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Hexofuranosyladenine Nucleosides as Substrates and Inhibitors of Calf Intestinal Adenosine Deaminase

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A series of hexofuranosyladenine nucleosides have been tested as substrates and inhibitors of adenosine deaminase from calf intestinal mucosa. The nucleosides differed from each other in configuration at the various carbon atoms of the hexose and had either a methyl group or hydroxymethyl group at the terminal position. It has been confirmed that the best substrates have the β-D or α-L configuration at the anomeric position and an hydroxyl group on the same side of the furanose ring as adenine. However, these properties are not minimal and other nucleosides will act as substrates even if they do not have the preferred configurations or groups available. The effect of having two hydroxyl groups in the same region of the molecule and in the preferred configurations was to greatly reduce V_{max}. Most structural changes resulted in changes in V_{max}, whereas K_M values remained fairly close. Only a change in configuration of the hydroxyl group at C-5′ caused a dramatic change in affinity, as reflected in the K_M. All nucleosides exhibited competitive inhibitory kinetics. In the latter studies also, a change of configuration at C-5′ greatly affected binding.

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) is a ubiquitous enzyme in mammalian tissues that catalyzes the deamination of adenosine to form inosine and ammonia. A large number of structural analogues of adenosine have been studied in order to ascertain the requirements for substrate activity or inhibitory activity. It has been a rather difficult task to clarify the main structural features that will make a substrate, particularly when the sugar moiety is considered. The minimal structural requirements for adenosine deaminase activity have been summarized by several investigators.[1-5] The preferred anomeric configuration was β-D or α-L (*R* configuration for C–N bond) and there had to be an hydroxyl group either at C-5′ or C-3′ in the "up" configuration, as shown in Chart I. However, a nucleoside lacking a 5′-OH group, 2,5′-anhydroformycin, was a substrate,[6,7] and in a recent paper we reported that a series of unsaturated adenine nucleosides were weak substrates for adenosine deaminase even though most of them lacked at least one

Chart I

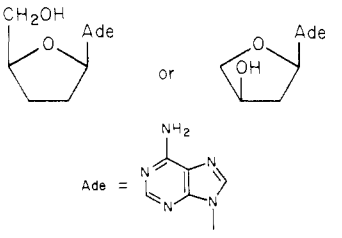

Table I. Activity of Hexofuranosyladenine Nucleosides with Adenosine Deaminase

no.	nucleoside	ref	K_M, μM	V_{max}, μmol min^{-1} mg^{-1}	K_i, μM
1	9-(6-deoxy-α-L-idofuranosyl)adenine	12	320	25	240
2	9-(α-L-idofuranosyl)adenine	13	260	0.178	180
3	9-(6-deoxy-α-L-talofuranosyl)adenine	14, 15, 16	21[a]	61[a]	32
4	9-(6-deoxy-β-D-allofuranosyl)adenine	16, 17	78[a]	0.792[a]	
5	9-(6-deoxy-β-D-galactofuranosyl)adenine	18	230	0.105	230
6	9-(6-deoxy-α-L-mannofuranosyl)adenine	19	670	0.326	260
7	9-(α-L-mannofuranosyl)adenine	20	280	0.092	120
8	9-(β-D-mannofuranosyl)adenine	21	*b*	*b*	670
9	9-(α-D-mannofuranosyl)adenine	22	*b*	*b*	240
10	9-(6-deoxy-α-D-mannofuranosyl)adenine	23	*b*	*b*	240
11	9-(β-L-gulofuranosyl)adenine	24	*b*	*b*	120
12	9-(6-deoxy-β-L-gulofuranosyl)adenine	12	*b*	*b*	200
13	9-(6-deoxy-β-L-galactofuranosyl)adenine	14	*c*	*c*	670
14	9-(6-deoxy-α-D-altrofuranosyl)adenine	14	*c*	*c*	350
15	9-(6-deoxy-β-L-glucofuranosyl)adenine	25	*c*	*c*	95
16	9-(6-deoxy-α-D-idofuranosyl)adenine	12	*c*	*c*	*d*
17	9-(α-D-idofuranosyl)adenine	26	*c*	*c*	*d*
18	9-(6-deoxy-β-D-gulofuranosyl)adenine	12	*c*	*c*	240

[a] Values obtained from ref 2. The values of V_{max} for 3 and 4 were calculated from information supplied in ref 2 using our value of V_{max} for adenosine (220 μmol min^{-1} mg^{-1}). This value is almost identical (217 μmol min^{-1} mg^{-1}) with that reported by Evans and Wolfenden.[27] [b] Very weak substrate. K_M and V_{max} could not be determined. [c] Not a substrate. [d] Not an inhibitor.

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of the two basic structural requirements for substrate activity; in some cases, all of the "requirements" were lacking.[8] In order to gain further knowledge of the workings of this enzyme, the substrate and inhibitory activity of a number of hexofuranosyl nucleosides have been examined.

Experimental Section

Calf intestinal adenosine deaminase, type I, was purchased from Sigma Chemical Co. A spectrophotometric assay[9] was used in which the change of absorbance at 265 nm was measured at 25 °C in cuvettes having a 1-cm light path. Into each cuvette was placed 3 mL of a solution of the nucleoside analogue in 0.05 M phosphate buffer at pH 7.0. For inhibitor studies, adenosine and the nucleoside analogue in buffer were added in required amounts and adjusted to 3 mL. The reactions were initiated by the addition of 0.1 mL of enzyme solution in buffer. In all substrate assays, the final spectra were obtained in order to verify that hypoxanthine nucleosides had been formed. The spectra were typical of N^9-substituted hypoxanthine derivatives with peaks at 249 nm, identical with solutions of inosine. Ultraviolet spectra and kinetic data were obtained with a Beckman Model 25 spectrophotometer. K_M, V_{max}, and K_i values were obtained from Lineweaver–Burk plots[10] using the Wilkinson weighted least-squares analysis[11] to obtain the best fit.

Results and Discussion

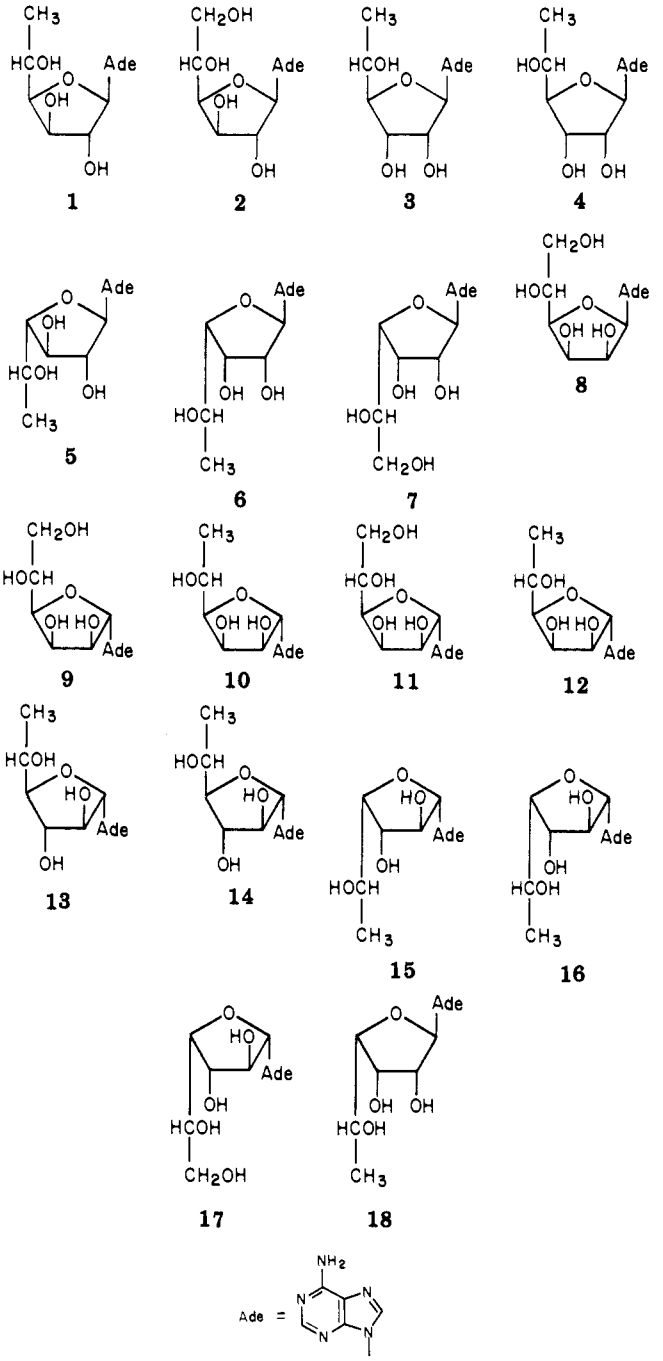
The names of the nucleosides used in this work are listed in Table I with their identifying numbers which correspond to the structures shown in Chart II. Table I also contains references to the original synthesis of each compound and the values for K_M, V_{max}, and K_i. Nucleosides **1–7** were substrates for adenosine deaminase. Nucleosides **1–5** possess the highly favorable structural features mentioned above as being required for best substrate activity. Each has a β-D or α-L configuration and an hydroxyl group on the same side of the furanose ring as adenine. Nucleosides **6** and **7** do not have the latter feature and yet were still substrates. Nucleosides **8–12** were also substrates but were so slowly deaminated that reproducible kinetic data could not be obtained. Compounds **9–12** have the "wrong" configuration at the anomeric carbon atom but in spite of this are capable of being deaminated. The results for nucleoside **8** were somewhat unexpected because even with the preferred structural features it was a very poor substrate.

With the exception of nucleosides **3** and **4**, the K_M values of the various substrates are rather close and the structural changes are reflected primarily in the values for V_{max}. It has been shown that the value of K_M with the calf intestinal enzyme is a good approximation of the dissociation constant for the enzyme–substrate complex.[28] Since all of the nucleosides except **16** and **17** were competitive inhibitors, the values for K_i can be compared with the values for K_M. The data show that a correlation exists. Because of this, a fairly good estimate of the K_M values for nucleosides **8–12** can be made. It can be seen that the structural changes do not significantly influence binding of these nucleosides to the enzyme; however, the breakdown of the enzyme–substrate complex to product is greatly affected. The exceptions were **3** and **4**, which had a greater affinity for the enzyme. Unfortunately, we did not have a sample of **4** to determine the K_i, but it can be seen in Table I that the difference in K_M of **3** from the other nucleosides is also reflected in the K_i. However, the change in configuration of the hydroxyl group at C-5′ in **3** and **4** results in a nearly 100-fold difference in V_{max}.

Nucleosides **1–3** have identical configurations at C-1′, C-2′, and C-5′, yet **3** is a better substrate for adenosine deaminase than **1** or **2**. The change in configuration at C-3′ apparently creates either a steric interaction or else enough of a conformational change in the nucleoside so that these molecules cannot bind as well. It is interesting to note the influence of the 6′-(hydroxymethyl) group of **2** on the value of V_{max}, where a tremendous reduction occurs. This same effect can be noted in nucleosides **6** and **7** as well, neither of which, incidentally, have the "minimum structural requirements" for substrate activity. It would be of interest to synthesize 9-α-L-talofuranosyladenine and compare its activity to **3**. Although nucleoside **5** has a favorable hydroxyl group in the "up" configuration at C-3′, the 5′, 6′ carbon atoms are "down". It has already been demonstrated that this configuration at C-4′ is unfavorable for good substrate activity, possibly due to steric considerations.[8,29]

It was expected that nucleoside **8** would be a fairly good substrate for adenosine deaminase. In fact, it was a very

Chart II



poor substrate. This result may demonstrate either an inability of the enzyme to accommodate the hydroxyl group at C-3′ (and C-2′ as well) due to steric crowding or may be a result of a conformational change in the nucleoside. A similar effect has been noted in going from the *xylo* configuration to the *lyxo* configuration in 9-β-D-pentofuranosyladenines.[3] Nucleosides **9–12** were very poor substrates; however, this cannot be ascribed simply to a change in anomeric configuration, because the structural features of the rest of the molecules are identical with **8** and yet there were no detectable differences in substrate activity. It is probable that the steric crowding in **8** is relieved somewhat when the adenine ring is in the α-D or β-L configuration, resulting in slightly better binding as reflected in the K_i values of **9–12** and **14**.

Nucleosides **13–17** were not substrates for adenosine deaminase. This was not too surprising, because no nucleosides having the arrangement of configurations shown at C-1′, C-2′, and C-3′ have ever acted as substrates for this enzyme. It would be very interesting to see what would happen if the adenine rings were cis to the hydroxyl groups at C-2′; however, none of these compounds have been prepared. Nucleosides **13–15** were competitive inhibitors, whereas **16** and **17** did not bind at all. The only difference between **15**, which was an inhibitor, and **16**, which was not, is the configuration of the hydroxyl group at C-5′. The result of changing the configuration at C-5′ was mentioned above during comparison of the activity of **3** and **4**, and this effect is illustrated again by comparison of the structure of **6**, which was a substrate, to **18**, which was not.

No simple explanation can delineate what elements of structure favor substrate activity over inhibitory activity, although it can be stated with some certainty that the best substrates are those having the previously mentioned structural requirements (Chart I). However, it is important to realize that other nucleosides will act as substrates too. The main effect noted is that lack of a 5′-OH group in the proper configuration results in a substantial decrease in binding, whereas other configurational changes only affect V_{max}. It should never be assumed that a given adenine nucleoside will not act as a substrate for adenosine deaminase simply because the structural features expressed in Chart I are lacking.

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