THE SPECIFIC RESISTANCE OF N-SUBSTITUTED INITIATOR METHIONYL-tRNA TO ENZYMATIC HYDROLYSIS

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E. coli contains an enzymatic activity which hydrolyzes N-substituted amino acid residues from N-substituted AA-tRNA's* but does not attack unsubstituted AA-tRNA's[1-3]. Among the substrates shown to be hydrolyzed are a number of N-formylated, N-acetylated and N-carbobenzyloxylated AA-tRNA's, and oligopeptidyl-tRNA's[2-4]. We have reported elsewhere, however, that there is a significant exception to this general specificity. F-met-tRNA, the initiator of protein synthesis, is not hydrolyzed although it is an N-substituted AA-tRNA[3].

Cuzin et al. have suggested that this enzyme may function in the terminal step of protein synthesis by releasing completed polypeptide chains from tRNA[2]. We have pointed out that the specificity of the enzyme is also compatible with an additional or alternative function, namely, to safeguard the fidelity of protein synthesis in the cell, in the following sense[3]. A number of N-substituted AA-tRNA's and oligopeptidyl-tRNA's appear able to replace F-met-tRNA as chain initiators in vitro[1,5-8]. If such species should ever appear in the cell, unattached to ribosomes, they might cause errors in protein synthesis by initiating synthesis at incorrect starting points. It might be a function of the enzyme in question to destroy such species while leaving the correct initiator species, F-met-tRNA, intact.

The initiator molecule, however, is one of two separable species of met-tRNA, met-$tRNA_F$, which is formylated by a specific enzyme in the cell. The other species, met-$tRNA_M$, is not formylated by this enzyme and does not initiate protein synthesis[9].

*Abbreviations used: tRNA, transfer RNA; AA-, aminoacyl; F-, N-formyl; Ac-, N-acetyl; Cbz-, N-carbobenzyloxy; met-, methionyl; leu-, leucyl; ala-, alanyl.

Our earlier experiments were performed with unfractionated tRNA and did not differentiate between the two species or, in general, clarify whether the tRNA moiety of the substrate plays a role in resistance. They showed that neither the blocking group alone nor the amino acid alone confers resistance, since both F-leu-tRNA and Ac-met-tRNA were hydrolyzed[3]. However, the latter substrate was only partially cleaved, about 50% remaining intact even after prolonged incubation with the hydrolytic enzyme. Since the chemically acetylated substrate contained both Ac-met-$tRNA_F$ and Ac-met-$tRNA_M$, the results suggested that only one of the two species was susceptible to enzymatic cleavage.

We have now separated and examined the two species of met-tRNA. Our results, reported here, show that certain N-substituted met-$tRNA_F$ species resist hydrolysis, while the corresponding derivatives of met-$tRNA_M$ are readily cleaved. Thus, it appears to be the initiator tRNA moiety which confers resistance to the hydrolytic enzyme.

MATERIALS AND METHODS

Transfer RNA and the hydrolytic enzyme were prepared from the ribonuclease I-deficient strain E. coli MRE-600[10] as described elsewhere[3]. The enzymatic hydrolysis of N-substituted met-tRNA's was assayed at an enzyme concentration of 200 µg protein/ml as previously described[3], except that 25 µg of uncharged carrier tRNA was added to each 50 µl reaction mixture. The two species of methionine-specific tRNA, $tRNA_F$ and $tRNA_M$ were separated by chromatography on benzoylated DEAE-cellulose[11]. They were charged with C^{14}-methionine and subjected to enzymatic formylation as described elsewhere[3]. In addition, both species were substituted chemically by : (a) formylation with the formyl ester of N-hydroxysuccinimide[12] (4 hours at room temperature in dimethylsulfoxide-H_2O (15:1, v,v); (b) acetylation with acetic anhydride[13]; and (c) carbobenzyloxylation with carbobenzyloxy chloride[1]. The degree of N-substitution was measured by hydrolyzing the C^{14}-methionine residue from the tRNA moiety with NH_3, separating the N-substituted and unsubstituted residues by paper electrophoresis[3] or chromatography (butanol, acetic acid, H_2O; 5:1:1), and estimating them[3]. In all cases the chemical substitution reactions were 80% or more effective (i.e., 20% or less of the radioactivity migrated as unsubstituted methionine), and the corresponding derivatives of met-tRNA's $_F$ and $_M$ gave electrophoretically identical radioactive hydrolysis products.

The R_f of the carbobenzyloxy derivatives was the same as that of authentic N-carbobenzyloxy-methionine (plus a small amount of what appeared to be N-carbobenzyloxy-methionine sulfoxide). The acetyl derivatives were not compared with authentic N-acetyl-methionine. The commercial (Cyclo Chemical Corp.) specimen of N-formyl-methionine employed as standard gave an elongated spot on paper electrophoresis. All formyl derivatives migrated to the same zone; however, the enzymatically formylated product (of met-$tRNA_F$) migrated somewhat faster than the chemical products, which were identical for met-$tRNA_F$ and met-$tRNA_M$. The nature of this difference is not yet clear.

The met-$tRNA_M$ preparation did not undergo enzymatic formylation, showing it to be essentially free of met-$tRNA_F$[9]. The enzymatic formylation of the met-$tRNA_F$ preparation was 70% effective. The experimental results presented below indicate that this is due to incomplete enzymatic formylation rather than to contamination with met-$tRNA_M$. Because the hydrolytic enzyme cleaves F-met-$tRNA_M$ very much faster than F-met-$tRNA_F$ (see Results), chemically formylated F-met-$tRNA_F$ would be cleaved faster than enzymatically formylated F-met-$tRNA_F$ if there were significant contamination with met-$tRNA_M$. In fact, both preparations were cleaved at the same rate (see Results), indicating that the met-$tRNA_F$ preparation contained little, if any, met-$tRNA_M$.

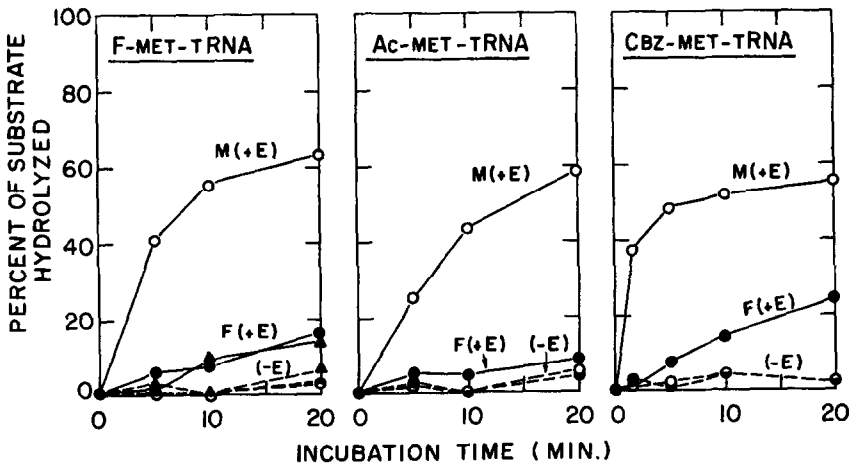

Fig. 1. The action of the hydrolytic enzyme on various N-substituted derivatives of met-$tRNA_F$ and met-$tRNA_M$. The same enzyme preparation was used in all cases at a concentration of 200 µg protein/ml. The assay is described elsewhere[3]. Open circles, chemical derivatives of met-$tRNA_M$; closed circles, chemical derivatives of met-$tRNA_F$; triangles, enzymatically formylated F-met-$tRNA_F$. Unbroken lines (+E), enzyme present; broken lines (-E), enzyme absent. The amount of substrate per 50 µl reaction mixture ranged from 2500 to 3500 cts/min for different substrates.

RESULTS AND DISCUSSION

Fig. 1 shows the rates at which the hydrolytic enzyme cleaved several substrates. The N-formyl, N-acetyl and N-carbobenzyloxy derivatives of met-$tRNA_M$ were all hydrolyzed much more rapidly than the corresponding derivatives of met-$tRNA_F$. We conclude that the tRNA moiety of the substrate is the major factor in causing resistance to the hydrolytic enzyme, the initiator species met-$tRNA_F$ being uniquely resistant.

The nature of the amino acid and the blocking group appear to be far less important in determining resistance. It may be noted, however, that the N-carbobenzyloxy derivative of met-$tRNA_F$ appears somewhat less resistant than the formyl and acetyl derivatives. Preliminary tests with more complex blocking groups (e.g. Cbz-ala-met-$tRNA_F$), to be reported elsewhere, indicate that some of these derivatives of met-$tRNA_F$ are hydrolyzed quite rapidly. It is possible, therefore, that resistance may result from an interaction between the $tRNA_F$ moiety and the N-substituted aminoacyl residue, with different residues being effective to different degrees. This is now under investigation.

This is the second instance in which the initiator species met-$tRNA_F$, as distinct from met-$tRNA_M$ and other species of aminoacyl-tRNA, exhibits a unique relationship to an enzyme. First, it is the only species formylated by the specific formylase of E. coli.[9] Second, as shown here, once formylated, it appears to be the only N-substituted aminoacyl-tRNA likely to be present in E. coli which is not cleaved by the hydrolytic enzyme. The possible physiological significance of this is indicated in the introduction to this communication and has been discussed in detail elsewhere.[3]

Acknowledgements

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