ARGINYL-tRNA SYNTHETASE FROM *BACILLUS STEAROTHERMOPHILUS*: HEAT INACTIVATION AND SUBSTRATE INDUCED PROTECTION

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1. Introduction

Among all the known aminoacyl-tRNA synthetases, the ones specific for arginine, glutamine and glutamate differ from the others with respect to their requirement for tRNA to catalyse an amino acid dependent ATP/[^{32}P]PP_i exchange reaction. Recent reports concerning glutamyl-tRNA synthetase from *E. coli* [1] and our results with arginyl-tRNA synthetase from *Bacillus stearothermophilus* [2] have suggested that these particular synthetases catalyse the aminoacylation of tRNA via a concerted reaction. These findings are consistent with the hypothesis first proposed by Loftfield and Eigner [3]. In a previous report [2] we have shown that there is a sequential addition of substrates to arginyl-tRNA synthetase from *B. stearothermophilus*, in which the binding of tRNA and ATP is prerequisite for the binding of arginine to the enzyme. The aim of the present work was to establish whether the binding of arginine could be related to a conformational change of the protein caused by tRNA and ATP. We have studied heat inactivation of our enzyme alone or when combined with its substrates. Our results show that the binding of arginine to arginyl-tRNA synthetase in the presence of tRNA and ATP induces an important synergistic protection of the enzyme against heat inactivation, already at very low arginine concentrations (2 μM).

2. Results

In a first set of experiments, we have determined the range of thermostability of our enzyme and the optimal temperature for the aminoacylation reaction it catalyses. The enzyme was incubated in HEPES buffer (0.1 M, pH 7.4) containing 1 mM dithioerythritol and the temperature of the incubation mixture was raised gradually. Aliquots of enzyme were removed every 5°, diluted in cold buffer containing 100 μg/ml serum albumin and the enzyme activity was measured at 37° in the standard reaction mixture described previously [2]. These experiments show (fig. 1) that the enzyme is stable up to 70° and in inactivated to 50% at 74.5° under these conditions.

Fig. 2 shows the rate of aminoacylation as a function of temperature. Limiting amounts of enzyme were incubated at different temperatures in the standard reac-

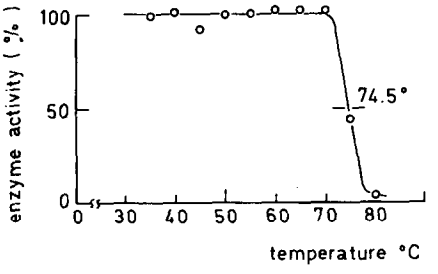

Fig. 1. Thermostability of arginyl-tRNA synthetase from *Bacillus stearothermophilus* (EC 6.1.1. a). The enzyme (20 μg) was incubated in 240 μl of 0.1 M HEPES buffer (Sigma) pH 7.4 containing 1 mM dithioerythritol. The temperature was raised gradually at the rate of 1°/min. Aliquots of enzyme were removed every 5° and diluted in the same cold buffer, containing 100 μg/ml of serum albumin. Enzyme activity was assayed in the standard aminoacylation reaction mixture described previously [2], using limiting amounts of enzyme. (o—o—o) Initial rate of aminoacylation in %.

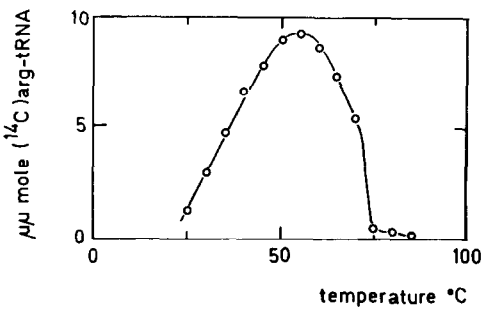

Fig. 2. Initial rate of arginyl-tRNA formation catalysed by arginyl-tRNA synthetase at temperatures ranging from 25° to 85°. Aliquots of the standard reaction mixture containing limiting amounts of enzyme were incubated for 10 min at each temperature and the amount of formed [^{14}C]arg-tRNA was measured.

tion mixture. The maximal rate of reaction is achieved around 55°. Above 70° this rate drops rapidly and becomes very low at 74.5°. Heat inactivation of arginyl-tRNA synthetase was studied at this latter temperature (74.5°). Fig. 3 shows typical first order inactivation of arginyl-tRNA synthetase incubated at 74.5°. When the enzyme is incubated alone or with 2 μM arginine, only 5% of the initial enzyme activity is recovered after 5 min. When the same amount of arginine is combined with 2 mM ATP and 0.9 mg/ml

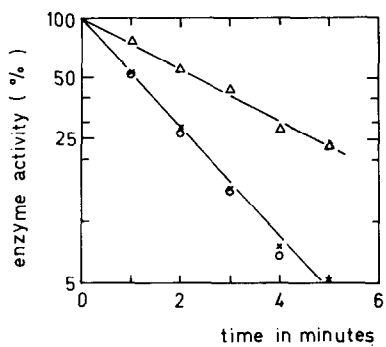

Fig. 3. Heat inactivation of arginyl-tRNA synthetase. The incubation mixture contained 0.1 M HEPES buffer pH 7.4, 1 mM dithioerythritol, 7.5 mM $MgCl_2$, 1 μM enzyme and substrates as indicated below. The incubation was carried out at 74.5° and the remaining enzyme activity measured every minute, after cold dilution as described in fig. 1. (X—X—X) enzyme alone; (○—○—○) plus 2 μM arginine; (△—△—△) plus 2 μM arginine, 2 mM ATP and 0.9 mg/ml of total tRNA.

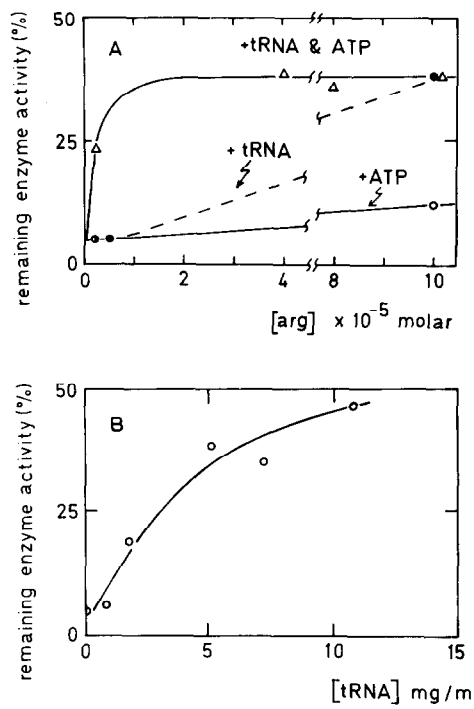

Fig. 4. Substrate induced protection of arginyl-tRNA synthetase against heat inactivation. The incubation mixture was as described in fig. 3. The incubation was carried out for 5 min at 74.5°. The remaining enzyme activity was assayed after cold dilution as described in fig. 1. (A) % of remaining enzyme activity after incubation with varying amounts of arginine: (○—○—○) arginine alone or + 2 mM ATP; (●—●—●) arginine + 0.9 mg/ml of total tRNA from *B. stearothermophilus* (1.6 μM $tRNA^{ARG}$); (△—△—△) arginine + 2 mM ATP and 0.9 mg/ml of total tRNA. (B) % of remaining enzyme activity after incubation with varying amounts of total tRNA from *B. stearothermophilus*.

of tRNA, the remaining enzyme activity is of 25% after the same incubation time.

Fig. 4 summarizes a series of similar inactivation plots of arginyl-tRNA synthetase in the presence of different substrates, for several arginine concentrations (4A) and for different tRNA concentrations (4B). Protection due to tRNA becomes effective only at concentrations above 1 mg/ml (4B). With arginyl-tRNA synthetase from *E. coli* Mitra et al. [4] have observed a synergistic protection of the enzyme due to the combined effect of arginine and tRNA, for arginine concentrations of 0.1 mM or more.Under those conditions ATP had no effect on the observed synergistic protection. Our experiments

Table 1
Association constants for arginyl-tRNA synthetase and its different substrates.

Substrate	K_a calculated from heat inactivation experiments	K_a calculated from substrate-binding experiments [2]
tRNA	2×10^{-6} M ($tRNA^{arg}$)	–
ATP	–	4×10^{-7} M
Arginine (2 mM ATP and 1.6 μM $tRNA^{arg}$ present)	7×10^{-7} M	2×10^{-7} M

with arginyl-tRNA synthetase from *B. stearothermophilus* (fig. 4A) also reveal a synergistic protection when the enzyme is incubated with an amount of tRNA unable to protect when present alone, and 0.1 mM arginine. But at lower arginine concentrations, such as 2 μM, the presence of arginine and tRNA does not increase enzyme protection. The synergistic protection now appears only when ATP is added to the system. (fig. 4A). These experiments show that if the synergistic protection can be observed with only tRNA and arginine for relatively high arginine concentrations (0.1 mM), the presence of ATP is absolutely required to induce synergistic protection at lower arginine concentrations such as 2 to 5 μM, where the enzyme is already saturated with arginine in a system containing all the substrates [2]. The amount of ATP engaged in these experiments conferred no protection at all when present alone and did not increase the protection due to tRNA. The low rate of aminoacylation at 74.5° shown in fig. 2 and the low extent of the reaction at this temperature [5], both indicate that the stable form of the enzyme in the presence of its three substrates is most probably the quaternary enzyme–substrate complex formed prior to the aminoacylation.

According to Changeux [6] a system like ours, where the enzyme alone is inactivated and the enzyme–substrate complex is the stable form, permits the determination of the association constant, K_a, of this enzyme–substrate complex. In table 1, the K_a values calculated from our data for arginine (in the complete system containing tRNA, ATP and arginine) and for tRNA are given and compared to the corresponding constants calculated from binding experiments described previously [2]. The similitude of the K_a's for arginine derived from these two systems indicates that the same enzyme–substrate complex was considered in both cases.

The sequential addition of tRNA, ATP and arginine to arginyl-tRNA synthetase has been shown to lead to the formation of a quaternary enzyme–substrate complex [2]. The results described above show that the system in this complex is strongly protected against heat inactivation. These results might be explained by a stabilization of the active form of the enzyme due to the presence of the substrates, or by a transformation process of the enzyme occurring during the binding of the substrates and leading to the active form of the synthetase.

The question remains open whether this stabilization or transconformation of the enzyme is achieved in one step during the binding of the last substrate (arginine) or whether each substrate contributes to a partial change, leading to the final active form. Further experiments are in progress in order to answer this question.

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