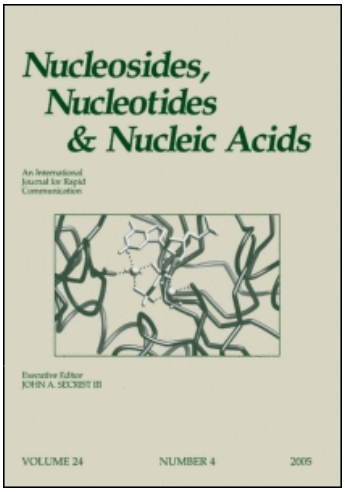



**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# MECHANISM OF INACTIVATION OF *S*-ADENOSYL-L-HOMOCYSTEINE HYDROLASE BY 5'-DEOXY-5'-*S*-ALLENYLTHIOADENOSINE AND 5'-DEOXY-5'-*S*-PROPYNYLTHIOADENOSINE

G. Guillerm[a]; D. Guillerm[a]; C. Witkowski-Vandenplas[a]
[a] Université de Reims, Reims, Cédex 2, France

Online publication date: 31 March 2001

**To cite this Article** Guillerm, G. , Guillerm, D. and Witkowski-Vandenplas, C.(2001) 'MECHANISM OF INACTIVATION OF *S*-ADENOSYL-L-HOMOCYSTEINE HYDROLASE BY 5'-DEOXY-5'-*S*-ALLENYLTHIOADENOSINE AND 5'-DEOXY-5'-*S*-PROPYNYLTHIOADENOSINE', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 685 — 688
**To link to this Article: DOI:** 10.1081/NCN-100002351
**URL:** http://dx.doi.org/10.1081/NCN-100002351

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 685–688 (2001)

# MECHANISM OF INACTIVATION OF *S*-ADENOSYL-L-HOMOCYSTEINE HYDROLASE BY 5′-DEOXY-5′-*S*-ALLENYLTHIOADENOSINE AND 5′-DEOXY-5′-*S*-PROPYNYLTHIOADENOSINE

**G. Guillerm,* D. Guillerm, and C. Witkowski-Vandenplas**

Laboratoire des Réactions Sélectives et Applications - UMR 6519,
Université de Reims, BP 1039, 51687 Reims Cédex 2, France



## ABSTRACT

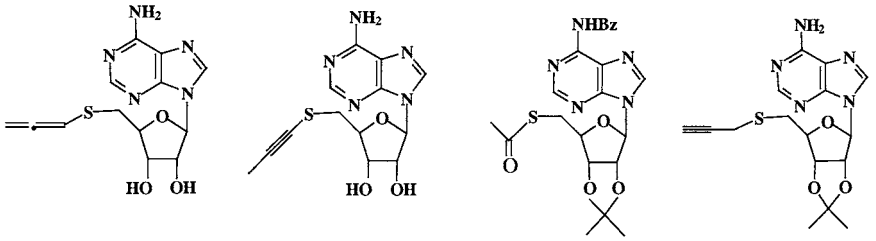
5′-Deoxy-5′-*S*-allenylthioadenosine **1** and 5′-deoxy-5′-*S*- propnylthioadenosine **2**, derived from adenosine, were prepared. **1** and **2** caused irreversible inactivation of AdoHcy hydrolase. ESI mass spectra analysis of the inactivated enzyme demonstrated that **1** and **2** were type II "mechanism-based" inhibitors.

Inhibition of the cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase results in intracellular accumulation of AdoHcy leading to a feed back inhibition of *S*-adenosylmethionine (AdoMet) dependent methylation reaction (i.e. viral mRNA methylation which are essential for viral replication). Therefore, AdoHcy hydrolase has become an attractive target for developing antiviral agents (1).

A number of inhibitors which function as substrates for the "3′-oxidative activity" of the enzyme and irreversibly keep AdoHcy hydrolase in its inactive NADH form has been identified (2). A second type of mechanism-based inhibitors (covalent) utilized the same 3′-oxidative activity of the enzyme (3) or its "hydrolytic activity" to generate an electrophilic site on the inhibitor which can then bind an active site nucleophile (4).

Recent results from our laboratory (5) led us to hypothezise that a new series of thionucleosides **1** and **2** (Fig. 1) might serve as novel type of irreversible

*Corresponding author.




1 2 3 4

*Figure 1.*



inhibitors of AdoHcy hydrolase. A direct method for the preparation of **1** and **2** has been developed starting from 6-N-benzoyl-2′,3′-isopropylidene-5′-deoxy-5′-*S*-acetylthioadenosine **3** readily available from adenosine (6). Treatment of **3** with sodium methoxide and propargyl bromide gave the 5′-deoxy-5′-*S*-propargylthioadenosine **4** (7). Under controled based-catalysed isomerisation with sodium tert-butoxide in tert-butanol, **4** was quantitatively converted into 5′-deoxy-5′-*S*-allenylthioadenosine **1** (8) (55%) or 5′-deoxy-5′-*S*-propynylthioadenosine **2** (9) (58%).

Pure **1** and **2** obtained this way were assayed for their inhibitory activity on recombinant human placental AdoHcy hydrolase (10a), purified to homogeneity, as already described (10b). AdoHcy hydrolase activity was assayed in the direction of AdoHcy synthesis using (8-$^{14}$C) adenosine (11).

Incubation of **1** and **2** with AdoHcy hydrolase resulted in time and concentration dependent irreversible inactivation of the enzyme. The Kitz and Wilson method (12) was used for kinetic constants determination. The values obtained ($\mathbf{1}: \mathrm{Ki} = 16 \pm 1\ \mu\mathrm{M}, \mathrm{K_{inact}} = 0.12\ \mathrm{min}^{-1}$ ; $\mathbf{2}: \mathrm{Ki} = 15 \pm 1\ \mu\mathrm{M}, \mathrm{K_{inact}} = 0.062\ \mathrm{min}^{-1}$) were in the range of those found for other inactivators recently described (4). The inactivation was concomitant with the formation of adenosine or AdoHcy from **1** and **2** when the enzyme was incubated in the absence or in the presence of homocysteine. These results indicated that the inhibition process was competing with substrate activity. The partition ratio (90/10) was in favor of inhibition.

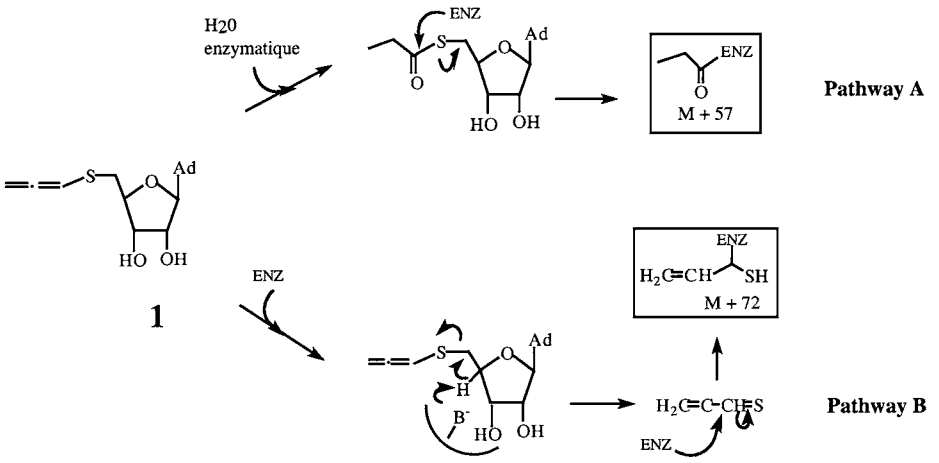
$NAD^+$/NADH content of the enzyme was not affected during the inactivation process with **1** or **2**.

The mechanism of inactivation was further investigated. Scheme 1 represents two plausible mechanisms by which **1** and **2** inactivate AdoHcy hydrolase. In pathway **A**, addition of the enzyme's sequested water on the S-allenyl group of **1** or S-propynyl group of **2** might generate the formation of the corresponding quite reactive thioester. Attak by amino acid functionalities of the active site might cause type II (covalent binding) inhibition of the enzyme.

A process involving a $\beta$-elimination, without prior oxidation at C-3′ (pathway B), yielding an allenyl or a propynyl mercaptan group should not be excluded. Under their tautomeric thioaldehyde forms these intermediates are highly reactive acylating agents which might irreversibly acylate nucleophilic residues involved in the catalytic process of AdoHcy hydrolase.



Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016




***Scheme 1.***

These two possibilities have been examined with compound **1**, using homogenous purified human placental recombinant AdoHcy hydrolase. ESI-MS analysis of AdoHcy hydrolase inactivated by **1** supports the mechanistic proposal described (pathway **A**). After incubation with **1**, each inactivated enzyme subunit was detected at a molecular weight of $47659.6 \pm 3.0$ Da (native enzyme $47604.8 \pm 1.5$ Da) showing that inhibition of AdoHcy hydrolase is accompanied by a covalent linkage of $54.8 \pm 4.5$ Da. Additional MALDI-TOF experiments concerning the Endo-LysC proteolytic cleavage fragments of inactivated AdoHcy hydrolase, located the labelling on the C terminal peptide of AdoHcy hydrolase subunit (20 last amino acids of the protein). Work is in progress using Nano ESI-$MS^n$ experiments to identify the amino acid residue involved in this inactivation process.

## ACKNOWLEDGMENTS

The authors express their thanks to Pr. Michael S.Hershfield and Dr. Franscisco Arredondo (Duke University School of Medecine) for providing sample of *E. Coli* transformed with a plasmid encoding for human placental Ado Hcy hydrolase, Dr Helene Rogniaux and Dr Alain Van Dorsselaer (University Louis Pasteur, France) for carrying out the mass spectra analysis in this study.

## REFERENCES

1. Liu, S.; Wolfe, M. S.; Borchardt, R. T. *Antiviral Research,* **1992**, *19*, 247–265.
2. Yuan, C. S.; Liu, S.; Wnuk, S. F.; Robins, M. J. ; Borchardt, R. T. in *Advances in Antiviral Drug Design*; De Clercq, E., Ed, JAI Press: Greenwich, **1996**, *2*, 41–88.
3. Parry, R. J ; Muscate, A. ; Askonas, L. J. *Biochemistry*, **1991**, *30*, 9988–9997.

4. Wnuk, S. F.; Mao, Y.; Yuan, C. S.; Borchardt, R. T.; Andrei, G.; Balzarini, J.; De Clercq, E. ; Robins, M. J. *J. Med. Chem.*, **1998**, *41*, 3078–3083. - Yuan, C. S.; Wnuk, S. F.; Robins, M. J. J. *Biol. Chem.*, **1998**, *273*, 18191–18197.
5. Muzard, M.; Vandenplas, C.; Guillerm, D.; Guillerm, G. *J. Enz. Inh.*, **1998**, *13*, 443–456.
6. Houston Jr, M. E.; Vander Jagt, D. L.; Honek, J. F. *Bioorg. Med. Chem. Lett.* **1991**, *11*, 623–628.
7. **4** : mp 87-89°C - HRMS (DCI/$NH_3$) 322.084 $(MH)^+$, calc. 321.299 -$[\alpha]_D^{20}$ −20.5° (c 0,23; $CHCl_3$/MeOH: 4/1). I.R.(KBr) 2170, 3281 $cm^{-1}$ - $^1$H NMR, $CDCl_3$, $\varepsilon$ $CD_3OD$ $\delta$ (ppm), J(Hz): 2.27 (t, 1H, C≡C<u>H</u>, J 2.67); 3.01 (dd, 1H, H5′$\alpha$, J 14.11, 6.1); 3.12 (dd, 1H, H5′$\beta$, J 14 .11, 4.57); 3.28 (d, 2H, C<u>H$_2$</u>C≡C, 2.67); 4.21 (m, 1H, H-4′); 4.29 (dd, 1H, H-3′, J 5.34, 5,72); 4.46 (dd, 1H, H-2′, J 5.34, 3.81); 5.81 (d, 1H, H-1′, J 3.81); 8.09 and 8.21 (2 s, 2H, H-2 and H-8) -$^{13}$C NMR, $CDCl_3$ $\varepsilon$ $CD_3OD$, $\delta$ (ppm) : 20.0 (<u>C</u>H$_2$C≡C); 33.3 (C-5′); 71.6(<u>C</u>≡CH); 72.3 (C-4′); 74.2 (C-3′); 80.0 (C≡<u>C</u>H); 83.9 (C-2′); 89.6 (C-1′); 120.0 (C-6); 139.3 and 152.8 (C-2 and C-8); 149.1 and 155.9 (C-4 and C-5).
8. **1** mp 90-92°C - HRMS (DCI/$NH_3$) 322.098 $(MH)^+$, calc.321.299 -$[\alpha]_D^{20}$ −18° (c 0.18; $CHCl_3$/MeOH: 4/1) - I.R.(KBr) 1944 $cm^{-1}$ (weak) - $^1$H NMR, $CDCl_3$, $\varepsilon$ $CD_3OD$ $\delta$ (ppm), J(Hz): 2.93 (dd, 1H, H-5′$\alpha$, J 14.5, 4.96); 3.05 (dd, 1H, H-5′$\beta$, J 14.5, 4.96); 4.21 (m, 1H, H-4′); 4.22 (dd, 1H, H-3′, J 6.1, 4.95); 4.49 (dd, 1H, H-2′, J 4.95, 4,2); 4.94 (d, 2H, C<u>H</u>2=C=CHS, J 6,48); 5.70 (dd, 1H, CH2=C=C<u>H</u>S, J 6.48); 5.86 (d, 1H, H-1′, J 4.2); 8.05 and 8.15 (2 s, 2H, H-2 and H-8) -$^{13}$C NMR, $CDCl_3$ $\varepsilon$ $CD_3OD$, $\delta$ (ppm) : 34.2 (C-5′); 72.1 (C-4′); 74.2 (C-3′); 81.0 (<u>C</u>H2=C=CHS); 83.0 (C-2′); 87.0 (CH2=C=*C*HS); 89.3 (C-1′); 119.4 (C-6); 139.1 and 152.2 (C-2 and C-8); 148.7 and 155.3 (C-4 and C-5); 205.6 (CH2=<u>C</u>=CHS).
9. **2** mp 95-97°C - HRMS (DCI/$NH_3$) 322.306 $(MH)^+$ , calc.321.299 - $[\alpha]_D^{20}$ +22.5 (c 0.020; $CHCl_3$/MeOH: 4/1) - $^1$H NMR, $CDCl_3$, $\varepsilon$ $CD_3OD$ $\delta$ (ppm), J (Hz): 1.87 (s, 3H, C≡CC<u>H$_3$</u>) ; 3.02 (dd, 1H, H-5′$\alpha$ J 13,72, 6,5); 3.13 (dd, 1H, H-5′$\beta$, J 13.72, 6,1); 4.31 (m, 1H, H-4′); 4.32 (dd, 1H, H-3′, J 2,27, 5,2) 4.39 (dd, 1H, H-2′, J 5.2, 3,6); 6.04 (d, 1H, H-1′, J 3.6); 8.19 and 8.29 (2 s, 2H, H-2 and H-8) - $^{13}$C NMR, $CDCl_3$, $\varepsilon$ $CD_3OD$, $\delta$ (ppm): 4.4 (C≡C<u>C</u>H$_3$,) ; 39.2 (C-5′); 68 (<u>C</u>≡CCH$_3$) ; 73.8 (C-4′); 74.8 (C-3′); 84.8 (C-2′); 89.0 (C≡<u>C</u>CH$_3$); 90 (C-1′); 119.4 (C-6); 141.2 and 153.8 (C-2 and C-8); 150 and 157.3 (C-4 and C-5).
10. (a) Coulter-Karis, D. E.; Hershfield, M. S. *Ann. Hum. Genet.* **1989**, *53*, 169–175. (b) Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. *J. Biol. Chem.* **1993**, *268*, 17030–17037.
11. Della Ragione, F.; Pegg, A. E. *Biochem. J.* **1983**, 210–429.
12. Kitz, K.; Wilson, I. B. *J. Biol. Chem. 237*, **1962**, 3245–3249.






MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

# Request Permission or Order Reprints Instantly!