DEFORMYLATION OF N-FORMYLMETHIONINE BY ESCHERICHIA COLI EXTRACTS

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There are increasing data supporting the role of formylmethionyl-sRNA as an initiator of protein synthesis in Escherichia coli (1,3,4, 5,6,10). In vitro studies using either viral RNA (1,5,11), or synthetic polynucleotides (4,6) as messengers have provided the best evidence for the initiator function of F-Met-sRNA. However, it is known that although cell-free synthesis of R17 and f2 phage coat protein shows F-Met-ala-ser at the N-terminal locus, intact R17 and f2 phage protein have alanine-ser in the N-terminal position (1). In addition the earlier work of Waller (10) showed that Met (45%), alanine (30%) and serine (15%) were the amino acids usually present in the N-terminal position of E. coli protein.

These results suggest the obvious possibility that mechanisms exist for the removal of both the formyl moiety and of N-formylmethionine from the newly synthesized protein. Capecchi (3) has discussed these alternatives in some detail, and has provided further evidence that formylmethionine is the initiator of most of the newly synthesized protein in an E. coli in vitro system. He also demonstrated that there is a preferential release of formate from the newly synthesized protein.

We would like to report the presence of an enzyme in extracts of E. coli which removes the formyl group from N-formylmethionine but not from other N-formyl amino acids.

E. coli B cells, harvested at the mid-log stage, were obtained from Grain Processing Corporation, Muscatine, Iowa. The cells were suspended in 2 volumes of 0.05 M potassium phosphate buffer pH 6.8, containing 0.01 M mercaptoethanol, and disrupted by sonication at 10 kc in a Raytheon apparatus. The suspension was centrifuged at 100,000 x g and the supernatant fraction treated with solid ammonium sulfate. The proteins that precipitated between 30 and 60% saturation were dissolved in 0.05 M phosphate buffer pH 6.8 containing 0.01 M mercaptoethanol and dialyzed against this buffer for 16 hrs. The protein concentration was about 37 mg/ml.

Radioactive N-formyl-L-methionine was synthesized by a modification of the procedure described by Sheehan and Yang (8). Ten μmoles of the L-methionine-$^{14}CH_3$ (25 μc) were dissolved in 1.5 ml of 90% formic acid. Acetic anhydride (1.5 ml) was added and the mixture kept at 23° for 2 hrs. The reaction mixture was diluted with 3 ml of H_2O and passed through a small column of Dowex-50 H^+ (1/2 x 5 cm). The resin was washed with 2 ml H_2O and the pass through and wash were combined and lyophilized. The N-formylmethionine was not retained by the resin although the labelled amino acid was removed. A 70-90% yield of the formylated amino acid was obtained. Radioactive N-formylmethionine was further identified using paper and gas chromatography.[1] Nonradioactive N-formyl-L-amino

[1]The authors express their gratitude to Dr. John Pisano for his aid in identifying synthetic N-formylmethionine-$^{14}CH_3$ by gas chromatographic separation.

acids were obtained from Cyclo Chemical Corporation. N-Acetyl-L-methionine-$^{14}CH_3$ was prepared by a similar procedure used for the synthesis of the N-formyl derivative except that glacial acetic acid was used in place of formic acid.

The standard incubation to show deformylation of N-formyl-methionine contained, in a total volume of 0.2 ml, enzyme (50-250 μg protein), 10 μmoles potassium phosphate buffer pH 6.7, and 160 mμmoles N-formylmethionine-$^{14}CH_3$ (400-500 cpm/mμmole). Incubations were for periods up to 20 min at 37°. The reactions were stopped by the addition of 0.8 ml of cold water, and passed through a Dowex 1-cl resin (1/2 x 3 cm) to remove the N-formyl-Met. The resin was washed with 1.0 ml of water and the effluent and wash (containing radioactive methionine) collected in a scintillation vial containing 10 ml of a counting fluid (2). Radioactivity was measured in a Packard Scintillation spectrometer.

In other experiments the formate which was released was assayed by the procedure of Rabinowitz and Pricer (7). This method was employed for substrates which were not available in radioactive form.

Methionine formed enzymatically from N-formylmethionine was identified by its paper chromatographic properties after purification on a Dowex-50-H resin.

As seen in Table 1 an ammonium sulfate preparation from a supernatant fraction of *E. coli* B catalyzed the deformylation of N-formyl-L-methionine-$^{14}CH_3$ as measured by the release of free ^{14}C-methionine. The reaction proceeded best between pH 6.5 and 7.0 and no cofactor requirements were observed. The amount of formate released, measured enzymatically (2), was shown to agree with the ^{14}C-methionine formed

(Table 2). The rate of deformylation of N-formylmethionine was linear with time and proportional to protein concentration up to 1.0 mg per ml. Several other formylated compounds were tested as substrates. The following were shown to be inactive as measured by formate release: N-formylmethionylalanine and the N-formyl

Table 1

Enzymatic Deformylation of N-Formylmethionine

	mμmoles methionine formed
Complete system	25
Complete system (0°)	0
Boiled enzyme or minus enzyme	0

A 10-min incubation containing 185 μg of protein in the reaction mixture was employed.

Table 2

Deformylation of N-Formylmethionine

Experiment No.	mμmoles methionine formed	mμmoles formate released
1	31	31
2	42	46
3	63	68

The details of the incubations are described in the text.

derivatives of glycine, alanine, proline, valine, leucine, phenylalanine and tyrosine.[2] Until other substrates can be tested it is not known whether this enzyme activity is responsible for the release of formate from newly synthesized E. coli protein.

Since 35% of E. coli protein contains alanine at the N-terminal position (10), N-formylmethionine may arise by being released intact from the newly formed protein. As yet free N-formylmethionine has no known function in protein synthesis, and the present enzyme could function to regenerate both methionine and formate for use by the cell.

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[2] The ammonium sulfate preparation employed also catalyzes the deacylation of N-acetyl-L-methionine. It is known that N-acetyl-L-methionine can be deacetylated by N-acetylornithinase (9) and evidence that N-formylmethionine may also be a substrate for this enzyme has been obtained by J. Adams (personal communication).