THERMAL DENATURATION AND AMINO ACID BINDING ABILITY OF SOLUBLE RNA. (*)

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Several recent studies on heat denaturation of soluble RNA have demonstrated that amino-acid incorporating ability is not destroyed by heat treatment followed by quick cooling (1-2).

This was generally interpreted as indicative of the fact that a specific secondary structure of soluble RNA is not necessary for the enzymatic formation of amino-acyl RNA.

However, the possibility cannot be excluded that the portion of the RNA molecule reacting with the enzyme is renaturated by cooling.

Ofengand et al.(3) reported that with a 20-50 fold excess of leucyl and valyl RNA synthetases from E.coli, the yield of leucyl and valyl RNA differed little at temperatures ranging from 25 to 55° C. At 55° in Na cacodylate buffer without Mg^{++} there was almost one half the maximal hyperchromic shift.

In this case also the authors observed that the possibility could not be eliminated that the activating enzymes reacted with that portion of the molecules with the native configuration.

In the course of some studies on the thermostability of amino-acid activating enzymes from a thermophilic microorganism B.stearothermophilus, (4) we found that isoleucine activating enzyme still catalyzed the pyrophosphate exchange at a temperature of 80° C.

This result was not altered by the addition of s.RNA to the reaction mixture.

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On the other hand the thermal stability of soluble RNA from B.stearothermophilus was investigated and compared with that of soluble RNA from E.coli; in the same experimental conditions (5-6) no significant differences were evident (7). The hyperchromic effect in the presence of concentrations of Mg ions as high as those used in amino-acid activation experiments is reported in figure 1 from a work by Mangiantini, Tecce and Toschi (8).

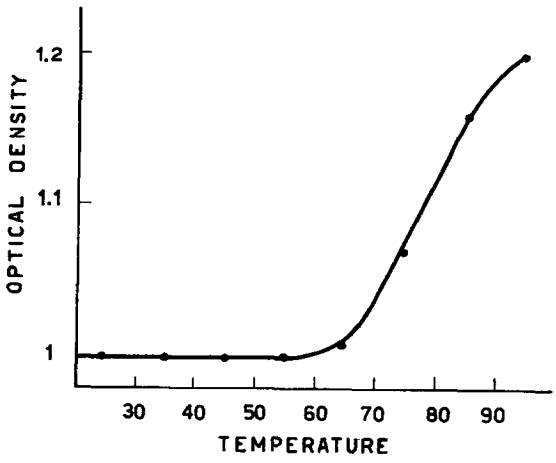

Figure 1. Increase in optical density of sol. RNA at 260 mμ as a function of temperature in Tris-HCl buffer pH 7.2 in the presence of 0.03 M $MgCl_2$.

The results mentioned above provide a good possibility to investigate the problem of amino-acid incorporation by denaturated RNA.

The incorporation of C^{14} L-isoleucine into soluble RNA by extracts of B.stearothermophilus was therefore studied as a function of temperature following the method reported by Berg et al. (10). Results are reported in figure 2. The curve shows an abrupt stoppage at 80° C.

If the isoleucyl-RNA synthetase is the same enzyme catalyzing isoleucine dependent pyrophosphate exchange, which is generally accepted and supported by purification studies (10,11,12,13,14), then the lack of incorporation into soluble RNA at 80°C cannot be ascribed to enzyme denaturation.

Several experiments were therefore performed to investigate the motives for this stoppage.

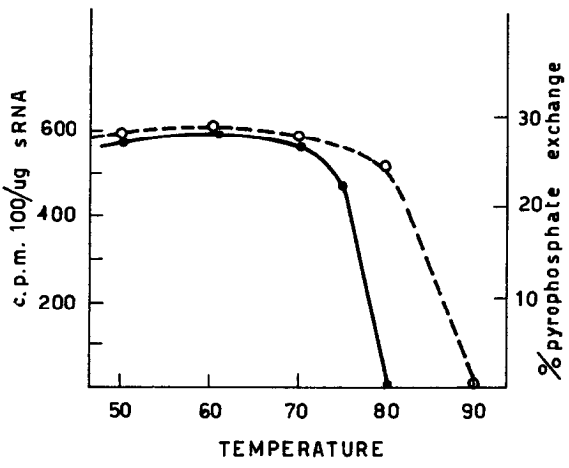

Figure 2. Incorporation of C^{14} L-isoleucine into soluble RNA (full line. For assay conditions see table 1) and pyrophosphate exchange (dotted line) as a function of temperature. Assay conditions for pyrophosphate exchange: 80 µmoles Tris-maleate buffer pH 7.2, 10 µmoles ATP, 10 µmoles $MgCl_2$, 20 µmoles L-isoleucine, 1 mg homogenate protein, 10 µmoles ^{32}PP in 1 ml. Radioactivity in ATP was determined by the method of Demoss and Novelli (9).

Neither pre-incubation of the enzyme at 80°C, nor pre-incubation of the sRNA (with the complete assay mixture) for 10' at the same temperature were found to affect isoleucine incorporation at 50°C. The results of these experiments are reported in Table I.

TABLE I

Incorporation of L-isoleucine into soluble RNA at 50°C

	c.p.m./100µg sRNA
Complete mixture	585
Minus ATP	76
Homogenate pre-incubated at 80°C	482
Complete mixture pre-incubated at 80°C. New enzyme added at 50°C.	560

Complete mixture: 5 µmoles ATP, 40 µmoles Tris-maleate buffer pH 7.2, 15 µmoles $MgCl_2$, 250 µg soluble RNA prepared following the method reported by Boman et al. (15), homogenate containing about 1 mg protein, 0.23 µm C^{14} L-isoleucine (specific activity 2.10^6 c.p.m./ µmole) to a final volume of 0.5 ml. After 3' incubation, RNA was precipitated, washed (10) and dissolved in 1 ml. tri-ethyl-amine. Aliquots were plated and counted with a windowless gas flow counter.

On the other hand the stability of isoleucyl-RNA was studied isolating isoleucyl-RNA and incubating it without enzyme at va-

rious temperatures. The results reported in figure 3 show that isoleucyl-RNA is practically stable at 30° but undergoes non-enzymatic cleavage at higher temperatures. Similar results were obtained with sRNA from E. coli.

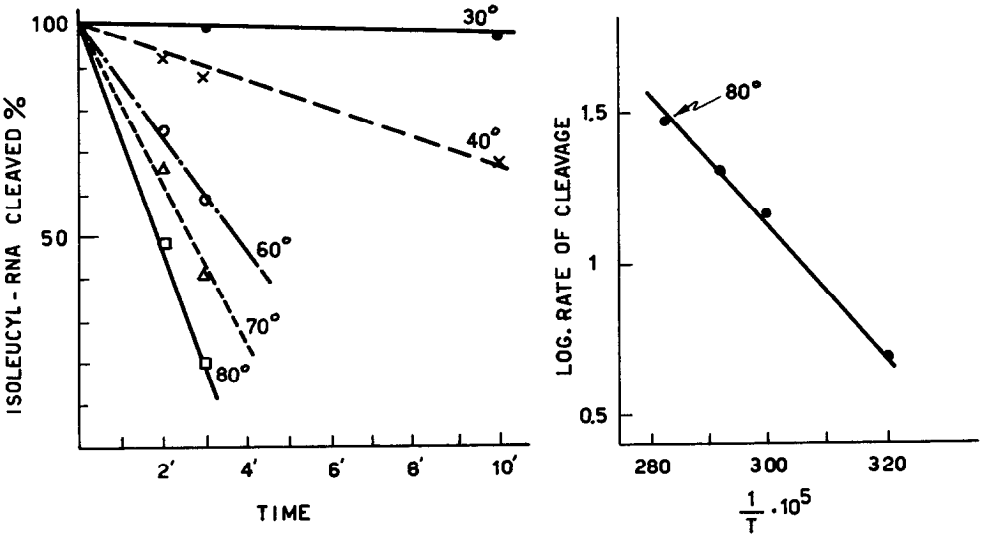

Figure 3. Time course of isoleucyl-RNA cleavage and relative Arrhenius plot (Rate expressed as % isoleucyl-RNA cleaved in 1'). Assay conditions: 2.3 μ moles C^{14} L-isoleucine (specific activity 2.10^6 c.p.m./ μ mole), 50 μ moles ATP, 400 μ moles Tris-maleate buffer pH 7.2, 150 μ moles $MgCl_2$, 3 mg sol RNA, 10 mg of extract protein in a final volume of 5 ml incubated for 10' at 50°C. Isoleucyl-RNA was isolated by adding NaCl and ethanol according to the method of Berg et al. (10). The final product was dissolved in 0.1 M Tris-maleate buffer pH 7.2 containing 0.03 M $MgCl_2$ and incubated at various temperatures. Aliquots were taken at different times and the amount of isoleucyl-RNA was determined by the standard method.

These results, which will be fully discussed in a later publication can be seen to fit the Arrhenius law. It is not probable, therefore, that the abrupt fall between 75° and 80° can be ascribed only to the increased rate of cleavage. On the other hand, as the hyperchromic effect at 75° is 37% and at 80° is 55% of the maximal hyperchromic shift, the lack of isoleucine incorporation at 80° could be explained if a certain degree of secondary structure were necessary for amino-acid incorpora-

tion. This hypothesis is not inconsistent with the results of other authors (2,3), as in our case amino acid acceptor activity ceases suddenly at a critical temperature at which the hyperchromic effect (more than 50% of the maximum hyperchromic shift) is higher than that observed by the authors mentioned for amino acid accepting RNA.

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