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ENZYMATIC HYDROLYSIS OF *N*-SUBSTITUTED AMINOACYL-tRNA

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1. Introduction

An enzyme which hydrolyzes the ester linkage between *N*-acetyl-aminoacids and tRNA's has been characterized in our laboratory [1]. It was shown that this hydrolase isolated from *E.coli* was also active with peptidyl-tRNA. In several cases which were studied, only low levels of specificity with respect to particular aminoacids were observed.

In this paper are reported the results concerning the influence of the nature of the amino blocking group and the structural modifications of the tRNA on enzymatic hydrolysis. It is shown that in general the rate of hydrolysis of *N*-acetyl derivatives is higher than that of *N*-formyl derivatives. The α-hydroxyl derivatives of aminoacyl-tRNA are not hydrolyzed or only very slowly. The results also show that the enzyme has lower affinity for the denatured tRNA and no affinity at all for the chemically modified *N*-acetyl-aminoacyl-tRNA.

The behaviour of one of the two methionyl-t-RNA's compared to other tRNA's is peculiar. It was observed that the rate of enzymatic hydrolysis of *N*-substituted methionyl-$tRNA_M$ is slower than that of the corresponding derivative of $tRNA_F$.

2. Material and methods

Hydrolase was prepared from a 105 000 g supernatant of MRE 600 *Escherichia coli.* The following steps were used: elimination of nucleic acids by protamine; precipitation by ammonium sulfate 40 to 60% of saturation; filtration through a DEAE-cellulose column at pH 7.4 (Tris-HCl 1.10^{-2} M); chromatography of the filtrate on Cm-cellulose (Tris-maleate 1.10^{-2}M, pH 6.5); the enzyme is eluted with a NaCl gradient (from 0.05 M to 0.3 M at pH 7.4); a second filtration of the active fraction on a DEAE-cellulose column. The specific activity of the purified fraction was 500 times higher than the activity of the crude extract.

N-substituted ^{14}C-aminoacyl-tRNA's were prepared as already described [2,3]. α-hydroxyl derivatives of ^{14}C-aminoacyl-tRNA were obtained by action of nitrous acid on the ^{14}C-aminoacyl-tRNA in conditions where only about 20% of the charging activity is lost [4]. After 8 hr treatment of *N*-acetyl-^{14}C-aminoacid-tRNA's by 3 M sodium nitrite at pH 3.2, partly deaminated derivatives in the polynucleotide chain were obtained. Their recharging capacity was completely lost. Denatured *N*-acetyl-^{14}C-leucyl-tRNA was prepared by treatment with EDTA [5].

Pure *E.coli* B $^{met}tRNA_F$ was a gift from Dr.Grunberg-Manago. Both ^{met}tRNA's were charged in the presence of pure methionyl-tRNA synthetase offered by Dr. Waller. Enzymatically formylated ^{14}C-methionyl-tRNA was obtained from Dr.Revel. Poly-valyl-^{14}C-valyl-tRNA was offered by Dr. Gottikh.

The products of hydrolysis were separated by paper electrophoresis in 0.5 M formic acid and 20 v/cm during 90 min and their radioactivity was determined as previously described [1].

3. Results and discussion

3.1. *Hydrolysis of N-substituted derivatives*

Fig. 1 shows that the rate of hydrolysis of *N*-formyl-valyl-tRNA was lower than the corresponding *N*-

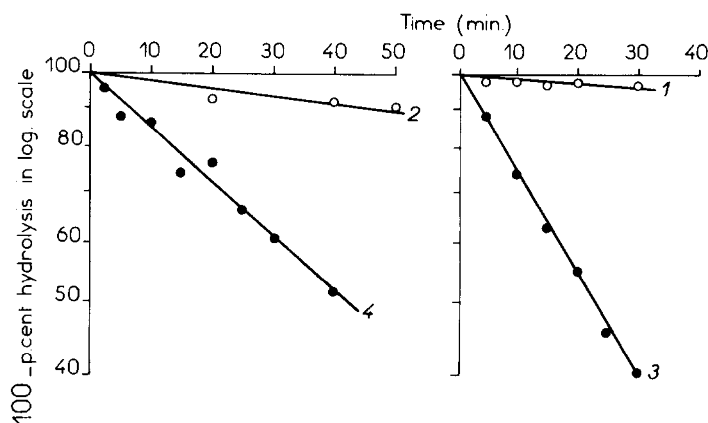

Fig. 1. Enzymatic hydrolysis of *N*-formyl-valyl-tRNA (curve 4) and *N*-acetyl-valyl-tRNA (curve 3). Incubation mixtures containing barbital-acetic acid pH 8, 10 μmoles; *N*-acetyl or *N*-formyl-^{14}C-valyl-tRNA, 150 $\mu\mu$moles and enzyme (15 μg of protein) were incubated at 37°C. At different times aliquots were taken and *N*-substituted ^{14}C-valyl derivatives were separated by paper electrophoresis. Curves 1 and 2 are controls without enzyme.

acetyl derivative. Separate experiments showed that α-hydroxyl derivatives of valyl or leucyl-tRNA were hydrolyzed at least 20 times slower than the *N*-acetyl-valyl-tRNA or *N*-acetyl-leucyl-tRNA.

Among seven other derivatives studied: *N*-acetyl-^{14}C-phe-tRNA; *N*-acetyl-phe-^{14}C-phe-tRNA; *N*-acetyl-phe-^{14}C-val-tRNA; *N*-acetyl-leu-gly-^{14}C-phe-tRNA; poly-val-^{14}C-val-tRNA; ^{14}C-phe-^{14}C-phe-tRNA and ^{14}C-phenyllactyl-^{14}C-phe-tRNA, all are hydrolyzed in the presence of enzyme. The rate was faster for the derivatives which do not contain a free amino group; for example, it was sensibly the same for *N*-ac-phe- and *N*-ac-phe-phe-tRNA and approximately double the rate of hydrolysis of phe-phe-tRNA.

3.2. *Hydrolysis of denatured or modified N-acetyl-aminoacyl-tRNA's*

The results reported in table 1 show that the hydrolysis of denatured *N*-acetyl-aminoacyl-tRNA was very slow and that the partly deaminated *N*-acetyl-val-tRNA by nitrous acid was practically not hydrolyzed. No significant hydrolysis was observed with *N*-acetyl-^{14}C-met-pentanucleotides prepared by the action of ribonuclease T_1 on *N*-acetyl-^{14}C-met-tRNA [6]. In all three cases it was shown that the integrity of the RNA structure is necessary for enzymatic hydrolysis.

Table 1
Hydrolysis of denatured and modified *N*-acetyl-aminoacyl-tRNA.

RNA	Treatment	Percent hydrolysis in 30 min
N-ac-val-tRNA	0	90
N-ac-val-tRNA	Nitrite 8 h	4
N-ac-met-tRNA	0	70
N-ac-met-tRNA	Nitrite 8 h	2
N-ac-met-tRNA	RNase T_1	4
N-ac-leu-tRNA	0	84
N-ac-leu-tRNA	EDTA	32

3.3. *Hydrolysis of two N-substituted met-tRNA's*

The rate of hydrolysis of *N*-acetyl-met-$tRNA_F$ is similar to that of the other *N*-acetyl-aminoacyl-tRNA's studied (val; leu; phe; ser; and thr) (fig.2 A). The formyl derivative is also rapidly hydrolyzed. However when the total *E.coli* *N*-acetyl-met-tRNA was incubated with enzyme, the kinetic studies showed two components, one of which is hydrolyzed slowly.

The ratio of these two components and the rate of their hydrolysis were determined as shown in fig. 2 B. It was deduced that the slow component which represents 50% should correspond to the *N*-acetyl-met-

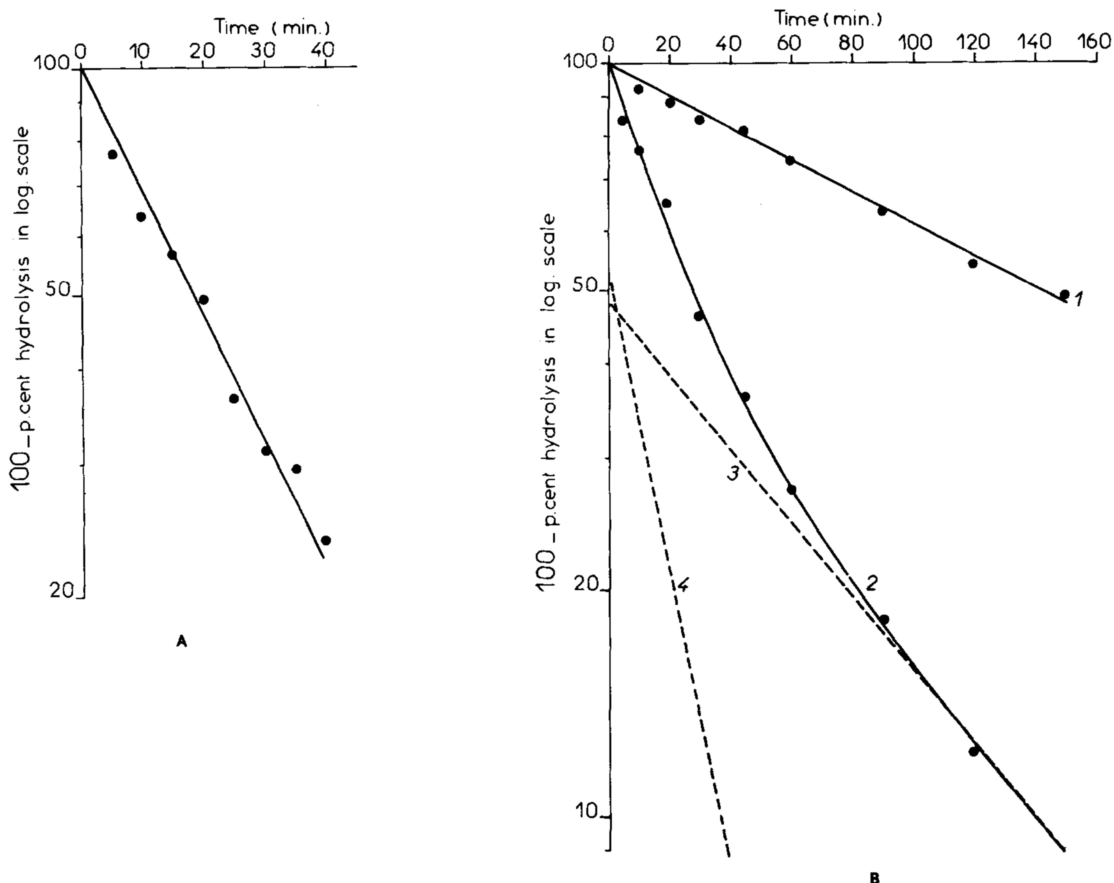

Fig. 2. Hydrolysis of *N*-acetyl-met-tRNA. (A) Pure *N*-acetyl-^{14}C-met-tRNA$_F$. (B) Crude *N*-acetyl-^{14}C-met-tRNA.
1. Hydrolysis in the absence of enzyme.
2. Hydrolysis in the presence of enzyme.
3. Rate of hydrolysis of the slow component.
4. Rate of hydrolysis of the fast component, which is the same as the rate observed for pure *N*-acetyl-^{14}C-met-tRNA$_F$ (A).

tRNA$_M$. This conclusion is in contradiction with the results reported by Rajbhandary and Kössel [7] in which *E.coli* hydrolase was active only on *N*-substituted met-tRNA$_M$. It seems unlikely that the fact that different strains were used could explain these opposite results. Our preliminary studies of the hydrolase which is present in all the biological material tested (beef spleen; rabbit reticulocytes; wheat germ; plant leaves and several bacterial species), showed little if any species specificity at least for *N*-acetyl-val-tRNA. The enzyme is also present in *Bacillus subtilis*; this indicates that it is different from the D-aminoacyl-tRNA hydrolase characterized by Calendar and Berg [8] which is absent in this bacteria.

References

[1] F.Cuzin, N.Kretchmer, R.E.Greenberg, R.Hurwitz and F. Chapeville, Proc. Natl. Acad. Sci. U.S. 58 (1967) 2079.
[2] Y.Lapidot, N.De Groot, M.Weiss, R.Peled and Y.Wolman, Biochim. Biophys. Acta 138 (1967) 241.
[3] A.L.Haenni and F.Chapeville, Biochim.Biophys. Acta 114 (1966) 135.
[4] G.Herve, Thèse de Doctorat, Université de Paris (1966).
[5] D.D.Henley, T. Lindahl and J.R.Fresco, Proc. Natl. Acad. Sci. U.S. 55 (1966) 191.
[6] K.Marcker, J. Mol. Biol. 14 (1965) 63.
[7] U.L.Rajbhandary and H. Kössel, Fed. Proc. 27 (1968) 296.
[8] R.Calendar and P.Berg, J.Mol. Biol. 26 (1967) 39.