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UNUSUAL SUBSTRATES FOR ADENOSINE DEAMINASE FROM CALF INTESTINAL MUCOSA

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Summary

A number of adenine nucleosides with exocyclic double bonds either at the 4′,5′ position in pentofuranosyl nucleosides or at the 5′,6′ position of hexofuranosyl nucleosides have been found to act as substrates for adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) from calf intestinal mucosa. Most of the results obtained are contrary to the accepted minimal structural requirements for substrate activity. These nucleosides had either incorrect anomeric configurations or no hydroxyl group at C-5′ or C-3′ in the proper configuration; some compounds incorporated both structural changes. There is a possibility that the unsaturated group has a special role in binding to the enzyme.

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), the enzyme that catalyzes the deamination of adenosine to inosine appears to be virtually ubiquitous, but the isoenzyme most frequently studied has been the one from calf intestinal mucosa, probably due to its commercial availability. A good deal of work has been reported in which the structural requirements for substrate activity have been determined [1—8]. In addition to a number of variations in structure allowed in the nitrogenous base [7,9,10], a number of structural changes in the sugar are permitted [2,3,5,6,8]. From these data a number of conclusions have been drawn regarding requirements for substrate activity. These "minimal requirements" have been stated to consist of a β-D or α-L configuration at the anomeric carbon atom of the nucleoside (i.e. an R configuration) and an hydroxyl group at C-5′ or C-3′ in the "up" configuration as shown in Fig. 1 [5,8,11—13].

Faith in the above principles led to their recent utilization to help prove the anomeric configuration of new nucleoside analogs in cases where other physical methods such as NMR, polarimetry, or chemical derivatization failed

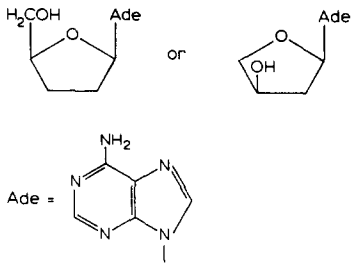

Fig. 1. Proposed minimal structural requirements for substrate activity for adenosine deaminase from calf intestinal mucosa.

to provide unequivocal results [14,15]. Indeed, Coddington [1] had used adenosine deaminase to show that cordycepin [9-(3′-deoxy-β-D-ribofuranosyl) adenine] had the β-D configuration. During routine assays of this type it was discovered that nucleosides having exocyclic unsaturation were slowly deaminated even when they did not have the "minimal structural requirements" for substrate activity.

The unsaturated nucleosides used in this study were all recently prepared in this laboratory and their structures are shown in Fig. 2. The names and references for each nucleoside are: 9-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)adenine (I) [16], 9-(5-deoxy-β-L-*erythro*-pent-4-enofuranosyl)adenine (II) [16], 9-(5,6-dideoxy-β-D-*xylo*-hex-5-enofuranosyl)adenine (III) [17,18], 9-(5,6-dideoxy-β-D-*ribo*-hex-5-enofuranosyl)adenine (IV) [19], 9-(5,6-dide-

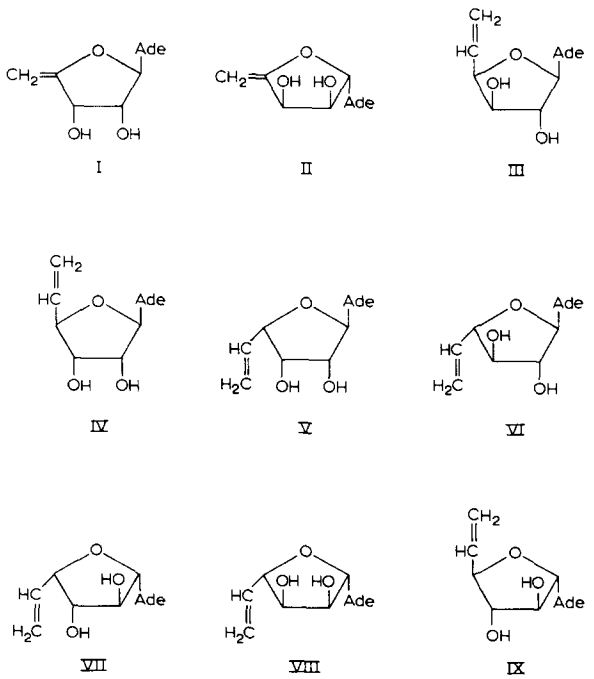

Fig. 2. Structures of unsaturated nucleosides.

oxy-α-L-*lyxo*-hex-5-enofuranosyl)adenine (V) [17], 9-(5,6-dideoxy-α-L-*arabino*-hex-5-enofuranosyl)adenine (VI) (Srivastava, V.K. and Lerner, L.M., manuscript in preparation), 9-(5,6-dideoxy-β-L-*xylo*-hex-5-enofuranosyl)adenine (VII) [17], 9-(5,6-dideoxy-β-L-*ribo*-hex-5-enofuranosyl)adenine (VIII) [19], and 9-(5,6-dideoxy-α-D-*arabino*-hex-5-enofuranosyl)adenine (IX) (Srivastava, V.K. and Lerner, L.M., manuscript in preparation). Adenosine was purchased from Sigma Chemical Company and was recrystallized three times from water before use.

Adenosine deaminase, Type 1, was purchased from Sigma Chemical Company. The assay procedure was based upon that of Kaplan [20] in which the change of absorbance at 265 nm was measured in 0.05 M phosphate buffer at pH 7.0 and 25° C. The cuvettes had a 1-cm path and contained 3.0 ml of adenosine and/or nucleoside analog solutions in buffer to which were added 0.1 ml of the enzyme solution also in buffer. The ultraviolet spectra and kinetic data were obtained with a Beckman DK-2 spectrophotometer. The deamination of the nucleoside analogs was verified in two different lot numbers of the enzyme preparation. In all cases the final spectra were typical of N^9-substituted hypoxanthine nucleosides, with λ_{max} 249 nm, identical to solutions of inosine obtained by enzymatic deamination of adenosine. Values for K_m, V and K_i were determined from plots of reciprocal velocity versus the reciprocal of the substrate concentration [21] and the best fit was obtained by using the Wilkinson weighted least square analysis [22].

With the exception of nucleoside VII, the unsaturated nucleosides were substrates for adenosine deaminase (Table I). However, one of these, IV, reacted so slowly that it was not possible to obtain reliable kinetic constants. Nucleosides III—VI and IX were competive inhibitors of the deamination of adenosine. It is interesting to note that nucleosides I, II, VII, and VIII exhibited noncompetitive inhibitory kinetics in spite of the fact that all, except VII, were substrates. One possible interpretation could be that I, II, and VIII are able to bind to more than one site on the enzyme, the second site not being near the active site. Generally, most of these nucleosides were weak in-

TABLE I

ACTIVITY OF UNSATURATED ADENINE NUCLEOSIDES WITH ADENOSINE DEAMINASE FROM CALF INTESTINAL MUCOSA

K_m for adenosine was 50 μM; V = 220 μmol/min per mg. NC, noncompetitive kinetics.

Nucleoside	K_m (μM)	V (μmol/min per mg)	K_i (μM)
I	87	0.39	172 (NC)
II	206	0.065	481 (NC)
III	183	3.54	96
IV	[a]	[a]	605
V	318	0.014	65
VI	636	0.14	494
VII	[b]	[b]	165 (NC)
VIII	146	0.065	442 (NC)
IX	130	0.018	485

[a] Substrate, but too slow to determine kinetic values accurately.
[b] Not a substrate.

hibitors with only V and III demonstrating moderately low K_i values.

From the results reported in Table I it can be concluded that the "rules" concerning minimal structural requirements for substrate activity of calf intestinal adenosine deaminase do not hold up, at least not for certain classes of compounds. The current problem is whether or not exocyclic unsaturation has a role to reorient these molecules in the active site of the enzyme, or are the generally accepted structural requirements not really limiting. First, consider the requirement that only β-D or α-L (R configuration) nucleosides are deaminated. In reviewing the literature, a major basis for this conclusion was the failure of two nucleosides, 9-α-D-arabinofuranosyladenine and 9-α-D-xylofuranosyladenine to react when exposed to mouse tissue adenosine deaminase [23]. It was shown too, that the calf intestinal enzyme did not deaminate 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine [1], 9-(2-deoxy-β-L-*erythro*-pentofuranosyl)adenine [5,6], or 9-α-D-arabinofuranosyladenine [24]. Other nucleosides studied were either hexofuranosyl nucleosides or hexopyranosyl nucleosides, and indeed, in the latter case, no pyranosyl nucleoside has ever served as a substrate. There was hardly enough examples recorded to make such a sweeping generalization concerning the configuration. The requirement for an hydroxyl group in the "up" configuration at C-5′ or C-3′ has already been shown not to be absolutely necessary. 2,5′-Anhydroformycin, a cyclonucleoside lacking the 5′ OH group has been demonstrated to be a substrate for calf intestinal adenosine deaminase [25,26]. In the present case also a number of the nucleosides lack this hydroxyl group and are still substrates, although the fastest reacting substrate was III which did have the 3′ OH in the "up" configuration. However, VI has the same structure except for the configuration at C-4′ and yet it is a very poor substrate in comparison. The fact that the 5′,6′ carbons are "down" may be important, possibly implicating a steric interaction, since all of the nucleosides with this configuration at C-4′ were relatively poor substrates and VII was not a substrate at all. It had previously been reported that 9-α-L-arabinofuranosyladenine, which also has a similar configuration at C-4′, was not a substrate [24]. The failure of VII to act as a substrate and the poor substrate property of VIII are probably a reflection of the additional problem in having a less favorable anomeric configuration. It is interesting to note that VIII and the pentose nucleoside II, which have identical structures in the carbohydrate ring, have identical V values and both exhibit noncompetitive inhibition with very close K_i values. It is somewhat of a problem to account for IV being such a poor substrate considering the closeness of its structure to I. The problem cannot be steric in origin because other nucleosides, such as III and IX, as well as three 6′-deoxyhexofuranosyl nucleosides, 9-(6-deoxy-β-D-allofuranosyl)adenine, 9-(6-deoxy-α-L-talofuranosyl)adenine, and 9-(6-deoxy-α-L-idofuranosyl)adenine were substrates [12,14,15]. However, among the 6′-deoxyhexofuranosyl nucleosides, the only ones which are substrates do have the previously recognized structural requirements [12,14,15].

We conclude from our experiments that (1) the requirement for the β-D or α-L configuration is not absolute and any attempts to prove an anomeric configuration with this enzyme have to be evaluated with caution and in conjunction with other chemical and physical data, (2) there is no absolute re-

quirement for the hydroxyl group at the C-5′ or C-3′ position in the "up" configuration, (3) there seems to be a limitation to having a large group in the "down" configuration at C-4′, (4) the exocyclic unsaturated group may have a role in binding to the enzyme. The latter may be as a hydrophobic bond or even as an electron donor·acceptor complex. It has not been established if unsaturation has a direct role or if a saturated group such as the ethyl at C-4′ could play the same role. It will be necessary to design further experiments to determine answers to these questions. In the meantime, it is doubtful that "minimal structural requirements" is a term that is meaningful with this enzyme preparation.

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