TWO ENZYMATICALLY ACTIVE FORMS OF LYSYL-tRNA SYNTHETASE FROM *E. coli* B

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1. Introduction

In a number of communications a non-homogeneity of the aminoacyl-tRNA synthetases (EC 6.1.1) from various multicellular organisms and of different specificity was reported [1–5]. In prokaryotes, however, attempts to find multiple forms of some of the synthetases have often been unsuccessful [6–8].

With lysyl-tRNA synthetase from *E. coli* a non-homogeneity of the enzyme was noticed after fractionation on hydroxylapatite [9, 10] and TEAE-cellulose [11] columns. However, others [12, 13] isolated this enzyme in an apparently homogeneous state.

In the present communication isolation of two enzymatically active forms of lysyl-tRNA synthetase from *E. coli* is described. Both forms have similar values for K_m with lysine and tRNA but differ in K_m for ATP and in some other properties.

2. Materials and methods

Lysyl-tRNA-synthetase from *E. coli* B (harvested at late log-phase) was isolated by means of a modification of the known method [12] described in detail elsewhere [14].

Initial rate of [^{14}C]lysyl-tRNA formation was taken as a measure of enzyme activity. The incubation mixture contained in a total volume of 0.1 ml (pH 8.0) 10 μmole Tris-HCl, 1 μmole $MgCl_2$, 0.25 μmole ATP, 0.1 μmole ethylenediaminetetra acetate, 10 nmoles L-[^{14}C]lysine, 0.4–0.8 nmoles of total tRNA and a rate-limiting amount of the enzyme protein. After 4 min at 30° the reaction was terminated with an equal volume of cold 10% trichloroacetic acid. After washing, the precipitate was collected on the nitrocellulose filter (RUFS, Chemapol, Czechoslovakia) and counted in a liquid scintillation spectrometer Intertechnique SL-40 with ~70% efficiency. Transfer RNA was isolated from *E. coli* B as described [15]. Hydroxylapatite was prepared after Lewin [16].

3. Results and discussion

Table 1 summarizes the purification scheme for isolation of two forms of lysyl-tRNA synthetase (Peaks I and II). After chromatography of the enzyme preparation in a linear salt gradient on the hydroxylapatite column two peaks of activity could be seen (fig. 1). Rechromatography of Peak I or Peak II on the same or similar column gave a single symmetric peak without splitting. The same two peaks could be found at earlier stages of isolation, too, i.e. after the calcium phosphate gel fractionation step.

Both enzyme forms gave similar homogeneous peaks after sucrose density gradient centrifugation (fig. 2) and after gel-chromatography on the Sephadex G-200 [14]. Molecular weights of both active forms are 100,000 (calculated after Martin and Ames [17]). From gel chromatography data with three marker proteins the values are 90,000 (calculated after Andrews [18]) [14].

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Table 1
Purification of the lysyl-tRNA synthetase from *E. coli*.

No.	Fraction	Volume (ml)	Protein (mg)	Activity (units)*	Specific activity (units/mg)	Recovery (%)
1	Crude extract	7250	50750**	289.3	1.4	
2	Streptomycin supernatant	8550	35400**	523.9	3.7	100
3	$Ca_3(PO_4)_2$ gel	1100	4400	626.1	35.2	119
4	Combined DEAE-Sephadex fractions	607	667	226.8	100.0	50
5	Hydroxylapatite eluate					
	Peak I	63	20	21.6	270.0	4.1
	Peak II	88	13	23.4	500.0	4.5
	Other fractions***	57	31	41.8	337.5	8.0
6	Sephadex G-100 eluate of Peak I	34	6	9.8	405.0	1.9

Starting material 0.8 kg wet weight.
* Unit of activity was defined as micromoles of [^{14}C]lysyl-tRNA formed during 1 min. A rate-limiting amount of enzyme fraction was used each time.
** Tentative determinations since no correction for interfering substances was made.
*** Fractions could be rechromatographed on the same column to increase the yield of the pure enzyme forms.

Table 2
Apparent values of K_m at 30° for lysyl-tRNA synthetase from *E. coli* B.

Substrate	K_m (M)	
	EI	EII
Aminoacylation assay		
L-Lysine	6.6×10^{-6}	5.0×10^{-6}
ATP	3.3×10^{-4}	3.7×10^{-5}
tRNA	2.0×10^{-6}	5.7×10^{-6}

Table 3
Comparison of the two forms of lysyl-tRNA synthetase from *E. coli* B.

Common properties	Distinctive properties
Molecular weight	Affinity to hydroxylapatite
K_m for lysine	K_m for ATP
Thermostability	Sensitivity to KCl

These values are in accord with calculations made from analytical centrifugation measurements [10] and sucrose gradient centrifugation [13] but differ slightly from that obtained from polyacrylamide gel electrophoresis (85,000) [11].

Apparent K_m values for substrates of lysyl-tRNA synthetase are given in table 2. The K_m for L-lysine is practically the same for both enzymatic forms and similar to the value reported earlier by Stern and Mehler [12] but differs from the data obtained by others [19] with another strain of *E. coli*. K_m for ATP with enzyme peak II is close to that of Waldenström [10].

However, the K_m with enzyme form I is one order of magnitude higher than with form II. Thus it seems that the affinity of the two enzymatic forms for ATP is not equal.

An attempt was made to find other differences between the two forms. Fig. 3 shows the thermostability of both enzymatic forms to be the same. The

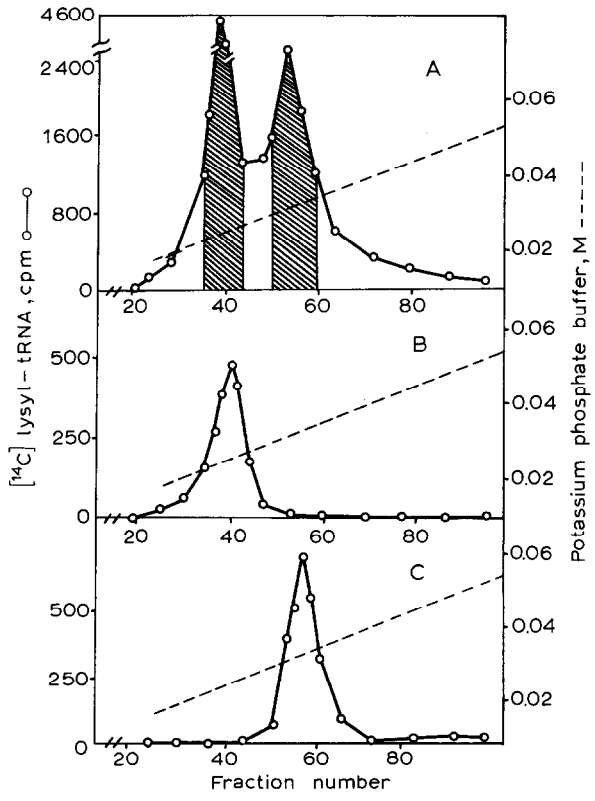

Fig. 1. Chromatography (A) and rechromatography (B and C) of the enzyme preparation on a hydroxylapatite column. Column 2.4 × 13 cm. Elution with linear gradient of 0.5 l of 0.01 M potassium phosphate buffer, pH 6.5, plus 0.5 l of 0.12 M potassium phosphate buffer, pH 7.5. (o—o—o): [^{14}C] lysyl-tRNA formation, (- - - -): gradient of pH and ionic strength. For rechromatography aliquots of the peak I and peak II were taken.

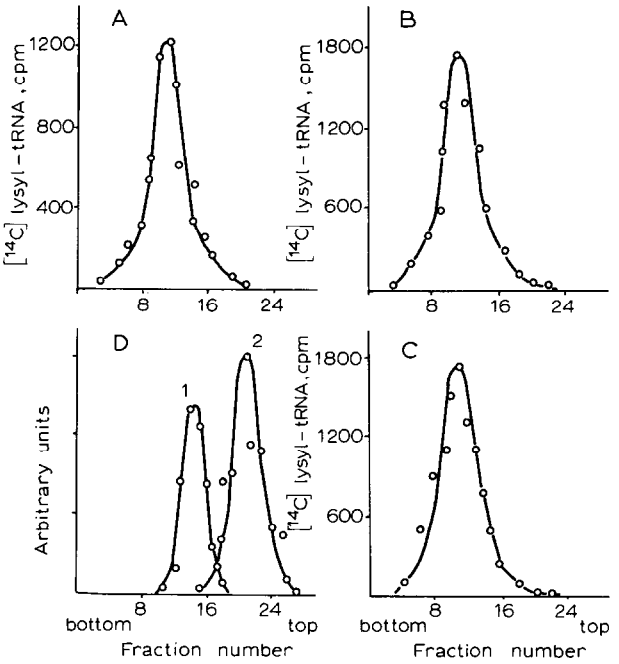

Fig. 2. Comparison of the two enzymatic forms of lysyl-tRNA synthetase by sucrose gradient centrifugation. 20–5% sucrose gradient, Spinco L-50, SW-39, 0°, 20 hr at 39,000 rpm. 0.2 mg of the preparation was applied to the gradient, 3-drop aliquots (~0.2 ml) were collected and 1 μl of each fraction was taken for activity determinations. A) Enzyme preparation after $Ca_3(PO_4)_2$ fractionation step (both forms were present); B) enzyme form I; C) enzyme form II, D) positions of marker proteins; 1: aspartate transaminase; 2: pancreatic ribonuclease. Two identical runs were made: A, B and C, and B, C and D.

presence of 2-mercaptoethanol in the incubation mixture in 0–0.05 M concentration range does not influence either form I or form II.

Presence of KCl in the incubation mixture causes a progressive inhibition of activity (fig. 4) which proceeds somewhat differently with enzyme I and enzyme II. The difference in KCl sensitivity between these forms, although relatively small, is fully reproducible.

Table 3 summarizes the common and distinctive properties of the two forms of lysyl-tRNA synthetase.

It is evident that the difference between forms I and II is not attributable to the number of subunits as has been shown for beef pancreas tryptophanyl-tRNA synthetase [20].

Since the enzyme preparations contained practically no other synthetases and part of the enzyme activity determinations was done in the presence of 18 cold amino acids, it is most unlikely that we are dealing with two aminoacyl-tRNA synthetases differing in amino acid specificity. It should be mentioned in this respect that the behaviour of both forms with various lysine analogs is indistinguishable [21].

The similarity in the molecular weights, thermostability, and behaviour at some stages of purification demonstrates the close relationship between the two forms and it is probable that the differences shown up are due to some modification of part of the enzyme molecules after biosynthesis.

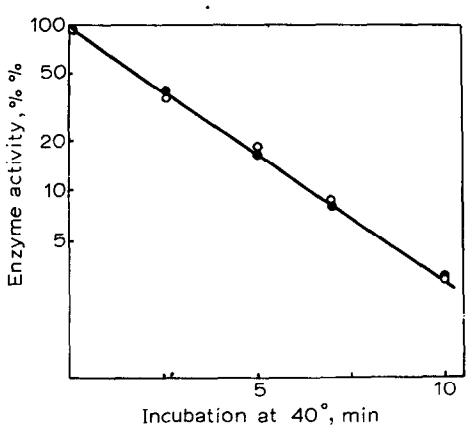

Fig. 3. Comparison of the thermostability of enzyme I and enzyme II forms of lysyl-tRNA synthetase at 40°. (●—●—●): enzyme form I, (○—○—○): enzyme form II. Concentration of the enzyme protein during thermal inactivation was 1.26 mg/ml for both forms.

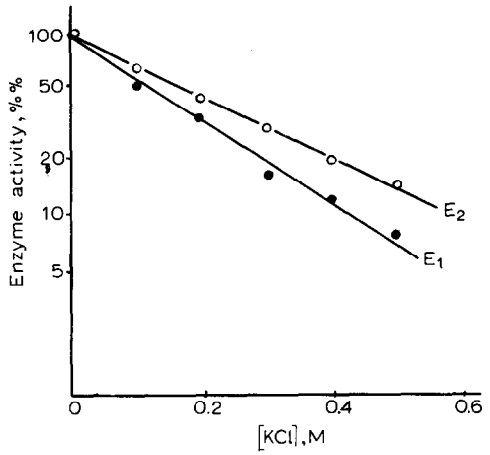

Fig. 4. Influence of the presence of KCl in incubation mixture on the activity of lysyl-tRNA synthetase. Concentration of each form in incubation mixture, 1.6 μg/ml; (●—●—●): enzyme form I, (○—○—○): enzyme form II.

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