THE AFFINITY OF FLUOROPHENYLALANINES TO PHENYLALANINE-tRNA LIGASES FROM *ESCHERICHIA COLI* AND BAKER'S YEAST

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1. Introduction

In our earlier studies [1] it was found that the formation of phenylalanine hydroxamate and phenylalanyl-tRNA catalyzed by phenylalanine-tRNA ligase from *E. coli* MRE-600 is competitively inhibited by mono- and difluorophenylalanines. A correlation was found between the affinity for the enzyme of substituted phenylalanines and the electron density distribution in their aromatic ring. The data presented in the present paper demonstrate that mono- and difluorophenylalanines are real substrates of phenylalanine-tRNA ligases both from *E. coli* MRE-600 and baker's yeast. The correlation is the same in the case with ligase from baker's yeast and the latter enzyme has a significantly greater affinity for fluorinated phenylalanines than the enzyme from *E. coli.*

2. Materials and methods

Crude phenylalanine-tRNA ligases were obtained from *E. coli* MRE-600 [2] and baker's yeast [3]. Transfer RNA preparations from *E. coli* MRE-600 and form baker's yeast were obtained as previously described [4]. ^{14}C-Phenylalanyl-tRNA was determined by the method of Muench and Berg [5]. Monofluorophenylalanines were obtained as described in [6]. Difluorophenylalanines were prepared from the corresponding fluorinated aldehydes by the azlactone method [7]. ^{14}C-3,4-Difluorophenylalanine was obtained in the same manner as the unlabelled compound [7] starting with ^{14}C-glycine.

3. Results and discussion

To determine whether fluorinated phenylalanines are substrates or competitive inhibitors of phenylalanine-tRNA ligases, the maximum incorporation of ^{14}C-phenylalanine into tRNA was determined in the presence and absence of analogues using phenylalanine-tRNA ligases from *E. coli* MRE-600 and baker's yeast. The data are presented in table 1.

It is seen that the yield of ^{14}C-phenylalanine-tRNA decreases in the presence of fluorinated analogues with both the ligases.

In the case of 3,4-difluorophenylalanine, the formation of aminoacyl-tRNA was also directly demonstrated using the ^{14}C-labelled compound (fig. 1).

To measure the affinity of fluorinated analogues for the enzyme, the dependence of the ^{14}C-phenylalanyl-tRNA formation rate on the concentration of fluorinated analogues (S_2') was determined at different concentrations of ^{14}C-phenylalanine (S_2) both for the enzyme from *E. coli* and from baker's yeast. The Michaelis constants K_2' for the analogues were calculated according to the usual equation for competitive substrates:

$$ v = \frac{V_m}{1 + (1 + S_2'/K_2') K_2/S_2} $$

where v is the initial reaction rate, V_m the maximum reaction rate and K_2 the Michaelis constant for phenylalanine.

The ratios of Michaelis constants (K_2'/K_2) thus obtained are presented in table 2.

Table 1

The incorporation of ^{14}C-phenylalanine into tRNA in the presence of its fluorinated analogues (% of maximum incorporation of phenylalanine in the absence of its analogues).

Fluorophenyl-alanine	Enzyme from E. coli		Enzyme from yeast	
	S'/S	Incorporation (%)	S'/S	Incorporation (%)
4-Fluoro	120	57	100	10
3-Fluoro	135	70	100	25
2-Fluoro	200	67	100	32
3,4-Difluoro	500	14	500	8
2,4-Difluoro	500	55	500	16
3,5-Difluoro	500	55	500	27
2,5-Difluoro	500	64	500	30
2,6-Difluoro	500	100	500	49

Composition of the reaction mixture: ATP 2 mM, ^{14}C-phenylalanine 0.4×10^{-3} mM (S), fluorinated analogues 2×10^{-3} – 200×10^{-3} mM (S'), tRNA 0.5 mg/ml, enzyme preparation 0.5–1 A_{280} units/ml, $MgSO_4$ 6 mM, tris-HCl 25 mM, pH 7.5, 25°.

Table 2

The Michaelis constants ratio (K'_2/K_2) for fluorinated analogues.

Fluorophenyl-alanine	K'_2/K_2	
	Enzyme from E. coli	Enzyme from yeast
4-Fluoro	18	1.5
3-Fluoro	67	3.0
2-Fluoro	104	4.5
3,4-Difluoro	750	9.8
2,4-Difluoro	1680	17.5
2,5-Difluoro	1730	48.0
3,5-Difluoro	2080	128.0
2,6-Difluoro	–	160.0

Composition of the reaction mixture: ATP 2 mM, ^{14}C-phenylalanine 0.1–1.6 $\times 10^{-3}$ mM, tRNA 0.5 mg/ml, enzyme preparation 0.5–1 A_{280} units/ml, $MgSO_4$ 6 mM, tris-HCl 20 mM, 25°, pH 7.5.

It is seen that the affinity of fluorinated analogues for the ligase from baker's yeast is significantly greater $(K'_2/K_2$ significantly lower) compared with ligase from E. coli MRE-600. 2,6-Difluorophenylalanine does not compete with ^{14}C-phenylalanine in the case of the E. coli MRE-600 enzyme, but shows a measurable affinity for the enzyme from baker's yeast. 2,3,5,6-
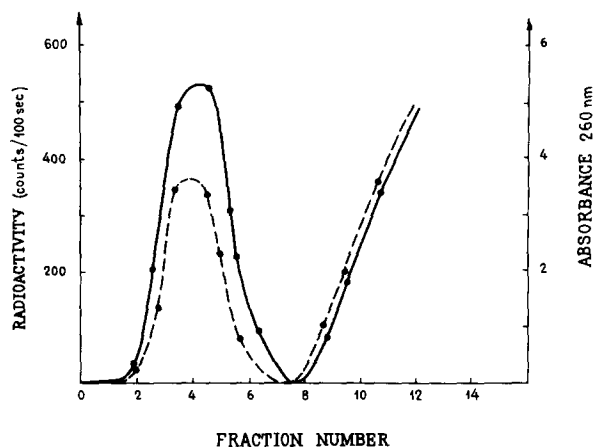

Fig. 1. Separation of ^{14}C-3,4-difluorophenylalanyl-tRNA from substrates on Sephadex G-50 (fine). (●———●) absorbance 260 nm, (● - - - - ●) ^{14}C-radioactivity. Composition of the reaction mixture: ATP 2 mM; $MgCl_2$ 6 mM; ^{14}C-3,4-difluorophenylalanine 0.2 mM, tRNA 2 mg/ml; enzyme preparation from yeast A_{280} units/ml.

Tetrafluorophenylalanine, 2,3,4,6-tetrafluorophenylalanine, and pentafluorophenylalanine do not compete with ^{14}C-phenylalanine with either of the ligases. In the case of baker's yeast ligase the lack of affinity for pentafluorophenylalanine was demonstrated earlier [8].

The dependence of $\log(K'_2/K_2)$ on the excess of a positive charge (σ) on the C^1 atom in the aromatic ring of the corresponding fluorobenzenes, calculated by the MO-method [9], is presented in fig. 2. Two separate linear correlations are seen for the analogues with and without fluorine atoms in the 2-position in the amino acid benzene ring. The slopes are the same for both the series of analogues and for both the enzymes.

It was shown [10, 11] that the grouping $C^2 = C^1 - C^\beta$ is essential for the affinity of phenylalanine analogues for phenylalanine-tRNA ligases. The correlation obtained suggests that the electron density on C^β significantly affects the affinity. The separate correlation obtained with 2-fluorophenylalanines may be due to the additional effect of the fluorine atom in the ortho position in the phenylalanine-aromatic ring upon the substrate-enzyme interaction.
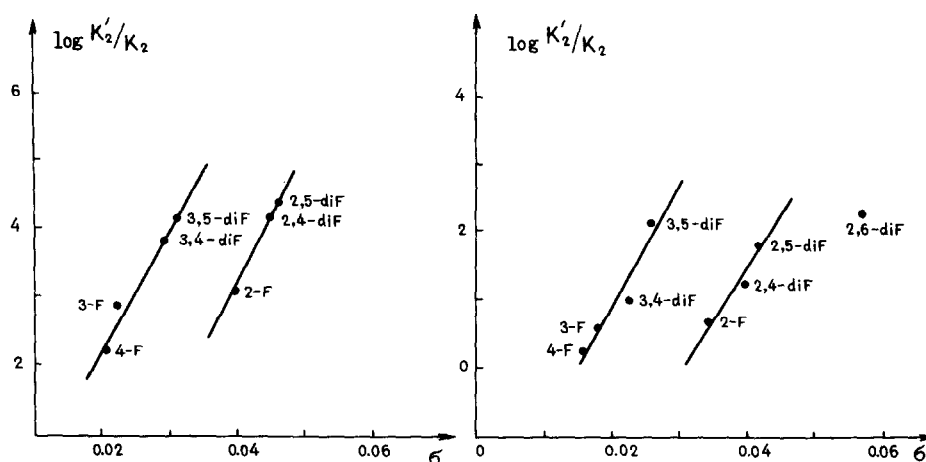
Fig. 2. Dependence of $\log(K_2'/K_2)$ on σ. (a) For the enzyme from *E. coli* MRE-600. (b) For the enzyme from yeast.

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