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 (aminalkyl- and aminophosphonyl adenylates with $K_i \approx 0.1 \mu M$) as well as the diadenosine 5',5',5',5'-tetraphosphate (Ap4A) phosphonoanalogues, were for the first time used for the Ap4A biosynthesis regulation. Effects of a set of such compounds on lysyl-, phenylalanyl- and alanyl-tRNA synthetases from E. coli, capable of synthesizing Ap4A in the presence of Zn2+ ions and pyrophosphatase, have been studied. The adenylate analogues were found to inhibit the Ap4A and Ap3A formation ($I_{50} \approx 6 \text{ mM}$). Aminophosphonic and aminophosphonous acids are not involved in Ap4A and Ap3A biosynthesis and inhibited it at high concentrations. The Ap4A phosphonanalogues slightly inhibited the major reactions of ARSases, as well as the biosynthesis of Ap4A and Ap3A, at a concentration of 5 mM.

Ap4A synthesis; Aminoacyl-tRNA synthetase; Aminoalkyl adenylate; Aminophosphonyl adenylate; Aminophosphonic acid; Aminophosphonous acid; Ap4A phosphonoanalogue

1. INTRODUCTION

Dinucleoside oligophosphates, in particular, Ap4A, 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].

Ap4A 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 Ap4A formation could be a possible way to regulate the level of Ap4A 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 Ap4A 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 Ap4A 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.

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Abbreviations: Ap4A, diadenosine 5',5',5',5'-tetraphosphate; Ap3A, diadenosine 5',5',5'-triphosphate

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2. MATERIALS AND METHODS

2.1. Chemical compounds

Phosphorus-containing analogues of amino acids and aminocarboxylic acids were prepared as described elsewhere [9,10]. Aminoaarkyl adenylates were synthesized as in [11]. AP4A phosphonate analogues were prepared as described in [12,13]. AP4A and AP3A were from P.-L. Biochem. (U-14]C-ATP (507 Ci/mmol) 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 l-lysyl- and alanyl-tRNA synthetases were purified to a homogeneity of 70 and 75%, respectively, as in [15,16]. E. coli B tRNA^Phe 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 ATP-PPi exchange and tRNA aminoacylation as in [19]. The enzyme-catalysed synthesis of AP4A and AP3A 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 23°C).

3. RESULTS AND DISCUSSION

The analogues of aminocarboxyl adenylates, intermediate compounds in the enzyme-catalysed reaction, are the most active and specific inhibitors of ARSases [10,11]. Since aminocarboxyl 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 AP4A synthesis. However, both AMP aminoalkyl esters (II) and aminophosphonyl adenylates (II) turned out to be weak and nonspecific inhibitors of AP4A and AP3A synthesis which had been catalysed by ARSases used in the experiments. As can be seen in Fig. 1, for phenylalanine-tRNA synthetase, phenylalaninol-AMP (Iia), aminocarboxyl phosphonate adenylate (IIa), aminophosphonyl phosphonoyl adenylate (IIb) and lysinol-AMP (IIIb) in an identically weak manner inhibit AP3A and AP4A synthesis at a 1 mM concentration. Nevertheless, in the normal reaction of tRNA^Phe enzyme-catalysed aminoacylation, the compounds (IIia) and (IIib) selectivity inhibit the synthetase activity with the $K_i = 10^{-7}$ M, whereas compounds (IIa) and (IIib) whose structure is quite different from that of phenylalanine adenylate, inhibit it with $K_i = 10^{-3}$ M. Similar results have been obtained for other ARSases. These data are indicative of fundamental differences between the normal reaction of tRNA aminoacylation and AP4A synthesis in the intermediate steps of the enzyme-catalysed reaction.

It is known that aminophosphonic acids* (Iib,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, L-aminoisobutyl phosphonic and L-amino-3-methylthiopropylphosphonous acids, can substitute for valine and methionine in the reactions of ATP-PPi exchange and tRNA aminoacylation as in [19]. The enzyme-catalysed synthesis of AP3A and AP4A 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 23°C).

*In the present paper we used the trivial name 'aminophosphonic acid' instead of 'aminophosphonic acid' that was recommended by the IUPAC Nomenclature of Organic Chemistry.
Fig. 2. Express analysis of labeled nucleotides from the reaction mixture for Ap4A and Ap4A 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.)(-)+ 6 mM ATP, ADP, AMP, (Ha) and (IVb) (2 mM) (Lb); C: (S.R.M.)(-)'+ 2 mM ATP, ADP, AMP, (Ia) and (IVb) (2 mM) each in 50 mM Tris-HCl, pH 7.8 + 10 mM MgCh) were applied to the plate margins as a standard.

So far, the effect of Ap4A was studied only with rat liver lysyl-tRNA synthetase [22] where Ap4A acted as a competitive (with respect to ATP) inhibitor of tRNA\textsubscript{Lys} aminoacylation with the \( K_i = 2.5 \mu M \). 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 Ap4A 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, which the O-atoms between the phosphorous atoms 1 and 3 were replaced by CH\(_2\), were not prone to chemical regulation.

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REFERENCES