BBA 63313

Competitive inhibition of adenosine deaminase by purine and pyrimidine bases

The factors underlying the specificity of the binding of bases, ribosides, ribotides and base-containing coenzymes to enzyme proteins, as well as related chemical mechanisms are still largely unknown One approach to this problem is through the study of enzymes which use simple purine and pyrimidine derivatives as substrates[1] For this study we have used adenosine deaminase (adenosine aminohydrolase, EC 3.5 4.4) from calf intestinal mucosa Adenosine, 2,6-diaminopurine and 6-chloropurine ribosides are substrates for the enzyme[2,3] The natural ribosides inosine, guanosine, xanthosine and cytidine do not inhibit the enzyme activity, while purine and 6-methylaminopurine ribosides are inhibitors[3].

A study of the inhibitory action of the purine and pyrimidine bases on adenosine deaminase activity is reported in this paper The direct utilization of the bases rather than the ribosides, allows one to compare the K_i values of the bases whose ribosides are enzyme substrates, with those of the bases whose ribosides are competitive inhibitors. Furthermore, it seems to be easier to correlate the degree of inhibition of the various bases with their chemical structure.

Adenosine deaminase has been purified according to the method of BRADY AND O'CONNELL[4] The enzyme activity was measured spectrophotometrically[5] in 0.05 M phosphate buffer at pH 7 and 20°. The K_i values have been calculated from Lineweaver–Burk and from Dixon plots. For the noninhibitory compounds, the lower boundary of the K_i values is reported

In Table I the K_i values of a series of bases and the corresponding ribosides are reported The bases of the ribosides which are enzyme substrates or competitive inhibitors are competitive inhibitors. The lack of the ribose moiety greatly decreases

TABLE I

KINETIC CONSTANTS FOR SUBSTRATES AND INHIBITORS OF ADENOSINE DEAMINASE

Ribosides	K_i *or* K_m $\times 10^5$ *(M)*	*Bases*	K_i $\times 10^4$ *(M)*
Adenosine	4 (K_m)	Adenine	2 8
2-Aminoadenosine	2 (K_m)	2-Aminoadenine	1 7
6-Chloropurine riboside	64 (K_m)*	6-Chloropurine	13 0
N^6-Methyladenosine	0 5	N^6-Methyladenine	3 7
Purine riboside	0 7	Purine	9 0
		2-Aminopurine	3 2
		2-Methyladenine	1 5
		2-Hydroxyadenine	2 4
Inosine	>30	Hypoxanthine	>30
Guanosine	>30	Guanine	>30
Xanthosine	>30	Xanthine	>30
6-Mercaptopurine riboside	>30	6-Mercaptopurine	>30
		2-Amino-6-mercaptopurine	>30
		2-Hydroxy-6-mercaptopurine	>30

* From ref 6

the affinity for the enzyme, however the bases show a measurable inhibitory effect Hypoxanthine, guanine, and xanthine, whose corresponding ribosides do not inhibit the enzyme activity, are not inhibitors (K_i over $30 \cdot 10^{-4}$ M) It appears that adenosine deaminase has different affinities for the bases tested, however it does not appear that an amino group at the 6-position of the purine nucleus is necessary for the binding at the active site, since purine, 2-aminopurine and 6-chloropurine are relatively good inhibitors The substitution of the hydrogen atom in the 2-position of adenine with an amino, hydroxyl or methyl group leads to a decrease of the K_i values with respect to that of adenine.

The mechanism whereby the enzyme discriminates between the classes of inhibitory compounds and noninhibitory compounds is not immediately apparent However a striking structural diversity exists between the bases which are inhibitors and the compounds which have K_i values over $30 \cdot 10^{-4}$ M. All inhibitor compounds bear a double bond between N-1 and C-6 atoms of the purine nucleus, while in aqueous solution hypoxanthine and 6-mercaptopurine are most prevalent in the tautomeric keto and thione form, respectively[7,8]; furthermore, the most basic nitrogen is N-1 for the inhibitors and N-7 for the other compounds[7,9] As Table II shows, there is a corre-

TABLE II

BASICITY OF ADENOSINE DEAMINASE INHIBITORS

Bases	$K_i \times 10^4$ *(M)*	pK_a*	*Position of the most basic nitrogen**
2-Methyladenine	1 5	5 1**	N-1
2-Aminoadenine	1 7	5 1	N-1
2-Hydroxyadenine	2 4	4 5	N-1
Adenine	2 8	4 2	N-1
2-Aminopurine	3 2	3 8	N-1
Purine	9 0	2 4	N-1
6-Chloropurine	13 0	<2 0	N-1
Hypoxanthine	>30	2 0	N-7
Guanine	>30	3 3	N-7
Xanthine	>30	0 8	N-7
6-Mercaptopurine	>30	<2 5	N-7
2-Amino-6-mercaptopurine	>30		N-7

* From refs 7–10
** pK_a value of 2-methyl-6-methylaminopurine

lation between the basicity of the N-1 and the K_i values, independent of the nature of the substituent. The purine compounds in which the most basic nitrogen is N-7 show K_i values higher than 30 10^{-4} M

The presence of a methyl group or a methylated substituent in the 6-position leads to noninhibitory compounds: 6-methyl-, 6-methoxy-, 6-methylthio- 6-dimethylaminopurine, which have a pK_a of 2 6, 2.2, 0 and 3.9, respectively[10], exhibit inhibition constants higher than $30 \cdot 10^{-4}$ M We think that the lack of inhibition can be ascribed to the low polarity of the methyl-, methoxy-, methylthio- and dimethylamino radicals rather than to a steric hindrance, since 6-methylaminopurine (pK_a of 4.2) is a good inhibitor

The basicity of N-1 does not seem to be the only factor responsible of the binding of the purine. Other results show the importance of the imidazole moiety of the purine nucleus. Although imidazole (20 mM) does not inhibit the enzyme, the modifications of the imidazole ring of the purine nucleus result in a decrease of the affinity for adenosine deaminase, 8-azaadenine (pK_a of 2 6), in which the most basic nitrogen is N-1 (ref 8) does not inhibit the enzyme activity. Cytosine in which the most basic nitrogen is N-3 of the pyrimidine ring, with a pK_a close to that of adenine[8], shows a K_i value higher than $30 \cdot 10^{-4}$ M. However when the basicity of the pyrimidine compounds increases, we again find good competitive inhibitors This is the case with 2,4,6-triaminopyrimidine which has a pK_a of 6 8 and a K_i value of $4 \cdot 10^{-4}$ M

We have also studied the effect of the protonation of 2,4,6-triaminopyrimidine, 2-aminoadenine and 2-methyladenine on the K_i values of these bases. The K_i values of the three compounds increase with decrease of pH and the increase of the K_i values parallels the protonation of the inhibitor However, constant K_i values are found when they are calculated from the concentration of the deprotonated form of the inhibitors at the various pH's tested The protonation of N-1 of the purine ring and of N-3 of the pyrimidine ring results in noninhibitory compounds

Although the data reported in this paper suggest that the N-1 of the purine nucleus plays a role in the binding of the inhibitors and substrates to the active site of adenosine deaminase, at the present time a direct demonstration has not been made.

Compounds in which the various nitrogen atoms of the purine nucleus are substituted by a carbon atom are now under investigation

The authors wish to thank Dr L B Townsend for the gift of N^6-methyladenosine and Mr R Chiti for technical assistance. This work was supported by the Italian C.N.R

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Received December 27th, 1967