Adenylate Deaminase (5′-Adenylic Acid Deaminase, AMPDA)-Catalyzed Deamination of 5′-Deoxy-5′-Substituted and 5′-Protected Adenosines: A Comparison with the Catalytic Activity of Adenosine Deaminase (ADA)

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The enzyme adenylate deaminase (AMPDA) is able to catalyze the hydrolytic deamination of 5′-substituted and 5′-protected 5′-deoxyadenosines, whereas limited or no activity is shown by adenosine deaminase (ADA) towards the same substrates.

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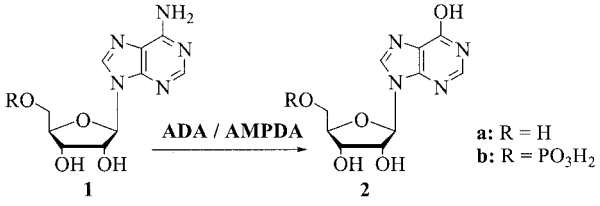
Introduction

Adenosine deaminase (ADA, EC 3.5.4.4) and adenylate deaminase (5′-adenylic acid deaminase, AMPDA, EC 3.5.4.6) catalyze the deamination of adenosine (**1a**) and 5′-phosphate adenosine (adenylic acid, AMP, **1b**) to the corresponding inosines **2a** and **2b** (Scheme 1).



Scheme 1. ADA- and AMPDA-catalyzed deamination of adenosine (**1a**) and 5′-adenylic acid (**1b**)

ADA can already be considered a valuable biocatalyst for the biotransformation of a wide range of structurally modified purine nucleosides,[1] provided that a hydroxy group is present at the 5′-position.[2] Additionally, it has been shown that some flexibility is possible at the 2′,3′-positions.[2,3] In previous papers,[4,5] we have shown that steric hindrance at positions 2′ and 3′ is well tolerated by both enzymes, since 2′,3′-*O*-diacetyladenosine (**3**) and 2′,3′-*O*-isopropylideneadenosine (**4a**) may be converted into the corresponding inosine derivatives (Figure 1).

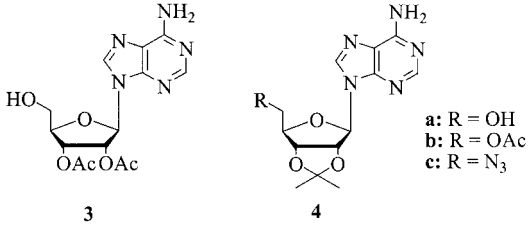


Figure 1. Structure of 2′,3′-*O*-diacetyladenosine (**3**) and 2′,3′-*O*-isopropylideneadenosines **4a**−**c**

Compared to ADA, applications of AMPDA are less frequent and only recently this enzyme has been proposed for the biotransformation of substrates other than AMP **1b**.[6] We have previously observed that AMPDA is able to catalyze the fast deamination of 2′,3′-isopropylideneadenosine **4a** and of 5′-substituted 2′,3′-isopropylideneadenosines like **4b** and **4c** that are not substrates for ADA.[5] It seemed interesting to investigate the activity of AMPDA on a few 5′-substituted and 5′-protected 5′-deoxyadenosines **5a**−**e** and compare the activity of this deamination to inosines **6a**−**e** with that of the other deaminating enzyme, namely ADA (Figure 2).

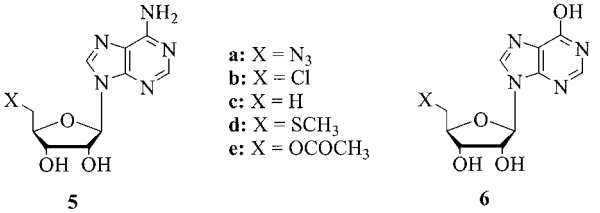


Figure 2. Structure of adenosine **5a**−**e** and inosine derivatives **6a**−**e**

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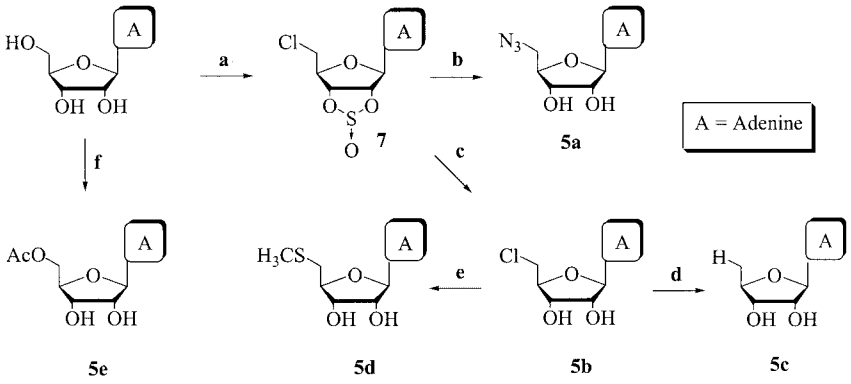

Scheme 2. Reagents and conditions: (a) $SOCl_2$, Py, CH_3CN, 0 °C; (b) NaN_3, DMF, 80 °C; (c) aq. MeOH, room temp; (d) $LiEt_3BH$, DMSO, room temp; (e) CH_3SNa, DMF, room temp; (f) *Candida antarctica*, vinyl acetate, THF, room temp.

Results and Discussion

Synthesis of 5′-Modified Adenosines 5a−e

For the synthesis of 5′-substituted 5′-deoxy derivatives **5a**−**d**, we started from the diastereomeric mixture of 5′-chloro-5′-deoxy-2′,3′-*O*-sulfinyladenosines (**7**) prepared from adenosine (**1a**) as described previously.[7] Treatment of the above intermediate **7** with sodium azide in dimethyl formamide at 80 °C directly afforded the deprotected azide **5a** (45 %). Compounds **5b**−**d** were prepared according to Robins and co-workers.[7] Thus, hydrolysis with aqueous methanol of the sulfinate moiety of **7** afforded the compound **5b** (98 %), that was in turn, reduced with lithium triethylborohydride (Superhydride®) to the 5′-deoxyadenosine (**5c**, 44 %) or transformed with sodium methanethiolate to the 5′-deoxy-5′-methylthioadenosine (**5d**, 50 %).

The acetate **5e** was preferably prepared by the previously described enzymatic acetylation of adenosine **1a** by means of the *Candida antarctica* lipase,[4] since any chemical approach would invariably yield mixtures of O- and N-acetates. The overall synthetic picture is summarized in Scheme 2.

AMPDA- and ADA-Catalyzed Deamination of 5′-Modified Adenosines 5a−e

The enzymatic deamination of compounds **5a**−**e** was carried out in a 3 % DMSO aqueous solution, conditions that do not influence ADA and AMPDA activity.[5] The commercially available preparation of AMPDA was less active than ADA (0.107 versus 1.5 units/mg solid) and we decided to use similar activity for both enzymes. However, due to problems of solubility in the medium for AMPDA, we finally adopted an enzyme/substrate ratio (mg/mg) of 1:1 and 0.1:1 for AMPDA and ADA, respectively. Results show that all adenosines **5a**−**e** were substrates for AMPDA-catalyzed conversion into the corresponding inosines **6a**−**e** in times varying between 3 and 150 minutes. For ADA-catalyzed deaminations, we followed the enzymatic transformation within a reaction time of 24 h. No transformation of compounds **5a** and **5d** was observed, whereas **5b**, **5c** and **5e** were only partially deaminated (Table 1). It should be pointed out that Robins and co-workers had already reported[8] that an α-amilase from *Aspergillus oryzae* (Sigma, USA) showed deaminating properties and was able to convert the thiomethyl derivative **5d** into the corresponding inosine **6d**. However, this "adenosine deaminase" activity was different from that of calf intestine ADA that did not transform the nucleoside **5d**. Since the AMPDA used by us is an enzymatic preparation from *Aspergillus species* purchased from Sigma (USA), there is the possibility that the enzyme used by Robins was not an adenosine deaminase, but rather an early preparation of AMPDA. In any event, we have confirmed that the enzymatic preparation of AMPDA used by us corresponds to a 5′-adenylic acid deaminase, since it is able to convert AMP (**1b**) to IMP (**2b**) in 10 min.

Table 1. ADA- and AMPDA-catalyzed deamination of adenosines **5a**−**e**

Substrate	AMPDA Reaction time (min)[a]	Product (yield,%)[b]	ADA (yield,%)[c]
5a	150	**6a** (94)	–
5b	30	**6b** (92)	26
5c	10	**6c** (91)	25
5d	3	**6d** (95)	–
5e	10	**6e** (97)	22

[a] Time to complete reaction. [b] Yield of isolated products. [c] Reaction stopped at 24 h.

Conclusions

Our results confirm that there might be a few differences in the binding pocket of the two deaminating enzymes ADA and AMPDA in spite of the similarity of the catalytic mechanism that has been proposed on the basis of kinetic considerations and the fact that deoxycoformycin derivatives are potent inhibitors of both enzymes.[9] This is reinforced by the observation that the two enzymes share some conserved sequence elements that may correspond to amino acid residues at the catalytic site.[10] Whereas the tridimensional structure is known for ADA,[11] the same infor-

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mation is not available for AMPDA and therefore a clear picture of the catalytic events for this enzyme is not available at present. From the results reported in this paper, AMPDA seems to be a biocatalyst that is more versatile than ADA in the nucleoside field, since it is able to catalyze fast conversion of all the compounds **5a**−**e** to the corresponding inosine derivatives **6a**−**e**. This result is even more intriguing if one considers that the residues present at the 5′ position in the ribose moiety of the above adenosines are virtually apolar, whereas at the pH conditions of the enzyme activity, the physiological substrate of AMPDA, i.e. AMP, bears the anionic form of a phosphate anion at the 5′ position. Further studies are needed in order to investigate the structure-activity relationship of AMPDA in more detail.

Experimental Section

General Remarks: Melting points were recorded with a Stuart Scientific SMP3 instrument and are uncorrected. ^{1}H NMR spectra were recorded at 303 K with a Bruker AM-500 spectrometer equipped with an Aspect 3000 computer, a process control and an array processor. The ^{1}H NMR chemical shifts are reported in parts per million, using the signal for residual solvent protons (δ = 3.30 ppm for CD_3OD) as an internal reference and coupling constants (*J*) are given in Hertz. NMR signals were assigned using ^{1}H-homodecoupling and COSY experiments. The progress of all reactions and column chromatography were monitored by TLC and HPLC. HPLC analyses were carried out with a Jasco HPLC instrument with an Uvidec 100 II UV detector using an Alltech Hypersil BDS C18 (4.6 mm × 250 mm). The eluent was a phosphate buffer pH 6.0/CH_3CN (70:30) with a (flow rate 1 mL/min; detection at 260 nm). Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F_{254} precoated plates with a fluorescent indicator. Flash chromatography[12] was performed using Merck silica gel 60 (230−400 mesh) and appropriate mixtures of CH_2Cl_2 and MeOH as eluents.

All reagents were obtained from commercial sources and used without further purification. Enzymes were obtained as follows: adenosine deaminase from calf intestinal mucosa (Sigma, type II, 1.5 units/mg solid), 5′-adenylic acid deaminase from *Aspergillus species* (Sigma, 0.107 units/mg solid).

The starting nucleoside **1a** was purchased from Aldrich. 5′-Chloro-5′-deoxyadenosine (**5b**), 5′-deoxyadenosine (**5c**) and 5′-deoxy-5′-methylthioadenosine (**5d**) were prepared according to literature procedures.[7] The enzymatic preparation of 5′-*O*-acetyladenosine (**5e**) has been described previously.[4]

5′-Azido-5′-deoxyadenosine (5a): Sodium azide (2.3 g, 35 mmol) was added portionwise to a solution of 5′-chloro-5′-deoxy-2′,3′-*O*-sulfinyladenosine (**7**) (2 g, 7 mmol) in DMF. The suspension was heated at 80 °C for 5 h, and the mixture cooled to room temperature. Excess sodium azide was removed by filtration. The resulting solution was diluted with ethyl acetate, washed with water, dried with Na_2SO_4, and the solvents evaporated to give a solid residue. The crude azide **5a** was purified by flash chromatrography (CH_2Cl_2/MeOH, 9:1) to give pure azide **5a** (0.9 g, 3.15 mmol, 45 %) as an amorphous solid. Attempts to crystallize the product failed.[13] ^{1}H NMR (CD_3OD): δ = 3.58 (dd, *J* = 4.4, 12.7 Hz, 1 H, H-5′b), 3.84 (dd, *J* = 7.0, 12.7 Hz, 1 H, H-5′a), 4.33 (dd, *J* = 4.0, 6.1 Hz, 1 H, H-3′), 4.53 (ddd, *J* = 4.4, 6.1, 7.0 Hz, 1 H, H-4′), 4.88 (dd, *J* = 4.0, 4.0 Hz, 1 H, H-2′), 5.99 (d, *J* = 4.0 Hz, 1 H, H-1′), 8.22 (s, 1 H, H-2), 8.27 (s, 1 H, H-8) ppm.

Enzymatic Deamination of Adenosine Derivatives 5a−e to Inosine Derivatives 6a−e: Compounds **5a**−**e** (0.02 g) in phosphate buffer (50 mM, 6 mL; pH 7.4 for ADA and pH 6.5 for AMPDA) containing 3 % DMSO were treated with ADA (2 mg) or AMPDA (20 mg) for the time indicated in Table 1. The progress of reactions was monitored by HPLC using the phosphate buffer pH 6.0/acetonitrile ratio as follows: **5a**, 70:30; **5b** and **5c**, 90:10, **5d**, 80:20; **5e** 85:15. When the reaction was complete, the solution was lyophilized to afford inosines **6a**−**e** as white solids.

5′-Azido-5′-deoxyinosine (6a): Attempts to crystallize the product failed [ref.[14] 161 °C (dec.)]. ^{1}H NMR (CD_3OD): δ = 3.57 (dd, *J* = 4.0, 12.4 Hz, 1 H, H-5′b), 3.81 (dd, *J* = 7.0, 12.4 Hz, 1 H, H-5′a), 4.34 (dd, *J* = 4.0, 4.0 Hz, 1 H, H-3′), 4.54 (ddd, *J* = 4.0, 4.0, 7.0 Hz, 1 H, H-4′), 4.86 (dd, *J* = 4.0, 4.0 Hz, 1 H, H-2′), 6.00 (d, *J* = 4.0 Hz, 1 H, H-1′), 8.08 (s, 1 H, H-2), 8.24 (s, 1 H, H-8) ppm.

5′-Chloro-5′-deoxyinosine (6b): M.p. 182−184 °C (from MeOH), [ref.[15] 191 °C (from MeOH)]. ^{1}H NMR (CD_3OD): δ = 3.92 (dd, *J* = 4.0, 11.4 Hz, 1 H, H-5′b), 3.93 (dd, *J* = 4.0, 11.4 Hz, 1 H, H-5′a), 4.26 (ddd, *J* = 4.0, 4.0, 4.0 Hz, 1 H, H-4′), 4.37 (dd, *J* = 4.0,4.0 Hz, 1 H, H-3′), 4.73 (dd, *J* = 4.0, 4.0 Hz, 1 H, H-2′), 6.02 (d, *J* = 4.0 Hz, 1 H, H-1′), 8.06 (s, 1 H, H-2), 8.24 (s, 1 H, H-8) ppm.

5′-Deoxyinosine (6c): M.p. 188−190 °C (from MeOH), ^{1}H NMR (CD_3OD): δ = 1.41 (d, *J* = 6.0 Hz, 3 H, CH_3), 4.05 (dd, *J* = 4.7, 5.4 Hz, 1 H, H-3′), 4.10 (dq, *J* = 5.4, 6.0 Hz, 1 H, H-4′), 4.65 (dd, *J* = 4.7, 4.7 Hz, 1 H, H-2′), 5.95 (d, *J* = 4.7 Hz, 1 H, H-1′), 8.05 (s, 1 H, H-2), 8.18 (s, 1 H, H-8) ppm. $C_{10}H_{12}N_4O_4$ (252.23): calcd. C 47.62, H 4.80, N 22.21; found C 47.72, H 4.74, N 22.10.

5′-Deoxy-5′-methylthioinosine (6d): M.p. 218−220 °C (from MeOH); {ref.[16] 223−224 °C (from water)}. ^{1}H NMR (CD_3OD): δ = 2.12 (s, 3 H, SCH_3), 2.86 (dd, *J* = 5.4, 14.0 Hz, 1 H, H-5′b), 2.92 (dd, *J* = 5.4, 14.0 Hz, 1 H, H-5′a), 4.22 (ddd, *J* = 5.4, 5.4, 5.4 Hz, 1 H, H-4′), 4.30 (dd, *J* = 5.4, 5.4 Hz, 1 H, H-3′), 4.71 (dd, *J* = 5.4, 5.4 Hz, 1 H, H-2′), 6.01 (d, *J* = 5.4 Hz, 1 H, H-1′), 8.05 (s, 1 H, H-2), 8.27 (s, 1 H, H-8) ppm.

5′-*O*-Acetylinosine (6e): M.p. 228−230 °C (from MeOH), ^{1}H NMR (CD_3OD): δ = 2.06 (s, 3 H, $OCOCH_3$), 4.25 (ddd, *J* = 3.2, 4.0, 4.0 Hz, 1 H, H-4′), 4.30−4.35 (m, 2 H, H-3′ and H-5′b), 4.37 (dd, *J* = 3.2, 12.0 Hz, 1 H, H-5′a), 4.68 (dd, *J* = 4.7, 4.7 Hz, 1 H, H-2′), 6.01 (d, *J* = 4.7 Hz, 1 H, H-1′), 8.04 (s, 1 H, H-2), 8.20 (s, 1 H, H-8) ppm. $C_{12}H_{14}N_4O_6$ (310.26): calcd. C 46.45, H 4.55, N 18.06; found C 46.52, H 4.48, N 18.12.

Acknowledgments

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