

Peptidyl-transfer RNA hydrolase prevents inhibition of protein synthesis initiation

PEPTIDYL-tRNA HYDROLASE (called hydrolase below) catalyses the hydrolysis of peptides from peptidyl-tRNAs that are not bound to ribosomes^{1,2} but will not hydrolyse amino acyl-tRNA or fMet-tRNA^{fMet}. It is interesting that hydrolase seems to be ubiquitous in nature; it has been found in all cells where it has been sought, including yeast³, bacteria, rat liver and jackbeans⁴. However, the metabolic role of this enzyme has not been established. It is required for normal function, as a mutant strain of *Escherichia coli* with a temperature-sensitive hydrolase cannot continue protein synthesis at the nonpermissive temperature⁵. Hydrolase itself is not required for initiation, elongation or termination phases of protein synthesis⁶. Menninger⁷ has shown that peptidyl-tRNA, formed on ribosomes, accumulates at nonpermissive temperatures in cells with the temperature-sensitive hydrolase. Hydrolase thus seems to be a scavenger enzyme of peptidyl-tRNAs normally aborted from ribosomes during protein synthesis. Of interest now is why peptidyl-tRNA is frequently prematurely released from ribosomes and why protein synthesis is inhibited in the mutant strain with a defective hydrolase⁵. I report here work on the latter problem and present evidence which indicates that peptidyl-tRNAs accumulate and compete with *N*-formyl-Met tRNA^{fMet} during initiation of protein synthesis.

Two possible mechanisms of protein synthesis inhibition at 42°C in the temperature-sensitive hydrolase strain are that one or more infrequently occurring tRNAs may become sequestered as peptidyl-tRNA, resulting in inhibition of polypeptide elongation or, on the other hand, that the only known product of the reaction (peptidyl-tRNA) may interfere with initiation of polypeptides. Peptidyl-tRNA is not unlike *N*-formyl-Met-tRNA^{fMet}. Thus, these two similar compounds may compete at initiation for the P-site on the ribosome.

When the hydrolase mutant strain is incubated at temperatures intermediate between permissive growth and nonper-

missive growth (40, 40.5, 41 °C), partial inhibition of growth is observed. This is interpreted as limited inactivation of hydrolase, resulting in a reduced rate of peptidyl-tRNA hydrolysis. If elongation rates are affected at these intermediate temperatures, protein polymerisation time should be increased. If initiation of protein synthesis is inhibited, however, the polymerisation rate will remain unchanged for a specific protein.

The time lag between the induction of the lactose operon and the first appearance of β -galactosidase activity above basal enzyme level was used to estimate the peptide chain elongation rate^{8,9}. It has been shown that this approach is not a reflection of changed mRNA synthesis times⁸. Hence a measure of polymerisation time of β -galactosidase can differentiate between the two possibilities outlined above.

The data presented in Table 1 indicate that the rate of peptide-chain elongation does not change significantly with increase in growth temperature in the hydrolase temperature-sensitive strain. The chain elongation rate is slightly less in the mutant strain at 40 °C than in the parent strain (20%), but nearly identical at the permissive temperature (30 °C). However, the growth rate of the mutant strain decreases considerably as the growth temperature is increased from 37 to 41.5 °C, reflecting inhibition of protein synthesis by steadily increasing concentrations of peptidyl-tRNA in the cell. Furthermore, the relative rate of polypeptide chain initiation is proportionally decreased (Table 1, column 5). These data are derived from the initial slope of the induction curve; they reflect the relative number of molecules synthesised per unit time. The relative number of chain initiations in strain AA-7852 (*pth*^{ts}) decreases by slightly more than half from 40 to 41.5 °C, and the doubling time decreases proportionally. No significant growth or β -galactosidase induction is observed at 42°C, reflecting complete inactivation of the hydrolase.

Other data suggesting that initiation of protein synthesis is inhibited in the hydrolase mutant strain at the nonpermissive temperature come from studies of the polysome distribution after shift to 43 °C. Menninger *et al.*⁶ found that polysomes slowly dissociated at 43 °C, forming 70S subunits. Elongation continues temporarily, resulting in dissociation of polysome structures. However, initiation only forms 70S units, possibly because of a peptidyl-tRNA in the P-site. This is consistent with the idea that some poison accumulates, very probably peptidyl-tRNA, but must reach a critical level before inhibition is seen. Protein synthesis is not inhibited in the hydrolase mutant until about 10 min after transfer to the nonpermissive temperature.

In conclusion these data show that the number of polypeptide chain initiations decreases as hydrolase is partly inactivated. On the other hand, the chain elongation rate is not affected. These findings imply that hydrolase plays an important part in cell metabolism by destroying spontaneously formed peptidyl-tRNAs which, in turn, inhibit protein synthesis initiation, probably by competing with *N*-formyl-Met-tRNA^{fMet} in the P-site of the ribosome.

This investigation was supported by the Iowa Agriculture and Home Economics Experiment Station and a grant from the NSF. I thank Mary Mascia for technical assistance.

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Received 29 June, accepted 8 September 1978.

Table 1 Amino acid polymerisation and growth rates at various temperatures

Strain	Growth temperature	Doubling time (min)	Polymerisation rate (amino acids s ⁻¹)	Initial slope of induction curve (nmol ONPG hydrolysed per min per 3 × 10 ⁸ cells)
AA-7852	30.0 ± 0.05	190 ± 5	5.6 ± 0.3	55
	37.0 ± 0.05	170 ± 5	6.8 ± 0.2	57
	40.0 ± 0.05	210 ± 8	7.5 ± 0.2	42
	41.0 ± 0.05	270 ± 10	7.3 ± 0.2	29
	41.5 ± 0.05	300 ± 10	7.3 ± 10	21
CP-78	30.0 ± 0.05	150 ± 5	6.1 ± 0.3	72
	40.0 ± 0.05	110 ± 5	9.1 ± 0.2	75

Cells were grown in M-9 medium with 0.4% glycerol and required amino acids in a gyrorotary shaking water bath at the specified temperature⁶. Strains CP-78 (a K12 strain of *E. coli*, *arg, his, leu, thr, thi*) and AA-7852 (a single-step mutant of strain CP-78 that is now temperature-sensitive for peptidyl-tRNA hydrolase (ref. 5) were induced for β -galactosidase synthesis by addition of 5 mM 3', 5'-cyclic AMP and 1 mM isopropylthiogalactoside after growth at the specified temperature for 2 hours. The cultures were sampled every 5 s by taking 0.5 ml into test tubes containing 0.50 ml of a solution (1 mg ml⁻¹) of chloramphenicol in distilled water⁸. These samples were assayed for β -galactosidase activity by cell lysis with toluene and hydrolysis of *o*-nitrophenol- β -D-galactopyranoside (ONPG) at 25 °C. Polymerisation rate was established by the time required for the first newly synthesised β -galactosidase molecules to appear^{8,9}. There are 1,021 amino acids in β -galactosidase¹⁰. A change of 0.004 A₄₂₀ units equals 1 nmol of ONPG¹¹.

- Lapidot, Y. & deGroot, N. *Prog. Nucleic Acid Res. molec. Biol.* **12**, 189-228 (1972).
- Menninger, J. R., Mulholland, M. C. & Stirewalt, W. C. *Biochim. biophys. Acta* **217**, 496-511 (1970).
- Jossel, H. & RayBhandary, U. L. *J. molec. Biol.* **35**, 539-560 (1968).
- Menninger, J. R., Deery, S., Draper, D. & Walker, C. *Biochim. biophys. Acta* **335**, 185-195 (1974).
- Atherly, A. G. & Menninger, J. R. *Nature new Biol.* **240**, 245-246 (1972).
- Menninger, J. R., Walker, C., Tan, P. F. & Atherly, A. G. *Molec. gen. Genet.* **121**, 307-324 (1973).
- Menninger, J. R. *J. biol. Chem.* **251**, 3392-3398 (1976).
- Galas, D. J. & Branscomb, E. W. *Nature* **262**, 617-619 (1976).
- Zengel, J. M., Young, R., Dennis, P. & Nomura, M. *J. Bact.* **129**, 1320-1329 (1977).
- Fowler, A. V. & Zabin, I. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1507-1510 (1977).
- Kennell, D. & Riezman, H. *J. molec. Biol.* **114**, 1-21 (1977).