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

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

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ABSTRACT

5'-Deoxy-5'-*S*-allenylthioadenosine **1** and 5'-deoxy-5'-*S*-propynylthioadenosine **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 hypothesize that a new series of thionucleosides **1** and **2** (Fig. 1) might serve as novel type of irreversible

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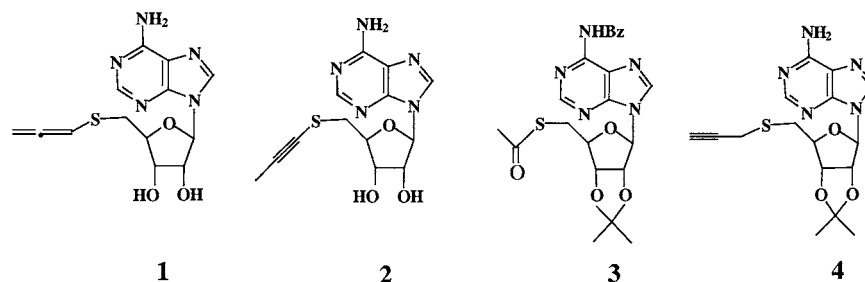


Figure 1.

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 controlled 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 (8-¹⁴C) adenosine (11).

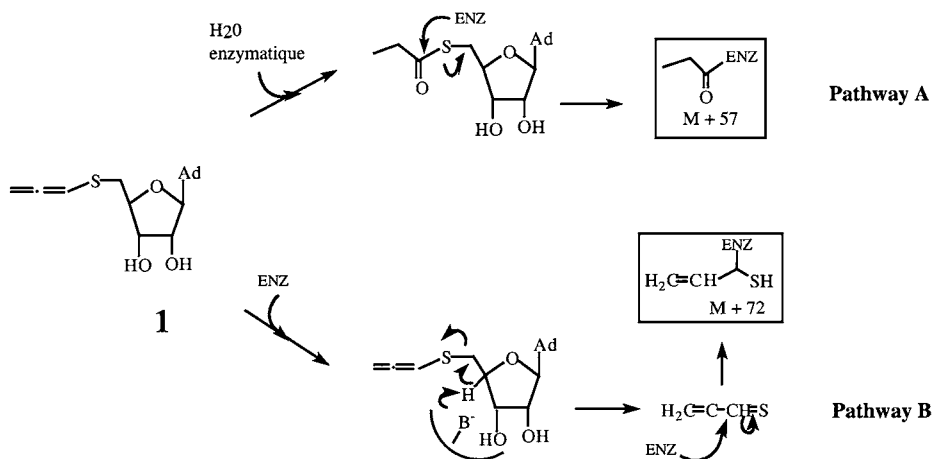
Incubation of **1** and **2** with AdoHcy hydrolase resulted in time and concentration dependent irreversible inactivation of the enzyme. The Kitz and Wilson method (12) was used for kinetic constants determination. The values obtained (**1**: $K_i = 16 \pm 1 \mu\text{M}$, $K_{\text{inact}} = 0.12 \text{ min}^{-1}$; **2**: $K_i = 15 \pm 1 \mu\text{M}$, $K_{\text{inact}} = 0.062 \text{ min}^{-1}$) 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 sequestered water on the S-allenyl group of **1** or S-propynyl group of **2** might generate the formation of the corresponding quite reactive thioester. Attack by amino acid functionalities of the active site might cause type II (covalent binding) inhibition of the enzyme.

A process involving a β -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.





These two possibilities have been examined with compound **1**, using homogeneous 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 ± 3.0 Da (native enzyme 47604.8 ± 1.5 Da) showing that inhibition of AdoHcy hydrolase is accompanied by a covalent linkage of 54.8 ± 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-MSⁿ experiments to identify the amino acid residue involved in this inactivation process.

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7. **4** : mp 87–89°C - HRMS (DCI/NH₃) 322.084 (MH)⁺, calc. 321.299 -[α]_D²⁰ -20.5° (c 0.23; CHCl₃/MeOH: 4/1). I.R.(KBr) 2170, 3281 cm⁻¹ - ¹H NMR, CDCl₃, ε CD₃OD δ (ppm), J(Hz): 2.27 (t, 1H, C≡CH, J 2.67); 3.01 (dd, 1H, H5'α, J 14.11, 6.1); 3.12 (dd, 1H, H5'β, J 14.11, 4.57); 3.28 (d, 2H, CH₂C≡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) - ¹³C NMR, CDCl₃ ε CD₃OD, δ (ppm) : 20.0 (CH₂C≡C); 33.3 (C-5'); 71.6(C≡CH); 72.3 (C-4'); 74.2 (C-3'); 80.0 (C≡CH); 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/NH₃) 322.098 (MH)⁺, calc. 321.299 -[α]_D²⁰ -18° (c 0.18; CHCl₃/MeOH: 4/1) - I.R.(KBr) 1944 cm⁻¹ (weak) - ¹H NMR, CDCl₃, ε CD₃OD δ (ppm), J(Hz): 2.93 (dd, 1H, H-5'α, J 14.5, 4.96); 3.05 (dd, 1H, H-5'β, 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, CH₂=C=CHS, J 6.48); 5.70 (dd, 1H, CH₂=C=CHS, 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) - ¹³C NMR, CDCl₃ ε CD₃OD, δ (ppm) : 34.2 (C-5'); 72.1 (C-4'); 74.2 (C-3'); 81.0 (CH₂=C=CHS); 83.0 (C-2'); 87.0 (CH₂=C=CHS); 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=C=CHS).
9. **2** mp 95–97°C - HRMS (DCI/NH₃) 322.306 (MH)⁺, calc. 321.299 - [α]_D²⁰ +22.5 (c 0.020; CHCl₃/MeOH: 4/1) - ¹H NMR, CDCl₃, ε CD₃OD δ (ppm), J (Hz): 1.87 (s, 3H, C≡CCH₃); 3.02 (dd, 1H, H-5'α J 13.72, 6.5); 3.13 (dd, 1H, H-5'β, 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) - ¹³C NMR, CDCl₃, ε CD₃OD, δ (ppm): 4.4 (C≡CCH₃); 39.2 (C-5'); 68 (C≡CCH₃); 73.8 (C-4'); 74.8 (C-3'); 84.8 (C-2'); 89.0 (C≡CCH₃); 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).
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