FORMYLATION OF AMINO ACID ANALOGUES OF METHIONINE sRNA

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Recent investigations have focused attention on N-formyl-methionine as an initiator of protein synthesis. In Escherichia coli, methionine is the major amino acid in the N-terminal position of soluble and ribosomal proteins (Waller, 1963). N-Formyl-methionyl-sRNA has been isolated from E. coli and yeast (Marcker and Sanger, 1964), and an enzyme from E. coli has been described that catalyzes the formylation of one of the two species of methionyl-sRNA (Marcker, 1965). This species of sRNA (Met-sRNA I, according to the terminology of Kellogg, et al, 1966) transfers methionine preferentially to the N-terminal position of proteins synthesized in vitro in response to poly UG or poly UAG, whereas the other species, Met-sRNA II, inserts methionine into internal positions and responds principally to poly UAG (Clark and Marcker, 1965; Clark and Marcker, in press; Kellogg et al, 1966).

As part of a general study on the characteristics of methionyl-sRNA transformylase, we have examined the specificity of the enzyme towards the amino acid moiety. Ethionine and norleucine are two

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methionine analogues that inhibit growth of E. coli and are incorporated into protein in place of methionine (Richmond, 1962). The present report demonstrates that both of these analogues can substitute for methionine in the acylation of sRNA, and that both can be formylated after attachment to sRNA.

MATERIALS AND METHODS

E. coli B sRNA was purchased from General Biochemicals, Inc. Uniformly-labeled L-methionine-^{14}C (198 mc per mmole), L-ethionine-ethyl-^{14}C (10.8 mc per mmole), DL-norleucine-1-^{14}C (4.4 mc per mmole), and sodium formate-^{14}C (50 mc per mmole) were obtained from New England Nuclear Corporation. ^{14}C-Methionine was diluted to 19.8 mc per mmole with ^{12}C-methionine before use. L-Ethionine and DL-norleucine were purchased from California Corporation for Biochemical Research.

Formate activating enzyme was obtained from Dr. T. Stadtman. ^{14}C-N^{5,10}-Methenyl-tetrahydrofolate was prepared using ^{14}C-sodium formate and the formate activating enzyme as described by Rabinowitz and Pricer (1962). Purification was achieved by the procedure of Huennekens et al (1963). The 100,000 x g supernatant fraction (S-100) used for charging sRNA was prepared from E. coli W 3100 as described by Nirenberg (1963), except that the preincubation and dialysis steps were omitted. Ammonium sulfate was added to the S-100 fraction and the protein that precipitated between 0 and 70% saturation was washed two times with a 70% saturated ammonium sulfate solution and then dissolved in "standard buffer" (Nirenberg, 1963) to give a final concentration of 15 mg/ml of protein. The transformylase preparation was a dialyzed protein fraction from E. coli K_{12} (50 mg protein/ml) that precipitated between 35-65% ammonium sulfate saturation (Dickerman and Weissbach, to be published).

The reaction mixture for charging sRNA contained in a total

volume of 0.05 ml: potassium cacodylate, pH 7.0, 0.1 M; magnesium acetate, 0.01 M; KCl, 0.01 M; ATP, sodium salt, 0.005 M; L-amino acid(s), as indicated; sRNA, 3.7 mμmoles; and 2 μl of the 0-70% ammonium sulfate fraction. Reactions were incubated for 15 minutes at 37°C. At the end of the incubation, sRNA was precipitated with 5% trichloroacetic acid. The precipitate was collected on a nitrocellulose filter (Millipore Corporation), and counted in a Packard Tri-Carb scintillation spectrometer.

In order to determine formylation, the charging was performed with ^{12}C-methionine, ethionine or norleucine. At the end of the 15-minute charging incubation, 0.37 mμmoles of ^{14}C-N^{5,10}-methenyltetrahydrofolate and 1 μl of the transformylase preparation were added. The reaction mixture was incubated for 5 more minutes, and sRNA was precipitated and counted as described above.

The concentration of sRNA was estimated on the basis of a value for E^{M}_{cm} equal to 6.40×10^{-5}, derived from data of Tissieres (1959). Protein was determined by the procedure of Lowry, et al (1951).

RESULTS AND DISCUSSION

As may be seen in Table I, when the charging reactions were run in the presence of 19 ^{12}C-amino acids (minus methionine), acceptance of ethionine and norleucine were almost equivalent to that of methionine. Charging with ^{14}C-ethionine was not affected by the omission of 19 ^{12}C-amino acids. However, acceptance of norleucine increased markedly in the absence of ^{12}C-amino acids. Additions of an equimolar amount of ^{12}C-methionine reduced the acceptance of ^{14}C-methionine by approximately 50%, as expected, whereas it completely inhibited ethionine acceptance. ^{14}C-Norleucine acceptance, however, was decreased by approximately 25%. Addition of ^{12}C-leucine further reduced norleucine acceptance, and all 20 natural amino acids gave

Table I

Amino Acid Acceptance Activity for Methionine, Ethionine and Norleucine

^{14}C-Amino Acid	^{12}C-Amino Acid(s) Added	mμmoles Accepted
Methionine	19(-Methionine)	.146
	None	.148
	Methionine	.071
Ethionine	19(-Methionine)	.128
	None	.134
	Methionine	.001
Norleucine	19(-Methionine)	.123
	None	.370
	Methionine	.272
	Leucine	.195
	Methionine + leucine	.092
	19(-Methionine) + Methionine	.008

Conditions of the assay are described in the text. ^{14}C-Methionine, ethionine and norleucine (L-isomer) were added at 10^{-5}M, 5×10^{-5}M and 5×10^{-4}M, respectively, to be in excess for the acylation of methionine sRNA. 19 Amino acids (-methionine) were added at a concentration of 10^{-4}M per amino acid, except in the case of ^{14}C-norleucine where this concentration was 2×10^{-4}M. ^{12}C-Methionine and leucine were added at the same concentrations as the ^{14}C-amino acid used.

almost total inhibition. It thus appeared that ethionine was transferred solely to methionine sRNA while norleucine was transferred to both leucine and methionine sRNA's and to a small extent to other sRNA species.

The data of Table II demonstrate that the capacity to accept formyl groups is approximately the same for methionyl-, ethionyl- and norleucyl-sRNA. In separate experiments it was established that when charging was performed in the absence of methionine or a methionine analogue, with or without the other 19 amino acids, little or no formylation occurred. In agreement with the results of Marcker (1965) and Adams and Capecchi (1966), formyl group acceptance was approximately 60% of amino acid acceptance.

It is evident that the methionine analogues, ethionine and

norleucine, when present at sufficient concentrations, will be accepted by methionine sRNA, and that the sRNA derivatives of these analogues are formylated as efficiently as methionyl-sRNA under the conditions of these experiments.

Table II

Formylation of Aminoacyl-sRNA

Amino acid	Charging mμmoles amino acid accepted	Formylation mμmoles formate accepted
Methionine	0.146	0.079
Ethionine	0.128	0.090
Norleucine	0.123	0.084

Two sets of tubes were incubated with the components for charging as described in the text and in the legend of Table I. One set, containing a ^{14}C-amino acid and 19 ^{12}C-amino acids (minus methionine) was assayed for amino acid acceptance. A second set, containing only a single ^{12}C-amino acid, was further incubated with the components required for formylation.

The failure of the transformylase to distinguish among methionyl-, ethionyl- and norleucine-sRNA, and its apparent ability to distinguish between the two species of methionine sRNA (Marcker, 1965) suggest that the enzyme recognizes only the sRNA portion of aminoacyl-sRNA.

On the basis of these findings one would expect norleucine and ethionine to replace methionine in N-terminal as well as in internal positions of proteins synthesized in the presence of these analogues. At very high concentrations, norleucine should also be incorporated into protein in place of leucine.

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