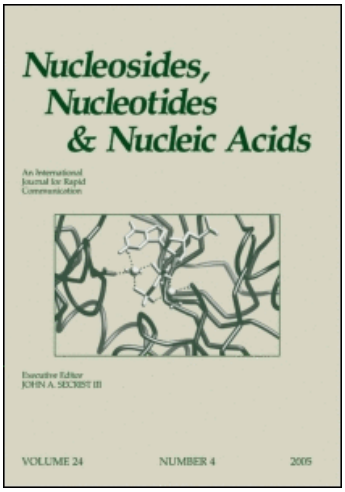

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Activity of Adenosine Deaminase and Adenylate Deaminase on Adenosine and 2', 3'-Isopropylidene Adenosine: Role of the Protecting Group at Different pH Values

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ACTIVITY OF ADENOSINE DEAMINASE AND ADENYLATE DEAMINASE ON ADENOSINE AND 2′, 3′-ISOPROPYLIDENE ADENOSINE: ROLE OF THE PROTECTING GROUP AT DIFFERENT pH VALUES

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□ *The deamination rate of 2′,3′-isopropylidene adenosine catalyzed by adenosine deaminase (ADA) from calf intestine and adenylate deaminase (AMPDA) from* Aspergillus *species has been evaluated and compared with that of the enzymatic reactions of adenosine, to elucidate the influence of the protecting group on ezyme activity.*

Keywords Adenosine; Adenosine deaminase from calf intestine (ciADA); Adenylate deaminase from *Aspergillus* species (AMPDA); 2′,3′-Isopropylidene adenosine

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 adenosine 5′-phosphate (adenylic acid, AMP, **1b**) to inosine (**2a**) and inosine-5′-phosphate (IMP, **2b**), respectively (Scheme 1).[1]

The enzyme-catalyzed deamination is irreversible and both enzymes can be advantageously used as biocatalysts for a fast and nearly quantitative biotransformation of a wide range of structurally modified purine nucleosides.[2] Compared to ADA, AMPDA-catalyzed biotransformations of substrates other than AMP (**1b**) have been reported more recently and AMPDA has immediately shown a greater versatility than ADA.[3] For instance, ADA cannot catalyze the deamination of AMP (**1b**), whereas AMPDA efficiently deaminates adenosine (**1a**). Biocatalytic applications of ADA are

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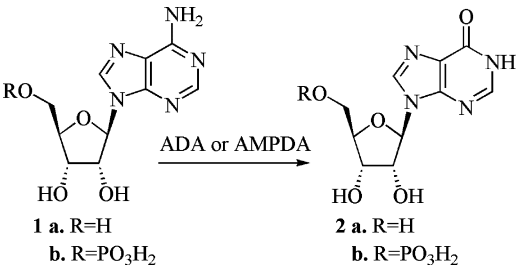


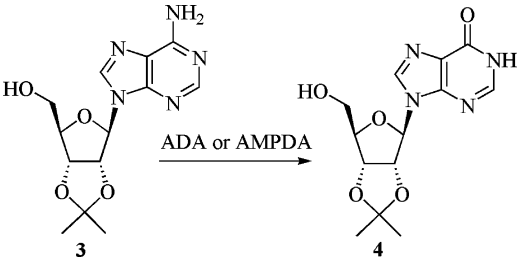
SCHEME 1 ADA and AMPDA-catalyzed deamination of adenosine (**1a**) and adenylic acid (**1b**).

somehow limited by the strict requirement that a hydroxy group has to be present at the 5′-position of the nucleoside that is deaminated,[4] whereas some flexibility is allowed at the 2′,3′-positions as shown for the efficient deamination of 2′,3′-isopropylideneadenosine (**3**) to the corresponding inosine derivative **4** (Scheme 2).[4]

For this reason, we have used the 2′,3′-isopropylidene adenosine analogues to study the influence of modifications at other positions of ribose on the activity of both deaminases.[5] In a recent paper,[6] we have reported that ADA- and AMPDA-catalyzed deamination of 2′,3′-isopropylidene adenosine-5′-carboxylic acid is influenced by the substrate ionization. In this context, we observed that activity of AMPDA on 2′,3′-isopropylidene adenosine (**3**) is not significantly influenced within the pH range 4–8, whereas ADA activity is considerably lower at pH 4.0. This drop of activity could be caused by the presence of the bulk and apolar protecting group and its interaction with aminoacid residues in the active site, that at this pH value, should be ionized at an extent different from that existing in the 6–8 pH interval. It became, therefore, interesting to compare enzymatic deaminations of 2′,3′-isopropylidene adenosine (**3**) with that on the natural, unprotected substrate adenosine (**1a**).

STRUCTURAL INFORMATION ON ADA AND AMPDA

For our studies on enzymatic deamination of adenosine (**1a**) and 2′,3′-isopropylidene adenosine (**3**), we have used commercial enzymes. AMPDA



SCHEME 2 ADA/AMPDA-catalyzed deamination of 2′,3′-isopropylidene adenosine (**3**) to inosine derivative **4**.

was from *Aspergillus* species and a clear picture of the active site of this enzyme is not available from X-ray studies. Only recently, the structure of a recombinant adenylate deaminase (named AMPD) expressed by the *Arabidopsis thaliana* embryonic factor 1 (FAC1) has been reported.[7] The commercially available adenosine deaminase from calf intestine (ciADA) is 85% homologous to murine adenosine deaminase (mADA),[8] for which a detailed catalytic mechanism was clarified nearly 15 years ago through X-ray studies of the enzyme complexed with inhibitors.[9–12] The structure of ciADA complexed with the inhibitor 6-hydroxy-1,6-dihydropurine riboside has been recently resolved and the similarity with mADA, including active site, has been confirmed.[13] Additional studies have shown a complex mechanism for ciADA recognition of inhibitors that involves open/closed conformations and hydrophobic or hydrophilic subsites at physiological pH.[14]

RESULTS AND DISCUSSION

Enzymatic reactions on 2′,3′-isopropylidene adenosine (**3**) and adenosine (**1a**) were carried out using the commercially available ciADA or AMPDA in buffer solutions at pH ranging from 4.0 to 8.0 containing 3% DMSO. The presence of the cosolvent was required to improve solubility of compound **3** but did not alter significantly the enzyme activity[15] and was maintained also for the deamination of adenosine (**1a**). The deamination progress of enzymatic reactions was monitored by HPLC.

Activity of ADA and AMPDA on Adenosine (1a) and 2′,3′-Isopropylidene Adenosine (3) at Different pH

Initially we followed the nearly quantitative transformation of substrates (95–98% of isolated products). In the presence of AMPDA this result can be obtained within 1 hour independently from the pH value (15 minutes for **1a** and 1 hour for **3**). ADA catalyzed the transformation of both substrates at an optimum pH interval 6–8 within 40 minutes and 1 hour, with no significant difference between **1a** and **3**.

We calculated the initial rate (V_0) from the initial linear portion of the kinetics, which were carried out at 25°C. The values for V_0, expressed as μmol product/min/mg of enzyme are collected in Tables 1 and 2.

TABLE 1 Initial rates (μmol product/min/mg enzyme) of deamination of substrates **1a** and **3** catalyzed by AMPDA from *Aspergillus* species at different pH values

Substrate	**AMPDA** pH = 4.0	**AMPDA** pH = 5.0	**AMPDA** pH = 6.0	**AMPDA** pH = 7.0	**AMPDA** pH = 8.0
1a	0.33	0.34	0.37	0.36	1.57
3	0.04	0.09	0.09	0.07	0.09

TABLE 2 Initial rate (μmol product/min/mg enzyme) of deamination of substrates **1a** and **3** catalyzed by calf intestinal ADA (ciADA) at different pH values

Substrate	**ADA** pH = 4.0	**ADA** pH = 5.0	**ADA** pH = 6.0	**ADA** pH = 7.0	**ADA** pH = 8.0
1a	0.2	0.5	1.0	1.0	1.1
3	0.06	0.5	0.6	0.9	0.8

The activity of AMPDA with adenosine (**1a**) is not influenced by variation of pH values between 4 and 7 and the highest value of V_0 observed at pH 8 (Table 1). Low V_0 values for compound **3** can be explained by interaction of apolar and relatively bulky isopropylidene group with charged lysine residues that have been reported in the AMPD catalytic site.[7] The repulsion may disfavor the proper nucleoside accomodation into the catalytic site and this becomes more evident at pH 4.

Results collected in Table 2 show that ciADA activity on adenosine (**1a**) at pH values between 6.0 and 8.0 remains constant and is lower at pH < 6. The activity is not influenced by the presence of the isopropylidene moiety in compound **3** within the pH 5–8 range. This result clearly shows that the 2′,3′-isopropylidene group can be advantageously used as scaffold to study ciADA-catalyzed biotransformations of adenosine analogues modified in ribose positions other than 2′ and 3′.[5]

At pH 4, reactions are slower for both substrates, but the difference is more relevant for compound **3**, suggesting a specific interference of the isopropylidene group with residues in ADA active site. Here, four acidic amino acids interact with the nucleoside and this involves syn/anti side chain conformations.[5]

CONCLUSIONS

Evaluation of rates of adenosine deaminase from calf intestine (ciADA) and adenylate deaminase from *Aspergillus* species (AMPDA) on adenosine (**1a**) and 2′,3′-isopropylidene adenosine (**3**) at varying pH values has evidenced a different mode of action of the two enzymes, especially on compound **3**. The presence of the isopropylidene group has a marked influence on AMPDA activity, whereas for ADA this is especially evident only at pH 4. Our results can be explained by different amino acid composition in the active site of the two enzymes.

MATERIALS AND METHODS

ADA from calf intestinal mucosa (type II, 2.2 units/mg protein) and AMPDA from *Aspergillus* species (0.107 units/mg protein) were available from Sigma (USA). Adenosine (**1a**) and 2′,3′-*O*-isopropylidene adenosine

(**3**) were purchased from Fluka (Switzerland). 2′,3′-*O*-isopropylidene inosine (**4**) for HPLC analysis was prepared from 2′,3′-*O*-isopropylidene adenosine (**3**) using ADA-catalyzed deamination as described in reference 15.

Enzymatic Deamination of Adenosine (1a) and 2′,3′-Isopropylidene Adenosine (3)

Compounds **1a** and **3** (20 mg) in buffer solutions at different pH (50 mM, 10 mL) containing 3% DMSO were treated with ADA (2 mg) or AMPDA (20 mg) at 25°C. Ten-microliter samples were taken from the reaction, diluted with mobile phase (0.3 mL), and analyzed by a Jasco HPLC instrument with an Uvidec 100 II UV detector operating at 260 nm using an Alltech Hypersil BDS C18 column (4.6 mm × 250 mm) and 1-mL/min flow rate. The eluant for HPLC analysis was a phosphate buffer at pH 6.0 containing 5% acetonitrile for the enzymatic reaction of **1a** (retention times: **1a** 12.2 minutes, **1b** 4.8 minutes) and 25% acetonitrile for reaction of **3** (retention times: **3**, 5.2 minutes; **4**, 2.8 minutes).

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