IRREVERSIBLE INACTIVATION OF ARGINYL-tRNA LIGASE BY PERIODATE-OXIDIZED tRNA

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1. Introduction

Aminoacyl-tRNA ligases play a crucial role in protein synthesis. Each enzyme esterifies one of the amino acids to the corresponding tRNA in the presence of ATP and Mg^{2+}. Periodate-oxidized tRNA is often used as a dead-end inhibitor in kinetic studies in this class of enzymes [1–5]. With the arginyl-tRNA ligase from *Escherichia coli* K12 we observed apparent mixed-type non-competitive inhibition with tRNA as the variable substrate. We also demonstrated an irreversible inactivation of the enzyme upon preincubation with periodate-oxidized tRNA. This finding indicates that special attention has to be paid to the interpretation of inhibition patterns obtained with this inhibitor.

2. Materials and methods

2.1. *Arginyl-tRNA ligase*

The enzyme was purified from crude extracts of *E. coli* K12. Details of the procedure followed will be published elsewhere. The purity of the preparation was $\geqslant 95\%$ based on polyacrylamide gel electrophoresis. The specific activity was determined to be 50 000 units/mg, under the conditions defined in [6].

2.2. *tRNA and periodate-oxidized tRNA*

Crude tRNA was isolated from *E. coli* K12 by phenol extraction followed by DEAE-cellulose chromatography [7] and stripping [8]. tRNA with the 3′-terminal *cis*-diol group oxidized by periodate to a dialdehyde function was prepared as in [9].

2.3. *Aminoacylation assay*

The catalytic activity of arginyl-tRNA ligase was assayed under optimum conditions for aminoacylation of tRNA (1 mg/ml) at 37°C in 100 μl or 200 μl containing 0.1 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes)–NaOH buffer (pH 8.35), 6.5 mM $MgCl_2$, 2.5 mM ATP and 12.5 μM [^{14}C]-arginine, except for fig.1. The kinetic experiments illustrated fig.1 were performed at pH 7.4 in order to reduce the non-enzymatic hydrolysis of arginyl-tRNA which cannot be neglected at pH 8.35. The fixed substrate concentrations were chosen about equal to the K_m values. At pH 7.4 the optimum $MgCl_2$ concentration was determined to be 1.5 mM in excess over ATP, instead of 4 mM at pH 8.35. Enzyme concentrations and times of incubation were chosen so that the measured activity was proportional to the concentration of active synthetase. Samples for liquid scintillation counting were prepared as described [6].

2.4. *Inactivation of arginyl-tRNA ligase*

Kinetics of inactivation were followed at 37°C or 50°C in 0.1 M Hepes–NaOH buffer (pH 7.4) containing 1.5 mM $MgCl_2$, 10 μM dithioerythritol and 50 μg/ml serum albumin, unless otherwise mentioned. Aliquots of the incubation mixtures were withdrawn at timed intervals and assayed directly as in section 2.3, using the aminoacylation assay.

3. Results

Figure 1 shows that periodate-oxidized tRNA apparently gives mixed type non-competitive inhibi-

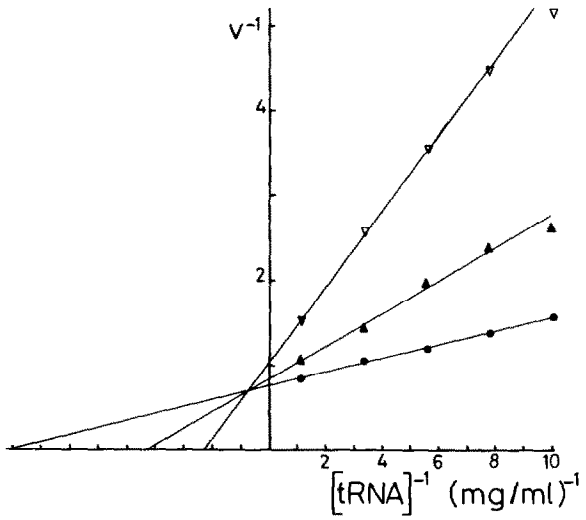

Fig.1. Double-reciprocal plot of arginylation of tRNA in absence (•) and in presence of periodate-oxidized tRNA: (▲) 0.28 mg/ml; (▽) 0.84 mg/ml). Data were obtained from the initial velocity of the aminoacylation assay: 0.1 M Hepes–NaOH (pH 7.4), 2.5 μM [^{14}C]arginine, 0.95 mM ATP, 2.45 mM $MgCl_2$, 0.1–0.9 mg/ml tRNA. Enzyme concentration was 2.2 ng/200 μl assay. Incubation for 2.5 min at 37°C.

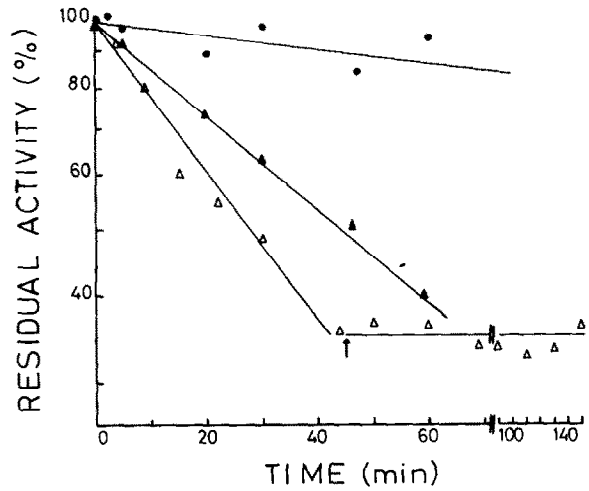

Fig.2. Time-dependent inactivation of arginyl-tRNA ligase at 37°C; (•) 11.2 ng/ml ligase in 0.5 ml buffer; (▲) 11.2 ng/ml ligase in 0.5 ml buffer with 0.7 mg/ml periodate-oxidized tRNA; (△) 112 ng/ml ligase in 0.1 ml buffer with 7.1 mg/ml periodate-oxidized tRNA; after 45 min (indicated by the arrow) the enzyme was diluted 50-fold with 0.1 M Hepes–NaOH buffer (pH 7.4), containing 0.2 mg/ml serum albumin and 1 mM dithioerythritol, and allowed to stand at 24°C.

tion with respect to tRNA in the arginyl-tRNA ligase system. The experiment was repeated several times and the double-reciprocal plots, calculated using the least square method, always intersected to the left of the $1/v$ axis.

Following this observation we investigated the effect of a preincubation of the ligase with the inhibitor on catalytic activity. It is obvious that if an inactivation of the enzyme occurs by reaction with the inhibitor during the assay, the concentration of active enzyme will be decreased in the experiments in which periodate-oxidized tRNA is added, causing the double-reciprocal plots not to intersect on the ordinate. Figure 2 demonstrates that at 37°C indeed such an

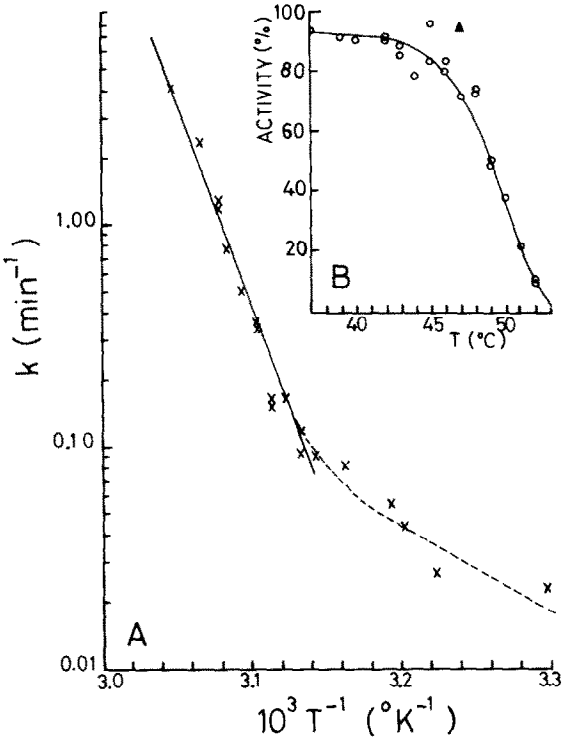

Fig.3. Heat inactivation of arginyl-tRNA ligase. The enzyme (0.6 μg/ml) was incubated in 0.5 ml 0.01 M Tris–HCl buffer (pH 7.4), containing 0.1 mM dithioerythritol at the temperatures indicated. (A) Arrhenius plot: the activation energy for the process, calculated with the first-order rate constants (k) of the decay curves in the region 45–55°C is 85 kcal/mol. (B) The percentage of the activity remaining after 2 min incubation at different temperatures; (▲) serum albumin (0.1 mg/ml) added.

inactivation occurs. Practically no loss of enzymatic activity is observed in the absence of the inhibitor or in the presence of untreated tRNA. When the inactivated synthetase was diluted with 50 vol. buffer, no recovery of activity could be demonstrated, neither by incubation at 0°C nor at 24°C.

At 50°C periodate-oxidized tRNA was found [10] protected to the same extent as untreated tRNA the ability of arginyl-tRNA ligase from *E. coli* B to catalyze an ATP–PP_i exchange. As can be seen from fig.3, the aminoacylation activity of arginyl-tRNA ligase from *E. coli* K12 is rapidly lost at 50°C. Serum albumin protects the enzyme against thermal inactivation, possibly by eliminating wall effects. tRNA specifically protects the enzyme [10]. We observed protection of enzymatic activity upon incubation at 50°C by periodate-oxidized tRNA as in [10], but to a much lower degree than is obtained with untreated tRNA at the same concentration which is assumed to saturate the enzyme (fig.4).

Table 1 shows the effect of the substrates of the enzyme on the inactivation process. High concentrations of tRNA and ATP clearly protect the enzyme against inactivation by periodate-oxidized tRNA; arginine on the other hand does not exhibit measurable protective effects.

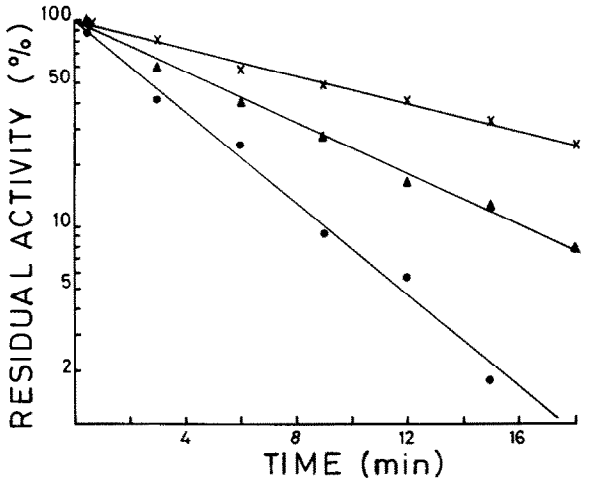

Fig.4. Time-dependent inactivation of arginyl-tRNA ligase (11.2 ng/ml) at 50°C in 0.5 ml buffer in the presence of 0.28 mg/ml tRNA (×), 0.28 mg/ml periodate-oxidized tRNA (▲) and without any tRNA (●).

Table 1
Effect of substrates on the inactivation of arginyl-tRNA ligase (11.2 ng/ml) by periodate-oxidized tRNA at 37°C

Additions				% act.
Oxidized tRNA (mg/ml)	tRNA (mg/ml)	Arginine (μM)	Mg^{2+}-ATP[a] (mM)	
–	–	–	–	93.4
–	0.28	–	–	95.8
0.7	–	–	–	59.8
0.7	0.28	–	–	66.0
0.7	2.8	–	–	87.1
0.7	–	3.0	–	66.0
0.7	–	25	–	60.6
0.7	–	–	1.3	68.3
0.7	–	–	9.7	81.8

[a] Added as an equimolar mixture of ATP and $MgCl_2$

The activity remaining after 30 min incubation was determined and is expressed as % of the original activity

4. Discussion

The experiments reported here demonstrate that periodate-oxidized tRNA not only inhibits the aminoacylation reaction catalyzed by arginyl-tRNA ligase by acting as a dead-end inhibitor, but also inactivates the enzyme. Binding of periodate-oxidized tRNA to the enzyme in addition yields protection against thermal denaturation. At 37°C the enzyme is heat-stable and the inactivating reaction can be observed. At 50°C the enzyme is heat-labile and the inactivation by periodate-oxidized tRNA is obscured by the stabilizing effect of the binding.

The inactivation by periodate-oxidized tRNA could occur through the formation of a Schiff's base between the reactive dialdehyde moiety of the modified tRNA and a functional group on the enzyme, such as the ε-amino group of a lysine residue. The fact that tRNA and ATP protect the enzyme from inactivation supports the hypothesis that the reacting group is in or near the active site of the enzyme. The possibility of an interfering reaction was mentioned in [11]. Recently, similar reactions were shown to occur in several other aminoacyl-tRNA ligases with pyridoxal 5′-phosphate [12] and with 2′,3′-ribose oxidized ATP [13], which were also assumed to be active site directed reagents.

The possible inactivation caused by periodate-treated tRNA stresses the importance of a careful evaluation of kinetic measurements in the presence of this reagent. Because of the concentration-dependent protection afforded by the substrates against the inactivation of arginyl-tRNA ligase, it was not possible to make accurate corrections for the observed reaction velocities. It is clear however that a mixed-type non-competitive inhibition pattern can be obtained unless the reaction conditions are such that the inactivation by periodate-oxidized tRNA proceeds only to a neglegible extent in the time period of the assay.

On the other hand, periodate-oxidized tRNA might prove useful as an active site labeling agent.

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