RENATURATION OF HEAT-INACTIVATED LEUCYL-tRNA SYNTHETASES FROM BACILLUS STEAROTHERMOPHILUS AND ESCHERICHIA COLI.

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Received May 14, 1968

Leucyl-tRNA synthetases(L-leucine:sRNA ligase(AMP).E.C.6.1.1.4) from E.coli 112-12 (Wollman,1953) and B.stearothermophilus are able to bind the same quantity of leucine per mg of tRNA[x] from both species.Yet,these enzymes differ in their resistance to denaturation by heat,the enzyme from B.stearothermophilus being the more stable (Vanhumbeeck et al.,1968).

Perrin and Monod (1963) showed that E.coli β-galactosidase can be partially reactivated by dialysis against 8 M urea after thermal denaturation and total loss of enzymatic activity.

The same procedure was adapted to the case of E.coli and B.stearothermophilus leucyl-tRNA synthetases.

Enzymatic activity was measured by the rate of formation of leucyl-tRNA with commercial E.coli B tRNA (Schwarz BioResearch). The reaction mixture contained per ml : Tris-HCl (Sigma) buffer pH 7.4,100 μmoles ; ATP disodium salt (Sigma),1 ; $MgCl_2$ (Merck),6;

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[x] The following abbreviations and symbols are used : tRNA,transfer RNA ; Tris,trishydroxymethylaminomethane ; DEAE, diethylaminoethyl ; $O.D._{260}$,optical density at 260 mμ.

reduced glutathione (Sigma),4 ; tRNA,9.6 $O.D._{260}$;leucine ^{14}C 305 mC/mM (The Radiochemical Centre,Amersham,England),2.5 μC. The amount of enzyme added was such that the initial rate of the reaction could easily be measured at 37°C.

Enzymatic extracts from E.coli and B.stearothermophilus partially purified by chromatography on DEAE-cellulose (Zubay,1962) and dissolved in 0.01 M Tris-HCl buffer pH 7.4 containing 0.001 M reduced glutathione,were kept for 10 minutes in a boiling water bath.There was an extensive clotting of proteins and a total loss of leucyl-tRNA synthetase activity : even with a large excess of the dispersed clot no activity could be detected.

The proteins (0.5 ml,3.3 to 7.5 mg of proteins per ml) were then dialysed for 14 hours at 4°C against 1 liter salt-free 8 M urea dissolved in 0.1 M Tris-HCl buffer pH 7.4 containing 0.001 M reduced glutathione and 0.01 M $MgCl_2$.

After that time,proteins dissolved completely.

The preparations were then dialysed against 1 liter 0.1 M Tris-HCl buffer pH 7.4 containing 0.01 M $MgCl_2$ and 1% mercaptoethanol at 4°C.

During the second dialysis some turbidity appeared ; the slightly turbid solutions showed up to 6% and 2.5% of initial activity with B.stearothermophilus and E.coli leucyl-tRNA synthetases respectively.Maximal recovery was attained after a dialysis of 4 hours.

When the extracts were further incubated for 16 hours at 28°C, the recovery reached a maximum of 10% of the activity of the native enzyme preparation for the B.stearothermophilus enzyme. There was no change for the E.coli enzyme.

If the dialysis against Tris-HCl buffer which removes urea was omitted,no enzymatic activity was observed with a sample rapidly

diluted in the assay mixture.

The data are summarized in Table 1.

Table 1.

Specific activity in μmoles of leucine esterified with tRNA/hour/mg of proteins x 10^2.

	E.coli	B.stearothermophilus
Native enzyme	5.1	5.6
Boiled enzyme solution	Not detectable	Not detectable
Boiled enzyme dialysed against 8 M urea and rapidly diluted in reaction mixture	Not detectable	Not detectable
Boiled enzyme dialysed against 8 M urea and then against 0.1 M Tris-HCl buffer	0.11	0.31
Boiled enzyme dialysed against 8 M urea then buffer and further incubated at 28°C for 14 hours	0.11	0.56

The ability of the renatured enzymes to form a leucyladenylate-enzyme complex was also investigated by complex isolation using molecular sieving on Sephadex G-50 according to Norris and Berg (1964).

As in the case of the native enzymes (Lurquin et al.1968) it was possible to isolate a labeled macromolecular fraction able to transfer its amino acid moiety to tRNA in the absence of ATP and without further addition of enzymes in a transfer mixture containing succinate buffer pH 6,50 μmoles ; $MgCl_2$,18 and

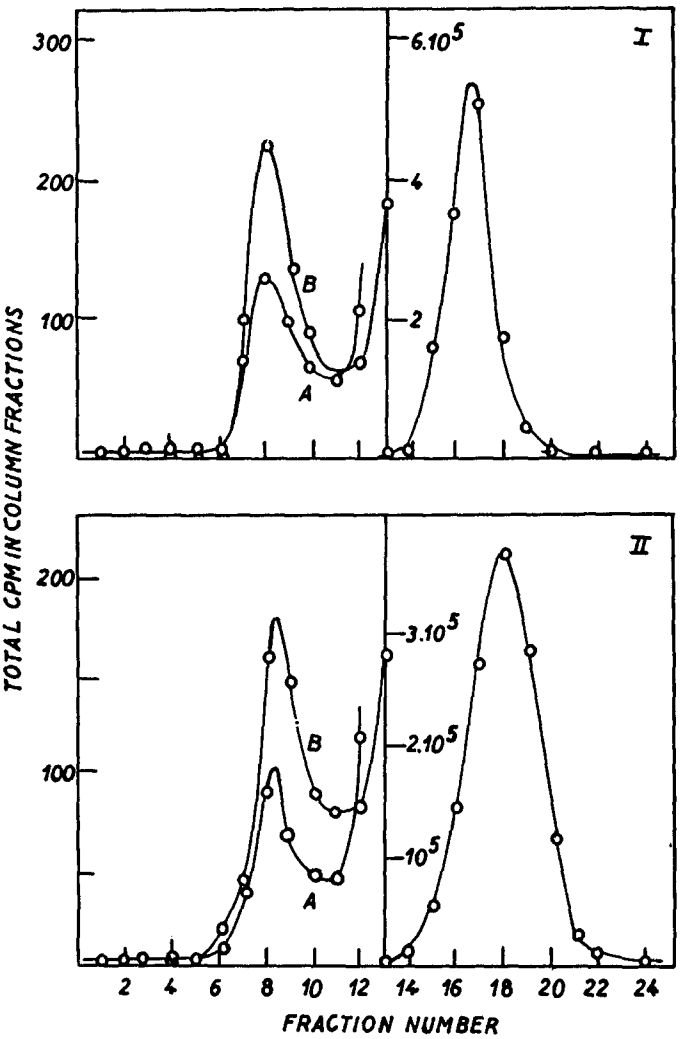

Figure 1.I.B.stearothermophilus enzyme.II.E.coli enzyme. Separation of leucyladenylate-enzyme complex on Sephadex G-50.The reaction mixture(final volume of 340 μl)contains: succinate buffer pH 6,4 μ moles;$MgCl_2$,1.2 μ mole;ATP disodium salt,0.6 μ mole;reduced glutathione,2 μ moles;leucine-^{3}H(The Radiochemical Centre,Amersham,England)1,000 mC/mM, 10 μC;renatured enzymatic extract,300 μ l.

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tRNA,9.6 $O.D._{260}$.The quantities of complexes obtained were in agreement with the levels of renaturation estimated from the kinetic assay.(Fig.1).

The thermal stability of renatured leucyl-tRNA synthetase from

The reaction mixture is incubated for 4 minutes at 37°C,then chilled and made 0.001 M in EDTA.The mixture is poured onto a Sephadex G-50 column(0.5 by 23 cm)previously equilibrated with succinate buffer 0.05 M pH 6 containing 0.05 M KCl and 0.001 M EDTA,and eluted with the same solution.Fractions of about 250 μ l were taken and assayed for enzyme activity and for radioactivity in a Nuclear Chicago Scintillation spectrometer using a toluene-triton based scintillating mixture.
Curve A : without ATP
Curve B : complete reaction mixture.

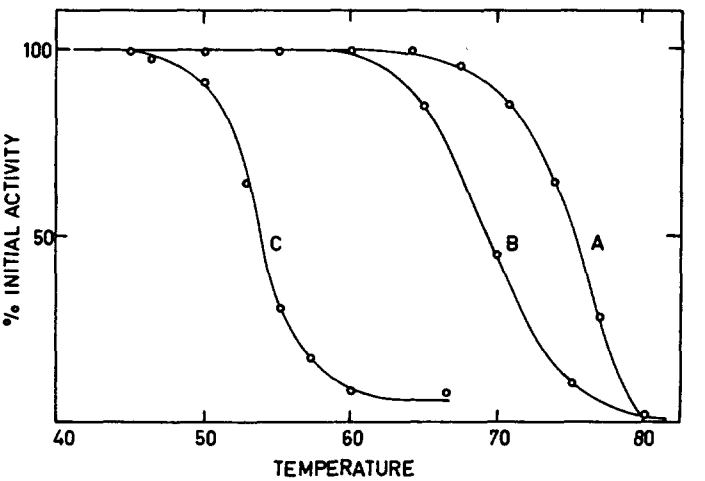

Figure 2.Thermal denaturation of the leucyl-tRNA synthetases. The enzymes(3.3 to 7.5 mg of proteins per ml)are incubated in a water bath which temperature increases by about 1°C/minute. Aliquots are taken and assayed for enzyme activity as described in text. Curve A:native enzyme from B.stearothermophilus
Curve B:renatured enzyme from B.stearothermophilus
Curve C:native enzyme from E.coli.

B.stearothermophilus was studied : it is not very different from that of the native enzyme as can be seen in Fig.2.

It is noticeable that the most heat-resistant enzyme recovers the highest activity upon dialysis against urea after heat inactivation.The renatured molecule from B.stearothermophilus must be structurally very close to the native enzyme since its exceptionnal thermostability has been maintained and is much higher than that of the native enzyme from E.coli.

Yet,it will be noticed from Fig.2 that the renaturation of the B.stearothermophilus enzyme is not perfect since its denaturation curve is not identical to that of the native enzyme.
The features responsible for exceptionnal thermostability have been retained in the renatured enzyme.

The authors hold a predoctoral fellowship from the I.R.S.I.A.

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