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3′-β-Ethynyl and 2′-Deoxy-3′-β-Ethynyl Adenosines: First 3′-β-Branched-Adenosines Substrates of Adenosine Deaminase

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Abstract—The 3′-C-branched-adenosine and 2′-deoxyadenosine analogues **1**–**7** were tested as substrate of adenosine deaminase. The 9-(3′-C-ethynyl-β-D-*ribo*-pentofuranosyl)adenine **1** and its 2′-deoxy analogue **7** were deaminated by the enzyme while the vinyl and ethyl derivatives **2** and **3** were not. The 9-(3′-C-branched-β-D-*xylo*-pentofuranosyl)adenines **4**–**6** were deaminated by the deaminase.

Adenosine deaminase (EC 3.5.4.4) plays an important regulatory role in purine metabolism. It catalyses the irreversible hydrolysis of (2′-deoxy)adenosine to (2′-deoxy)inosine and ammonia. It is well established that some adenosine analogues of chemotherapeutic interest are rapidly deaminated to inactive or less active inosine derivatives.[1] Therefore the factors governing the enzyme specificity are of considerable importance for the design of new compounds with preserved chemotherapeutic efficacy. We present here our study into the action of adenosine deaminase on the 3′-branched adenosine and 2′-deoxyadenosine analogues **1**–**7**.

Preparation of C-3′ substituted nucleosides **1**, **2**, **4**, **5** and **7** has been described elsewhere.[2,3] Compounds **3** and **6** were prepared via catalytic hydrogenation of the ethynyl compounds **1** and **4**, respectively, in almost quantitative yield.[4,5]

The effect of the C_{sp}, C_{sp2} and C_{sp3} substituent at the C-3′ stereocenter (*ribo* **1**–**3** versus *xylo* **4**–**6**) and of the 2′ hydroxyl group in compound **7**, on adenosine deaminase activity was studied. The different analogues were tested on purified adenosine deaminase to compare their kinetic properties against those of adenosine and of 2′-deoxyadenosine (Table 1). Among the compounds with *ribo*-configuration, only the C-3′-ethynyl derivative **1** was a substrate of adenosine deaminase. The K_m was only about 2- to 3-fold higher than that for adenosine. On the other hand the V_{max} was 50-fold lower than the V_{max} of adenosine hydrolysis. Compounds **2** and **3** were resistant to deamination as previously observed with 3′-β-methyl-adenosine, and both were weak inhibitors.[8] In contrast all the *xylo* compounds **4**–**6** were deaminated by the enzyme. The K_m of the three *xylo* compounds were similar, about 3- to 4-fold higher than the K_m of adenosine. The rate of hydrolysis was 3- to 5-fold lower. These compounds would be transformed in vivo to their corresponding inosine derivatives. Compound **7**, the 2′-deoxy analogue of compound **1**, was also a substrate of the enzyme but the affinity was too low to determine the kinetic constants (K_m and V_{max}). The rate of hydrolysis was proportional to the concentration of compound **7**, showing first order kinetics as observed at substrate concentrations much lower than the K_m.[9] The efficacy of the enzymatic reaction shown by the ratio (V_{max}/K_m) is higher for the ethynyl deoxy compound **7** than for the ethynyl compound **1**. Compounds **1** and **7** are the first 3′-β-branched adenosines (*ribo* and *erythro*) to be substrates of adenosine deaminase.

Adenosine analogues modified on the furanose ring or on the purine ring have been reported to be substrates of adenosine deaminase.[10–12] At C-3′ position, analogues, in the *ribo*- and in the *xylo*-configuration, where the hydroxyl group has been replaced by a hydrogen, an amino group, an azido group or by a halogen atom

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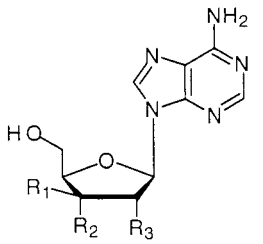

Ribo		R_1 = -C ≡ CH	***Xylo***		R_1 = OH	***Erythro***		R_1 = -C ≡ CH
	1	R_2 = OH		**4**	R_2 = -C ≡ CH		**7**	R_2 = OH
		R_3 = OH			R_3 = OH			R_3 = H
	2	R_1 = -CH = CH_2		**5**	R_1 = OH			
		R_2 = OH			R_2 = -CH = CH_2			
		R_3 = OH			R_3 = OH			
	3	R_1 = -CH_2- CH_3		**6**	R_1 = OH			
		R_2 = OH			R_2 = -CH_2- CH_3			
		R_3 = OH			R_3 = OH			

have been shown to be deaminated by the enzyme.[12] The C-methyl- and C-hydroxylmethyl-3′-β-adenosine derivatives are described to be resistant to deamination.[11] On the other hand the C-methyl- and the C-hydroxymethyl-3′-α-adenosine derivatives are reported to be susbstrates of adenosine deaminase.[11] From these results it appears that the nature of the C-3′ substituents has a dramatic effect on their recognition by the enzyme.

In the present work, we have shown that the three *xylo* compounds **4**–**6** were deaminated by adenosine deaminase. The K_m and the V_{max} values were similar to those reported in the literature for other *xylo*-derivatives.[10–12] Among the tested nucleosides in the *ribo* series, the ethynyl analogue **1** was surprisingly substrate of the enzyme. The K_m was only 3-fold higher than the K_m of adenosine, suggesting that analogue **1** fitted well in the active site cavity of the enzyme. The deamination rate was 50-fold lower than that of adenosine. The C-6 carbon of the base in the ethynyl compound may not be ideally situated in relation to the catalytic groups of the enzyme. The corresponding methyl,[11] ethyl and vinyl derivatives were not substrates. These observations raise the question of why the enzyme tolerates the ethynyl compound but not the three other derivatives.

Table 1. Kinetic constants for the deamination of adenosine, 2′-deoxyadenosine and compounds **1**–**7** by adenosine deaminase.[6]

Compounds	K_m (μM)	V_{max}[a]	Relative V_{max}	V_{max}/K_m
Adenosine	42	289	100	6.9
1	110	6	2	0.05
2	NS[b]	NS[b]	NS[b]	NS[b]
3	NS[b]	NS[b]	NS[b]	NS[b]
4	140	95	33	0.7
5	140	75	26	0.5
6	160	55	19	0.3
2′-Deoxyadenosine	31	353	122	11.4
7	—[c]	—[c]	—[c]	2.6

[a]μmoles/min/mg prot.
[b]Not substrate.
[c]First order kinetics were observed $\{v = (V_{max}/K_m)\,[S]\}$.

Nucleosides may take several conformations in rapid equilibrium.[13] Major factors influencing the conformational flexibility are the following: (i) rotation around the glycosidic N9–C1′ bond (*syn*/*anti* orientation), (ii) ribose puckering, (iii) rotation around the C4′–C5′ bond. The conformational requirements for an active enzyme–substrate complex can be deduced from the three dimensional structure of the binary complex (adenosine deaminase-inhibitor).[14] Adenosine would adopt the anti-conformation (χ values from −106 to −110°), with the C4′–C5′ bond in a +sc orientation (γ values from 54 to 57°) and with a 3′-*endo* sugar pucker (North).[14] Therefore only the compounds with the proper requirements would bind to the active site. As the tested analogues contain all the sites required for binding to the enzyme,[14] the absence of recognition could be due to an inappropiate conformation of the analogues. Substituents at position C-3′ were reported to increase the conformational rigidity of the furanose ring.[15,16] This could hinder the productive binding of the nucleoside analogue to the active site. In the case of 3′-β-methyl-adenosine, known to be resistant to deamination, it was proposed that steric hindrance of the C-8 proton with the bulky methyl group of the 3-*endo* conformer prevents the base from adopting the anti-conformation required for an enzyme–substrate complex.[17]

Preliminary NMR conformational analyses showed no significant differences between the *ribo*-derivatives **1**–**3**. They had, as reported for 9-(3′-*C*-methyl-β-D-*ribo*-furanosyl)-adenine,[18] a coupling constant $J_{H1'-H2'}$ of about 8 Hz.[3,4] Moreover, on irradiation of H-8,[19] comparable NOE intensities for H1′ and H2′ (6.2–6.7% and 3.5–4.2%, respectively)[20] were measured from the adiabatic off resonance ROESY.[21] So the sugar moiety of the three *ribo* derivatives adopted predominantly a South

conformation to limit the sterical interactions.[22] In this puckering mode, the C-substituent was located in a pseudo-equatorial location where its influence on the *syn* and *anti* equilibrium is the weakest. On the other hand, the $J_{H1'-H2'}$ coupling constants for the *xylo*-derivatives **4**–**6** were about 0 to 1 Hz,[3,5] suggesting that they exist predominantly, as 9-(3′-*C*-methyl-β-D-*xylo*-furanosyl)-adenine, in the North conformation.[18] These results did not show conclusively why compound **1**, in contrast to compounds **2** and **3**, was recognized by adenosine deaminase. Additional NMR, molecular modeling and crystallographic studies (structure of the complex of adenosine deaminase with 3′-β-C-ethynyl-adenosine or 3′-β-C-ethynyl-6-hydroxyl-1,6-dihydropurine ribonucleoside) should help to explain why the enzyme deaminated compound **1** and not compounds **2** and **3**.

The very high activity of the 3′-C-ethynyl-nucleosides as anticancer agents is now well established.[23,24] The adenosine analogue was less efficient than the uridine and cytidine derivatives.[3] This could be due to the in vivo deamination of compounds **1** and **7** by adenosine deaminase. Addition of adenosine deaminase inhibitors could be essential to potentiate the effectiveness of adenosine analogues **1** and **4**–**7** as anticancer reagents.

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4. 9-(3′-C-ethyl-β-D-ribo-furanosyl) adenine **3**. 9-(3′-C-ethynyl-β-D-ribo-furanosyl) adenine **1**[3] (0.37 g; 1.25 mmol) was hydrogenated overnight in methanol (73 mL) at 25 °C and 1 bar, in the presence of palladium on charcoal (10%, 0.15 g). The catalyst was removed by filtration on Celite (2×6 cm, methanol). The solution was concentrated to 2 mL then acetonitrile (10 mL) was added. Evaporation of the solvents gave compound **3** quantitatively. Mp = 193–194 °C; UV(MeOH): λ_{max} = 214 nm; ε = 14000 M^{-1} cm^{-1}; λ_{max} = 262 nm; ε = 13600 M^{-1} cm^{-1}; IR (KBr) 3422, 3339, 2993, 1674 cm^{-1}; $[\alpha]_D^{24}$ −63 (*c* = 1; DMSO); ^{1}H NMR (DMSO-d_6) δ 8.35 (s; 1H; H_2); 8.12 (s; 1H; H_8); 7.39 (s; 1H; NH_2); 5.96 (s; 1H; OH); 5.88 (d; $J_{H'1H'2}$ = 7.9 Hz; 1H; H′$_1$); 5.40 (s; 1H; OH); 4.60 (s; 1H; OH); 4.50 (d; $J_{H'1H'2}$ = 7.9 Hz; 1H; H′$_2$); 3.91 (m; 1H; H′$_4$); 3.61 (m; 2H; H′$_5$); 1.73 (q; *J* = 7.5 Hz; 2H; CH_2); 0.97 (t; *J* = 7.5 Hz; 3H; CH_3); ^{13}C NMR (DMSO-d_6) δ 156.2 (C_6); 152.0 (C_2); 149.0 (C_4); 140.6 (C_8); 119.5 (C_5); 87.8 (C′$_4$); 87.7 (C′$_1$); 78.2 (C′$_3$); 76.5 (C′$_2$); 61.3 (C′$_5$); 25.5 (CH_2); 7.7 (CH_3). Anal. calcd for $C_{12}H_{17}N_5O_4$: C 48.81, H 5.76, N 23.73. Found: C 48.98, H 5.93, N 23.55.; SM (CI) 296 (MH^+; 100).
5. 9-(3′-C-ethyl-β-D-*xylo*-furanosyl) adenine **6**. Compound **6** was synthesized from 9-(3′-C-ethynyl-β-D-*xylo*-furanosyl) adenine **4**[3] as described above for compound **3**. Mp = 206–208 °C; IR (KBr) 3476, 3303, 2975, 1679 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.37 (s; 1H; H_2); 8.20 (s; 1H; H_8); 5.96 (s; 1H; H′$_1$); 4.81 (s; 1H; H′$_2$); 4.03 (m; 1H; H′$_4$); 3.93 (m; 2H; H′$_5$); 1.83 (m; 1H; CH_2); 1.62 (m; 1H; CH_2); 1.02 (t; *J* = 7.2 Hz; 3H; CH_3); ^{13}C RMN (DMSO-d_6) δ 156.0 (C_6); 152.2 (C_2); 148.6 (C_4); 139.8 (C_8); 118.85 (C_5); 90.41 (C′$_1$); 86.47 (C′$_4$); 80.9 (C′$_2$); 80.5 (C′$_3$); 60.2 (C′$_5$); 24.5 (CH_2); 7.8 (CH_3); SM (CI; NH_3) 296 (MH^+; 40), 295 (M^+; 63), 135 (BH^+; 100).
6. Adenosine deaminase from calf spleen was purchased from Sigma. The activity was measured in a 50 mM sodium phosphate buffer pH 7.5 at 30 °C by following the decrease of the absorbance at 262 nm. To determine the kinetic constants (K_m and V_{max}), the concentration of adenosine and its analogues was varied between 0.05 and 0.1 mM according to the procedure of Murphy et al.[7] All the double reciprocal substrate–initial velocity plots were linear. Enzyme (0.14 μg for adenosine and 2′-deoxy-adenosine; 1.4 μg for compounds **4**–**7**; 5.6 μg for **1** and until 22.4 μg for **2** and **3**) was added to initiate the enzymatic reaction.
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