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A comparative study of the relationship between thermostability and function of phenylalanyl-tRNA synthetases from *Escherichia coli* and *Thermus thermophilus*

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The relationship between thermostability and functional activities of phenylalanyl-tRNA synthetases (EC 6.1.1.20) from *E. coli* and *Thermus thermophilus* has been studied. In the case of the *E. coli* enzyme, the activity decreased after the 43°C treatment, both in the $[^{32}P]PP_i$–ATP exchange reaction and the overall aminoacylation reaction, due to thermo-inactivation of the phenylalanyl-tRNA synthetase, whereas $tRNA^{Phe}$ preserved its native structure. In the *Th. thermophilus* system, the enzyme showed extreme thermostability (up to 90°C), and the reduction in the tRNA aminoacylation rate after the 78°C treatment was ascribed to denaturation of the $tRNA^{Phe}$. Since the enzyme did not lose the $[^{32}P]PP_i$–ATP exchange activity up to 85°C, the observed lower thermo-resistance of the tRNA is evidence that the native structure of ribonucleic acids should be one of the most difficult to stabilize at high temperatures.

Protein biosynthesis; Phenylalanyl-tRNA synthetase; tRNA; Thermostability

1. INTRODUCTION

Amino-tRNA synthetases are key enzymes of protein biosynthesis that provide the high accuracy of genetic information translation. The catalytic responsibility of these enzymes is the aminoacylation of tRNA via the formation of an enzyme–aminoacyladenylate complex (E·AA~AMP) followed by the transfer of the activated aminoacylic residue on tRNA:

$$E + ATP + AA \rightleftharpoons E \cdot AA\sim AMP + PP_i \quad (1)$$
$$E\cdot AA\sim AMP + tRNA \rightleftharpoons AA\sim tRNA + AMP + E \quad (2)$$

To elucidate the effect of temperature on the catalytic function of aminoacyl-tRNA synthetases and their relationship with thermostability is of great importance for understanding the regulation of protein biosynthesis at high temperatures. Since intensive investigations of extreme thermophilic systems of protein biosynthesis have just started, only a few data concerning tRNA and rRNA [1–3] are available. As to the enzyme components of protein biosynthesis systems, there are no data whatsoever. In the present work, our aim was to investigate the relationship between function and thermostability of hetero-oligomeric enzymes of protein biosynthesis which have a quaternary structure of the $\alpha_2\beta_2$-type, specifically the phenyl-alanyl-tRNA synthetases from *E. coli* and an extreme thermophile, *Thermus thermophilus*.

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2. MATERIALS AND METHODS

tRNA from *E. coli* MRE-600 containing 2.5% $tRNA^{Phe}$ was purchased from NPO Biolar (Latvia). tRNA from *Th. thermophilus* HB8 containing 2–3% $tRNA^{Phe}$ was obtained as in [2]. The phenylalanyl-tRNA synthetases from *E. coli* MRE-600 and *Th. thermophilus* HB8 were purified according to [4].

The thermo-inactivation curves for the enzymes incubated in a 25 mM Tris–HCl buffer (pH 8.0) at different temperatures were obtained by measuring residual tRNA aminoacylation activity at 25°C. The concentration of the incubated proteins was 10–80 μg/ml. During the incubation, aliquots were taken out, cooled rapidly to 25°C and the enzymatic activity was measured in the reaction of $tRNA^{Phe}$ aminoacylation as in [4].

The electrophoretic separations of the incubated enzyme solutions were done by a Phast System (Pharmacia, Sweden) using Phast Gel Gradient 8-25 which allowed separation under native conditions in an 8–25% PAAG gradient.

To characterize the rate of amino acid activation (Equation 1), the isotopic exchange of ATP-$[^{32}P]$pyrophosphate was used. To reveal the temperature dependences of $[^{32}P]PP_i$–ATP exchange rates the reaction mixtures involved 12.5 mM ATP, 0.33 mM ammonium $[^{32}P]$pyrophosphate, 2.5 mM EDTA, 24 mM $MgCl_2$, 0.5 mM L-phenylalanine, 25 mM Tris–HCl (pH 8.0), 3.5 μg/ml phenylalanyl-tRNA synthetase. The mixtures were pre-incubated without enzyme at a certain temperature for 3 min. After addition of enzyme the aliquots were taken out at certain time intervals and then added to a stop-mixture (5% TCA, 10 mM sodium pyrophosphate, 1% activated charcoal). After vigorous stirring the samples were filtered through nitrocellulose filters and washed with water (5 × 10 ml). The activated charcoal was fixed on the filters by polyvinylic alcohol. The filters were dried and their radioactivity measured.

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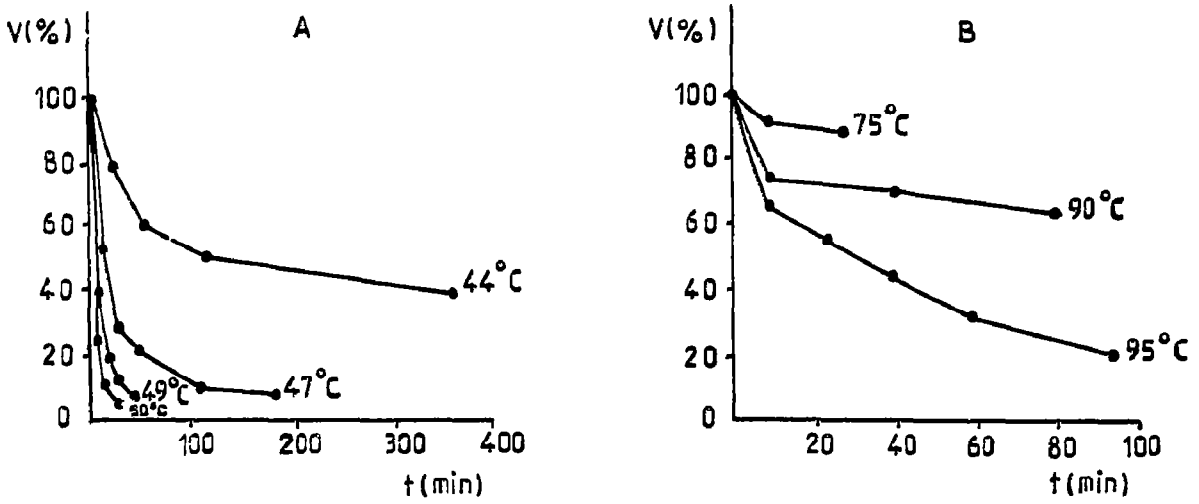

Fig. 1. Thermo-inactivation curves of phenylalanyl-tRNA synthetases from *Escherichia coli* (A) and *Thermus thermophilus* (B), where v(%) is the rate of tRNA aminoacylation reaction (100% corresponds to that of a sample without incubation).

3. RESULTS

The curves of thermo-inactivation of the mesophilic (A) and thermophilic (B) phenylalanyl-tRNA synthetases are shown in Fig. 1. To compare the data obtained for both enzymes, it was necessary to find a standard parameter characterizing the enzymes' thermostability. Here we have chosen the value of residual enzymatic activity in tRNA aminoacylation at 25°C after 10 min incubation of the enzyme solution at certain temperatures. The data are given in Fig. 2.

Temperature dependencies of the $[^{32}P]PP_i$–ATP exchange rates for both phenylalanyl-tRNA synthetases were defined. As can be seen in Fig. 2, the *E. coli* enzyme dependence has a marked maximum at about 43°C, while that of the thermophilic enzyme shows a steady increase up to 85°C.

The results of native gradient electrophoresis of the mesophilic (A) and thermiphilic (B) enzymes after the incubation at denaturing temperatures are shown in Fig. 3. For the *E. coli* enzyme, we observed the bands corresponding to β-monomers and β_2-dimers, while the thermophilic enzyme gave a single band of a native oligomer.

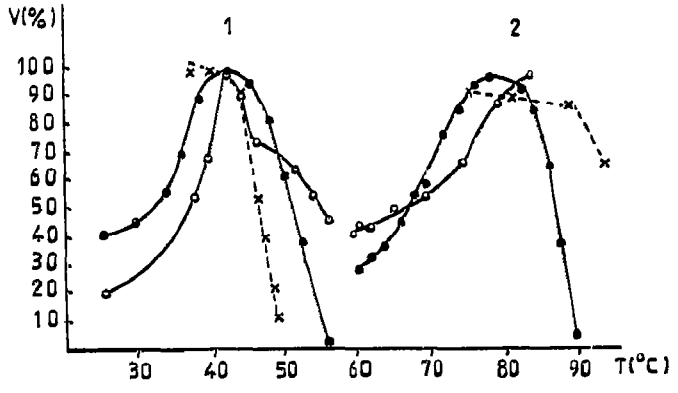

Fig. 2. Temperature dependences of tRNA aminoacylation [5] and $[^{32}P]PP_i$–ATP exchange rates and thermostability curves for phenylalanyl-tRNA synthetases from *E. coli* (1) and *Thermus thermophilus* (2). (●) tRNA aminoacylation activity; (○) $[^{32}P]PP_i$–ATP exchange activity; (×) thermostability.

4. DISCUSSION

The temperature dependencies of the tRNA aminoacylation rates have already been determined [5]. For convenience, they are also given in Fig. 2. The thermostability curve of the mesophilic enzyme correlates well with the activity in both the $[^{32}P]PP_i$–ATP exchange and the total aminoacylation reaction at elevated temperatures. Since the melting temperature of the $tRNA^{Phe}$ *E. coli* is known as 75°C [2], the decrease in the enzymatic activity after 43°C treatment is due to the thermal denaturation of the mesophilic phenyl-alanyl-tRNA synthetase.

To find the cause of the enzyme denaturation we carried out electrophoretic separations of the incubated mixtures (Fig. 3A). The bands corresponding to different forms of β-subunits indicate that the mesophilic oligomer dissociates at high temperature. That we failed to observe any α-subunit is likely to be due to its strong tendency to aggregate [6], which could have increased with temperature. It is interesting that, even after the incubation at 95°C accompanied by thermal denaturation, the thermophilic enzyme did not produce any bands except that of the native oligomer, whose intensity decreased with the time of incubation (Fig. 3B). This means that the phenylalanyl-tRNA synthetase from *Th. thermophilus* has much stronger intersubunit contacts.

Comparison of the data on thermostability and catalytic activity in the case of the thermophilic enzyme

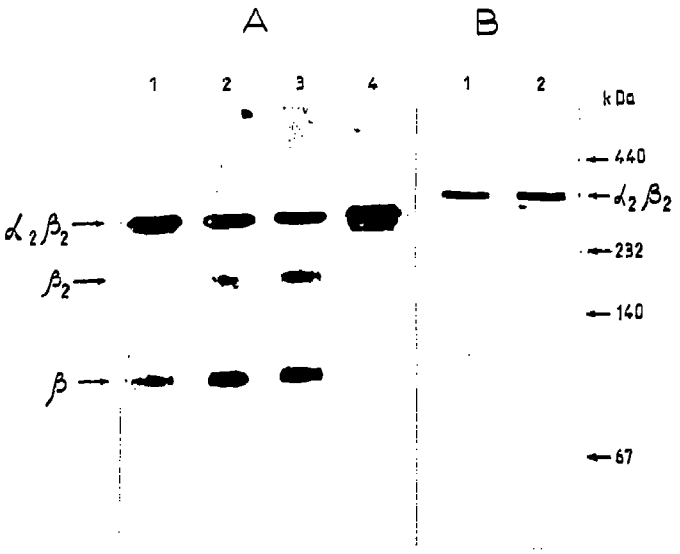

Fig. 3. Native gradient electrophoresis of phenylalanyl-tRNA synthetases from *E. coli* (A) and *Thermus thermophilus* (B) after incubation under denaturing temperatures (47 and 90°C, respectively). A, incubation for 10 min (lane 1), 45 min (lane 2), 60 min (lane 3) and 0 min control (lane 4). B, incubation for 100 min (lane 1) and 0 min control (lane 2).

leads to a quite different result (see Fig. 2). Up to 90°C, the phenylalanyl-tRNA synthetase from *Th. thermophilus* was fairly stable (not less than 90% of initial values) and could catalyze the $[^{32}P]PP_i$–ATP exchange up to at least 85°C. Watanabe et al. [2] obtained the melting curves of the tRNA from *E. coli* and *Th. thermophilus* under similar conditions, including the data for $tRNA^{Phe}$. The thermophilic $tRNA^{Phe}$ starts melting at 78°C, with a melting temperature of 85°C. These data agree well with our tRNA aminoacylation curve. Thus, one can consider that the thermo-dissociation of the *E. coli* enzyme was the cause of the loss of its catalytic activity, while in the thermophilic system, where the enzyme itself is very thermostable, it is the denaturation of tRNA that was the factor limiting the aminoacylation by 78°C.

We believe these properties are important in order to understand the main principes of thermostabilization, as well as to estimate possible thermostability limits of protein biosynthesis. Evidently, the problem here is the tRNA which can hardly resist the high temperature by increasing GC-content and methylation just because it is shorter than the DNA. As to rRNA, according to [1] its thermostabilization is achieved by forming a complex with ribosomal proteins. Hence, it may be assumed that, in extreme thermophiles, it is the ribonucleic components that are the least protected from the elevated temperatures.

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