N-FORMYLMETHIONINE: THE N TERMINUS OF *CLOSTRIDIUM PASTEURIANUM* RUBREDOXIN

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Received July 20, 1970

SUMMARY. The N terminal amino acid of *C. pasteurianum* rubredoxin has been determined to be N-formylmethionine.

Rubredoxin from *Clostridium pasteurianum* is a small electron transfer protein that contains a single methionine residue (1). The primary amino acid sequence is now being investigated (2). The amino acid sequences of two other anaerobe rubredoxins have been determined (3, 4). In each of these methionine is the N terminal amino acid. Analysis of DNP amino acids following reaction of *C. pasteurianum* rubredoxin with dinitrofluorobenzene and hydrolysis yielded only DNP methionine. The yield however was less than 10% of the theoretical value. We now wish to report the isolation of a peptide, N-formylmethionyllysine, from tryptic digests of aminoethylrubredoxin.

METHODS. *Preparation of aminoethylrubredoxin*: Rubredoxin was isolated from *Clostridium pasteurianum* by the method of Lovenberg Williams (1). Aporubredoxin was prepared by incubating 20 μmoles of rubredoxin for 3 hours at 60° in 20 ml of a solution containing 2 ml, 2-mercaptoethanol (Eastern Organic Chemicals), 200 mg, 1,10-phenanthroline (Fisher Scientific Company) and enough NH_4OH to bring the pH to 9.5. The aporubredoxin was then separated from the low molecular weight substances by gel filtration on G-25

medium (Pharmacia). This whole procedure was repeated twice. The protein was then dissolved in a solution containing 6 M urea, 1 M Tris, pH 8.6, and .15 M 2-mercaptoethanol and reduced under N_2 for 4 hours. The reduced aporubredoxin was aminoethylated by the addition of two 2 ml portions of ethyleneimine (Pierce Chemical Co.) and then separated from the reagents by gel filtration.

Isolation of methionine containing peptide: Ten μmoles of aminoethylated rubredoxin were digested with 2% TPCK-trypsin (Worthington) for 24 hours in 0.1 M potassium phosphate, pH 8.2. The digested aminoethylated protein was applied to a 1.5 x 300 cm column of Sephadex G-25 medium which had been equilibrated with .18 M pyridine acetate buffer, pH 5.4. The effluent was collected in 5 ml fractions. Fractions 37-40 (low molecular weight peptides) were pooled, lyophilized, redissolved and applied to a column (0.9 cm by 65 cm) of Dowex 50 X-4. The column was developed with a linear pyridine acetate gradient at 50°C (0.2 M pyridine, pH 3.1, to 2 M pyridine, pH 5.0, with 250 ml each in the reservoir and mixing chamber). Fractions of 4.4 ml were collected and the methionine containing peptide was found in fractions 26 and 27. Formic acid or formate buffers were not used in any of the preparative procedures.

RESULTS. Hydrolysis of the methionine peptide with constant boiling HCl for 12 hours at 110° yielded the amino acids methionine and lysine in a ratio of .86 to 1.0. An aliquot of the peptide was digested with carboxypeptidase B (Worthington) in 0.1 M N-ethylmorpholine acetate buffer, pH 8.6, using an enzyme substrate ratio of 0.015:1 for one hour at 37°. Amino acid analysis of the digest revealed that nearly all the lysine was liberated while there was no detectable free methionine, methionine sulfone or sulfoxide, suggesting the presence of an N terminal blocking

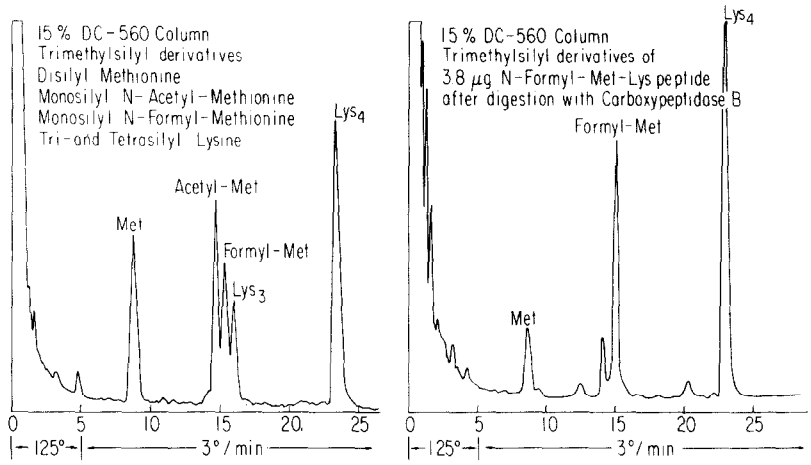

Fig. 1. The identification of N-formylmethionine by gas liquid chromatography. Lys_3 and Lys_4 refer to tri- and tetrasilyl lysine.

Table I. Identification of N-Formylmethionine by paper chromatography*.

Sample	R_f Value
N-Acetylmethionine	.94
N-formylmethionine	.86
Methionine	.56
Peptide	.55
Digested Peptide	.84

*The chromatography was performed in butanol:acetic acid:water, 4:1:1 solvent using Whatman 3MM paper. The sulfur containing amino acids and peptide were located by using the KI -chloroplatinic acid-starch stain (5).

group. Also indicative that the N-terminus is blocked was the observation that the peptide was electrophoretically neutral.

To identify the nature of the blocking group, aliquots of

the carboxypeptidase B digest were examined by gas-liquid and paper chromatography. The digest contained a substance which had identical retention times and R_f values to N-formylmethionine (Fig. 1 and Table I). The N-terminal peptide therefore appeared to be N-formylmethionyllysine.

In an attempt to determine what percentage of the polypeptide molecules had the N terminus blocked, several different preparations of aminoethylrubredoxin were digested with aminopeptidase M. The amount of free methionine liberated varied from 5 to 20% of theoretical yield suggesting that the protein normally occurs mainly in the formylated form.

DISCUSSION. The unique initiator of polypeptide synthesis in bacterial systems is N-formylmethionyl-t-RNA (6). Most native proteins that have been examined do not contain N-formylmethionine at the N terminus indicating that they are usually deformylated or otherwise modified with proteases. N-formylmethionine has been observed in phage nucleic acid directed protein synthesis (7) both *in vitro* and *in vivo*. Clostridial rubredoxin is to our knowledge, however, the first normal cell constitutive protein found to retain the initiator amino acid. Although rubredoxin is very acidic the amino terminal end of the protein contains the sequence N-f-MET-LYS-LYS (2). It is possible that this positively charged sequence plays a part in preventing the deformylating reaction.

ACKNOWLEDGEMENT

We wish to thank Dr. John Pisano and Mr. Thomas Bronzert for advice and help with the gas chromatography experiments.

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