ENZYMATIC HYDROLYSIS OF N-SUBSTITUTED MET-tRNA$_M$ AND MET-tRNA$_F$

Y.LAPIDOT, D.INBAR and N.de GROOT
Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

and

H.KÖSSEL
Institut für Genetik der Universität Freiburg, Freiburg, Germany

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1. Introduction

Several laboratories have reported on the enzymatic hydrolysis of *N*-acetylaminoacyl-tRNA [1–3] and of peptidyl tRNA [2,4,5]. The enzyme responsible for the hydrolysis was obtained from E. coli supernatant and from *E. coli* ribosomes. This enzyme hydrolyzes the ester linkage between the *N*-blocked amino acid (or the peptide) and the tRNA, and does not hydrolyze unsubstituted aminoacyl-tRNA. It was shown that the rate of hydrolysis of oligopeptidyl-tRNA, with two or more peptide bonds, is higher than that of *N*-acylaminoacyl-tRNA [5]. Kössel et al. [2] and Vogel et al. [3b] found that *N*-acetyl- and *N*-formyl-met-tRNA$_F$ are not cleaved to any appreciable extent by the above-mentioned enzyme as compared to the corresponding met-tRNA$_M$ derivatives. Paulin et al. [6] on the other hand claimed that the rate of the enzymatic hydrolysis of *N*-substituted met-tRNA$_M$ is slower than that of the corresponding derivative of met-tRNA$_F$.

In the present communication we wish to report on a study of the enzymatic hydrolysis of *N*-substituted met-tRNA$_F$ and met-tRNA$_M$ using a highly purified enzyme preparation. It is shown that the rates of hydrolysis of gly$_2$met-tRNA$_F$ and formylmet-tRNA$_F$ are much slower than that of gly$_2$met-tRNA$_M$ and formylmet-tRNA$_M$. At low enzyme concentration, at which there is significant hydrolysis of gly$_2$met-tRNA$_M$, the substituted met-tRNA$_F$ is practically resistant.

2. Materials and Methods

Peptidyl-tRNA hydrolase was purified from *E. coli* B by the following 4 steps: 1) Filtration of a 100,000 × *g* supernatant, through a DEAE cellulose column at low ionic strength. 2) Absorption on a CM cellulose column followed by elution with 0.3 M KCl. 3) Chromatography on a Sephadex G 100 column. 4) Chromatography on a CM cellulose column using a linear gradient of KCl. The enzyme obtained was purified 1220 times as compared to the crude extract. As judged from polyacrylomide gel electrophoresis at pH 4.5 the enzyme was essentially homogeneous. Details of the purification procedure will be published elsewhere [7].

E. coli tRNA$_F^{Met}$ and tRNA$_M^{Met}$ were prepared by chromatography of crude tRNA (Calbiochem) on a DEAE Sephadex column followed by chromatography on a benzoylated DEAE cellulose column according to Seno et al. [8].

A mixture of partially purified aminoacyl-tRNA synthetases was prepared by chromatography of a 100,000 × *g* supernatant on a DEAE cellulose column, according to Muench and Berg [9];

Formylmet-tRNA was prepared by the reaction of

the *N*-hydroxysuccinimide ester of formic acid and met-tRNA [10]. Gly_2met-tRNA was prepared by condensing *N*-hydroxysuccinimide ester of *N*-monomethoxytritylgly$_2$ with met-tRNA and subsequent removal of the *N*-blocking group by treatment with 5% dichloroacetic acid [11].

Assay for enzymatic activity: An incubation mixture of 250 μl contained: 0.1 M sodium cacodylate (pH 6.9); 0.01 M $MgCl_2$ or Mg acetate; 0.01 M 2-mercaptoethanol; 0.01 M KCl; 25 μg bovine serum albumin. The various amounts of enzyme and radioactive substrates used are given in the legends to the figures. All incubations were carried out at 37°C. Samples of 50 μl were removed at various time intervals and assayed for residual acid-insoluble radioactivity according to Hoskinson and Khorana [12]. The radioactivity was counted in a Packard liquid scintillation counter. The specific activity of [^{14}C] methionine was 57 mc/mmole (Radiochemical Centre, England).

3. Results

As can be seen from fig. 1a, formylmet-tRNA$_M$ and gly$_2$met-tRNA$_M$ are cleaved by the peptidyl-tRNA hydrolase, while formyl- and gly$_2$-met-tRNA$_F$ are almost resistant. When the enzyme concentration was increased (fig. 1b), the *N*-substituted met-tRNA$_F$

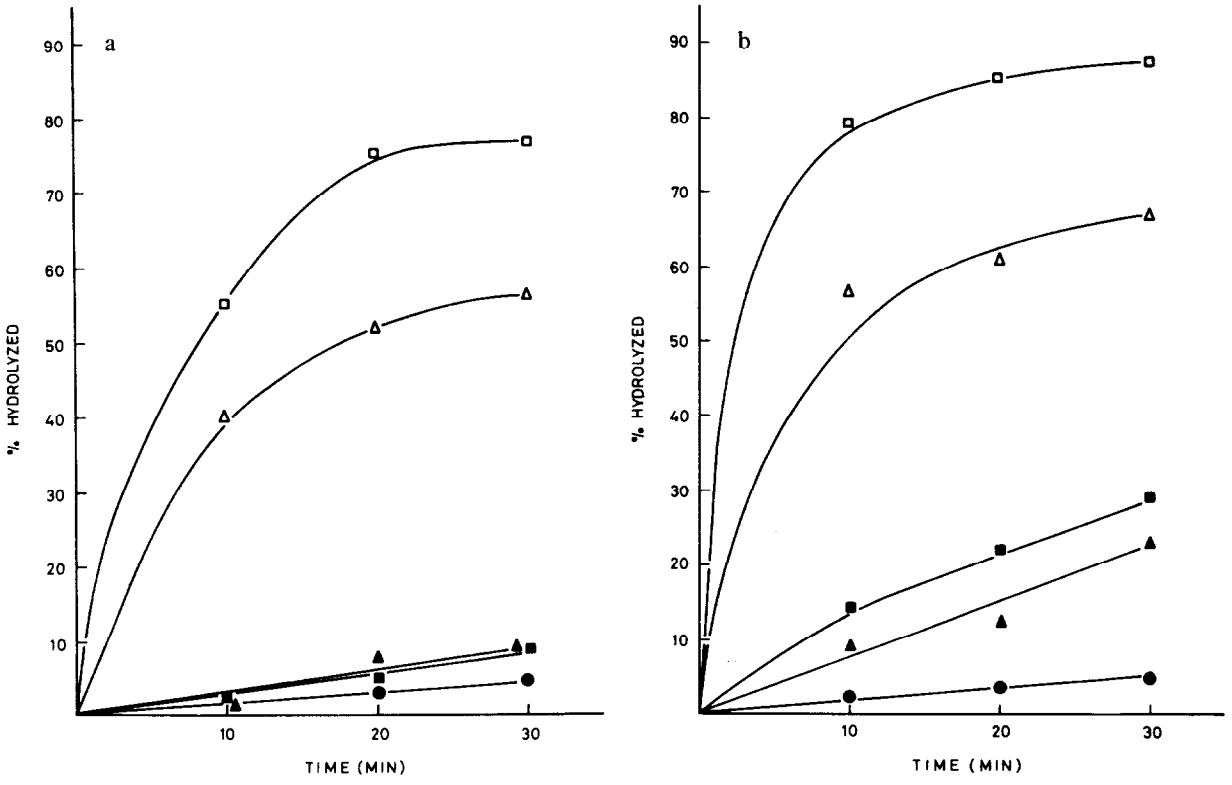

Fig. 1a and b. Kinetics of enzymatic hydrolysis of *N*-substituted met-tRNA$_M$ and met-tRNA$_F$. The reaction mixtures (250 μl) contained: 0.1 M sodium cacodylate pH 6.9; 0.01 M $MgCl_2$; 0.01 M 2-mercaptoethanol; 0.01 M KCl; 25 μg bovine serum albumin; about 5000 counts/min of substrate; peptidyl-tRNA hydrolase 0.56 μg protein (a) and 2.80 μg protein (b). Aliquots of 50 μl were removed at various time intervals for measurement of acid precipitable radioactivity. For details see Methods. □—□ gly$_2$[^{14}C] met-tRNA$_M$; ■—■ gly$_2$[^{14}C] met-tRNA$_F$; △—△ F[^{14}C] met-tRNA$_M$; ▲—▲ F[^{14}C] met-tRNA$_F$; ●—● substrates without enzyme. (The rates of the non-enzymatic hydrolysis of all the substrates used were practically the same.)

derivatives were hydrolyzed to a small extent, but still the rates of hydrolysis of the *N*-substituted met-tRNA$_M$ were much higher. It is interesting to note that the rate of hydrolysis of gly$_2$met-tRNA$_M$ is higher than that of formylmet-tRNA$_M$. This result is in agreement with the general phenomena found in this laboratory [11], in which the rate of hydrolysis of tripeptidyl-tRNA is higher than that of the corresponding *N*-acylamino-acyl-tRNA.

Fig. 2 shows that the rate of hydrolysis of formyl- and gly$_2$-met-tRNA$_F$ depends on the enzyme concentration.

4. Discussion

It is clear from the above results that the rate of hydrolysis of *N*-substituted met-tRNA$_F$ is much slower than that of *N*-substituted met-tRNA$_M$. Yet the *N*-substituted met-tRNA$_F$ is not absolute resistant to enzymatic hydrolysis and the rate of hydrolysis depends upon the enzyme concentration. These results are in agreement with those reported by Kössel and RajBhandary [2] and Vogel et al. [3], but are contradictory to the results reported by Paulin et al. [6]. Paulin et al. claimed that the rate of hydrolysis of formylmet-tRNA$_F$ is higher than that of formylmet-tRNA$_M$.

The fact that at low enzyme concentration gly$_2$-met-tRNA$_F$ too is resistant to the enzymatic hydrolysis by peptidyl-tRNA hydrolase proves that the formyl group can be substituted by other acyl groups without impairing this property. It seems therefore that the structure of the tRNA$_F^{Met}$ proper mainly is responsible for the above mentioned resistance although at high enzyme concentration gly$_2$met-tRNA$_F$ is cleaved somewhat faster than formylmet-tRNA$_F$.

The results described in this paper are in agreement with the proposed function of the peptidyl-tRNA hydrolase namely to regenerate tRNA from those peptidyl-tRNA molecules which were released from the ribosomes carrying immature protein.

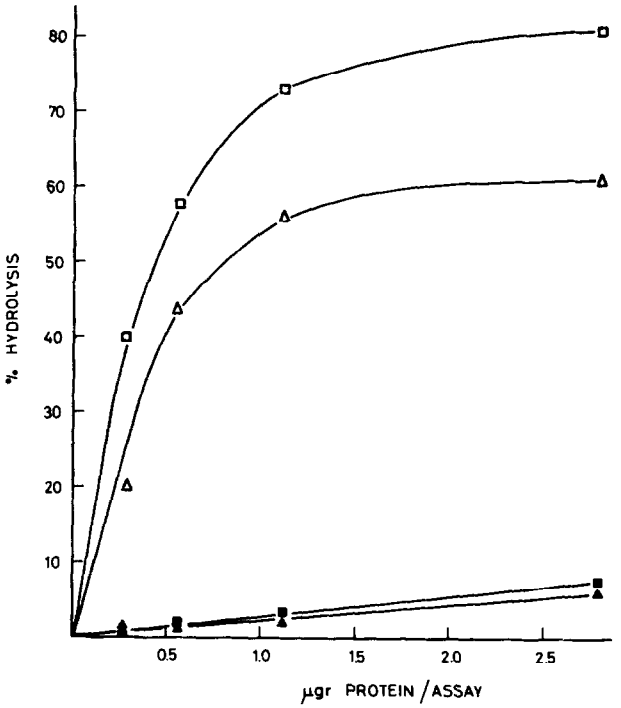

Fig. 2. Enzymatic hydrolysis of *N*-substituted met-tRNA$_F$ and met-tRNA$_M$ after 10 min incubation as function of enzyme concentration. The results shown were obtained by subtracting the non-enzymatic hydrolysis from the experimental hydrolysis rates. For experimental details see legend to fig. 1. □—□ gly$_2$[^{14}C] met-tRNA$_M$; ■—■ gly$_2$[^{14}C] met-tRNA$_F$; △—△ F[^{14}C] met-tRNA$_M$; ▲—▲ F[^{14}C] met-tRNA$_F$.

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References

[1] F.Cuzin, N.Kretchmer, R.E.Greenberg, R.Hurwitz and F.Chapeville, Proc. Natl. Acad. Sci. U.S. 58 (1967) 2079.
[2] H.Kössel and U.L.RajBhandary, J. Mol. Biol. 35 (1968) 539.
[3] a) Z.Vogel, A.Zamir and D.Elson, Proc. Natl. Acad. Sci. U.S. 61 (1968) 701.
b) Z.Vogel, A.Zamir and D.Elson, Biochem. Biophys. Res. Commun. 33 (1968) 94.
[4] N.de Groot, A.Panet and Y.Lapidot, Biochem. Biophys. Res. Commun. 31 (1968) 37.
[5] N.de Groot, Y.Groner and Y.Lapidot, Biochim. Biophys. Acta, in press.
[6] D.Paulin, P.Yot and F.Chapeville, FEBS Letters 1 (1968) 163.

[7] H.Kössel, manuscript in preparation.
[8] T.Seno, M.Kobayashi and S.Nishimura, Biochim. Biophys. Acta 169 (1968) 80.
[9] K.H.Muench and P.Berg, in: Progress in Nucleic Acid Research, eds. F.L.Cantoni and D.R.Davies (Harper and Row, New York, 1966) p. 375.
[10] Y.Lapidot, N.de Groot and I.Fry-Shafrir, Biochim. Biophys. Acta 145 (1967) 292.
[11] Y.Lapidot, N.de Groot and S.Rappoport, Biochim. Biophys. Acta 182 (1969) 105.
[12] R.M.Hoskinson and H.G.Khorana, J. Biol. Chem. 240 (1965) 2129.