SUBSTRATE SPECIFICITY OF LYSYL-tRNA SYNTHETASE FROM *E. coli* B

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1. Introduction

The specificities of many aminoacyl-tRNA synthetases (EC 6.1.1) with respect to their amino acid substrates have been widely studied (for reviews see [1–3]). The broad variability revealed in structural requirements of aminoacyl-tRNA synthetases for various amino acid analogues and derivatives showed that in each case a special analysis is required. Thus we decided to continue the study of amino acid specificity of these enzymes taking as an example the lysyl-tRNA synthetase from *E. coli* B. This enzyme was previously the subject of this type of investigation in other laboratories [4–6].

In this paper the effect of a number of lysine analogues (D-lysine, amide and hydrazide of L-lysine, N^{α}-formyl-L-lysine, methyl and ethyl esters of L-lysine) on the activity of the purified lysyl-tRNA synthetase from *E. coli* B is reported.

2. Materials and methods

Transfer RNA was isolated from *E. coli* B [7]. Lysyl-tRNA synthetase almost free of other aminoacyl-tRNA synthetases was isolated from *E. coli* B (harvested at late log phase) as described in detail elsewhere [8]. Two enzymatically active forms obtained were enriched 80- and 120-fold, respectively. Enzyme activity was determined from the initial rate (4 min, 30°) of the acylation of total tRNA with [^{14}C]lysine in the presence or absence of analogues. All details are given elsewhere [8, 11]. L-[^{14}C]lysine (Czechoslovakia) was checked for its purity chromatographically and specific activity (16 ml/mmole) was established through isotope dilution technique. Methyl and ethyl esters as well as N^{α}-formyl-L-lysine were synthesized by published procedures [9, 10]. D-Lysine was purchased from Reanal (Hungary).

For preparation of hydrazide of L-lysine as trihydrobromide, N^{α},N^{ϵ}-dicarbobenzoxy-L-lysine was synthesized [9]; to 1 g of the latter 25 ml of 37% HBr in glacial acetic acid was added and stirred for 30 min. After stirring, 120 ml of dry ether was added, the precipitate formed was decanted and washed several times with dry ether followed by removal of ether by filtration and drying in a vacuum-desiccator over P_2O_5. The yield was 0.8 g (90%), melting point 165–168° (decomposes with foaming), $[\alpha]_D^{25}$ = +20.0° in 0.1% aqueous solution.

For synthesis of L-lysine amide as the dihydrobromide to 0.28 g (1 mmole) of N^{ϵ}-carbobenzoxy-L-lysine amide obtained as described below 20 ml of 37% HBr in glacial acetic acid was added; further treatments are described above. The yield was 0.25 g (81%), melting point 232–234°, $[\alpha]_D^{25}$ = +17.0° in 0.1% aqueous solution. For synthesis of N^{ϵ}-carbobenzoxy-L-lysine amide 2.8 g (10 mmoles) of N^{ϵ}-carbobenzoxy-L-lysine was placed together with 70 ml of tetrahydrofuran and a stream of phosgene was passed through the vessel with constant stirring for 5 hr. The temperature of the reaction mass was kept at 40°. After standing overnight the undissolved residue was removed by filtration. The excess phosgene was removed and the dry material dissolved in tetrahydrofuran was added drop-wise to a solution of NH_3

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in the same solvent precooled to –25°. The mixture was stirred for 3 hr and within that time the temperature of the reaction mixture reached 20°–25°. The mixture was evaporated in vacuo and the residue was dried in a vacuum-desiccator over P_2O_5/KOH. The product was extracted with ether and the undissolved part extracted with water. The filtrate was evaporated in vacuo, dissolved in a minimal amount of water and passed through a column of Dowex 1 X 8. The fractions collected were evaporated in vacuo and dried over P_2O_5/KOH in a vacuum-desiccator. The yield was 0.85 g (30%), melting point 93°.

The purities of all analogues synthesized were checked by high voltage electrophoresis (V = 4000 V, t = 15 min, pH 6.4, FNN 11 paper). In all cases the possible amount of L-lysine in the analogues was < 1%. Analogues were neutralized with NH_3 just before the experiments.

3. Results and discussion

The affinity of the analogues to the lysyl-tRNA synthetase was calculated as K_i values from Lineweaver-Burk plots [12] using several concentrations of both substrate and analogues. Fig. 1 shows the depedence of 1/[V] on 1/[S] in the presence of analogues in S_i concentration.

As could be seen a linear dependence was found for all the L-lysine derivatives tested. Intersection of all these curves at one point on the ordinate demonstrated the competitive nature of the interrelationship between L-lysine and its analogues in the aminoacylation reaction.

Table 1
Apparent K_m and K_i values for L-lysine and its analogues with lysyl-tRNA synthetase from *E. coli* B.

Substrate or analogue	K_i (M)
L-Lysine	2.5×10^{-6} (K_m)
D-Lysine	2.2×10^{-4}
L-Lysine amide	7.7×10^{-6}
L-Lysine hydrazide	4.0×10^{-4}
L-Lysine methyl ester	1.0×10^{-4}
L-Lysine ethyl ester	6.6×10^{-5}

The mean values taken from 3 repeated determinations with 2 forms of the enzyme [11] with 2–3 concentrations of the analogue (cf. fig. 1) are given.

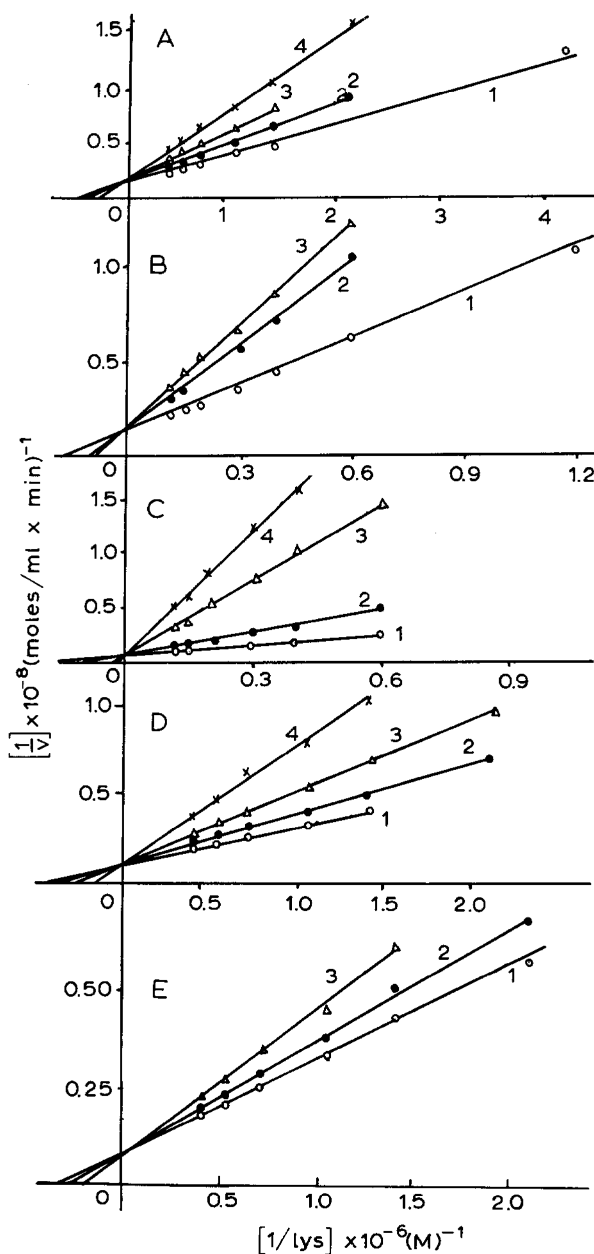

Fig. 1. Effect of L-lysine analogues on the formation of lysyl-tRNA catalyzed by lysyl-tRNA synthetase from *E. coli* B (enzyme form II). In all figures curve 1 represents [^{14}C]-lysyl-tRNA formation in the absence of analogue. A) With D-lysine (curve 2, 9.5×10^{-5} M; curve 3, 2.8×10^{-4} M; curve 4, 4.7×10^{-4} M). B) With L-lysine methyl ester (curve 2, 7.6×10^{-5} M; curve 3, 1.1×10^{-4} M). C) With L-lysine ethyl ester (curve 2, 7.6×10^{-5} M; curve 3, 1.9×10^{-4} M; curve 4, 3.0×10^{-4} M). D) With L-lysine amide (curve 2, 2×10^{-6} M; curve 3, 1.0×10^{-5} M; curve 4, 2.0×10^{-5} M). E) With L-lysine hydrazide (curve 2, 5.0×10^{-5} M; curve 3, 2.0×10^{-5} M).

An attempt to determine the inhibitor activity of formyl-L-lysine was unsuccessful in our hands: even a 200-fold excess of this analogue with respect to L-lysine does not measurably change the kinetics of the aminoacylation reaction.

From the inhibition data (fig. 1) we calculated K_i for the lysine analogues from

$$V = \frac{V_{max} \cdot S}{1 + (1 + S_i/K_i)K_m}$$

The K_i values are summarized in table 1.

We found that D-lysine possesses a certain inhibiting activity ($K_i = 2.25 \times 10^{-4}$ M), however some uncertainty exists since this value may be affected in some degree by the presence of traces of L-lysine in the commercial preparation of the D-form.

The high affinity of L-lysine amide which is only 3 times lower than the affinity of the natural substrate is very demonstrative indeed. The affinities of the methyl and ethyl esters are also high but that of the hydrazide is markedly lower, probably due to the presence of the strong positive charge on the hydrazide group.

The data presented here showed that considerable substitution at the carboxylic end of the amino acid substrate, both in volume and charge of the substituting groups, does not lead to loss of affinity for the enzyme. This fact supports the conclusions made earlier (for reviews see [1–3]) concerning the secondary importance of the COOH-group of the substrate in binding with the aminoacyl-tRNA synthetases.

The wide range of changes in the carboxylic end of lysine which do not prevent the interaction of the COOH-substituted lysine derivatives with the lysyl-tRNA synthetase open up new experimental possibilities. In particular, active derivatives could be synthesized which are able to react with functional groups of the enzyme protein at the active center.

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