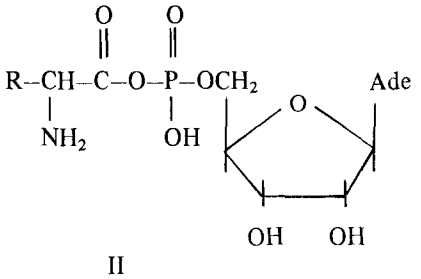
TRANSITION-STATE ANALOGUES OF AMINOACYL ADENYLATES

A. I. BIRYUKOV, B. Kh. ISHMURATOV and R. M. KHOMUTOV*
Institute of Molecular Biology, USSR Academy of Sciences, Moscow 117312, USSR

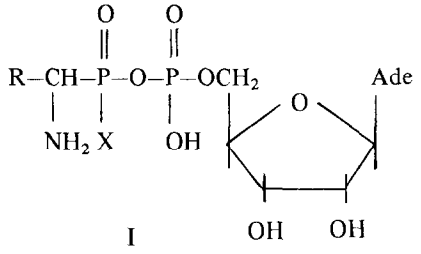
Received 6 May 1978

1. Introduction

There is only one type of highly specific inhibitor known for aminoacyl-tRNA-synthetases, i.e., aminoalkyl adenylates [1]. This paper presents data on the interaction of mixed anhydrides of AMP and aminophosphonic acids, Ia–d, with valyl-, phenylalanyl- and methionyl-tRNA-synthetases from *E. coli* B, and demonstrates that I is a new class of effective and selective inhibitors of synthetases.



II



I

(a) R = $-CH(CH_3)_2$; X = OH
(b) R = $-CH_2C_6H_5$; X = OH
(c) R = $-CH_2CH_2SCH_3$; X = OH
(d) R = $-CH(CH_3)_2$; X = OC_2H_5

* To whom reprint requests should be addressed

2. Materials and methods

2.1. *Chemical compounds*

Aminophosphonyl adenylates (I) were synthesized as in [2]. Their solutions were freshly prepared prior to use. Standard ^{14}C-labelled amino acids were purchased from Chemapol (Czechoslovakia) and Amersham (England), and sodium [^{32}P]pyrophosphate was obtained from Isotope (USSR).

2.2. *Valyl-, phenylalanyl- and methionyl-tRNA-synthetases*

These were isolated from *E. coli* B according to [3–5], respectively. Total tRNA of *E. coli* B was prepared by the method in [6]. *E. coli* B $tRNA^{Val}$, $tRNA^{Phe}$ and $tRNA^{Met}$ were purified as in [7].

2.3. *Determination of synthetase activity*

The activity of synthetases in the ATP–PP_i exchange reaction was determined in the conditions of [8]. Samples were analysed as in [5]. Aminoacylation of tRNA was conducted at 37°C for 10 min. The reaction mixture (0.5 ml) contained (μmol): Tris/HCl, pH 8.0, 50; $Mg(CH_3COO)_2$, 5; KCl, 5; ATP, 1; β-mercaptoethanol, 2; ^{14}C-labelled amino acid, 0.001; tRNA, 0.01; and 0.005–0.01 mg appropriate enzyme. The yield of ^{14}C-labelled aminoacyl-tRNA was determined as in [9].

2.4. *The effect of Ia–d on the activity of synthetases*

(a) An aliquot of a freshly prepared solution of Ia–d was introduced at a concentration of 10^{-2}–10^{-9} M into standard samples for ATP–PP_i exchange directly prior to the addition of the enzyme. The samples were incubated and analysed as described in section 2.3.

(b) Changes in the rate of tRNA aminoacylation under the action of Ia–d were determined in a similar way by introducing an aliquot of the solution of Ia–d. The kinetics of tRNA aminoacylation was analysed by taking samples as indicated in [9]. The inhibition constants, K_i, were obtained by double-reciprocal plots [10] varying the amounts of L-amino acid or ATP at fixed levels of other substrates.

2.5. *Inhibition as a function of time*

The extent of inhibition as a function of time was studied in a standard mixture for aminoacylation. The original level of inhibition was 40–50% at 1×10^{-6} M I. The kinetics of inhibition was analysed by taking samples after definite intervals of time within 0.5–3.0 h.

In the control experiment, 1×10^{-3} M Ia–d were incubated in Tris/HCl buffer, pH 7.5 for 2 h, then aliquots were taken and assayed according to section 2.4 (b).

2.6. *Interaction of I with $^{32}PP_i$ in the presence of the enzyme*

Ia–d, 4×10^{-4} M, were added to the reaction samples for ATP–PP_i exchange (ATP and L-amino acid were excluded), either in the presence of Mg^{2+} ions or in their absence. The formation of [^{32}P]ATP was determined as indicated in section 2.3.

2.7. *Interaction of I with tRNA in the presence of the enzyme*

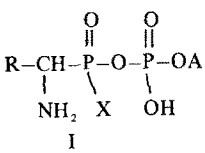
Aminophosphonylation of tRNA was performed under standard conditions for tRNA aminoacylation, ATP and a ^{14}C-labelled amino acid being excluded. Compounds Ia–d at 1×10^{-5} M were added to the reaction mixture with subsequent incubation at 37°C for 30 min. The amount of tRNA remaining free was determined from the acceptance of the ^{14}C-labelled amino acid in the presence of ATP according to section 2.3.

3. Results and discussion

The anhydrides I similar in structure to certain aminoacyl adenylates II were found to be effective inhibitors of the corresponding synthetases in both reactions, ATP–PP_i exchange and tRNA aminoacylation (see table 1). The inhibition was specific enough: e.g., Ia, a formal analogue of valyl adenylate inhibited the valine enzyme by 4 orders of magnitude more strongly than other synthetases. The inhibition was competitive with respect to the substrate amino acids and ATP (fig.1), thus suggesting that the inhibitors were bound in the active centre.

The level of inhibition almost did not change when Ia–d were incubated with the enzymes in the presence of tRNA or in its absence. Subsequent addition of the excess of substrate amino acid restored the enzyme activity. In the control experiment, the incubation of I without the enzyme for 2 h resulted in a decrease of the inhibition by 1–2 order of magnitude. Therefore, I acted as reversible inhibitors and the synthetases not only catalysed the hydrolysis of the anhydride

Table 1

	α-Aminophosphonyl-5′-adenylates	Inhibition[a] K_i (M)					
		Valyl-tRNA-synthetase		Phenylalanyl-tRNA synthetase		Methionyl-tRNA synthetase	
	 I	ATP–PP_i exchange	tRNA amino-acylation	ATP–PP_i exchange	tRNA amino-acylation	ATP–PP_i exchange	tRNA amino-acylation
1	Ia	1.4×10^{-7}	2.8×10^{-7}	3.5×10^{-3}	3.0×10^{-3}	6.0×10^{-3}	4.0×10^{-3}
2	Ib	5.1×10^{-3}	3.4×10^{-3}	2.0×10^{-7}	5.0×10^{-7}	2.4×10^{-3}	3.5×10^{-3}
3	Ic	3.4×10^{-3}	6.2×10^{-3}	4.1×10^{-4}	2.0×10^{-3}	4.0×10^{-6}	2.0×10^{-7}
4	Id	4.2×10^{-6}	2.7×10^{-6}				

[a] Since I are derivatives of racemic aminophosphonic acids, the inhibition will be in fact still higher

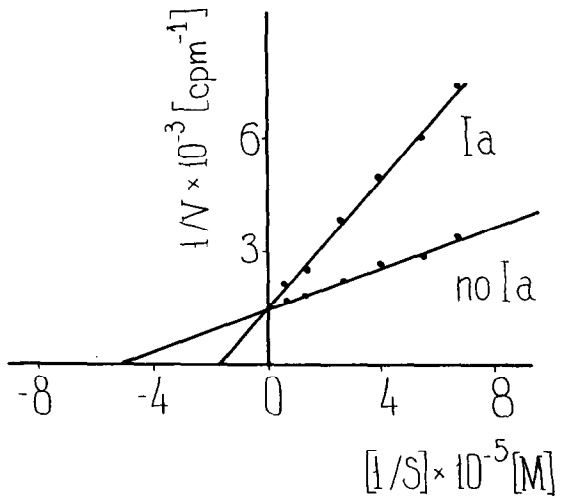


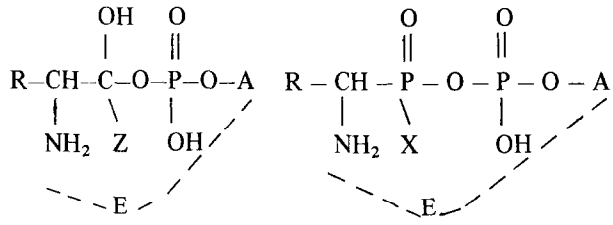
Fig.1. Lineweaver-Burk plot for the inhibition of $tRNA^{Val}$ aminoacylation by Ia at a concentration of 1×10^{-7} M measured by varying the [^{14}C]valine concentration in the presence of saturating amounts of ATP (2×10^{-3} M). The conditions for inhibition are described in section 2.

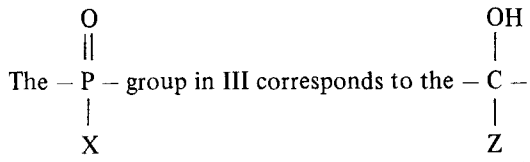
bond of the inhibitors in the presence of tRNA but also stabilized the inhibitors.

As was shown with the anhydride of CMP and β-aminethylphosphonic acid, an analogue of cytidyl diphosphoethanolamine, this pyrophosphonate could enzymatically phosphonylate hydroxyl groups and undergo pyrophosphorolysis [11]. In the case of synthetases and I, similar reactions could be the formation of ATP and aminophosphonyl-tRNAs.

However, no [^{32}P]ATP was detected upon incubation of Ia or Ib with $^{32}PP_i$ and the appropriate synthetases in the presence of Mg^{2+} ions or in their absence. Similarly, if tRNA and I were preincubated in the presence of the enzyme and then the complete reaction was conducted, the level of aminoacylation did not change, i.e., aminophosphonylation of tRNA was not observed. The latter result could not be attributed to the labile nature of aminophosphonyl-tRNA since 2′(3′)-aminophosphonyl esters of AMP were stable in the conditions of aminoacylation [12]. Thus, I possessing an unusually high affinity* towards synthetases and a potential active anhydride bond were not substrates in any of the reactions catalysed by synthetases.

These properties of the anhydrides I would be difficult to be interpreted in terms of their being considered simply as aminoacyl adenylates II in which the –C(O)– group is substituted by a >P(O)–X fragment. The electron and spatial parameters of these groups are so different that such an analogy would be formal. The inhibiting properties of I can be better accounted for if one considers the enzyme–inhibitor complexes, III, as analogues of tetrahedral products of addition at the carbonyl group of aminoacyl adenylates, IV.





group in IV, that is consistent with both the absence of the substrates properties of I and the high affinity of Id.

Jenks [13] was one of the first to point out transition-state analogues to be useful in enzymatic investigations. Independently, in the case of pyridoxal enzymes, we suggested to use the analogues of intermediate coenzyme–substrate complexes, N_α-pyridoxyl-5′-phosphoamino acids [14,15]. A number of effective inhibitors, transition-state analogues, are known [16]. However, for enzymatic activation of carboxylic acids involving ATP, anhydrides like I seem to be the first example when a phosphorous-containing structure was employed to model the transition state. Apparently, this approach will be helpful in other cases as well since, for instance, 2′(3′)-aminophosphonyl esters of AMP were shown to be specific inhibitors of aminoacyl-tRNA

* For aminophosphonic acids and their derivatives, $K_i = 10^{-2}$ M and higher, except for α-amino-β-phenylethyl phosphonic acid. However, the properties of Ib did not differ from other I as was shown in this work

deacylation catalysed by synthetases [12] whereas 2′- and 3′-acylaminophosphonyl esters of AMP inhibited acylaminoacylation of aminoacyl-tRNA in a cell-free system with ribosomes [17].

Acknowledgement

The authors gratefully acknowledge the contribution of T. I. Osipova and I. A. Gandurina who prepared the anhydrides I.

References

[1] Cassio, D., Lemoine, F., Waller, J.-P., Sandrin, E. and Boissonnas, R. (1967) Biochemistry 6, 827–835.
[2] Khomutov, R. M., Osipova, T. I., Biryukov, A. I. and Ishmuratov, B. Kh. (1978) Bioorg. Khim. in press.
[3] Yaniv, M. and Gros, F. (1969) J. Mol. Biol. 44, 1–15.
[4] Stulberg, M. P. (1967) J. Biol. Chem. 242, 1060–1064.
[5] Lemoine, P., Waller, J.-P. and Van Rapenbusch, R. (1968) Eur. J. Biochem. 4, 213–221.
[6] Gutcho, S. (1968) Biochim. Biophys. Acta 157, 76–82.
[7] Gillam, J. C. and Tener, G. M. (1971) Methods Enzymol. 20, 55–70.
[8] Bergmann, F. H., Berg, P. and Dieckmann, M. (1961) J. Biol. Chem. 236, 1735–1740.
[9] Kelmes, A. D., Nevelli, G. D. and Stulberg, M. P. (1965) J. Biol. Chem. 240, 3979–3983.
[10] Lineweaver, H. and Burk, D. (1934) J. Am. Chem. Soc. 56, 658–666.
[11] Tamari, M., Cassaigne, A., Lacoste, A.-M. and Neuzil, E. (1975) Biochimie 57, 97–103.
[12] Osipova, T. I., Biryukov, A. I., Gandurina, I. A., Tarusova, N. B. and Khomutov, R. M. (1978) Bioorg. Khim. in press.
[13] Jenks, W. P. (1966) Current Aspects of Biochemical Energetics (Kaplan, N. O. and Kennedy, E. P. eds) pp. 273–298, Academic Press.
[14] Khomutov, R. M. (1967) Symp. Structure and Function of Peptides and Proteins, abstr. p. 69, Riga.
[15] Khomutov, R. M. (1968) 5th Int. Symp. Chemistry of Natural Products, abstr. 210–211, London.
[16] Wolfenden, R. (1976) Ann. Rev. Biophys. Bioenerg. 5, 271–306.
[17] Tarusova, N. B., Kukhanova, M. K. and Khomutov, R. M. (1978) Bioorg. Khim. in press.