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Abstract

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STUDY OF THE ACTIVATION OF CERTAIN ANALOGUES OF AMINO ACIDS IN THE PRESENCE OF THE TOTAL FRACTION OF pH 5 ENZYMES OF *E. coli* B

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It was shown quite some time ago that certain analogues of natural amino acids can be incorporated into proteins of cells and tissues ([1]). With the discovery of the process of amino acid activation as the first stage of protein synthesis ([2]), it became clear that the incorporation of amino acid analogues into proteins must be preceded by their activation, the result of which is the formation of the corresponding aminoacyl adenylate, i.e., a mixed anhydride of an amino acid and adenylic acid.

At the present time, data have been obtained on the possibility of activating a number of amino acid analogues, but the contradictory and incomplete information for some of them requires further investigation of this question. Thus, directly opposite results were obtained by Rendi ([3]) and Glenn ([4]) regarding the possibility of activating ethionine. The question has not been investigated as to which natural amino acid aminoacyl-RNA synthetase activates norleucine. Little data have been presented on the kinetic constants characterizing the activation processes of natural substrates and their analogues.

In the present work we present the results of a study of the possibility of activation of eight amino acid analogues not previously investigated from this point of view, as well as more detailed studies of participation in the first stage of protein biosynthesis of amino acid analogues, the very fact of whose activation had been established earlier.

Activation of amino acids and their analogues was determined by the hydroxamate method according to Hoagland ([5]) in the presence of the total pH 5 fraction isolated from *E. coli* B. The composition of the samples (total volume 1.2 ml) was as follows: the amino acid or amino acid analogue under study, the amounts of which are indicated in the text and in the corresponding graphs, 15 μmoles ATP, 30 μmoles $MgSO_4 \cdot 7H_2O$, 1300 μmoles free base hydroxylamine, and such an amount of enzyme preparation as contained ~1 mg protein. Solutions of amino acids, amino acid analogues, and ATP were preliminarily adjusted with 1 N caustic potassium to pH 7.2. The samples were incubated for 1 hour at 37°.

Fig. 1

Figure 1: Fig. 1

The aminonhydroxamic acids formed were determined by means of the color reaction with $FeCl_3$ on an SF-5 spectrophotometer at 520 mμ. As controls, samples were used to which the amino acid or amino acid analogue was added after incubation, immediately before addition of $FeCl_3$. An equimolar mixture of alanyl-, tyrosyl-, and tryptophanylhydroxamic acids served as the standard for determining aminohydroxamic acids.

In the work, the possibility of activation and the effect on the activation process of the following 15 amino acid analogues*: *DL*-methionine sulfoximine, *DL*-methioninamide, *DL*-phenylalaninamide, β-2-thienylserine, *DL*-β-2-thienyl-β-alanine, *DL*-*p*-dimethylaminophenylalanine, *DL*-*p*-diethyl-

* *DL*-methionine sulfoximine, *DL*-methioninamide, *DL*-phenylalaninamide, *L*-tyrosinamide, and *L*-3-fluorotyrosine were synthesized in the Laboratory of Technological Synthesis, Institute of Chemistry of Natural Compounds, under the direction of G. N. Kosheleva. *DL*-β-2-thienyl-α-alanine, *DL*-β-2-thienyl-β-alanine, and β-2-thienylserine were synthesized by B. P. Fabrichnyi in the Laboratory of Heterocyclic Compounds, Institute of Organic Chemistry.

aminophenylalanine, hydroxycinnamic and phenyllactic acids, *L*-ethionine, *DL*-norleucine, *L*-3-fluorotyrosine, *L*-tyrosinamide, *DL*-β-2-thienyl-α-alanine, and *DL*-phenylserine. We found no data in the literature on studies of the activation of the first eight compounds.

The amounts of *L*-3-fluorotyrosine, *DL*-β-2-thienyl-α-alanine, *L*-ethionine, and *DL*-norleucine used in the various experiments are indicated* in Fig. 1.

Fig. 1. Dependence of the rate of the enzymatic activation reaction of natural amino acids and their analogues on substrate concentration.
1 —tyrosine, 2 —3-fluorotyrosine, 3 —tyrosine + 3-fluorotyrosine, 4 —phenylalanine, 5 —β-2-thienyl-α-alanine, 6 —phenylalanine + β-2-thienyl-α-alanine, 7 —methionine, 8 —ethionine, 9 —methionine + ethionine, 10 —norleucine, 11 —methionine + norleucine.

In the study of the remaining amino-acid analogues, the amino-acid amides were introduced into the samples in an amount of 20 μmoles, and the other compounds in an amount of 40 μmoles.

Of the amino-acid analogues indicated above, only four proved capable of being activated: *L*-ethionine, *DL*-norleucine, *L*-3-fluorotyrosine, and *DL*-β-2-thienyl-α-alanine. One of the compounds studied, namely *L*-tyrosinamide, in agreement with the literature data (6), is a competitive inhibitor of tyrosine activation. All the other amino-acid analogues were inert both with respect to their ability to be activated and with respect to their effect on the activation process of the natural substrates.

The absence of an ability of the phenylalanine and methionine amides to inhibit the activation of the corresponding amino acid indicates that, for this effect as well, there is specificity of the corresponding enzyme.

For those amino-acid analogues capable of being activated, it was studied by which aminoacyl-RNA synthetase present in the pH 5 fraction of *E. coli* B the given analogue is activated. To answer this question, the mixed-substrate method (7) was used.

Data were obtained on the dependence of the rate of the enzymatic activation reactions of *L*-3-fluorotyrosine, *DL*-β-2-thienylalanine, *L*-ethionine, *DL*-norleucine, and the homologous natural amino acids on substrate concentration. As is seen from Fig. 1, during joint incubation of a natural amino acid with the corresponding analogue, there was no summation of the aminohydroxamic acids formed in each individual case. This indicates that the activation of both compounds is catalyzed

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* The amounts of β-2-thienyl-α-alanine, methionine, and norleucine indicated in the figures correspond to the *L* form of these amino acids. During joint incubation of the natural amino acid and its analogue, the natural amino acid was used in the amounts indicated on the abscissa, and the amino-acid analogue in an amount sufficient for saturation of the enzyme (3-fluorotyrosine 6 μmoles, β-2-thienyl-α-alanine 5 μmoles, ethionine 40 μmoles, norleucine 20 μmoles).

by one and the same aminoacyl-RNA synthetase. Thus, 3-fluorotyrosine is activated by tyrosyl-RNA synthetase, β-2-thienyl-α-alanine by phenylalanyl-RNA synthetase, and ethionine and norleucine by methionyl-RNA synthetase. The results of experiments with the first two compounds are consistent with the literature data (6, 8). The conclusions regarding ethionine confirm Glenn' s data (4), while the fact that activation of norleucine is catalyzed by methionyl-RNA synthetase is a fact established for the first time.

On the basis of the data obtained, several kinetic characteristics of the above-mentioned amino-acid analogs and of the corresponding natural amino acids were determined. To calculate the Michaelis constants K_m and the maximum reaction rates V, the Lineweaver–Burk double-reciprocal method was used. The results obtained are presented in Table 1.

Table 1

Values of the Michaelis constants K_m and maximum reaction rates V for several natural amino acids and their analogs

Compound	$K_m \cdot 10^{-3}$, M	$V \cdot 10^{-5}$, M/min	Compound	$K_m \cdot 10^{-3}$, M	$V \cdot 10^{-5}$, M/min
L-tyrosine	1.43	3.2	*DL*-methionine *	0.92	2.3
L-3-fluorotyrosine	2.50	2.8	*L*-ethionine	10.30	1.6
L-phenylalanine	0.45	1.2	*DL*-norleucine *	3.31	1.3
DL-β-2-thienyl-α-alanine *	1.48	1.3			

* In calculating K_m for *DL*-methionine, *DL*-β-2-thienyl-α-alanine, and *DL*-norleucine, the amount of the L-form of the amino acid was taken into account.

It follows from the data in Table 1 that methionyl-RNA synthetase has a greater affinity for norleucine than for ethionine, but the maximum rates of the activation reaction of both compounds are approximately the same and amount to $\sim$ 70% of the maximum rate of the methionine activation reaction. For 3-fluorotyrosine and β-2-thienyl-α-alanine it was found that the affinity of the enzymes activating them is lower than for the natural substrates, but the maximum rates of the activation processes for the natural amino acid and its analog are the same in both cases.

Thus, the results of our experiments have once again shown that the specificity of aminoacyl-RNA synthetases is great, but not absolute. Thus, certain changes in the alkyl chain of amino acids of the aliphatic series do not prevent their activation by methionyl-RNA synthetase. Limited changes in the ring of aromatic amino acids or a change in the nature of the ring are possible. Conversely, the introduction of a hydroxyl group in the α-position relative to the aromatic ring prevents the activation process. The presence and position of the amino group have a decisive influence on the reaction of enzymatic activation of amino acids. The same is true of the carboxyl group; moreover, certain amides of amino acids are competitive inhibitors of the activation of the corresponding amino acid. Naturally, additional studies are necessary for broader generalizations.

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Note: Figure translations are in progress. See original paper for figures.