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9-[(2'S,3'S)-3'-FORMYL-2',3'-DIHYDROXYPROPYL]ADENINE: A FACILE AFFINITY-LABELING PROBE OF HUMAN S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE

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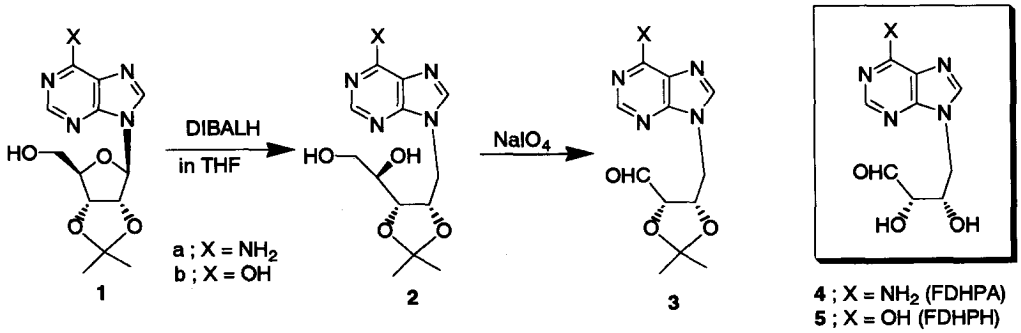
Abstract: Treatment of human recombinant *S*-adenosyl-L-homocysteine (SAH) hydrolase with 9-[(2'S,3'S)-3'-formyl-2',3'-dihydroxypropyl]adenine (FDHPA) caused irreversible inactivation in a time- and concentration-dependent manner (K_i = 8.8 μM, k_{inact} = 0.09 min^{-1}). FDHPA behaved as a facile affinity-labeling probe of SAH hydrolase.

The cellular enzyme *S*-adenosyl-*L*-homocysteine (SAH) hydrolase (EC 3.3.1.1) has emerged as a target enzyme for the molecular design of anti-viral agents.[1] Inhibition of SAH hydrolase results in cellular accumulation of SAH, which is a potent product inhibitor of *S*-adenosyl-L-methionine-dependent biological methylation. We have recently found a convenient method for the direct preparation of 9-ribitylpurines (**2**) by the reductive cleavage of purine nucleosides (**1**) with diisobutylaluminum hydride (DIBALH).[2] Various acyclic nucleosides such as *(S)*-9-(2,3-dihydroxypropyl)adenine [*(S)*-DHPA],[4] (D)-eritadenine,[5] and *(RS)*-3-adenin-9-yl-2-hydroxypropanoic acid [*(RS)*-AHPA][6] have been found to exhibit a broad spectrum of antiviral



Scheme 1

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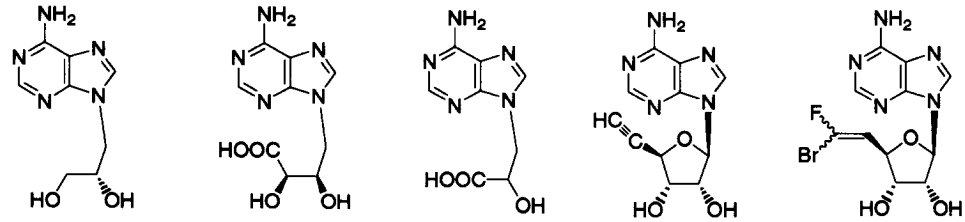
properties due to the inhibition of SAH hydrolase. Affinity-labeling probes such as acetylenic adenosine[3a] and BDDFHA[3b,c] were prepared for the elucidation of the molecular mechanism of SAH hydrolase (Scheme 2).



Scheme 2

During the course of studies on the preparation of biologically important nucleosides[7,8] using the reductive cleavage of purine nucleosides,[2] a DHPA analogue possessing a formyl group at the 3'-position has been designed as a possible affinity-labeling probe for the elucidation of the catalytic site of SAH hydrolase. In this paper, we describe a method for the preparation of a facile affinity-labeling probe and its biological properties against human recombinant SAH hydrolase.

Thus, the possible affinity-labeling probe, 9-[*(2'S,3'S)*-3'-formyl-2',3'-dihydroxypropyl]adenine (**4**; FDHPA), was prepared from naturally-occurring nucleosides (**1**) *via* reductive cleavage with DIBALH (Scheme 1). Subsequent oxidation of **2** with $NaIO_4$ gave the corresponding formyl derivatives (**3**) quantitatively. Careful deprotection of **3** with 2% trifluoroacetic acid gave FDHPA (**4**) in good yield.[9]

The cDNA[10] for human SAH hydrolase has been cloned from human hepatoma cell HepG2 by RT-PCR and the obtained human recombinant SAH hydrolase was purified according to the literature.[11] Incubation with FDHPA (**4**) caused inactivation of the human SAH hydrolase and the activity was not recovered by dialysis. The inactivation was followed by the pseudo-first-order reaction (Figure 1). However, k_{app} increased in a dose-dependent manner and reached maximum at 100 μM FDHPA (**4**) (Figure 1, inset). The values of K_i and k_{inact}, which are useful values to evaluate the affinity and reactivity of an affinity-labeling reagent,[3c,12] were 8.8 μM and 0.09 min^{-1}, respectively.

To elucidate the FDHPA acts as an affinity-labeling probe, a hypoxanthine derivative of FDHPA (**4**), 9-[*(2'S,3'S)*-3'-formyl-2',3'-dihydroxypropyl]hypoxanthine (**5**; FDHPH), was also synthesized and compared. Despite its structural similarity with FDHPA (**4**), FDHPH (**5**) showed only minimal inactivation effect (Figure 2). Furthermore, SAH hydrolase activity was completely protected from the inactivation by FDHPA (**4**) with

co-existence of 1 mM adenosine (data not shown).

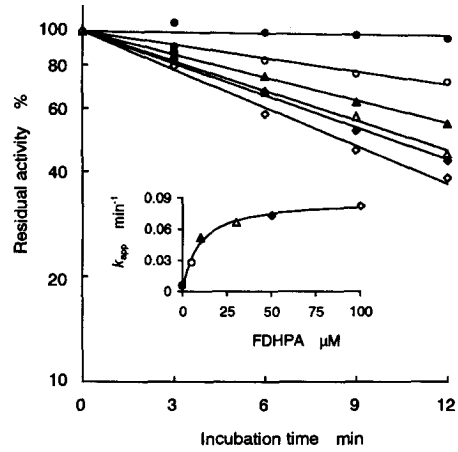


Figure 1. Concentration-dependent inactivation of SAH hydrolase by FDHPA (**4**) SAH hydrolase was incubated with or without each concentration of FDHPA (**4**) at 30°C (0 μM (●), 5 μM(○), 10μM(△), 30 μM(▲), 50 μM(◆), 100 μM(◇)). Aliquots of the reaction mixture were removed for evaluating the enzymatic activity at indicated intervals. Inset indicates the secondary plot of FDHPA (**4**) concentration versus apparent pseudo-first-order rate of inactivation.

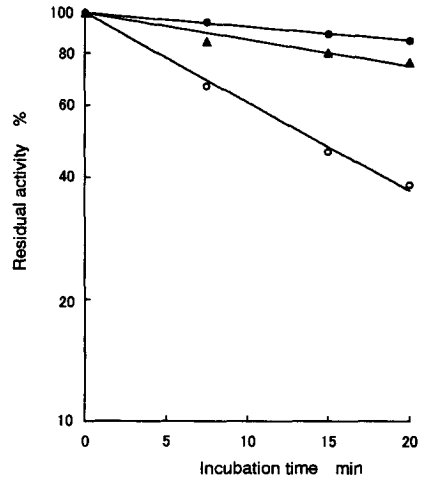


Figure 2. Inhibitory effect of FDHPA (**4**) on the SAH hydrolase activity SAH hydrolase was incubated under the absence (●) or presence of 0.1 mM FDHPA (**4**) (▲) and 0.1 mM FDHPH (**5**) (△) at 30°C.

Taking the above results into consideration, it is deduced that FDHPA (**4**) functions as a useful affinity-

labeling probe of SAH hydrolase and provides a clue to elucidation of the molecular mechanism of SAH hydrolase aiming at the molecular design of anti-viral drugs.

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12. The data were analyzed by Kitz and Wilson plot, fitting to the following equation: $k_{app} = k_{inact}[I] / (K_i + [I])$ in this equation k_{app}, k_{inact}, K_i and I mean pseudo-first-order rate of inactivation, maximum rate of inactivation, inhibition constant and concentration of FDHPA (**4**), respectively. The value of k_{app} was determined from a plot of the residual activity versus incubation time. The values of K_i and k_{inact} were obtained using a curve-fitting program CurveExpert. The proposed equilibrium is as follows: **I**-CHO + H_2N-**E** ⟶ **I**-CH=N-**E**. **I**-CHO and **E**-NH_2 mean FDHPA (**4**) and SAH hydrolase, respectively.