Influence of the aminoacyl-tRNA synthetase inhibitors and the diadenosine-5′-tetraphosphate phosphonate analogues on the catalysis of diadenosyl oligophosphates formation

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Well-known aminoacyl-tRNA synthetase (ARSase) inhibitors, namely the analogues of amino acids and aminoacyl adenylates (aminoalkyl- and aminophosphonyl adenylates with $K_i \simeq 0.1$ μM) as well as the diadenosine 5′,5‴-p^1,p^4-tetraphosphate (Ap_4A) phosphonoanalogues, were for the first time used for the Ap_4A biosynthesis regulation. Effects of a set of such compounds on lysyl-, phenylalanyl- and alanyl-tRNA synthetases from *E. coli*, capable of synthesizing Ap_4A in the presence of Zn^{2+} ions and pyrophosphatase, have been studied. The adenylate analogues were found to inhibit the Ap_4A and Ap_3A formation ($I_{50} \simeq 6$ mM). Aminophosphonic and aminophosphonous acids are not involved in Ap_3A and Ap_4A biosynthesis and inhibited it at high concentrations. The Ap_4A phosphoanalogues slightly inhibited the major reactions of ARSases, as well as the biosynthesis of Ap_3A and Ap_4A, at a concentration of 5 mM.

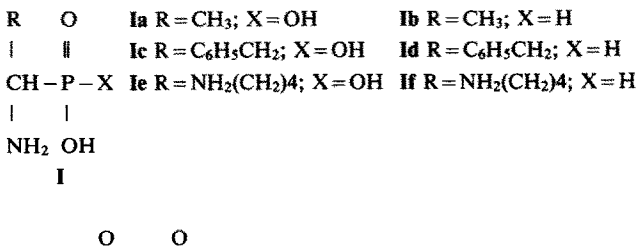
Ap_4A synthesis; Aminoacyl-tRNA synthetase; Aminoalkyl adenylate; Aminophosphonyl adenylate; Aminophosphonic acid; Aminophosphonous acid; Ap_4A phosphonoanalogue

1. INTRODUCTION

Dinucleoside oligophosphates, in particular, Ap_4A, are involved in metabolic processes such as cell proliferation and DNA replication (for review see [1]), RNA processing [2], blood clotting [3], heat shock and oxidative stress [4], and the transformation of purine nucleotides [5].

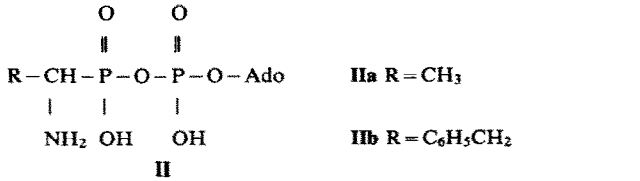
Ap_4A can be synthesized by various aminoacyl-tRNA synthetases [6–8] under certain conditions which differ noticeably from those involved in normal aminoacylation. Apparently, an intermediate aminoacyl adenylate interacts with ATP because the synthesis occurs in the presence of a substrate amino acid.

The inhibition of enzyme-catalyzed Ap_4A formation could be a possible way to regulate the level of Ap_4A in the cell. However, the possibility of inhibition of this reaction, particularly through the use of specific inhibitors for ARSases, has not been adequately studied, although the peculiarities of Ap_4A enzyme-catalysed synthesis have been discussed in detail [6–8]. The present work demonstrates that the known inhibitors of ARSases, namely, phosphoanalogues of substrate amino acids (**Ia–f**) and aminoacyl adenylates (**IIa,b**; **IIIa,b**) as well as Ap_4A phosphonate analogues (**IVa–d**), can inhibit, in a weak or nonspecific manner, enzymatic synthesis of diadenosine oligophosphates catalysed by lysyl-, phenylalanyl- and alanyl-tRNA synthetases from *E.coli*.



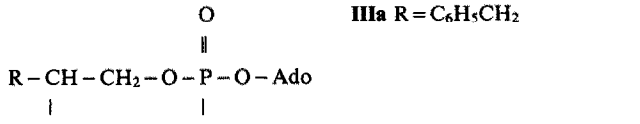
I

Ia R = CH_3; X = OH **Ib** R = CH_3; X = H
Ic R = $C_6H_5CH_2$; X = OH **Id** R = $C_6H_5CH_2$; X = H
Ie R = $NH_2(CH_2)4$; X = OH **If** R = $NH_2(CH_2)4$; X = H



II

IIa R = CH_3
IIb R = $C_6H_5CH_2$



III

IIIa R = $C_6H_5CH_2$
IIIb R = $NH_2(CH_2)_4$

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Abbreviations: Ap_4A, diadenosine 5′,5‴-p^1,p^4-tetraphosphate; Ap_3A, diadenosine 5′,5‴-p^1,p^3-triphosphate

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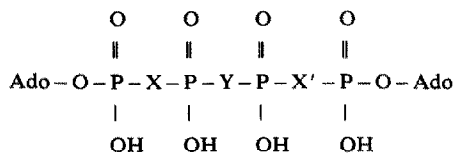

IV

IV	Ap_4A	(X = X' = Y = O)
IVa	$ApCH_2pppA$	(X = CH_2; X' = Y = O)
IVb	$AppCH_2ppA$	(X = X' = O; Y = CH_2)
IVc	AppCHBrppA	(X = X' = O; Y = CHBr)
IVd	$ApCH_2ppCH_2pA$	(X = X' = CH_2; Y = O)

2. MATERIALS AND METHODS

2.1. *Chemical compounds*

Phosphorus-containing analogues of amino acids and aminoacyl adenylates were prepared as described elsewhere [9,10].Aminoalkyl adenylates were synthesized as in [11]. Ap_4A phosphonate analogues were prepared as described in [12,13]. Ap_4A and Ap_3A were from P.-L. Biochem. [U-^{14}C]ATP (507 Ci/mol) was purchased from Amersham.

2.2. *Enzymes and their activity assay*

Homogeneous *E. coli* MRE-600 phenylalanyl-tRNA synthetase was prepared as in [14]. *E. coli* B lysyl- and alanyl-tRNA synthetases were purified to a homogeneity of 70 and 75%, respectively, as in [15,16]. *E. coli* B $tRNA^{Lys}$ and $tRNA^{Ala}$ were purified according to [18]. *E. coli* MRE-600 $tRNA^{Phe}$ was prepared as described elsewhere [17]. The activity of ARSases was assayed using a standard procedure in the reactions of isotope [^{32}P]PP_i-ATP exchange and tRNA aminoacylation as in [19]. The enzyme-catalysed synthesis of Ap_3A and Ap_4A was determined as described earlier [7]. The radioactivity of TLC plates was determined by express analysis using a set of instruments including a counter, a computer, a display or a plotter as described in [20]. Yeast inorganic pyrophosphatase was obtained from P.-L. Biochem. (spec. act. 200 units/mg at 25°C).

3. RESULTS AND DISCUSSION

The analogues of aminoacyl adenylates, intermediate compounds in the enzyme-catalysed reaction, are the most active and specific inhibitors of ARSases [10,11]. Since aminoacyl adenylates are presumed to be formed as intermediate compounds in the biosynthesis of diadenosine oligophosphates, these inhibitors also should be expected to have strongly suppressed Ap_4A synthesis. However, both AMP aminoalkyl esters (**III**) and aminophosphonyl adenylates (**II**) turned out to be weak and nonspecific inhibitors of Ap_4A and Ap_3A synthesis which had been catalysed by ARSases used in the experiments. As can be seen in Fig. 1, for phenylalanyl-tRNA synthetase, phenylalaninol-AMP (**IIIa**), aminoethylphosphonyl adenylate (**IIa**), aminophenylethylphosphonyl adenylate (**IIb**) and lysinol-AMP (**IIIb**) in an identically weak manner inhibit Ap_3A and Ap_4A synthesis at a 1 mM concentration. Nevertheless, in the normal reaction of $tRNA^{Phe}$ enzyme-catalysed aminoacylation, the compounds (**IIIa**) and (**IIb**) selectivity inhibit the synthetase activity with the $K_i \simeq 10^{-7}$ M, whereas compounds (**IIa**) and (**IIIb**) whose structure is quite different from that of phenylalanyl adenylate, inhibit it with $K_i \simeq 10^{-3}$ M. Similar results have been obtained for other ARSases. These data are indicative of fundamental differences between the normal reaction of tRNA amino-acylation and Ap_4A synthesis in the intermediate steps of the enzyme-catalysed reaction.

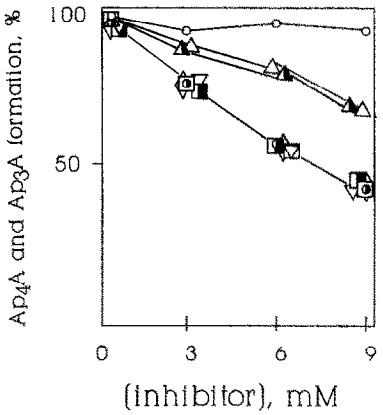

Fig. 1. Inhibition by adenylates (**IIa,b**), (**IIIa,b**), phosphorus-containing L-phenylalanine analogues (**Ic,d**) and a phosphonate Ap_4A analogue (**IVb**) of Ap_4A and Ap_3A synthesis catalysed by *E. coli* MRE-600 phenylalanyl-tRNA synthetase. The reaction mixture had the same composition as indicated in [20]. 5 μl aliquots of the reaction mixture were applied to plates every 30 min and, after TLC, were analysed as described in section 2 (○) (S.R.M.) = standard reaction mixture without inhibitors; (▽) (**IIa**); (□) (**IIb**); (■) (**IIIa**); (◇) (**IIIb**); (△) (**Ic**); (▲) (**Id**); (◑) (**IVb**).

It is known that aminophosphonic acids (**Ia,c,e**) are not substrates in the overall reaction and, with the exception of 1-amino-2-phenylethylphosphonic acid (**Ic**), show a low affinity for synthetases. In contrast, α-aminophosphonous acids* (**Ib,d,f**) have an elevated affinity for these enzymes, as a rule, close to that of a substrate amino acid. Some of them, in particular, α-aminoisobutylphosphonous and α-amino-γ-methylthiopropylphosphonous acids, can substitute for valine and methionine in the reactions of ATP-PP_i exchange which have been catalysed by the corresponding ARSases [21]. However, aminophosphonous and aminophosphonic acids cannot substitute for substrate amino acids in Ap_4A synthesis and virtually do not inhibit the reaction at a 10^{-2} M concentration or more.

In contrast to AMP aminoalkyl esters (**III**), aminophosphonyl adenylates (**II**) contain an active anhydride bond which, in principle, makes an enzyme-catalysed reaction of (**II**) with ATP and the formation of Ap_4A possible. Nevertheless, none of the synthetases that have been studied, including alanyl-tRNA synthetase (Fig. 2), catalyses this process, just a compound (**II**) does not react with PP_i in the normal synthetase reaction [19].

*In the present paper we used the trivial name 'aminophosphonous acid' instead of 'aminophophinic acid' that was recommended by the IUPAC Nomenclature of Organic Chemistry.

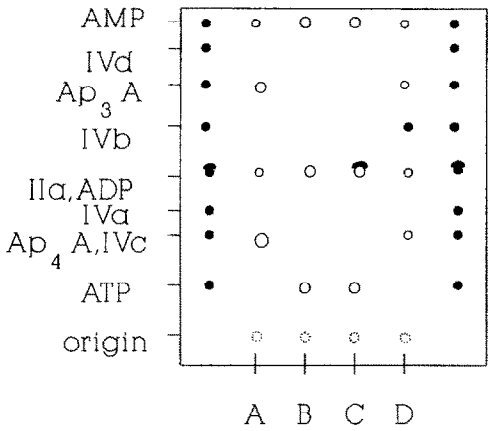

Fig. 2. Express analysis of labeled nucleotides from the reaction mixture for Ap_4A and Ap_3A synthesis catalysed by *E. coli* B alanyl-tRNA synthetase after TLC on PEI-cellulose plates (see section 2). Figure of a plotter indicating relative radioactivity distribution in nucleotide spots on the chromatogram after the reaction mixture was incubated at 37°C for 180 min. Relative nucleotide mobility corresponded to that indicated in [23]. $(S.R.M.)^{(-)}$= standard reaction mixture without L-alanine. (A) (S.R.M.); B: $(S.R.M.)^{(-)}$+2 mM (**Ia**) or 2 mM (**Ib**); C: $(S.R.M.)^{(-)}$+2 mM (**IIa**); D: $(S.R.M.)^{(-)}$+6 mM (**IVb**). ATP, ADP, AMP, (**IIa**) and (**IVb**) (2 mM each in 50 mM Tris-HCl, pH 7.8 + 10 mM $MgCl_2$) were applied to the plate margins as a standard.

So far, the effect of Ap_4A was studied only with rat liver lysyl-tRNA synthetase [22] where Ap_4A acted as a competitive (with respect to ATP) inhibitor of $tRNA^{Lys}$ aminoacylation with the $K_i = 2.5$ μM. We found a similar situation with alanyl-tRNA synthetase although the K_i was greater by two orders of magnitude (0.4 mM). In the case of other studied ARSases, we found that Ap_4A and its metabolically stable analogues, in which the O-atoms between the phosphorous atoms 1 and 2 or 2 and 3 were substituted by CH_2 or CHBr groups, did not compete with ATP for the sites of binding on the enzyme (Fig. 3) and weakly inhibited the main reaction. Moreover, those compounds used at a 3 mM concentration weakly inhibited the synthesis of Ap_4A, which indicated that this reaction in general was not prone to chemical regulation.

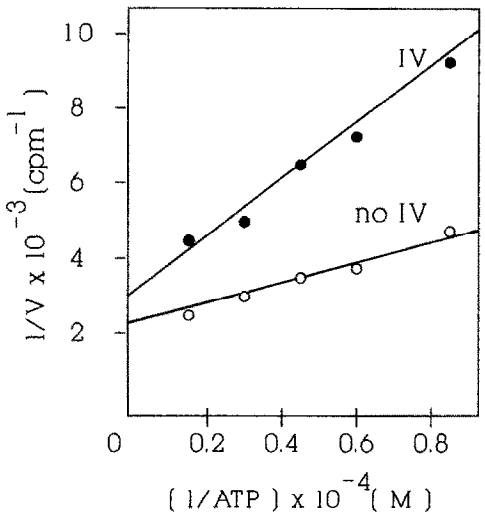

Fig. 3. Inhibition of lysyl-$tRNA^{Lys}$ synthesis by Ap_4A. $tRNA^{Lys}$ was aminoacylated at saturating concentrations of tRNA (40 μM) and L-lysine (30 μM with sp. act. 240 Ci/mol) and different ATP concentrations. Ap_4A concentrations: (○) = 0 mM; (●) = 3 mM.

It would be relevant to note that the Ap_4A analogues used in this work are potent inhibitors (the $K_i \simeq 0.1$–5 μM) of some Ap_4A-phosphohydrolases [23]. Therefore, stable Ap_4A analogues can be used for studying the function of Ap_4A in the cell since they weakly affect the synthesis of Ap_4A, but prevent its degradation in catabolic processes.

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