ACTIVATION OF LEUCYL-tRNA FORMATION IN PREPARATIONS FROM RAT LIVER

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1. Introduction

Although aminoacyl-tRNA synthetases each recognise specifically only one naturally occurring amino acid, many have been found to recognise a wide variety of amino acid analogues [1]. Some of these analogues such as L-norleucine [2] and D-tyrosine** [3] can be attached to tRNA in the place of the natural amino acid, while others simply compete with the amino acid at the substrate binding site on the aminoacyl-tRNA synthetase.

We report here that leucyl-tRNA synthetase from rat liver can recognise a wide variety of amino acid analogues and that these analogues may allosterically interact with the enzyme. We have found that a number of amino acid analogues cause an activation of the extent of aminoacylation of rat liver tRNA with leucine. A study of the kinetic parameters of the activation of leucyl-tRNA formation by one of these analogues, D-methionine, indicates that the leucyl-tRNA synthetase recognises this analogue at a site other than the leucine binding site.

2. Materials and methods

L-*S*-Ethylcysteine was a gift from Dr J. S. Morley, Imperial Chemical Industries Ltd., Pharmaceuticals Division. DL-Homocysteine was purchased from Koch Light. All other amino acid analogues were purchased from Sigma. L-[^{14}C]leucine (331 mCi/mmol) was purchased from The Radiochemical Centre, Amersham. tRNA was prepared from rat liver by the method of Delihas and Staehelin [4]. A rat liver aminoacyl-tRNA synthetase fraction was prepared according to the procedure described by Nishimura and Weinstein [5]. The enzyme fraction was stored in small aliquots in liquid nitrogen.

The aminoacylation of rat liver tRNA was assayed by a modification of the method described by Hoskinson and Khorana [6]. The basic reaction mixture consisted of 25 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.5 mM EDTA and contained 0.15 mg of tRNA, 0.5 nmol of L-[^{14}C]leucine (100 μCi/μmol) and 0.19 mg of rat liver enzyme in a total volume of 250 μl. Control experiments were performed in which tRNA was omitted from the reaction mixture. The reaction was initiated by the addition of enzyme and the reaction mixtures were incubated at 37°C. To measure the extent of aminoacylation of tRNA, a 200 μl aliquot was removed after 25 min incubation, and transferred to two Whatman 3 MM filter paper discs pinned together which were then immersed in cold trichloracetic acid (5%) for 15 min. The filter papers were washed twice with fresh cold trichloracetic acid (5%), once with a cold mixture of ethanol and ether (1:1, v/v) and finally once with cold ether, each for 15 min at 4°C. The filter papers were dried in the air and assayed for radioactivity in a Beckman liquid scintillation counter.

3. Results

The data in fig.1 show the effect of varying D-norleucine and L-methionine concentrations on the

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** For convenience, in this paper we have referred to D-amino acids as amino acid analogues, although we realise that in other contexts this might not be strictly correct.

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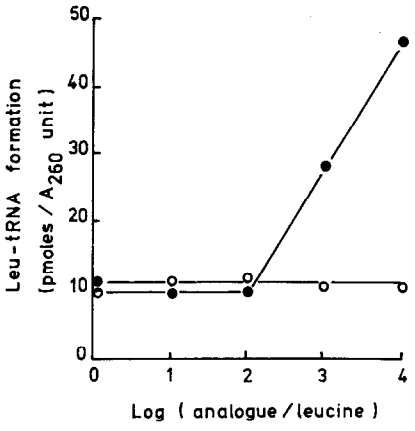

Fig.1. The effect of D-norleucine (•) and L-methionine (○) on the extent of leucyl-tRNA formation in a rat liver in vitro system. Basic reaction mixtures contained increasing amounts of D-norleucine or L-methionine to give a range of analogue to [^{14}C]leucine ratios from 1:1 to 10 000:1. The reaction mixtures were incubated at 37°C for 25 min and the extent of leucyl-tRNA formation determined as described in Materials and methods.

extent of leucyl-tRNA formation. High concentrations of D-norleucine caused an increase in the extent of leucyl-tRNA formation from 11 to 48 pmol of leucine incorporated per A_{260} unit of tRNA. L-Methionine as a control had no effect on the extent of leucyl-tRNA formation. A number of amino acid analogues and D-amino acids were tested for an effect on the extent of leucyl-tRNA formation and the results of these experiments are presented in table 1. L-Norvaline, L-*S*-Methyl cysteine and L-methionine were found to have no effect on the extent of leucyl-tRNA formation. L-Ethionine and D-leucine were found to have an inhibitory effect which in the case of the latter possibly could have been caused by a slight contamination of the D-isomer with L-leucine. All the other analogues increased the extent up to a level of 48 pmol of leucine per A_{260} unit of tRNA.

It is interesting to note that the results obtained here in a rat liver system differ from those obtained in similar experiments in an *Escherichia coli* system [7]. In the *E. coli* system L-norvaline, L-ethionine and L-norleucine inhibited leucyl-tRNA formation in a similar manner to that previously shown for L-norleucine by Trupin et al. [8], whereas L-*S*-ethyl-cysteine and DL-homocysteine had no effect on leucyl-tRNA formation.

The data in fig.2 show the effect of one of the analogues, D-methionine, at an analogue to [^{14}C] leucine ratio of 10 000 to 1 on the rates of leucyl-tRNA formation at different enzyme concentrations. D-Methionine was found to increase both the rate and the extent of leucyl-tRNA formation at the lower than optimum enzyme concentration of 0.19 mg of protein. At the optimum enzyme concentration of 1.4 mg of protein, D-methionine was found to increase only the rate of leucyl-tRNA formation.

Table 1
Effect of amino acids and analogues on the extent of Leu-tRNA formation in preparations from rat liver

Amino acid or analogue	Leucyl-tRNA formation	Amino acid or analogue	Leucyl-tRNA formation
None	10.8	L-Norvaline	11.3
L-Methionine	9.9	D-Norvaline	48.7
D-Methionine	46.3	L-Norleucine	33.0
D-Aspartic acid	47.2	D-Norleucine	47.8
D-Leucine	2.9	L-*S*-Ethylcysteine	46.6
D-Phenylalanine	42.3	DL-Homocysteine	48.8
D-Tryptophan	47.8	D-Ethionine	47.9
D-Valine	48.2	L-Ethionine	1.1
		L-*S*-Methylcysteine	11.2

The extent of leucyl-tRNA formation, expressed as pmoles of leucine incorporated per A_{260} unit of tRNA, was determined in the presence of each of the above amino acids or amino acid analogues at analogue to [^{14}C]leucine ratio of 10 000:1, as described in Materials and methods.

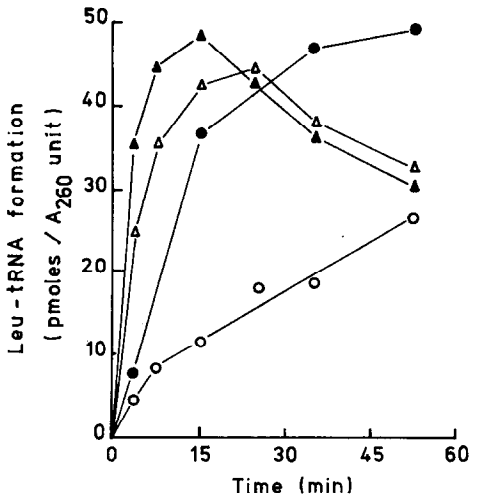

Fig.2. The effect of D-methionine on the rate of leucyl-tRNA formation at different enzyme concentrations in a rat liver in vitro system. Basic reaction mixtures containing D-methionine at an analogue to [^{14}C]leucine ratio of 10 000:1 and either 0.19 mg of enzyme (•) or 1.4 mg of enzyme (▲) were incubated for different times. Basic reaction mixtures without D-methionine and containing either 0.19 mg (○) or 1.4 mg (△) of enzyme were also incubated for different times. The amount of leucyl-tRNA formed in each reaction mixture was determined as described in Materials and methods.

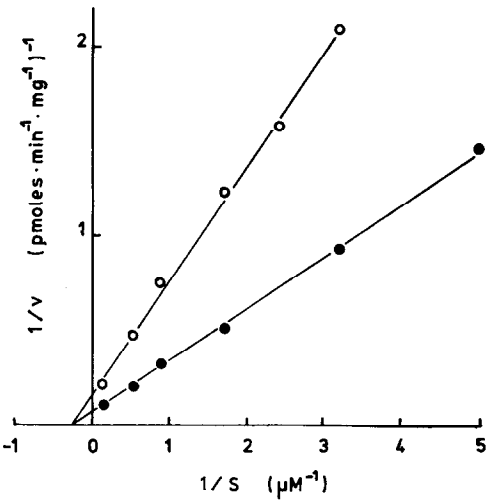

Fig.3. The effect of D-methionine on the initial rate of leucyl-tRNA formation in rat liver in vitro system. Basic reaction mixtures containing 0.3 mg of tRNA, 0.4 mg of enzyme and different amounts of [^{14}C]leucine were incubated with D-methionine (•) and without the analogue (○) at a 10 000:1 analogue to [^{14}C]leucine ratio. The amount of leucyl-tRNA formed in each reaction mixture was determined after 3 and 5 min incubation as described in Materials and methods.

The kinetic parameters of the activation by D-methionine of the initial rate of leucyl-tRNA formation were determined by the method of Lineweaver and Burk [9]. The rates of leucyl-tRNA formation were linear for the first 5 min for all the incubations and the reciprocal plots are shown in fig.3. The K_M for leucine was found to be 4.0×10^{-6} M and the maximum velocity was 7 pmoles of leucine incorporated into tRNA per min per mg of protein. In the presence of D-methionine the K_M remained unchanged but the maximum velocity increased to 14 pmol of leucine incorporated into tRNA per min per mg of protein.

4. Discussion

A number of amino acid analogues have been shown to increase the extent of leucyl-tRNA formation. This effect is observed if an enzyme concentration lower than the optimum enzyme concentration is used. The experiments with D-methionine show that the observed increase in the extent of leucyl-tRNA formation is the result of an increase in the rate of leucyl-tRNA formation and that the increase in the rate occurs at any enzyme concentration.

An increase in the rate of aminoacylation of tRNA can be caused by an increase in the ionic strength of the reaction medium [10]. However, it is unlikely that this is the cause in our case as L-methionine and L-norvaline have no effect. The increase is unlikely to be due to the mischarging of tRNA with leucine as the amount of mischarging would be expected to increase with an increase in enzyme concentration. The results in fig.2 show that the extent of leucyl-tRNA formation in the presence of D-methionine was the same at both enzyme concentrations. Also the fact that the maximum levels of leucyl-tRNA formation at the optimum enzyme concentration with and without D-methionine are similar does not support the mischarging of tRNA as the cause of the activation effect.

A study of the reaction kinetics of the initial rate of leucyl-tRNA formation showed that D-methionine altered the enzyme velocity but not the substrate binding affinity of the leucyl-tRNA synthetase. These reaction kinetics are characteristic of an allosteric system of enzyme control [11].

Thus rat liver leucyl-tRNA synthetase appears to recognise a number of amino acid analogues at a separate site from the substrate binding site. The recognition at this site would appear to be considerably less specific than at the leucine binding site, although, as will be seen from table 1, not all analogues (e.g. L-norvaline, L-*S*-methylcysteine and L-ethionine) nor other naturally occurring L-amino acids are recognised. However all of the D-amino acids examined do stimulate leucyl-tRNA formation and recognise this site on the enzyme. Further experiments are necessary to determine the nature of this recognition mechanism.

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