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Synthesis of Glutaminyl Adenylate Analogues that are Inhibitors of Glutaminyl-tRNA Synthetase

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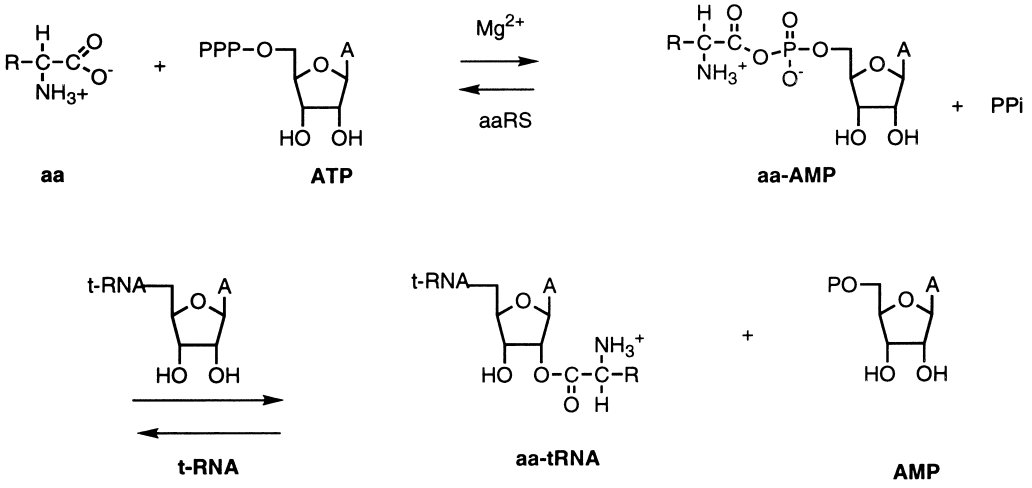
Abstract—Glutaminol adenylate **5** is a competitive inhibitor of glutaminyl-tRNA synthetase with respect to glutamine ($K_i = 280$ nM) and to ATP ($K_i = 860$ nM). The corresponding methyl phosphate ester **4** is a weaker inhibitor ($K_i \sim 10$ μM) with respect to glutamine.

Introduction

Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes involved in protein synthesis in all living organisms. They catalyze the esterification of a particular tRNA with its corresponding amino acid. It has been established that this reaction is a two-step event (Scheme 1).[1,2] In the first step, the appropriate amino acid (aa) is recognized by the enzyme and reacts with ATP to form an enzyme-bound mixed anhydride (aa-AMP, aminoacyl adenylate) with displacement of pyrophosphate (PP_i). In the second step, the activated amino acid is transferred to the CCA end of the cognate tRNA to form the aminoacyl-tRNA (aa-tRNA) and AMP.

Glutaminyl-tRNA synthetase (GlnRS) has the characteristic, which is shared by glutamyl- and arginyl-tRNA synthetases, of requiring the presence of its cognate tRNA to catalyze the activation of its amino acid substrate. Selective inhibition of microbial aaRS has proved



Scheme 1.

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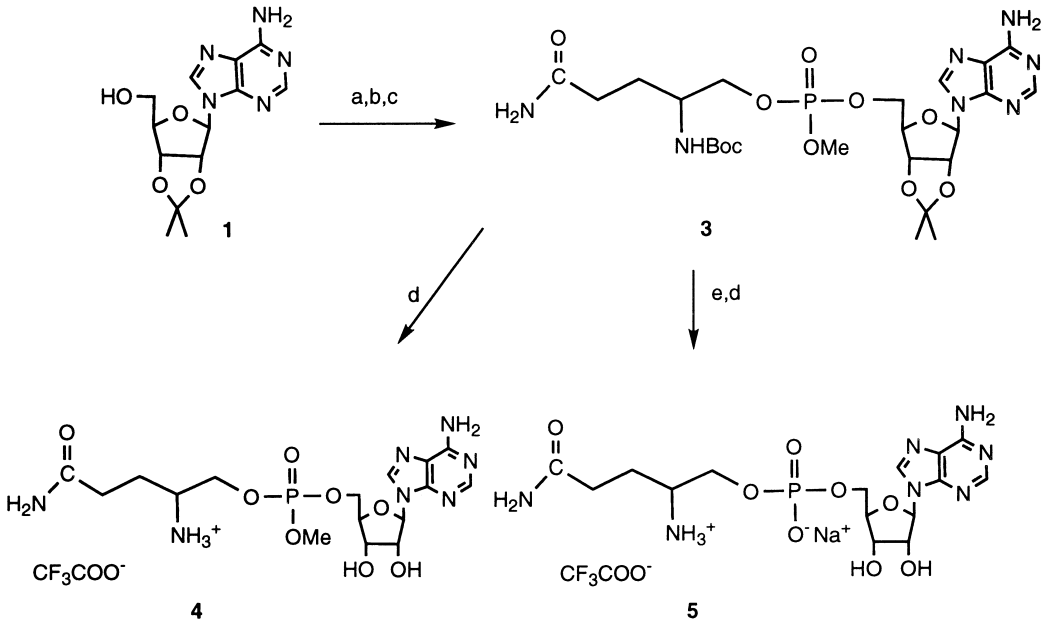

Scheme 2. Reagents: (a) $(i\text{Pr})_2\text{NP}(\text{OCH}_3)\text{Cl}$, $\text{EtN}(i\text{Pr})_2$, CH_2Cl_2, 15 min, 25 °C; (b) $\text{H}_2\text{N–C(=O)–(CH}_2)_2\text{–CH(NHBoc)CH}_2\text{OH}$ (**2**), 1*H*-tetrazole, THF, 12 h, 25 °C; (c) I_2, $\text{H}_2\text{O/THF/Pyr}$; (d) TFA, H_2O, 15 min, 25 °C; (e) NaI, 2-butanone, reflux 4 h.

to be a successful strategy for the production of antibiotics.[3] Pseudomonic acid, isolated from *Pseudomonas fluorescens*, is a highly potent and selective inhibitor of bacterial isoleucyl-tRNA synthetases, and plays an important clinical role.[4,5] Potent synthetic inhibitors of aminoacyl-tRNA synthetases are invariably analogues of aminoacyl adenylates (aa-AMP).[6–16] Such analogues have been useful to study the reaction mechanism of aaRSs and to help in the design of more potent inhibitors. For instance, the bacterial IleRS inhibitor isoleucinol-AMP was useful to identify the reaction cycle of this enzyme and the mode of binding of the reaction intermediate isoleucyl adenylate;[17] this information was then used in the rational design of femtomolar inhibitors of this enzyme.[18]

Synthesis of Glutaminyl Adenylate Analogues

The phosphoramidite-phosphite triester approach was used for the condensation between commercially available 2′,3′-isopropylidene adenosine **1** and glutaminol **2** (Scheme 2). Glutaminol **2** was obtained by reduction of commercially available *N*-*tert*-butyloxycarbonylglutamine with sodium borohydride via a mixed isobutyl carbonic anhydride.[19]

Compound **1** was first phosphorylated with *N*,*N*-diisopropylmethylphosphonamidic chloride in the presence of *N*,*N*-diisopropylethylamine in dry CH_2Cl_2. The intermediate phosphoramidite was coupled with glutaminol **2** in dry tetrahydrofuran using 1*H*-tetrazole as an activating agent and then the phosphite triester was oxidized to phosphate triester **3** by treatment with iodine in mixed solvents (water–tetrahydrofuran–pyridine).

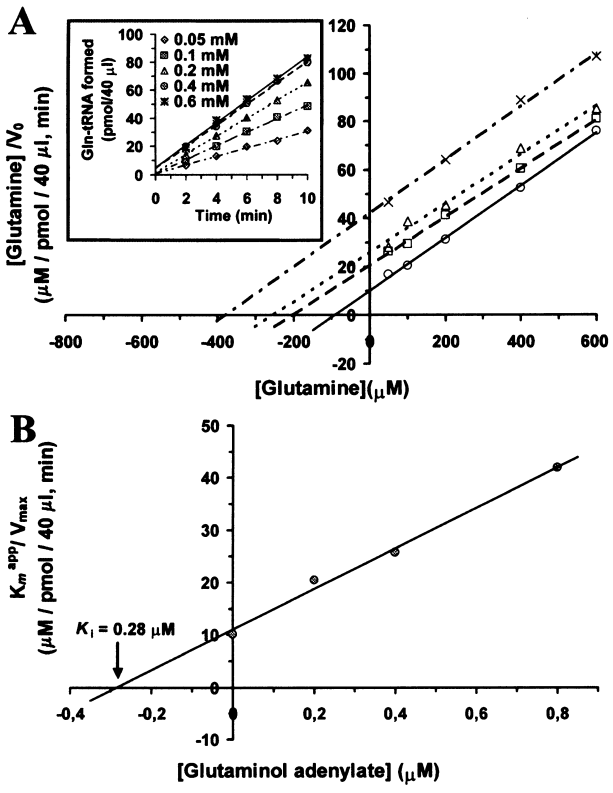

Figure 1. (A) Hanes plot representation of the determination of the apparent K_m^{Gln} of *E. coli* glutaminyl-tRNA synthetase (GlnRS) in the presence of various fixed glutaminol adenylate (**5**) concentrations: 0 μM (○), 0.2 μM (□), 0.4 μM (△), and 0.8 μM (×). The insert shows the initial velocity of tRNA^{Gln} aminoacylation by *E. coli* GlnRS in the absence of inhibitor at various glutamine concentrations. (B) Determination of the K_i value of glutaminol adenylate for *E. coli* GlnRS with respect to glutamine.

Treatment of **3** with wet trifluoroacetic acid (TFA) resulted in simultaneous cleavage of the Boc and isopropylidene groups to provide phosphate ester **4**. Also, phosphotriester **3** was deprotected by sequential treatment with sodium iodide in butanone and then with wet trifluoroacetic acid to yield **5**.

In phosphate triesters **3** and **4**, the phosphorus is a center of chirality and these compounds were obtained as a mixture of diastereoisomers. The nonequivalence (chemical shift difference of some groups) was observed in ^{1}H, ^{13}C and ^{31}P NMR spectra.

Inhibition of *Escherichia coli* GlnRS by Glutaminol Adenylate (5) and Glutaminol Adenylate Methyl Phosphate Ester (4)

As glutaminol adenylate (**5**) is an analogue of glutaminyl-AMP, the enzyme-bound intermediate resulting from the activation of glutamine in the presence of ATP and GlnRS, we studied its influence on the interaction of GlnRS with glutamine and with ATP.[20] The approach that we used was to look at the inhibition of glutaminyl-tRNA formation, first in the presence of various concentrations of glutamine and of saturating concentrations of ATP and tRNA, and then in the presence of various concentrations of ATP and of a saturating concentration of tRNA and of a nearly saturating concentration of glutamine (see below). In the absence of **5**, K_m values of 114 μM glutamine (Fig. 1) and of 100 μM ATP were obtained (Fig. 2), in agreement with previously reported values.[21]

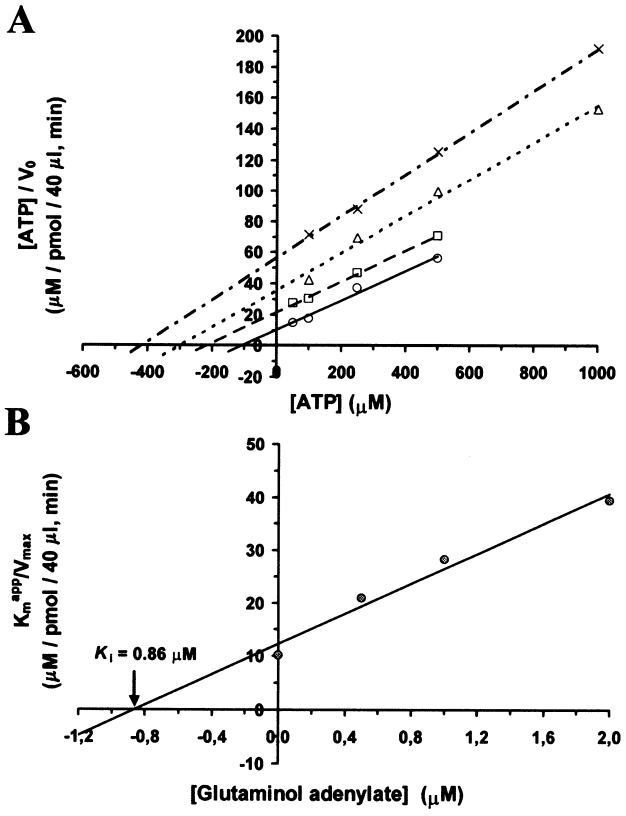

Figure 2. (A) Hanes plot representation of the determination of the apparent K_m^{ATP} of *E. coli* GlnRS in the presence of various fixed glutaminol adenylate concentrations: 0 μM (○), 0.5 μM (□), 1.0 μM (△), and 2.0 μM (×). The highest concentration of [^{14}C]glutamine allowing a precise measurement of the levels of [^{14}C]glutaminyl-tRNA under our experimental conditions was about 1 mM; therefore, we chose it as the fixed concentration of glutamine for the measurements of apparent K_m^{ATP} in the presence of glutaminol adenylate. The slight decrease in V_{max} (the slope of the curves in the Hanes plot is equal to $1/V_{max}$) with increasing inhibitor concentrations is attributed to the competition of glutaminol adenylate with glutamine at 1 mM glutamine (which is 10 × K_m^{Gln} of free GlnRS). (B) Determination of the K_i value of glutaminol adenylate for *E. coli* GlnRS with respect to ATP. According to a presumed competitive inhibition, V_{max} determined without glutaminol adenylate was used for all apparent K_m^{ATP}/V_{max} ratios to compensate for the effect of nonsaturating glutamine concentration at high inhibitor concentration (higher apparent K_m^{Gln}) described in (A).

The type of inhibition and the values of the inhibition constants (K_i) were obtained by analysis of the influence of various fixed concentrations of **5** on the rate of formation of glutaminyl-tRNA in the presence of variable and undersaturating concentrations of glutamine and of fixed and saturating concentrations of ATP and tRNA (Fig. 1); it revealed that **5** is a competitive inhibitor of *E. coli* GlnRS with respect to glutamine, with a K_i of 280 nM. When a similar experiment was conducted in the presence of variable and undersaturating concentrations of ATP and of fixed and saturating concentrations of tRNA and nearly saturating concentrations of glutamine (Fig. 2), the analysis also revealed a competitive inhibition with respect to ATP, with a K_i of about 860 nM.

Glutaminol adenylate (**5**) binds more strongly to GlnRS ($K_i = 0.28$ μM) than does 5′-*O*-[*N*-(L-glutaminyl) sulphamoyl] adenosine[22] (QSI) ($K_i = 1.3$ μM), another analogue of glutaminyl-AMP. As both of these inhibitors share intact adenosine and glutamine side chain moieties with glutaminyl-AMP, and as these moieties are separated by the same number of atoms, the fact that **5** is a significantly better inhibitor than QSI, in spite of the reduction of its carboxyl group, suggests that it is more

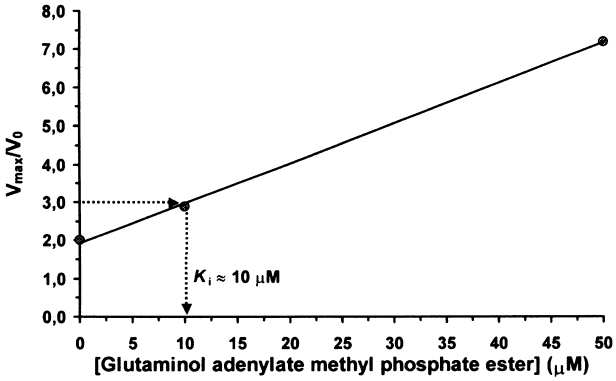

Figure 3. Estimation of the K_i value of glutaminol adenylate methyl phosphate ester (**4**) for *E. coli* GlnRS with respect to glutamine. According to a presumed competitive inhibition:

$$\frac{V_0}{V_{max}} = \frac{S}{S + K_m\left(1 + \frac{I}{K_i}\right)}$$

where V_0 is the initial velocity measured for a given I concentration and $V_{max} = 2 \times V_0$ measured at $[S] = K_m$ and $I = 0$ (so $\frac{V_{max}}{V_0} = 2$). Under those conditions, at $I = K_i$, $\frac{V_0}{V_{max}} = 33.3\%$, and thus $\frac{V_{max}}{V_0} = 3$.

similar to the transition state for glutamine activation than is QSI.

Glutaminol adenylate (**5**) differs from glutamol adenylate only at the end of the side chains of these amino alcohols. It inhibits GlnRS more efficiently (K_i = 0.28 μM) than glutamol adenylate does with glutamyl-tRNA synthetase[12] (K_i = 3 μM). This difference suggests that these evolutionarily closely linked enzymes[23] differ slightly in their interactions with their specific aminoacyl adenylates.

The corresponding methyl phosphate ester (**4**) is a weaker inhibitor (K_i∼10 μM) with respect to glutamine (Fig. 3). The 50-fold increase of K_i due to this phosphate methylation indicates that the charge of the phosphate group of glutaminol adenylate is important for its interaction with GlnRS; it is likely that the negatively charged phosphate makes a charged hydrogen bond with a residue of GlnRS, as that of tyrosyl adenylate does with the main chain NH of Asp 38 of *Bacillus stearothermophilus* tyrosyl-tRNA synthetase.[15,24]

Acknowledgements

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