris flavodoxin (9). The dimerization of Azotoflavin reported by Yoch (33) which results in inactivation of flavodoxin occurs through the oxidation of the cysteine residues present in position 69. Crystal X-ray diffraction studies of another group II flavodoxin, the Anacystis nidulans flavodoxin, are in progress in the laboratory of Dr. Martha Ludwig at the U. of Michigan and when this study is completed, a more definite picture of the 3D structure of a group II flavodoxin will be available.

ACKNOWLEDGEMENTS

The authors are grateful for the support of this research project by Grants GB 43448 from the National Science Foundation, National Institutes of Health Grant No. GM 16228 and NSF Grant BMS 71-01204 to D.I. Arnon.

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