4,5,6,7-TETRAFLUOROTRYPTOPHAN: A SUBSTRATE FOR TRYPTOPHAN-tRNA LIGASE

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1. Introduction

The substitution of hydrogen atoms by fluorine in amino acids permits the study of the effect of electronegative substituents on the ability of amino acids to participate in the enzymatic activation of amino acids and the acylation of tRNA. It was shown for fluorophenylalanines that the affinity of the amino acids to the enzyme decreases significantly with increasing numbers of fluorine atoms in the benzene ring [1]. Pentafluorophenylalanine is not activated at all by phenylalanine-tRNA ligase from yeast [2]. The results presented here indicate a certain possibility of the enzymatic acylation of tRNA with 4,5,6,7-tetrafluorotryptophan (F-Trp).

2. Materials and methods

The tryptophan-tRNA ligase from bovine pancreas (EC 6.1.1.) was purified as described earlier [3, 4]. The tRNA preparation from yeast was isolated according to Sandakchiev et al. [5]. Free hydroxylamine was obtained according to Beinert et al. [6]. The NH_2OH concentration was determined with hydroxyquinoline [7]. The accumulation of tryptophanylhydroxamates was determined after its sorption on CM-cellulose discs [8]. Aliquots of reaction mixtures were heated at 100° for 30 sec, 0.05 ml of supernatant was applied to the cellulose disc, dried, washed with distilled water, dried and counted with a toluene scintillator in the Tritiomatic Counter (Belgium). To determine ^{14}C-tryptophanyl-tRNA formation, cold 2 M sodium acetate, pH 5.5, and two volumes of ethanol were added to each aliquot. After repeated precipitation with 2 M HCl, the tRNA was collected from 0.2 M Na-acetate, pH 5.5, on nitrocellulose filters (AUFS, Czechoslovakia) [9]. The filters were washed, dried and counted with the toluene scintillator. 4,5,6,7-Tetrafluorotryptophan was prepared by alkylation of the acetamidomalonic ester with 4,5,6,7-tetrafluoro-3-(*N*-piperidylmethyl)-indole or its quaternary salt with dimethylsulfate, followed

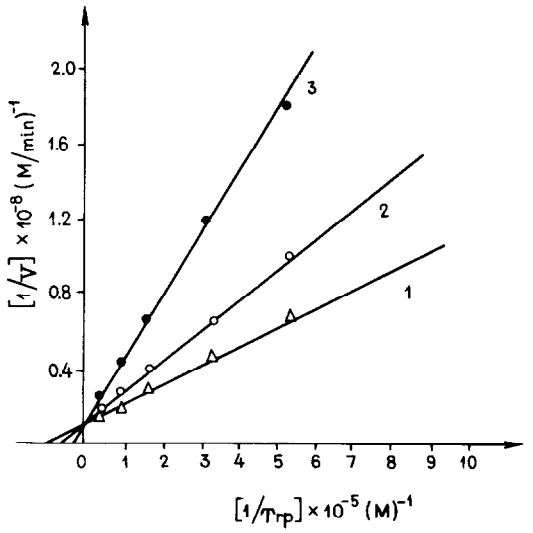

Fig. 1. Double-reciprocal plots of the rate of ^{14}C-tryptophanylhydroxamate formation at 25° at various F-Trp concentrations. 1) without an inhibitor; 2) 0.3 mM F-Trp; 3) 1.12 mM F-Trp. Concentrations of other reactants were NH_2OH 2000 mM; ATP 3 mM; $MgCl_2$ 15 mM; ^{14}C-Trp 0.001–0.1 mM (26.7 mCi/mmole); tris HCl 20 mM pH 7.5; enzyme preparation 1 μg/ml.

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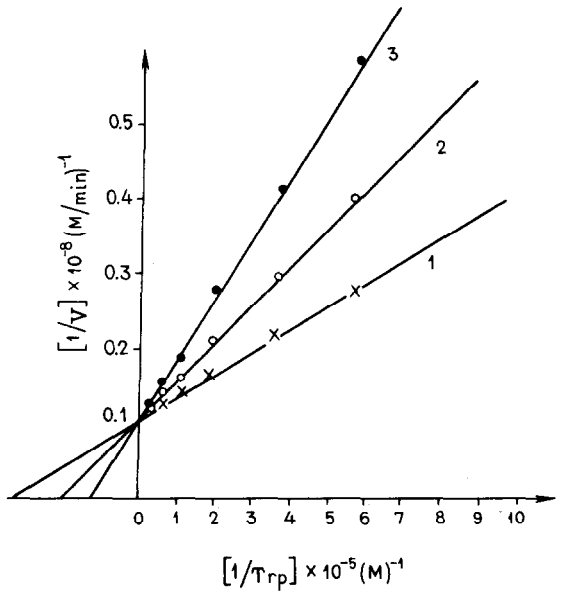

Fig. 2. Double-reciprocal plots of the rate of ^{14}C-tryptophanyl-tRNA formation at 25° at various F-Trp concentrations. 1) without an inhibitor; 2) 0.3 mM F-Trp; 3) 1.12 mM F-Trp. Concentrations of other reactants were the same as for the formation of hydroxamates (see fig. 1).

by acid hydrolysis [10]. Other experimental details are given in the legends to figures.

3. Results and discussion

To investigate the affinity of F-Trp to the tryptophan-tRNA ligase, the rate of enzymatic formation of ^{14}C-tryptophanylhydroxamate and ^{14}C-tryptophanyl-tRNA was measured in the presence of different F-Trp concentrations at various Trp concentrations. Figs. 1 and 2 show the data obtained as the dependence of the reciprocal rate (V) on the reciprocal Trp concentration (S_2) for several F-Trp concentrations (S_2'). The straight lines obtained and the position of the intercept show that there is a competition between Trp and its fluorinated analog in both the activation and the acylation processes.

The Michaelis constants (K_2) or inhibition constants (K_2') were calculated for the processes using the general equation:

$$V = \frac{V_{max}}{1 + (1 + S_2'/K_2)\, K_2/S_2}$$

The K_2 and K_2' values obtained and their ratio are presented in the table.

Table 1

Method of determining the rate	$K_2' \times 10^3$ (M)	$K_2 \times 10^5$ (M)	K_2'/K_2
Formation of ^{14}C-tryptophanylhydroxamate	0.544	1.11	49
Formation of ^{14}C-tryptophanyl-tRNA	0.740	0.363	204

Assuming K_2 as a measure of the affinity of F-Trp to the enzyme, it may be concluded that it is lower than the tryptophan affinity by two orders of magnitude. The decrease of affinity is of the same order of magnitude as when one fluorine atom is introduced into phenylalanine in the case of phenylalanine-tRNA ligase from *E. coli* MRE-600 [1]. The difference between the K_2'/K_2 ratio for the two reactions studied is probably due to the dependence of K_2 on the kinetic parameters of reaction of the intermediate enzyme–aminoacyladenylate complex with hydroxylamine and tRNA which may differ in their sensitivity to fluorine substitution [1].

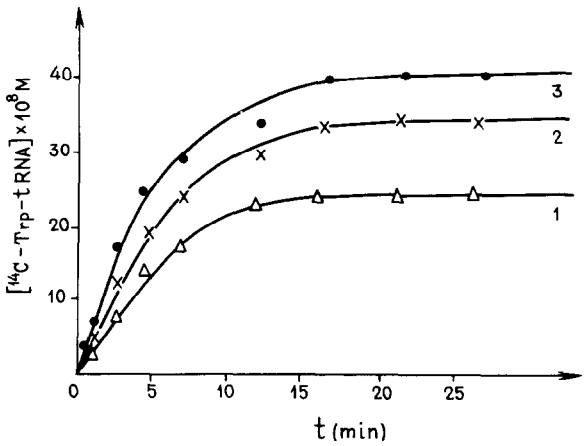

Fig. 3. Increased incorporation of ^{14}C-Trp by tRNA in the presence of F-Trp. 1) without F-Trp; 2) 2 mM F-Trp; 3) 4 mM F-Trp.

To establish whether F-Trp is a competitive substrate or inhibitor, the F-Trp-hydroxamate was identified in the mixture after incubation of F-Trp with the enzyme, ATP and NH_2OH by its reaction with $FeCl_3$, in 0.1 N HCl according to [11]. It was also shown that the increased incorporation of ^{14}C-Trp by tRNA in the presence of F-Trp decreases (fig. 3), thus indicating that F-Trp acylates tRNA.

Up to the present only monofluorinated derivatives of tryptophan have been investigated. It was shown that 6-fluorotryptophan and 5-fluorotryptophan formed hydroxamates and activated the ATP–PP exchange catalyzed by bovine pancreas tryptophan-tRNA ligase [12].

The results obtained show that even a high degree of substitution of hydrogen atoms with fluorine in the indole ring does not completely disrupt the interaction of the substrate with the enzyme. Therefore the investigation of polyfluorinated tryptophans may be an interesting approach to the study of interactions of enzymes with an aromatic ring.

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