COMPARATIVE STUDIES ON THE ISOLEUCYL- AND LEUCYL-tRNA SYNTHETASES FROM *BACILLUS STEAROTHERMOPHILUS* AND *ESCHERICHIA COLI*: THERMAL STABILITY OF THE AMINOACYLADENYLATE-ENZYME COMPLEXES

J. CHARLIER, H. GROSJEAN, P. LURQUIN, J. VANHUMBEECK and J. WERENNE
Laboratory of Biological Chemistry, Faculty of Sciences, University of Brussels, Brussels, Belgium

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1. Introduction

Bacillus stearothermophilus is a thermophilic microorganism that grows at temperatures as high as 65°.

The isoleucyl- (EC 6115) and leucyl-(EC 6114) tRNA synthetases extracted from this microorganism are particularly thermostable and are able to aminoacylate *in vitro* the transfer ribonucleic acids (tRNA) at temperatures above 70° [1].

In previous papers [2,3] we already mentioned that these enzymes can form quite stable enzyme-bound aminoacyladenylate complexes which can be isolated from a reaction mixture by gel filtration. Under appropriate experimental conditions these complexes are able to transfer their aminoacyl group onto specific tRNA's.

Because of the exceptional resistance to heat of the activation enzymes from *B. stearothermophilus*, it was of interest to compare the stabilities of the (aminoacyladenylate-enzyme) complexes formed by these enzymes with the corresponding complexes formed by the synthetases extracted from a mesophilic strain, namely *E. coli*.

In this paper we systematically compared the thermal stabilities of the isolated (aminoacyladenylate-enzyme) complexes formed with the isoleucyl- and leucyl-tRNA synthetases from *B. stearothermophilus* and *E. coli*.

The results indicate that some differences do exist between the stabilities of the complexes but no striking differences have been observed between the complexes formed either with the thermophilic or the mesophilic enzymes.

2. Materials and methods

Organisms, chemicals and the preparation of activation enzymes from *B. stearothermophilus* have been described elsewhere [2,3].

Activation enzymes from *E. coli* (AS2 fraction) were prepared according to Bergmann et al. [4].

B. stearothermophilus as well as *E. coli* tRNA were prepared by phenol extraction of the whole cells according to Brubaker and McCorquodale [5]. They were further purified by chromatography on DEAE-cellulose as described by Stephenson and Zamecnik [6].

^{14}C labelled aminoacids (150–300 mC/mM) were purchased from The Radiochemical Centre, Amersham (England).

The (aminoacyladenylate-enzyme) complexes were made and isolated by gel filtration on Sephadex G-50 essentially as described by Norris and Berg [7].

The enzyme-substrates complexes obtained from the major peak fractions eluted from the Sephadex columns were used for heat inactivation experiments in the following way: The isolated complexes (0.1 ml in 0.05 M sodium succinate buffer pH 6.0 containing 0.05 M KCl and 0.001 M EDTA) were incubated in a water bath at constant temperature for a given period of time. The samples were then chilled in ice and immediately assayed for enzymatic activity at 0° in mixtures containing 50 mM succinate buffer pH 6.0, 8 mM $MgCl_2$ and 1 mg/ml tRNA. The reaction mixtures were allowed to stand in an ice bath for about 45 min in order to achieve maximal transfer of the aminoacid to the tRNA. The amount of ^{14}C amino-

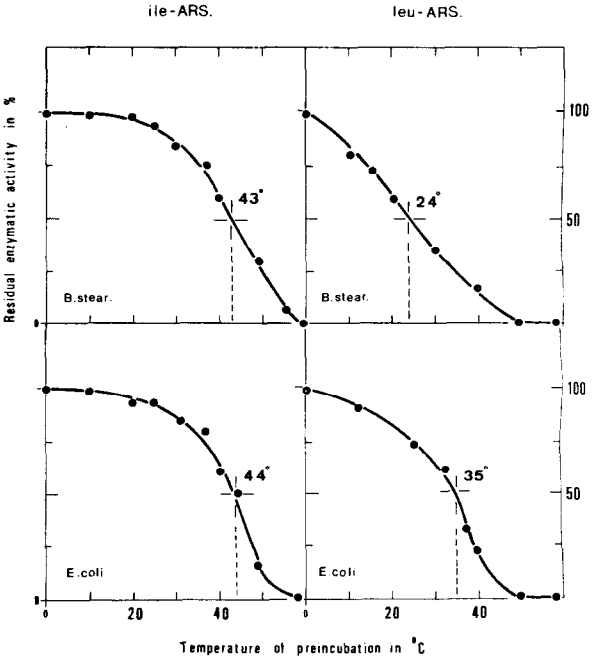

Fig. 1. Comparison between the thermal stabilities of the (aminoacyl-AMP-enzyme) complexes formed with the isoleucyl- and leucyl-tRNA synthetases from *B. stearothermophilus* and *E. coli*. The inactivation concerns the ability of the complexes to transfer their aminoacyl group onto tRNA after an incubation of 7 min at the different temperatures. ARS = aminoacyl-tRNA synthetase.

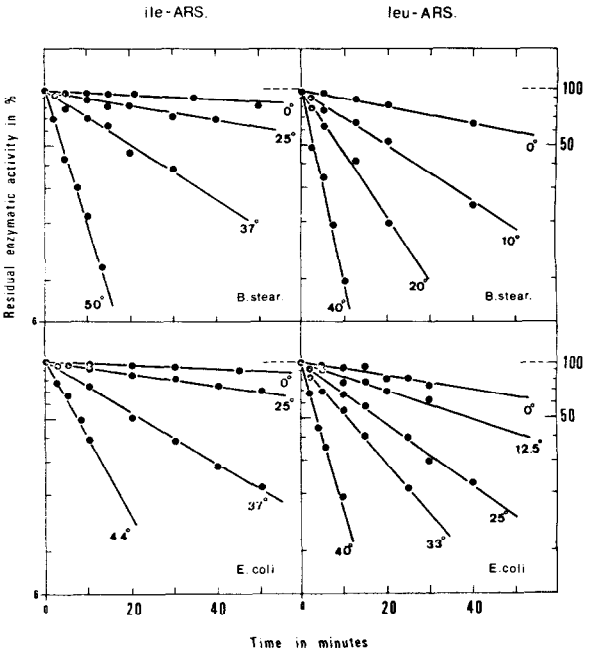

Fig. 2. Kinetics of heat inactivation of the (aminoacyl-AMP-enzyme) complexes formed with the isoleucyl- and leucyl-tRNA synthetases from *B. stearothermophilus* and *E. coli*. For details see Methods. ARS = aminoacyl-tRNA synthetase.

acid transferred to tRNA was determined by the radioactivity which was found associated with the nucleic acid after precipitation by cold trichloracetic acid (10% w/v final) and filtration on Millipore membranes.

The results are expressed as percent of the residual enzymatic activity after heat treatment compared to the enzymatic activity at zero time incubation.

3. Results and discussion

The isoleucyl- and leucyladenylate-enzyme complexes formed with the corresponding aminoacyl-tRNA synthetases from *B. stearothermophilus* and *E. coli* have been isolated by gel filtration and their stabilities have been characterized by their resistance to heat inactivation. Fig. 1 shows the results obtained when samples of the complexes were incubated for 7 min at different temperatures between 0° and 60°. From these experiments we determined temperatures of half-inactivation ($T_{\frac{1}{2}}$) corresponding to the temperatures at which the complexes have lost half of their enzymatic activity. We found that these temperatures were respectively 43° and 24° for the isoleucyl- and leucyl-adenylate-enzyme complexes from *B. stearothermophilus*.

Under the same experimental conditions the values obtained for the corresponding complexes formed with *E. coli* enzymes were respectively 44° and 35°.

Kinetics of inactivation by heat of the different (aminoacyladenylate-enzyme) complexes have also been compared.

In all cases inactivation followed first order kinetics (fig. 2). The relation between the rate constant and the temperature of incubation obeyed the Arrhenius law in the entire range of temperature explored (0° to 50°) (fig. 3).

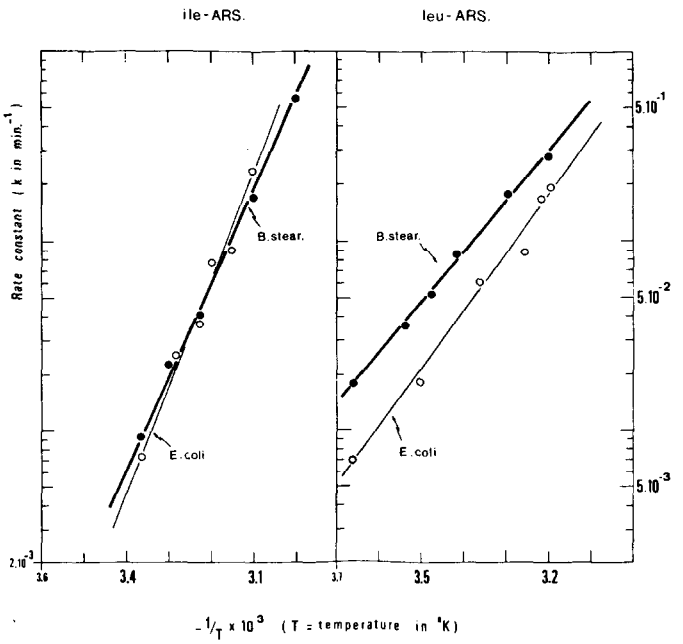

Fig. 3. Arrhenius plots relating the first order rate constants of thermal inactivation of the different (aminoacyl-AMP-enzyme) complexes to temperature. The values of k were derived from the kinetics of heat inactivation (fig. 2) using the relation $k\ (\text{min}^{-1}) = (\ln 2)/\tau$ where τ is the half time of the complex at a given temperature. ARS = aminoacyl-tRNA synthetase.

Table 1

Apparent activation energy (E) for the inactivation by heat of the different (aminoacyl-AMP-enzyme) complexes formed with the synthetases from *B. stearothermophilus* and *E. coli.*

	Enzyme	
Microorganism	Isoleucyl-tRNA synthetase	Leucyl-tRNA synthetase
B. stearothermophilus	23 ± 2	13 ± 2
E. coli	25 ± 2	14 ± 2

These values were calculated from the data obtained from fig. 3. The values of E are expressed in terms of kcal deg^{-1} mole^{-1}.

From these Arrhenius plots, an apparent activation energy (E) was calculated for the inactivation of the transfer capacity of each of these complexes. The results are listed in table 1.

Different values for E have been found for the isoleucyl- and leucyladenylate-enzyme complexes but a very good correspondence was found between the values of E for the same enzyme-substrates complex formed with either the synthetase from *B. stearothermophilus* or that one from *E. coli.*

These results clearly indicate that the different (aminoacyladenylate-enzyme) complexes possess different heat stabilities, but they also show that in no case the complexes formed with *B. stearothermophilus* activation enzymes have higher thermal stabilities than the corresponding complexes formed with *E. coli* enzymes.

Our results do not give any indication about the nature of the binding between the enzymes and their respective aminoacyladenylates; however, if we consider the good correspondence between the values of E obtained for the same aminoacyladenylate bound either to the synthetase from *B. stearothermophilus* or that one from *E. coli*, we can conclude that the transfer capacities of these complexes are equally sensitive to inactivation by heat despite the fact that *B. stearothermophilus* activation enzymes are very stable at high temperature.

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