same direction as our findings. A possible explanation of our results would be that the irradiation directly damages enzymic protein(s) or the structural integrity of the system (for example on the basis of the impairment of microsomal lipids[19,20]); alternatively enzyme activator(s) may be reduced. Finally, the decrease in the proteosynthesis of this enzymic system could be considered.

A possible effect on the pituitary, with the resulting changes in the functions of the adrenal cortex or the sexual glands, has also to be kept in mind[9,21].

Résumé. L'irradiation (800 R) entraîne une diminution de la déméthylation in vitro de la péthidine par la fraction microsomale du foie de Rat, du premier au cinquième jour après l'irradiation. Une réduction marquée a été aussi observée après 1000 R du premier au troisième jour et après 600 R –, le quatrième et le cinquième jour. Le taux du cytochrome P-450 ne change pas après ces doses dans les intervalles mentionnés. C'est un cas de manque de parallélisme entre le taux du P-450 et les changements de la fonction de la monooxygénase microsomale déjà connue.

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The Effects of Heat on the Interactions Between Leucyl-tRNA Synthetases and tRNA's

Several studies have revealed the importance of tRNA secondary structure for ensuring its aminoacylation by activation enzymes[1]. The thermostable leucyl-tRNA synthetase from *Bacillus stearothermophilus*[2,3] seemed to be particularly suitable for the investigation of the effects of heat on the biological properties of tRNA's in aminoacylation systems in such conditions that a direct effect of heat on enzyme stability can be, if not neglected, at least largely avoided. As cross-reactions between *B. stearothermophilus* and *Escherichia coli* tRNA's and leucyl-tRNA synthetases are complete[2], we were also able to study the effects of heat on heterologous systems.

Materials and methods. The enzymes used in this study were *E. coli* 112-12 AS3 fractions[4] and *B. stearothermophilus* highly purified enzyme fractions[3]. The preparation, purification and properties of tRNA's, labelled products, chemicals and standard reaction mixtures for the loading of tRNA and for the leucine-dependent ATP-PP_i exchange have been fully described elsewhere[3,5]. Melting of tRNA's were performed in standard reaction mixtures (in the presence of $6\times10^{-3}M$ $MgCl_2$) without addition of enzyme preparation.

Results and discussion. We reported earlier that *B. stearothermophilus* leucyl-tRNA synthetase is very resistant against denaturation by heat[2,3]: Figure 1 shows that its kinetics of denaturation are very slow at 68 °C, whereas the corresponding enzyme from *E. coli* is rapidly destroyed at 53 °C. The same figure also shows that both functions of these enzymes – the loading of leucine on tRNA and the ATP-PP_i exchange – possess the same heat stability. Figure 2 represents the effect of heat on the maximum amount of leucine that can be loaded on a limiting amount of tRNA: it can be seen that those loadings are affected differently by heat when *E. coli* or *B. stearothermophilus* tRNA's are allowed to react with the thermostable enzyme from the thermophilic bacteria. In the first case, there is a drop in amino acid accepting activity at 60 °C while this phenomenon only occurs at 65 °C in the second case. Furthermore, at 66.5 °C, the *B. stearothermophilus* tRNA's accept 95% of the amount of leucine loaded at 37 °C, whereas this value only reaches 52% for the *E. coli* tRNA's. The loss of activity occurs when the hyperchromicities of the unfractionated tRNA's are only 2 or 3%; at the T_m of the latter, the residual aminoacylation is almost negligible. The difference between the 2 tRNA species can still be increased when this time the effects of heat on the rates of the loading reactions are investigated. Figure 3 shows that in systems containing tRNA's from *E. coli* and activation enzymes either from *E. coli* or from *B. stearothermophilus*, there is a sharp drop of the initial velocity of the aminoacylation reaction at 30 °C. As the enzyme from *E. coli*

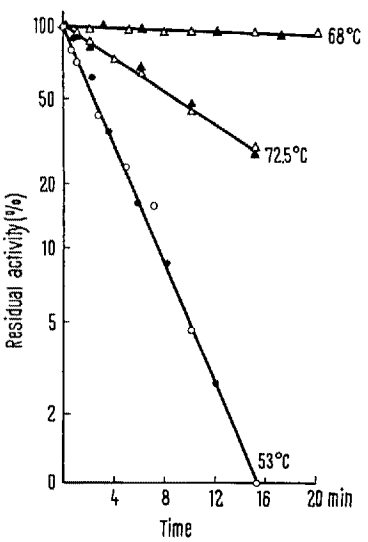

Fig. 1. Thermal stability of the leucyl-tRNA synthetases from *E. coli*: ○—○, loading of leucine on tRNA; ●—●, ATP-PP_i exchange; and from *B. stearothermophilus*: △—△, loading of leucine on tRNA; ▲—▲, ATP-PP_i exchange. The enzymes were incubated in 0.01 *M Tris*-HCl buffer pH 7.4 containing 10^{-3} M reduced glutathione. Aliquots were taken at various time intervals, cooled at 0 °C and assayed for enzyme activity at 37 °C in the standard reaction mixtures.

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The effect of heat on the protecting capacities of *E. coli* tRNA and ATP + leucine. Enzyme: *E. coli* leucyl-tRNA synthetase in 0.01 *M* *Tris*-HCl buffer pH 7.4 containing 0.01 *M* $MgCl_2$ and 0.001 *M* reduced glutathione

Temperature (°C)	k_P (tRNA 1 mg/ml)	k_{NP} (ATP 4×10^{-3} *M* + leucine 1.2×10^{-3} *M*)
48	0	0
50	0.57	0.02
53	0.70	0.12

k_P and k_{NP} are the first order rate constants of the thermal denaturation of the protected and unprotected enzymes respectively. When the protected enzyme is stable at a given temperature, the ratio is equal to 0; when the protected enzyme has the same thermal stability as the unprotected enzyme (loss of protecting capacity of the substrates), the ratio is equal to 1.

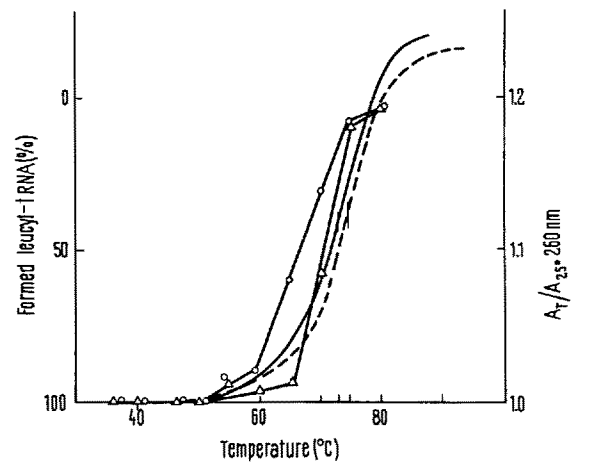

Fig. 2. Effects of heat on the maximum loading of leucine on limiting amounts of tRNA (200 µg/ml). The reaction mixtures were preheated for 5 min at the temperatures indicated; excess *B. stearothermophilus* enzyme was then added and the reaction was stopped 10 min later, after the equilibrium had been reached. ○—○, *E. coli* tRNA; △—△, *B. stearothermophilus* tRNA; —, crude *E. coli* tRNA melting curve in the reaction mixture; ---, crude *B. stearothermophilus* tRNA melting curve in the reaction system.

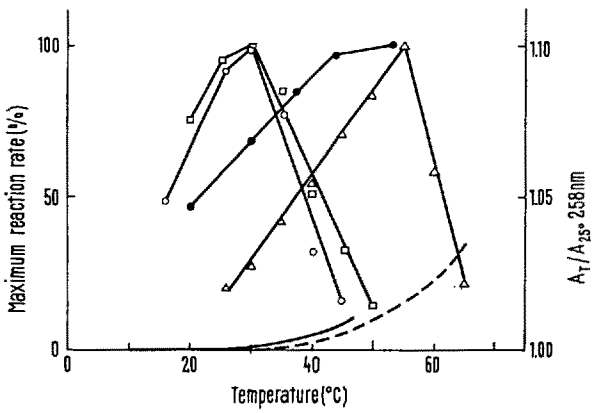

Fig. 3. Effects of heat on the initial velocity of the loading of leucine on tRNA. ○—○, *E. coli* homologous system; △—△, *B. stearothermophilus* homologous system; □—□, system containing *E. coli* tRNA and *B. stearothermophilus* leucyl-tRNA synthetase; ●—●, ATP-PP_i exchange catalyzed by the *E. coli* leucyl-tRNA synthetase; —, *E. coli* $tRNA_{leu}$'s melting curve in the reaction mixture; ---, *B. stearothermophilus* $tRNA'_{leu}$s melting curve in the reaction mixture.

catalyzes normally the ATP-PP_i exchange up to 40 °C, and the enzyme from *B. stsarothermophilus* up to 55 °C[3], it seems unlikely that the phenomenon would be due to enzyme denaturation, especially if we remember that in both systems, as mentioned above, the ATP-PP_i exchange and loading of leucine on tRNA have the same thermal stability. It can be seen from Figure 3 also that a comparable change of the initial velocity occurs in the homologous system from *B. stearothermophilus*, but at a temperature as high as 55 °C. This higher temperature of transition can be related to the high thermal stability of the $tRNA_{leu}$'s from *B. stearothermophilus* that we reported previously[5]. On the other hand, the Table shows that the leucyl-tRNA synthetase from *E. coli* can be protected against thermal denaturation by the presence of *E. coli* tRNA (1 mg/ml) in the incubation mixture. This protection is specific since rabbit reticulocytes tRNA's – which are not 'recognized' by the leucyl-tRNA synthetase from *E. coli*[6] – are inefficient.

It is observed that a little increase in temperature markedly affects the protective effect of tRNA; as this phenomenon almost does not occur when the enzyme is protected by the substrates of the activation reaction (ATP and leucine), it may be concluded that the primary effect of heat is to alter the protecting capacity of the substrate (tRNA) rather than the tRNA recognition site on the enzyme, since the 2 functions of the enzyme possess the same thermal stability and since neither ATP nor leucine are subject to changes at these temperatures.

The results presented in this study suggest that tRNA structure is of capital importance in order to allow efficient enzyme-tRNA interactions. Furthermore, the ability of tRNA to be recognized by the cognate activation enzymes can be altered by heat even before any important loss of tRNA secondary or secondary plus tertiary (folding of the clover-leaf structure – see LEVITT[7]) structure. Similar results were obtained with other systems[8,9] in which the tertiary structure has been shown to melt before the secondary structure ($tRNA^{ser}_{yeast}$) or cooperatively with it ($tRNA^{phe}_{yeast}$); in these cases also, there is a sharp drop in biological activity corresponding to temperatures at which very little hyperchromicity could be observed. It is thus clear that minor modification of the tRNA tridimensional structure are sufficient to hinder activation enzymes – tRNA interactions.

Résumé. La chaleur affecte considérablement l'activité acceptrice d'acide aminé des tRNA à des températures auxquelles leur structure spatiale est très peu modifiée. L'utilisation d'un enzyme thermostable permet de suggérer que la perte d'activité biologique des tRNA est liée à de minimes modifications de leur structure spatiale.

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