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DEAMINATION OF 2′,3′-*O*-ISOPROPYLIDENEADENOSINE-5′-CARBOXYLIC ACID CATALYZED BY ADENOSINE DEAMINASE (ADA) AND ADENYLATE DEAMINASE (AMPDA): INFLUENCE OF SUBSTRATE IONIZATION ON THE ACTIVITY OF THE ENZYMES

Pierangela Ciuffreda and Laura Alessandrini □ *Dipartimento di Scienze Precliniche LITA Vialba–Università degli Studi di Milano, Milano, Italy*

Radmila Pavlovic and Enzo Santaniello □ *Dipartimento di Medicina, Chirurgia e Odontoiatria–Università degli Studi di Milano, Milano, Italy*

□ *Adenosine deaminase (ADA) and adenylate deaminase (AMPDA) catalyze the deamination of 2′,3′-O-isopropylideneadenosine-5′-carboxylic acid to the corresponding inosine derivative and dependence of the rate of enzymatic reaction on the ionization degree of the substrate has been studied at different pH values.*

Keywords 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid; adenosine deaminase; adenylate deaminase

INTRODUCTION

Adenosine deaminase (adenosine aminohydrolase, ADA, EC 3.5.4.4) and adenylate deaminase (5′-adenylic acid deaminase, AMP deaminase, AM PDA, EC 3.5.4.6) are metalloenzymes that belong to the class of hydrolases and catalyze the irreversible elimination of ammonia from adenosine **1a** and adenosine 5′-phosphate (adenylic acid, AMP, **1b**) to the corresponding inosines **2a**, **b** (Scheme 1).[1]

Both enzymes are commercially available at a considerable level of purity and adequate activity and have been used as biocatalysts for chemo-enzymatic transformations in nucleoside chemistry.[2] For instance, starting from the initial observation that the steric hindrance at the positions 2′ and 3′ in 2′,3′-isopropylidene adenosine **3a** is well tolerated by the enzyme

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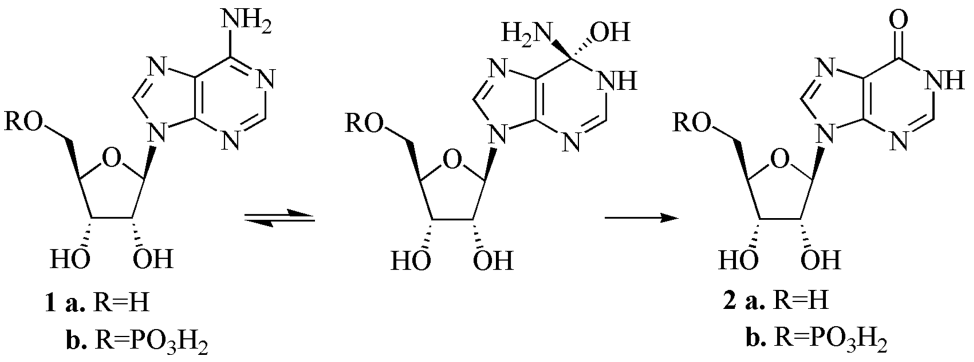
Address correspondence to Enzo Santaniello, Dipartimento di Medicina, Chirurgia e Odontoiatria—Università degli Studi di Milano, Via A. di Rudinì, 8-20142, Milano, Italy. E-mail: Enzo.Santaniello@unimi.it



SCHEME 1 ADA- and AMPDA-catalyzed deamination of adenosine (**1a**) and adenosine 5′-phosphate (**1b**).

ADA,[3] our group has studied ADA- and AMPDA-catalyzed deamination of a few 2′,3′-isopropylidene adenosines modified at position 5′ (compounds **3b–e**, Figure 1).[4,5]

The aforementioned results have shown that both enzymes can tolerate a substitution at the 2′,3′-positions. AMPDA seems a more versatile biocatalyst, since it converts all the compounds **3a–e** to the corresponding inosine derivatives in a time well suitable for preparative purposes. ADA instead requires at the 5′-position the presence of a hydroxy group for binding to the residue of Asp-19 through a hydrogen bond, which is essential for the catalytic mechanism.[6]

We have now investigated ADA and AMPDA activity with 2′,3′-isopropylideneadenosine-5′-carboxylic acid (**4a**) where the 5′-CH_2OH moiety of compound **3a** is substituted by a carboxylic group (Figure 2).

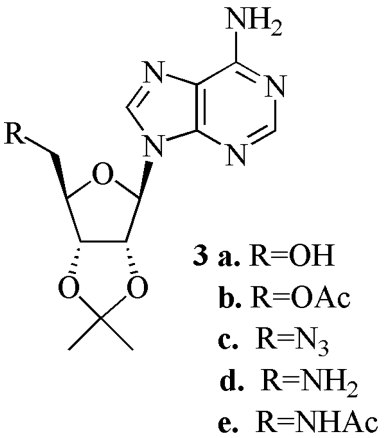


FIGURE 1 Structure of adenosine analogues **3a–e**

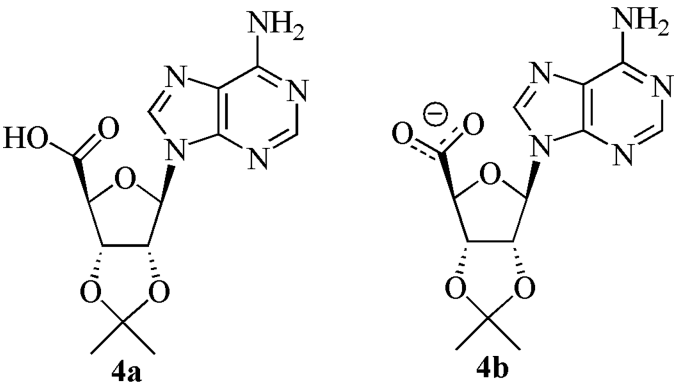


FIGURE 2 Structure 2′,3′-isopropylideneadenosine-5′-carboxylic acid in uncharged and charged forms (**4a** and **4b**).

However, aqueous solutions of compound **4a** should consist of charged and uncharged species (**4a** and **4b**) according to an equilibrium that should be pH-dependent. Because of the essential hydrogen bond with Asp-19, the ionization degree of carboxy group should influence ADA and AMPDA activity. Considering a pK of 4–5 for the COOH group, compound **4a** should prevail at pH values <4–5 and the carboxylate anion in **4b** should be more present at pH >5–6. On the other hand, AMPDA catalytic performance with compound **4a** could be influenced in a different manner by pH variations, since at the optimum value of pH 6.5, the physiological substrate 5′-adenylic acid should be almost exclusively present in an anionic form.

Here, we report the results of our studies on the activity of ADA and AMPDA on compound **4a** at different pH values of the enzymatic reaction medium, consisting in a 3% DMSO aqueous solution.[4]

RESULTS AND DISCUSSION

Activity of ADA and AMPDA on 2′,3′-Isopropylidene Adenosine 3a at Different pH

It cannot be excluded that, at various pH, ionization of amino acid residues of catalytic site or purine bases in nucleoside could influence the enzyme activity. Therefore, experiments at different pH values were preliminarily performed with both enzymes using 2′,3′-isopropylidene adenosine **3a** that contained a non-ionizable group at the critical 5′-position. We have found that, within a range of pH 6–8, ADA activity is not significantly affected, in agreement with previously reported observations.[7] Our data show that for this enzyme the lowest working acidity remains at pH 4 (24 hours for 20% conversion).

TABLE 1 Reaction time (hours for 100% conversion) of ADA and AMPDA catalyzed deamination of substrates **3a** and **4a** at different pH

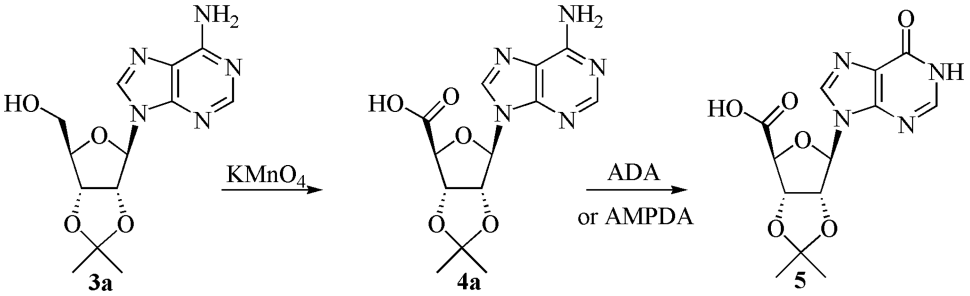
Substrate	ADA pH = 4.0	ADA pH = 6.0	ADA pH = 7.4	ADA pH = 8.0	AMPDA pH = 4.0	AMPDA pH = 6.0	AMPDA pH = 6.5	AMPDA pH = 8.0
3a	24[a]	0.5	0.25	1.0	1.5	0.75	0.75	1.0
4a	24[b]	3.0	7.5	9.0[a]	1.25	1.0	0.75	0.5

[a]20% conversion.
[b]46% conversion.

Additionally, AMPDA showed no appreciable variation of activity within a range going from pH 4.0 to 8.0 (Table 1). Our results suggest that the pH-dependent ionization of enzyme aminoacid or purine basic residues does not appreciably influence enzymatic rates of ADA and AMPDA. On these bases, we have undertaken studies on ADA- and AMPDA-catalyzed deamination of 2′,3′-isopropylidene adenosine 5′-carboxylic acid (**4a**) at different pH values.

ADA- and AMPDA-Catalyzed Deamination of 2′,3′-Isopropylideneadenosine-5′-Carboxylic Acid (4a) at Different pH Values

We have prepared compound (**4a**) from the corresponding adenosine derivative **3a** with excess $KMnO_4$ under described conditions[8] (Scheme 2).



SCHEME 2 Synthesis of 2′,3′-isopropylideneadenosine-5′-carboxylic acid (**4a**) and related enzyme-catalyzed deamination to inosine derivative **5**.

Results from ADA- and AMPDA-catalyzed reactions of deamination of 2′,3′-isopropylideneadenosine-5′-carboxylic acid (**4a**) at different pH values are shown in Table 1. At the optimum pH value 7.4, ADA-catalyzed deamination proceeded quantitatively in 7.5 hours, a reaction rate considerably lower than the corresponding adenosine isopropylidene derivative **3a** (0.25 hour). Interestingly, at slightly higher value (pH 8.0) the reaction rate was further lowered (20% conversion in 9 hours) while it was relatively higher

(3 hours) at pH 6.0. Although pH 4.0 is the lowest acceptable limit for ADA activity, it is interesting to observe that a significant 46% conversion was reached in 24 hours for 2′,3′-isopropylidene adenosine 5′-carboxylic acid (**4a**) (within the same time, 20% conversion of **3a** was obtained). These results clearly indicate that for ADA activity the presence of a negative charge at the critical position 5′ has a detrimental effect on the rate of the enzymatic reaction. Considering the active site described for ADA,[6] the catalytically crucial hydrogen bond between Asp-19 and the 5′-hydroxyl group is substituted by repulsion between Asp-19 and the 5′-carboxylate anion of **4b**. At more acidic pH the equilibrium is shifted towards the neutral form of the 5′-carboxylic group and compound **4a** is transformed by the enzyme, although at a lower rate than **3a**.

Compared to ADA, AMPDA activity with substrate **4a** is not significantly affected by pH values higher than the optimal 6.5 and is only slightly lowered at pH 4. Interestingly, AMPDA is more active than ADA at low pH values, although its activity with physiological substrate 5′-adenylate anion indicates a preference for ionized substrate **4b**.

EXPERIMENTAL

General

Melting points were recorded on SMP3 instrument (Stuart Scientific, UK) and are uncorrected. The NMR spectra were recorded on a Bruker AVANCE 500 spectrometer operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C. The central peak of DMSO-d_6 signals (2.49 ppm for ^{1}H and 39.50 ppm for ^{13}C) was used as internal standard. The chemical shifts are reported in parts per million and coupling constants (J) are given in Hertz. Mass spectra were recorded on Finnigan LCQ-Deca (Termoquest) in ESI negative-ion mode, KV 5.00, 225°C, 15 V. HPLC analyses were carried out on a Jasco HPLC instrument with an Uvidec 100 II UV detector operating at 260 nm using an Alltech Hypersil BDS C18 (4.6 mm × 250 mm). The eluant was phosphate buffer at pH 6.0 containing 20% or 25% acetonitrile for **3a** and **4a**, respectively, at a 1 ml/minute flow rate.

Enzymes were obtained from Sigma, specifically ADA from calf intestinal mucosa (type II, 2.2 units/mg protein) and AMPDA from *Aspergillus species* (0.107 units/mg protein). 2′,3′-*O*-isopropylideneadenosine **3a** was prepared according to a published procedure.[9] 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid (**4a**) was obtained from the corresponding adenosine derivative **3a** with excess $KMnO_4$ under described conditions:[8] mp 244–246°C (dec) (lit.[10] 246–249°C dec); ^{1}H NMR (DMSO-d_6) δ 8.25 (1H, s, H-8), 8.08 (1H, s, H-2), 7.35 (2H, s, NH_2), 6.32 (1H, bs, J = <1.0 Hz, H-1′), 5.52 (1H, dd, J = 2.0, 6.0 Hz, H-3′), 5.45 (1H, d, J = <1.0, 6.0 Hz, H-2′), 4.68 (1H, d, J = 2.0 Hz, H-4′), 1.50 (3H, s, CCH_3), 1.33 (3H, s, CCH_3).

^{13}C NMR (DMSO-d_6) δ 171.17 (COOH), 156.36 (C-6), 152.74 (C-2), 149.58 (C-4), 140.91 (C-8), 119.18 (C-5), 113.18 (CCH$_3$), 89.99 (C-1′), 85.88 (C-3′), 84.22 (C-2′), 83.85 (C-4′), 26.92 (CCH_3), 25.34 (CCH_3). *M/z* 320 (M-1)$^-$, 663 [(M-1)+(M-1)+Na]$^-$.

Enzymatic Deamination of Adenosine Derivatives 3a and 4a

Compounds **3a** and **4a** (20 mg) in phosphate buffer at different pH (50 mM, 10 mL) 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, as previously indicated. The solution was lyophilized and the residue crystallized from methanol as white solids. 2′,3′-*O*-isopropylideneinosine showed physical characteristics in agreement with published data.[11]

2′,3′-*O*-isopropylideneinosine-5′-carboxylic acid [12] (**5**): mp 252–254°C (dec) (lit.[12] 252°C); ^{1}H NMR (DMSO-d_6) δ 8.45 (1H, s, H-8), 8.01 (1H, s, H-2), 6.18 (1H, bs, J = <1.0 Hz, H-1′), 5.21–5.18 (2H, m, H-2′ and H-3′), 4.49 (1H, d, J = 2.0 Hz, H-4′), 1.49 (3H, s, CCH_3), 1.29 (3H, s, CCH_3). ^{13}C NMR (DMSO-d_6) δ 172.14 (COOH), 157.12 (C-6), 148.45 (C-4), 146.01 (C-2), 140.33 (C-8), 124.28 (C-5), 113.16 (CCH$_3$), 90.61 (C-1′), 87.09 (C-3′), 84.66 (C-2′), 84.30 (C-4′), 27.09 (CCH_3), 25.31 (CCH_3). *M/z* 321 (M -1)$^-$, 665 [(M-1)+(M-1)+Na]$^-$.

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