THE SPECIFICITY OF PEPTIDYL-tRNA HYDROLASE FROM *E. COLI*

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Received 9 June 1975

1. Introduction

Peptidyl-tRNA hydrolase is an enzyme which hydrolyses the ester bond between a peptide or a N-blocked aminoacyl residue and the 2′ or 3′OH group of the terminal ribose of tRNA [1]. The specificity of the enzyme isolated from *E. coli* has been studied in some detail. The enzyme does not hydrolyze methyl or ethyl esters of peptides [2], 2′ or 3′ adenosyl esters of peptides or unblocked aminoacyl-tRNA [3]. The enzyme is not specific for a certain tRNA since it is capable of hydrolysing all the N-blocked aminoacyl-tRNA's tested [4] and attacks peptidyl-tRNA's such as $(Gly)_2$-phe-$tRNA^{E.\ coli}$ and $(Gly)_2$-phe-$tRNA^{yeast}$ with the same efficiency [6]. There seems to be only one exception as Lapidot et al. [5] found, that the enzyme does not attack (or attacks only very slightly) peptide esters of the *E. coli* initiator tRNA, such as $(Gly)_2$-met-$tRNA_F^{met}$.

Recently, tRNA's modified in the 3′-terminal adenosine or in the penultimate cytidine nucleotides have been investigated; some of these tRNA's can be aminoacylated. One of these can be prepared by oxidizing the 3′-terminal ribose with periodate and reduction of the dialdehyde with sodium borhydride to tRNA-C-C-$A_{oxi\text{-}red}$ [7]. Other derivatives can be prepared by incorporating ATP analogues to tRNA-C-C or CTP analogues and ATP to tRNA-C with the tRNA-nucleotidyl transferase. In this work we describe the action of a purified *E. coli* peptidyl-tRNA hydrolase on several peptidyl-tRNA's prepared from these modified tRNA's, $tRNA^{phe}$C-C-3′deoxyA [8]; $tRNA^{phe}$C-C-3′amino-A [9]; $tRNA^{phe}$C-C-F [10]; (F = formycin), $tRNA^{phe}$C-i^5C-A [11] and $tRNA^{phe}$C-C-$A_{oxi\text{-}red}$. It was found that peptidyl-tRNA hydrolase can hydrolyse all the different peptidyl-tRNA's except peptidyl-$tRNA^{phe}$C-C-$A_{oxi\text{-}red}$.

2. Methods

The different tRNA derivatives from yeast were prepared as previously described [7–11] from yeast $tRNA^{phe}$.

E. coli peptidyl-tRNA hydrolase was prepared essentially according to Kössel [4]. The final enzyme preparation gave in a SDS acrylamide gel (12%) only one protein band.

The tRNA derivatives were aminoacylated with [^{14}C]phenylalanine using yeast phenylalanyl-tRNA synthetase [12] according to Chinali et al. [13], and the corresponding $(Gly)_3$-phe-tRNA's were prepared from them according to Lapidot and Rappoport [14]. In all the substrates prepared, at least 80% of the tRNA was aminoacylated (± 1300 pmol/A_{260} unit). Therefore, one could neglect the inhibition caused by small amounts of uncharged tRNA, which may be present in the substrate preparation [15]. The kinetic data obtained were used directly for the evaluation of the K_M and V_{max} values of the different substrates.

The enzyme activity was assayed by determining the increase of radioactivity in 5% trichloroacetic

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acid soluble products during incubation of the substrates with the enzyme. The values obtained were corrected for the non-enzymatic hydrolysis. Incubation was carried out in a final volume 0f 0.125 ml and contained triethanolamine buffer, pH = 8.0, 40 mM; magnesium acetate 5 mM; β-mercaptoethanol 5 mM; bovine serum albumin 0.05 mg; substrate as indicated in the figure and enzyme (0.05 μg – 0.5 μg protein according to the substrate). Samples (20 μl) were transferred at zero time and after one, two and three min of reaction into 100 μl of 6% aqueous trichloroacetic acid. The samples were centrifuged and the radioactivity in the supernatant was determined in a liquid scintillation counter according to Turner [16].

High voltage paper electrophoresis of the reaction mixture after the incubation showed that $(Gly)_3$-[^{14}C]phe is the only radioactive compound produced by the action of the enzyme on the substrates used in this paper.

3. Results and discussion

Fig.1 shows the kinetics of the hydrolysis of the different $(Gly)_3$-phe-$tRNA^{phe}$'s by the *E. coli* peptidyl tRNA hydrolase. From the data in fig.1 the K_M and V_{max} values were calculated. These values are summarized in table 1. From the results it is clear that only one of the peptidyl-tRNA's $(Gly)_3$-phe-$tRNA^{phe}$C-C-$A_{oxi-red}$ is not hydrolysed by the enzyme. $(Gly)_3$-$tRNA^{phe}$C-C-$A_{oxi-red}$ can, however, bind to the enzyme as it behaves like a competitive inhibitor, with an inhibition constant K_I = 10 μM. The same K_I value was obtained for bulk $tRNA^{phe}$C-C-A. From these results and from the K_M value for $(Gly)_3$-phe-$tRNA^{phe}$C-C-A (0.7 μM, table 1) one can conclude that peptide linked to an 'open terminal ribose ring', does not contribute to the binding of the compound to the enzyme and, that an intact ribose ring is necessary for the action of the enzyme on the substrate

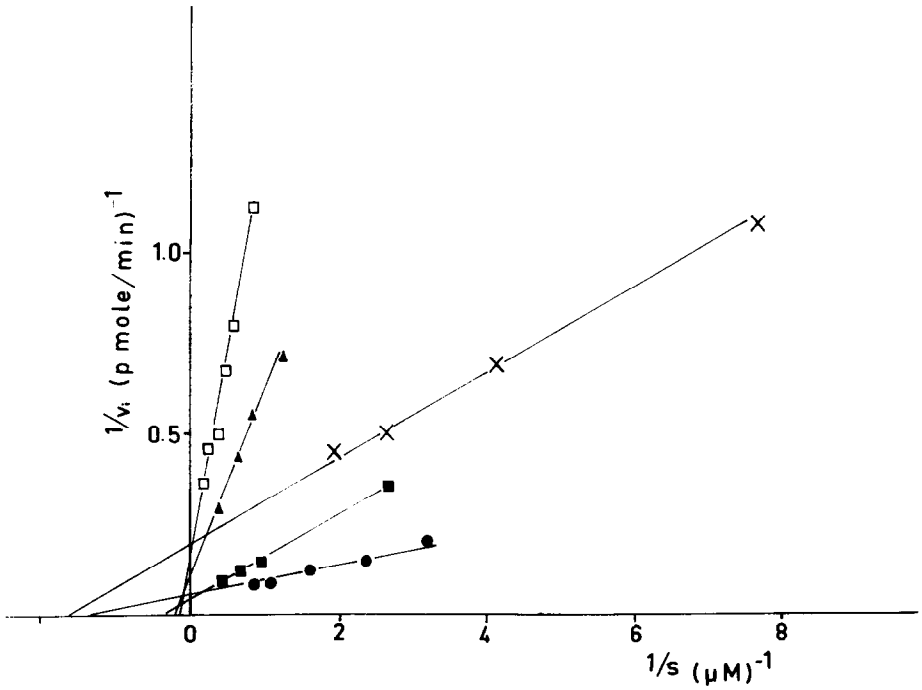

Fig.1. Lineweaver – Burk plots for the various substrates. Reaction mixtures as described in Methods, contained 0.05 μg protein of the purified enzyme for $(Gly)_3$-phe-$tRNA^{phe}$C-C-A (●——●) and for $(Gly)_3$-phe-$tRNA^{phe}$C-i^5C-A (■——■), 0.1 μg protein for $(Gly)_3$-phe-$tRNA^{phe}$C-C-3'deoxyA (▲——▲) and for $(Gly)_3$-phe-$tRNA^{phe}$C-C-F (×——×), and 0.5 μg protein for $(Gly)_3$-phe-$tRNA^{phe}$C-C-3'aminoA (□——□). All the velocity measurements were corrected for the same enzyme concentration. The experimental data concerning a straight line were fitted to the appropriate equation by the least-square method assuming equal variance for the velocities.

Table 1
K_M and V_{max} values for the various substrates determined from Lineweaver – Burk plots

Substrate	K_M (μM)	K_i (μM)	Relative V_{max}
$(Gly)_3$-phe-$tRNA^{phe}$C-C-A	0.7		100
$(Gly)_3$-phe-$tRNA^{phe}$C-C-F	0.63		25
$(Gly)_3$-phe-$tRNA^{phe}$C-i^5C-A	2.8		120
$(Gly)_3$-phe-$tRNA^{phe}$C-C-3′deoxyA	6.6		50
$(Gly)_3$-phe-$tRNA^{phe}$C-C-3′aminoA	7.0		29
$(Gly)_3$-phe-$tRNA^{phe}$C-C-$A_{oxi\text{-}red}$		8.0[a]	<3
$tRNA^{phe}$C-C-A		9.0	

[a] Only the K_I value for $(Gly)_3$-phe-$tRNA^{phe}$C-C-$A_{oxi\text{-}red}$ was determined as this compound is not hydrolysed at a detectable rate.

The reason for the unsusceptibility of the $(Gly)_3$-phe-$tRNA^{phe}$C-C-$A_{oxi\text{-}red}$ to the enzyme is not due to lack of hydroxyl group vicinal to the carbon atom carrying the peptidyl residue, as $(Gly)_3$-phe-$tRNA^{phe}$C-C-3′ deoxyA which also lacks the vicinal OH group, can be hydrolysed by the enzyme with a V_{max} close to that of the unmodified $(Gly)_3$-phe-$tRNA^{phe}$C-C-A. From this result one can conclude that the hydroxyl group vicinal to the peptide residue is not directly involved in the hydrolytic action of the enzyme. $(Gly)_3$-phe-$tRNA^{phe}$C-C-3′ aminoA can also be hydrolysed but the maximum velocity of the reaction is three to four times smaller than that of the normal substrate. It is clear, therefore, that peptidyl-tRNA hydrolase can hydrolyse amide bonds (although it does not hydrolyse the amide bonds in the peptide chain). In the phe-$tRNA^{phe}$C-C-3′aminoA, the substance from which $(Gly)_3$-phe-$tRNA^{phe}$C-C-3′aminoA was prepared by chemical peptidation, all the phenylalanyl residues are linked to the terminal ribose in an alkaline stable bond (data not shown), and therefore, 100% of the amino acid is linked in a peptide bond to the 3′amino group of the terminal ribose. Another conclusion which one can draw is, therefore, that peptidyl-tRNA hydrolase can release a peptide bound to the 2′ and to the 3′ position of the terminal ribose, because in $(Gly)_3$-phe-$tRNA^{phe}$ C-C-3′deoxyA the peptide is bound exclusively to the 2′ position and in $(Gly)_3$-phe-$tRNA^{phe}$ C-C-3′aminoA it is bound exclusively to the 3′position of the ribose.

The action of the peptidyl-tRNA hydrolase preparation on $(Gly)_3$-phe-$tRNA^{phe}$C-C-3′aminoA is not due to a proteolytic activity present in the purified enzyme preparation. When aliquots of this enzyme preparation were incubated with casein, no proteolytic activity could be measured, even if the aliquot contained an amount of enzyme a hundred times that normally used in our assay system (results not shown).

The peptidyl-tRNA hydrolase also hydrolyses $(Gly)_3$-phe-$tRNA^{phe}$C-C-F and $(Gly)_3$-phe-$tRNA^{phe}$C-i^5C-A. The affinity of the first substrate for the enzyme is of the same order of magnitude as the unmodified $(Gly)_3$-phe-$tRNA^{phe}$, however, the V_{max} is four times smaller. Similar results were obtained for the interaction between $tRNA^{phe}$C-C-F and the phe-tRNA synthetase, namely $tRNA^{phe}$C-C-F has the same K_M as $tRNA^{phe}$C-C-A, but the V_{max} of the aminoacylation reaction with $tRNA^{phe}$C-C-F is fifty times smaller than the reaction with the normal tRNA as substrate [10].

The V_{max} for the hydrolysis of $(Gly)_3$-phe-$tRNA^{phe}$C-i^5C-A is approximately equal to that of the 'normal' substrate, but the bulky iodine atom in the penultimate C seems to cause some hindrance in the tRNA–enzyme interaction, as the K_M value for this substrate is somewhat higher than that of the normal substrate.

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