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Stereoselective adenylate deaminase (5′-adenylic acid deaminase, AMPDA)-catalyzed deamination of 5′-alkyl substituted adenosines: a comparison with the action of adenosine deaminase (ADA)

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Abstract—The enzymes adenylate deaminase (AMPDA) and adenosine deaminase (ADA) are able to catalyze the stereoselective hydrolytic deamination of (5′*R*,*S*)-methyl-2′,3′-isopropylidene adenosine, but the 5′-butyl analog is a substrate only for AMPDA, which stereospecifically converts the (5′*S*)-isomer to the corresponding inosine derivative.

1. 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 adenylic acid (adenosine 5′-phosphate, AMP) **1b** to the corresponding inosines **2a**,**b** (Fig. 1).

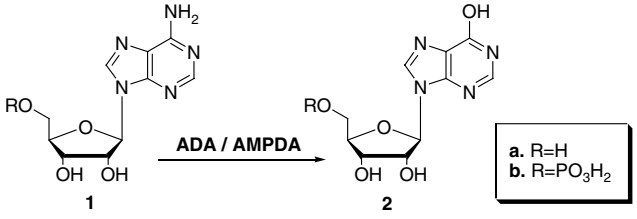

Figure 1.

Recently, we have reported that both enzymes are able to deaminate also 2′,3′-isopropylidene adenosine **3a** (Fig. 2), thus showing that some steric hindrance at the 2′,3′-positions is tolerated well by both enzymes.[1]

Taking advantage of the above observation, we prepared (5′*R*,*S*)-5′-methyl-2′,3′-isopropylidene adenosine **3b** and found that the deamination catalyzed by ADA proceeded stereoselectively and that the (5′*S*)-isomer

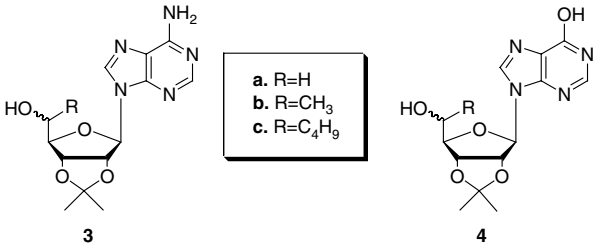

Figure 2.

was preferentially converted to the corresponding inosine **4b**[2] (Fig. 3).

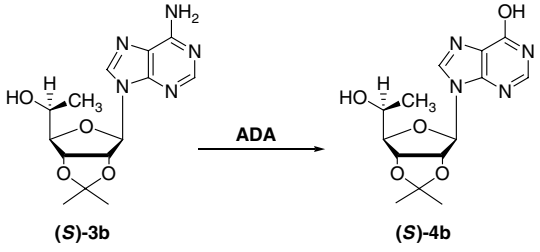

Figure 3.

We have now investigated the action of AMPDA on (5′*R*,*S*)-5′-methyl-2′,3′-isopropylidene adenosine **3b** and extended the study to another 5′-alkyl substituted derivative, namely (5′*R*,*S*)-5′-butyl-adenosine **3c** (Fig. 2). The AMPDA-catalyzed deamination to the corresponding inosine **4c** was also compared to the same reaction performed in the presence of ADA.

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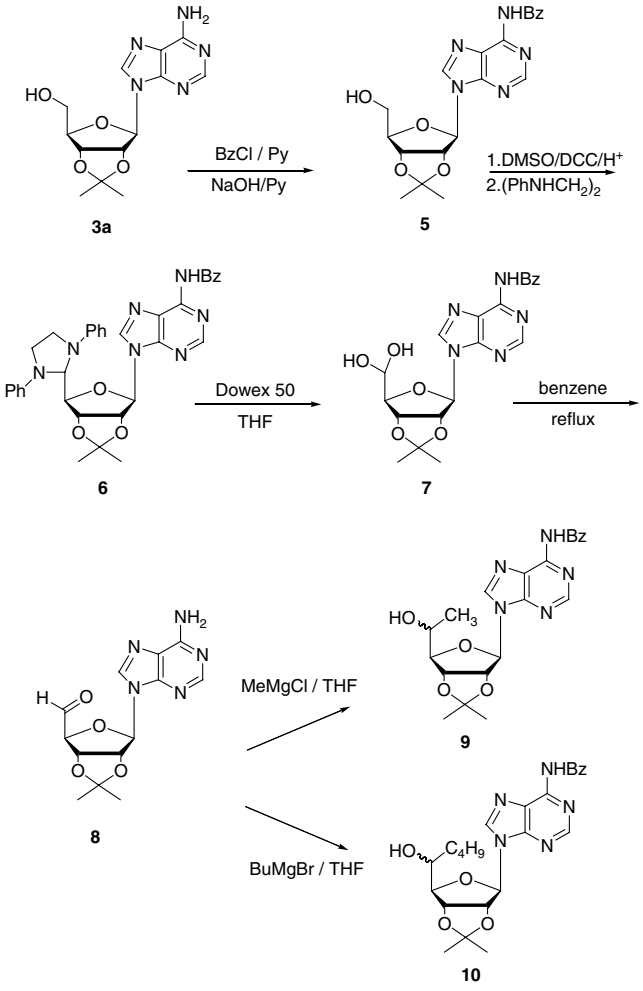
2. Results and discussion

2.1. Synthesis of (5′*R*,*S*)-5′-methyl- and (5′*R*,*S*)-5′-butyl-2′,3′-isopropylidene adenosine, 3b and 3c

For the synthesis of 5′-*C*-alkyl-adenosines **3b**,**c**, we required the corresponding N^7-protected 5′-aldehyde that can be obtained by oxidation of the N^7-benzoyl derivative **5**, in turn prepared from 2′,3′-isopropylidene adenosine **3a** by an in situ benzoylation–debenzoylation procedure.[3] This method has been described nearly four decades ago and we found that it is still the most reliable procedure for the preparation of the N^7-benzoyl derivative **5**[4] since final yields are superior or comparable to other methods.[5,6] For the oxidation of compound **5** to the aldehyde **8** the well established method described by Moffatt is preferable.[7] It should be briefly pointed out that this oxidation procedure is effected with dicyclohexylcarbodiimide–DMSO in the presence of *N*,*N*′-diphenylethylenediammine. This allows the isolation of the imidazoline **6**[8] that is further hydrolyzed to the corresponding aldehyde by treatment with a resin in the acid form. The aldehyde is isolated in the hydrate form **7** and only azeotropic distillation with benzene (or toluene) may remove water and afford the carbonyl form of the aldehyde **8**. However, the water removal generally causes extensive decomposition and yields of this critical step never exceeded 30–40%. The reaction of the aldehyde **8** with excess MeMgCl or BuMgBr allowed the preparation of the required (5′*R*,*S*)-5′-methyl and (5′*R*,*S*)-5′-butyl derivatives **9** and **10** (20% and 32% yield from compound **5**) (Scheme 1). Hydrolysis of the N^7-benzoyl group in compounds **9** and **10** to the required (5′*R*,*S*)-5′-methyl and butyl derivatives **3b** and **3c** may be quantitatively achieved by treatment with methanol solutions of aqueous ammonia.



Scheme 1.

2.2. AMPDA-catalyzed deamination of (5′*R*,*S*)-5′-methyl- and (5′*R*,*S*)-5′-butyl-2′,3′-isopropylidene adenosine, 3b and 3c

The 3:1 diastereomeric mixture of synthetic (5′*R*,*S*)-5′-methyl-2′,3′-isopropylidene adenosine **3b** was determined by HPLC and 500 MHz ^{1}H NMR analysis and the (5′*R*)-**3b** was the major isomer.[2] The AMPDA-catalyzed deamination of (5′*R*,*S*)-**3b** was carried out in a phosphate buffer at pH 6.5 and proceeded to stereoselectively transform in 10 min the minor isomer into the (5′*S*)-inosine derivative **4b**.[9] The unreacted (5′*R*)-adenosine **3b** required an additional 3 h to be deaminated to the corresponding (5′*R*)-inosine. With the substrate **3b** AMPDA behaves similarly to ADA,[2] taking into account the difference in rate to reach complete transformation of the (5′*S*)-isomer (10 min for AMPDA vs 1 h for ADA). A fast reaction of AMPDA took place as well with (5′*R*,*S*)-butyl-adenosine **3c** that had been synthesized as a mixture of two diastereomers in a ratio 4:1 (as established by HPLC and NMR analysis).[10] Interestingly, no reaction occurred with ADA, indicating that steric hindrance at position 5′ is not tolerated by the enzyme. On the contrary, AMPDA-catalyzed reaction completely transformed in 25 min, the less abundant isomer of the diasteromeric mixture (5′*R*,*S*)-5′-butyl-adenosine **3c** into the corresponding inosine **4c**.[11] The major isomer could be completely consumed after 4 h and the unreacted nucleoside was also used to establish the stereochemical outcome of the enzymatic reaction by the ^{1}H NMR method adopted to determine the absolute configurations of secondary alcohols.[12] Our results indicate that the unreacted diastereomer is (5′*R*)-5′-butyl-adenosine (5′*R*)-**3c** and that, consequently, AMPDA is able to catalyze the preferential deamination of (5′*S*)-5′-butyl-adenosine derivative (5′*S*)-**3c**. We have therefore established that AMPDA is able to catalyze the preferential deamination of (5′*S*)-5′-methyl and 5′-butyl-adenosine derivatives **3b**,**c** to the corresponding inosines **4b**,**c** (Fig. 4). The results so far obtained for AMPDA and ADA indicate that stereochemically pure (5′*R*)-5′-methyl-2′,3′-isopropylidene adenosine **3b** and the corresponding (5′*S*)-inosine **4b** may be prepared by the action of ADA and AMPDA. Whereas ADA is not suitable for the transformation of (5′*R*,*S*)-5′-butyl-2′,3′-isopropylidene adenosine **3c**, AMPDA-catalyzed deamination allows the preparation of stereochemically pure (5′*R*)-5′-butyl-2′,3′-isopropylidene adenosine (5′*R*)-

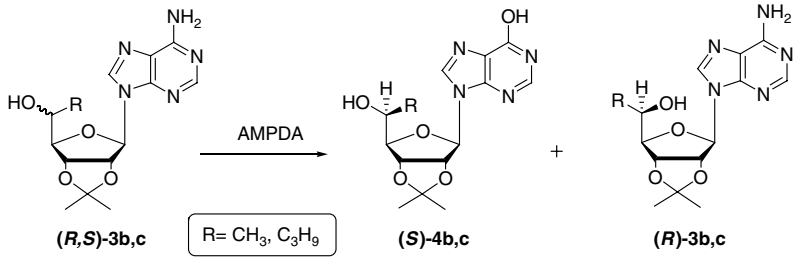


Figure 4.

3c and the corresponding (5′*S*)-5′-butyl-2′,3′-isopropylidene inosine (5′*S*)-**4c**.

3. Conclusions

Our results show that ADA and AMPDA are able to catalyze the deamination of a 2′,3′-isopropylidene adenosine in a stereoselective fashion when a methyl group is present at the 5′-position. For both enzymes, the (5′*S*)-isomer is preferentially deaminated to the corresponding inosine derivative and the unreacted (5′*R*)-adenosine may be recovered. This result may constitute an additional example of the application of two important biocatalysts in the nucleoside area for chemo-enzymatic preparation of modified purine nucleosides. In contrast to AMPDA, ADA seems more sensitive to the steric hindrance at the 5′ position, since this enzyme is unable to catalyze the deamination of (5′*R*,*S*)-5′-butyl derivative **3c**. However, the high reaction rate observed for AMPDA-catalyzed conversion of 5′-butyl derivative **3c** raises another interesting question. In fact, at the pH required for the enzymatic transformation of the physiological substrate of AMPDA, that is AMP, the phosphate at the position 5′ should be in the anionic form and AMPDA should accept ionic or polar substituents at that position. This is in contrast with the results obtained with the 5′-butyl derivative **3c** and other nucleosides bearing apolar substituents at the 5′-position.[1] The results obtained so far highlight also a main difference in the action of the AMPDA, compared to the other deaminating enzyme ADA.

Acknowledgements

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References and notes

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4. Compound **5**: ^{1}H NMR ($CDCl_3$) δ 9.29 (1H, s, NH), 8.74 (1H, s, H-8), 8.09 (1H, s, H-2), 8.00 (2H, d, $J = 7.7$, *o*-PhH), 7.58 (1H, dd, $J = 7.7$, 7.7 Hz, *m*-PhH), 7.42 (2H, t, $J = 7.7$ Hz, *p*-PhH), 5.95 (1H, d, $J = 5.6$ Hz, H-1′), 5.21 (1H, dd, $J = 5.6$, 5.6 Hz, H-2′), 5.09 (1H, dd, $J = 1.4$, 5.6 Hz, H-3′), 4.55 (1H, ddd, $J = 1.4$, 1.4, 2.1 Hz, H-4′), 3.98 (1H, dd, $J = 1.4$, 12.6 Hz, H-5′a), 3.80 (1H, dd, $J = 2.1$, 12.6 Hz, H-5′b), 1.63 (3H, s, CCH_3), 1.36 (3H, s, CCH_3).
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8. The most significant signals for compound **6** are: 8.70 (1H, s, H-8), 7.86 (1H, s, H-2), 6.16 (1H, d, $J = 2.1$ Hz, H-1′), 5.72 (1H, d, $J = 2.8$ Hz, H-5′), 5.20 (1H, dd, $J = 6.3$, 6.3 Hz, H-3′), 5.16 (1H, dd, $J = 2.1$, 6.3 Hz, H-2′), 4.62 (1H, dd, $J = 2.8$, 6.3 Hz, H-4′) 1.47 (3H, s, CCH_3), 1.31 (3H, s, CCH_3).
9. Compounds **3b**,**c** (0.02 g) in phosphate buffer (50 mM, 6 mL, pH 6.5) with 3% DMSO were treated with AMPDA (from *Aspergillus species*, Sigma, 0.107 units/mg solid, 20 mg) for the time indicated in the text. The progress of reactions was monitored by HPLC (**3b**: phosphate buffer pH 6.0/CH_3 CN, 8:2; **3c**: phosphate buffer pH 6.0/CH_3 CN, 7:3). When the reaction was complete, the solution was lyophilized to afford inosines **4b**,**c**.
10. **3c**, major diastereomer: HPLC $t_R = 18.5$ min (phosphate buffer pH 6.0/CH_3CN, 7:3); ^{1}H NMR (CD_3OD) δ 8.28 (1H, s, H-8), 8.19 (1H, s, H-2), 6.11 (1H, d, $J = 3.4$ Hz, H-1′), 5.25 (1H, dd, $J = 3.4$, 6.0 Hz, H-2′), 5.07 (1H, dd, $J = 2.7$, 6.0 Hz, H-3′), 4.15 (1H, dd, $J = 2.7$, 3.4 Hz, H-4′), 3.74 (1H, dt, $J = 3.4$, 4.0 Hz, H-5′), 1.60 (3H, s, CCH_3), 1.54–1.41 (m, 2H, CH_2), 1.35–1.23 (m, 4H, $CH_2 \times 2$), 1.38 (3H, s, CCH_3), 0.87 (3H, t, $J = 6.0$ Hz, CH_3); minor diastereomer: HPLC $t_R = 17.2$ min (phosphate buffer pH 6.0/CH_3 CN, 7: 3); ^{1}H NMR (CD_3OD) δ 8.36 (1H, s, H-8), 8.18 (1H, s, H-2), 6.13 (1H, d, $J = 3.4$ Hz, H-1′), 5.15 (1H, dd, $J = 3.4$, 6.0 Hz, H-2′), 5.00 (1H, dd, $J = 2.7$, 6.0 Hz, H-3′), 4.26 (1H, dd, $J = 2.7$, 3.4 Hz, H-4′), 3.74 (1H, dt, $J = 3.4$, 4.0 Hz, H-5′), 1.61 (3H, s, CCH_3), 1.54–1.41 (m, 2H, CH_2), 1.35–1.23 (m, 4H, $CH_2 \times 2$), 1.37 (3H, s, CCH_3), 0.90 (3H, t, $J = 6.0$ Hz, CH_3).
11. Compound (*S*)-**4c** HPLC $t_R = 10.1$ min (phosphate buffer pH 6.0/CH_3CN, 7:3); ^{1}H NMR (CD_3OD) δ 8.21 (1H, s,

H-8), 7.92 (1H, s, H-2), 5.87 (1H, d, $J = 3.4$ Hz, H-1′), 5.10 (1H, dd, $J = 3.4$, 6.0 Hz, H-2′), 5.02 (1H, dd, $J = 2.7$, 6.0 Hz, H-3′), 4.37 (1H, dd, $J = 2.7$, 3.4 Hz, H-4′), 3.75 (1H, dt, $J = 3.4$, 4.0 Hz, H-5′), 1.61 (3H, s, CCH_3), 1.59–1.48 (m, 2H, CH_2), 1.39–1.24 (m, 4H, $CH_2 \times 2$), 1.36 (3H, s, CCH_3), 0.86 (3H, t, $J = 6.0$ Hz, CH_3).

12. The configuration of the unreacted (*R*)-**3c** was assigned converting the compound into the N^6-benzoate **10** and preparing the corresponding (*S*)- and (*R*)-MTPA esters, that were analyzed by ^{1}H NMR according to the Mosher's modified method (Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096). The $\Delta\delta(\delta_S - \delta_R)$ values of the protons of the ribose moiety ($\Delta\delta < 0$) and the 5′-butyl chain protons ($\Delta\delta > 0$) were in agreement with the proposed *R*-configuration.