ENZYMATIC HYDROLYSIS OF PEPTIDYL-tRNA

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Introduction

Recently Cuzin et al. (1967) described an enzyme, obtained from 105.000 X g supernatant of E. coli, which hydrolyses N-substituted aminoacyl-tRNA. This enzyme did not attack unsubstituted aminoacyl-tRNA. The biological significance of this enzyme is not clear. Capecchi (1967) described an enzymatic factor from E. coli, which promoted the release of a peptide from the ribosome-messenger RNA-peptidyl-tRNA complex in collaboration with the terminating UAG triplet. But Capecchi's factor seems not to be identical with the enzyme described by Cuzin et al. (1967).

In this communication we report on the enzymatic hydrolysis of peptidyl tRNA by the supernatant of E. coli. The hydrolytic activity of the supernatant on the free peptidyl-tRNA is compared with its action on ribosomal bound peptidyl-tRNA.

Materials and Methods

L-[^{14}C] phenylalanine was obtained from the Radiochemical Centre, Amersham, England (specific activity 495 mc/mmole). [^{14}C] phenylalanyl-tRNA was prepared essentially according to Nathans and Lipmann (1961) from E. coli B t-RNA (Calbiochem). Acetyl [^{14}C] phenylalanyl-tRNA was prepared by reacting the N-hydroxysuccinimide ester of acetic acid with [^{14}C] phenylalanyl-tRNA as described in a previous publication (Lapidot, de Groot and Fry-Shafrir, 1967). Glycyl [^{14}C] phenylalanyl-tRNA was obtained by reacting the N-hydroxysuccinimide ester of N-monomethoxytritylglycine with [^{14}C] phenylalanyl-tRNA (Lapidot, de Groot, Hamburger and Rappoport, 1967), and glycylglycyl [^{14}C] phenylalanyl-tRNA was prepared in a similar way (Lapidot, de Groot and Rappoport in preparation).

Twice washed ribosomes and dialyzed 105.000 X g supernatant (S-100 fraction) were prepared from E. coli W. as described by Nirenberg (1963). The ribosomes and S-100 fraction could be stored at -60°C for several weeks before use without loss of activity.

Hydrolase activity assay: An incubation mixture of 0.1 ml contained: tris acetate pH 7.7, 5 μmoles; S-100 fraction 45 μg protein. The reaction was started by adding the substrate. After incubation at 30°C the reaction was stopped by adding 3.0 ml of cold 5% TCA (together with 0.5 mg RNA as carrier). The suspension was kept at 3°C for 30 min., filtered on a Millipore filter and washed three times with 3.0 ml 5% TCA. The filter was dried and the radioactivity was counted in a Packard liquid scintillation counter.

Results and Discussion

As can be seen from Fig. 1, the S-100 fraction hydrolyses peptidyl-tRNA and acetylphenylalanyl-tRNA. The rate of hydrolysis of glycylphenyl-

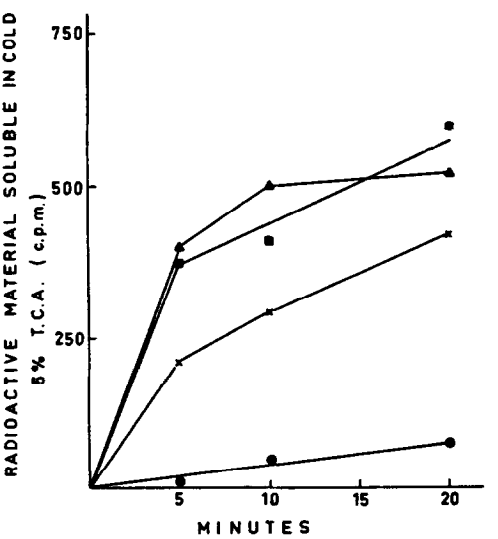

Fig. 1. The rate of enzymatic hydrolysis of different substrates. The data presented were obtained by subtracting the nonenzymatic hydrolysis from the total hydrolysis in the presence of the S-100 fraction. For experimental conditions see Methods.

▲——▲ glycylglycyl [^{14}C] phenylalanyl-tRNA (770 cpm added).
■——■ acetyl [^{14}C] phenylalanyl-tRNA (730 cpm added).
X——X glycyl [^{14}C] phenylalanyl-tRNA (1130 cpm added).
●——● [^{14}C] phenylalanyl-tRNA (710 cpm added).

alanyl-tRNA is considerably less than that of acetylphenylalanyl-tRNA and glycylglycylphenylalanyl-tRNA. The radioactive products obtained by the reaction of S-100 fraction on the different substrates were analyzed, after 20 min. of incubation, by high voltage paper electrophoresis at pH 2.5 (1.0 M acetic acid) 45 volts/cm for 3 hrs. In the case of acetyl [^{14}C] phenylalanyl-tRNA, two radioactive spots were obtained, one of them remained at the origin, corresponding to acetyl [^{14}C] phenylalanyl-tRNA and the second, corresponding to acetylphenylalanine. In the case of glycyl [^{14}C] phenylalanyl-tRNA and glycylglycyl [^{14}C] phenylalanyl-tRNA two radioactive spots were obtained in each case; one of them remaining at the origin and corresponding to unreacted peptidyl-tRNA and the second — corresponding to phenylalanine.

Notwithstanding that [^{14}C] phenylalanine was identified as the reaction product of the S-100 fraction on the peptidyl-tRNA's it seems very unlikely that [^{14}C] phenylalanyl-tRNA is an intermediate in the hydrolysis of the peptidyl-tRNAs. First of all the rate of hydrolysis of phenylalanyl-tRNA is much slower than that of the peptidyl-tRNAs (see Fig. 1). Secondly the following experiment was performed to support this assumption. Two hydrolase reaction mixtures, one with glycyl [^{14}C] phenylalanyl-tRNA and the second with glycylglycyl [^{14}C] phenylalanyl-tRNA were incubated for 5 min. at 30°C. The reactions were stopped by adding 3.0 ml of 5% TCA. The precipitates were isolated by centrifugation, washed with ethanol, and hydrolysed with 0.03 ml of 0.2 N NaOH for 1 hr. at 37°C. The hydrolyzate was analyzed by high voltage paper electrophoresis as described above. The radioactive spots were identified as glycyl [^{14}C] phenylalanine and glycyl-glycyl [^{14}C] phenylalanine respectively.

From these results we conclude that the action of the hydrolase activity is on the peptidyl-tRNA and that the S-100 fraction contains a peptidase which hydrolyses free glycylphenylalanine and glycylglycylphenylalanine, but does not act on these peptides when bound to tRNA.

From the rate of the nonenzymatic hydrolysis of the different substrates the half life time of hydrolysis can be calculated (Table 1)

Glycylphenylalanyl-tRNA hydrolyses faster than glycylglycylphenylalanyl-tRNA or acetylphenylalanyl-tRNA. At higher pH (pH 8.5) glycylphenylalanyl-tRNA is ever more rapidly hydrolyzed than phenylalanyl-tRNA (unpublished results).

Table 1. Half life time of nonenzymatic hydrolysis of phenylalanyl-tRNA and its derivatives

t-RNA derivative	Half life time (min)
[^{14}C] phenylalanyl-tRNA	43
acetyl [^{14}C] phenylalanyl-tRNA	170
glycyl [^{14}C] phenylalanyl-tRNA	53
glycylglycyl [^{14}C] phenylalanyl-tRNA	190

The half life time was measured at pH 7.7 (0.05 M tris acetate) at 30°C.

The enzymatic hydrolysis of phenylalanyl-tRNA is much enhanced by the increase of the magnesium ion concentration, but the hydrolysis of peptidyl-tRNA, on the other hand, is somewhat inhibited by raising the magnesium ion concentration (Table 2). These findings may indicate that the enzymatic hydrolyses of phenylalanyl-tRNA and peptidyl-tRNA are the result of the action of different enzymes.

Table 2. Influence of magnesium ion concentration on the enzymatic hydrolysis of phenylalanyl-tRNA and peptidyl-tRNA

Substrate	cpm added	Substrate hydrolysed (cpm)			
		Mg^{++} mMolar			
		1	6	16	31
[^{14}C] phenylalanyl-tRNA	475	15	40	260	268
glycylglycyl [^{14}C] phenylalanyl-tRNA	645	349	316	212	153

For experimental conditions see Methods. Incubation time — 5 min. The data presented (radioactive material soluble in cold 5% TCA) is obtained by subtracting the nonenzymatic hydrolysis from the hydrolysis in the presence of S-100 fraction.

Fig. 2 shows the rate of hydrolysis of ribosomal bound glycylglycylphenylalanyl-tRNA in the presence and in the absence of S-100 fraction, as compared to that of free peptidyl-tRNA. From the results, it can be concluded that in the

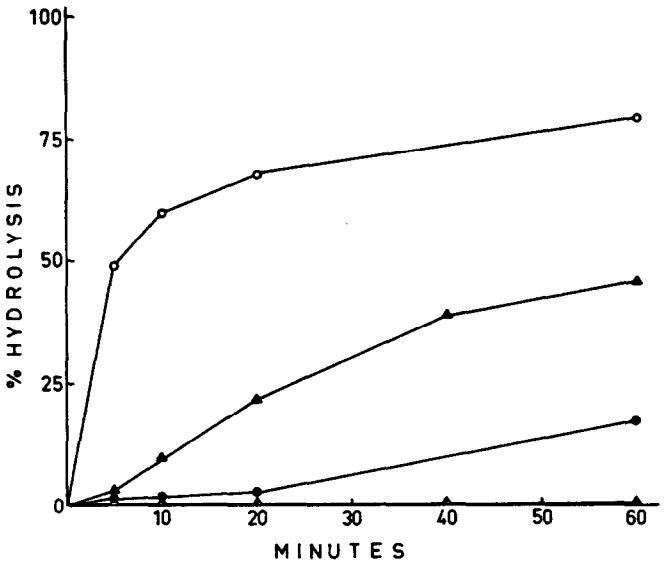

Fig. 2. The rate of hydrolysis of peptidyl-tRNA bound to ribosomes in the presence and absence of S-100 fraction.

a. Binding reaction was carried out in 0.6 ml volume which contained: 0.05 M Tris acetate buffer pH 7.7; 0.03 M KCl; 0.03 M magnesium acetate; 120 μg poly U; ribosomes 20 A_{260} mμ units. The reaction was started by adding glycylglycyl-[^{14}C] phenylalanyl-tRNA 12000 cpm. After 10 min. of incubation at 30°C the binding reaction was stopped by adding 10 ml of cold "washing buffer" which contained: 0.05 M Tris acetate pH 7.7; 0.03 M KCl; 0.015 M magnesium acetate. The ribosomes were sedimented by centrifugation at 105000 X g for two hours. The supernatant was discarded and the pellet was suspended in 0.54 ml of cold "washing buffer".

b. The enzymatic hydrolysis reaction was started by adding 0.06 ml of S-100 fraction (360 μg protein) and the mixture was incubated at 30°C. 0.1 ml aliquots were taken out into 3.0 ml of cold 5% TCA which contained 500 μg of RNA as carrier at different time intervals. An appropriate blank experiment was run in the absence of the S-100 fraction. For other experimental conditions, see Methods.

●—● - ribosomes; - supernatant (770 cpm added).
○—○ - ribosomes; + supernatant (770 cpm added).
▲—▲ bound to ribosomes; + supernatant (550 cpm added).
△—△ bound to ribosomes; - supernatant (550 cpm added).

absence of the S-100 fraction, ribosomal bound peptidyl-tRNA is not hydrolysed. Even after 60 minutes of incubation no hydrolysis of peptidyl-tRNA could be detected. Ribosomal bound phenylalanyl-tRNA was also immune to nonenzymatic hydrolysis under the experimental conditions (not shown in Fig. 2). Ribosomal

bound peptidyl-tRNA is much more stable against hydrolysis in the presence of the S-100 fraction than free peptidyl-tRNA. After 5 minutes of incubation 50% of the free peptidyl-tRNA is hydrolysed, but less than 5% of the ribosomal bound peptidyl-tRNA is hydrolysed. After one hour of incubation 50% of the ribosomal bound peptidyl-tRNA is hydrolysed. This result can be explained by assuming that ribosomal bound peptidyl-tRNA (obtained by binding peptidyl-tRNA to ribosomes) is not completely protected against the hydrolase activity.

Studies are now carried out in our laboratory for more details about the enzymatic hydrolysis of different chemically prepared peptidyl-tRNA's as compared to peptidyl-tRNA synthesized on the ribosomes in situ.

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