S-ADENOSYL-L-HOMOCYSTEINE DIALDEHYDE*: AN AFFINITY LABELING REAGENT FOR HISTAMINE-N-METHYLTRANSFERASE

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SUMMARY: S-Adenosyl-L-homocysteine (SAH) was converted to 2'-O-[(R)-formyl(adenin-9-yl)methyl]-3'-S-homocysteinyl-3'-deoxy-(R)-glyceraldehyde (SAH dialdehyde) by periodic acid oxidation. SAH dialdehyde was then reduced with sodium borohydride to the corresponding diol, 2',3'-acyclic SAH. SAH dialdehyde, but not 2',3'-acyclic SAH, was found to inhibit histamine-N-methyltransferase (HMT). Neither analog showed significant inhibitory activity toward other methyltransferases. The inhibition of HMT by SAH dialdehyde was irreversible with the inactivation following first-order kinetics. A kinetic analysis suggests the formation of a dissociable enzyme-inhibitor complex prior to inactivation. The enzyme could be protected from inactivation by inclusion of S-adenosyl-L-methionine in the preincubation mixture.

Histamine-N-methyltransferase (HMT) (E.C. 2.1.1.4) is an S-adenosylmethionine (SAM) dependent enzyme which plays an important role in the metabolism of histamine by catalyzing its N-methylation to form 1-methylhistamine (1-2). Similar to most SAM-dependent methyltransferases, HMT is sensitive to inhibition by the demethylated product S-adenosyl-L-homocysteine (SAH) (3-5). In earlier work from our laboratory we have described the synthesis of analogs of SAH, several of which proved to be fairly specific reversible inhibitors of HMT (e.g. S-adenosyl-D-homocysteine) (6-10). The data obtained from these studies yielded valuable information about those structural features of SAH which were necessary to produce maximum binding to HMT. As part of our continuing interest in this enzyme, we have also attempted to develop affinity

*The abbreviations used are: SAH dialdehyde, 2'-O-[(R)-formyl-(adenin-9-yl)methyl]-3'-S-homocysteinyl-3'-deoxy-(R)-glyceraldehyde; HMT, histamine-N-methyltransferase (E.C. 2.1.1.8); SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteine.

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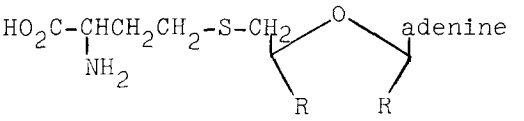
labeling reagents which would provide information about the nature of the active site of HMT. Dialdehyde systems such as those obtained by periodate oxidation of the 2',3'-cis-diol functionality of ribonucleosides are known to react with amino groups of proteins to form Schiff base adducts (11-14). In fact, the periodate oxidation products of 6-methylmercaptopurine ribonucleoside and a fluorescent analog were shown to inhibit E. coli RNA polymerase by covalently binding to an ε-amino group of a lysine residue at the enzyme's initiation site (15-17). Therefore, we have synthesized 2'-O-[(R)-formyl(adenin-9-yl)methyl]-3'-S-homocysteinyl-3'-deoxy-(R)-glyceraldehyde (SAH dialdehyde, 1) by periodate oxidation of SAH as a possible affinity label for HMT. SAH dialdehyde (1) was shown to produce rapid inactivation of HMT involving affinity labeling at the active site of the enzyme.



Compd	R=
1	-CHO
2	-CH_2OH

MATERIALS AND METHODS: HMT was purified from guinea pig brain (Pel-Freez Biologicals) according to the methods previously described by Brown et al. (2). The enzyme was purified through the dialysis step resulting in a preparation which contained 21 mg of protein per milliliter with a specific activity of 64 nmol of product/mg of protein/min with histamine as a substrate. The activity was determined using histamine and SAM-$^{14}CH_3$ (New England Nuclear, 55 mCi/mmol) according to a previously described radioassay (6-10). SAH dialdehyde (1) was prepared in 51% yield by oxidation of SAH using periodic acid in water (18-21). The SAH dialdehyde was further reduced to its corresponding dialcohol 2 using sodium borohydride.

A typical inactivation experiment was carried out in a total volume of 0.25 ml containing 40 mM phosphate buffer, pH 7.4, variable concentrations of SAH dialdehyde, and the enzyme preparation. The preincubation step was started by the addition of enzyme and incubation was carried out at 37°. In the protection experiments varying amounts of SAM or histamine were included during the preincubation. After the appropriate preincubation time the samples were assayed by addition of 0.05 μCi of SAM-$^{14}CH_3$ and SAM and/or histamine to give final concentration of 1 mM for each substrate. The assay mixtures were incubated for 15 min at 37° and the reaction stopped by addition of 0.25 ml of 0.25 M borate buffer (pH 10.0). The assay mixture was extracted with 10 ml of toluene-isoamyl alcohol (1:1) and after centrifugation a 5 ml aliquot of the organic phase was measured for radioactivity. The results were corrected using the appropriate histamine blank. The percent activity remaining at any given time was calculated relative to zero-time activity. The pseudo-first order kinetic constants of inactivation, K_{app}, were calculated from the slopes of the plots of log of percentage activity remaining vs. preincubation time (22,23).

RESULTS AND DISCUSSION: In preliminary experiments SAH dialdehyde (1) was found to be a potent inhibitor of the HMT catalyzed transmethylation (Table 1). 2',3'-Acyclic SAH (2) showed substantially less inhibitory activity. In contrast neither SAH analog showed inhibitory activity toward other methyltransferases [e.g. catechol-O-methyltransferase (E.C. 2.1.1.6); phenylethanolamine-N-methyltransferase (E.C. 2.1.1); hydroxyindole-O-methyltransferase (E.C. 2.1.1.8)]. The kinetics of SAH dialdehyde inhibition of HMT were found to be noncompetitive when SAM was the variable substrate, which is in sharp contrast to the competitive kinetic patterns observed for SAH or analogs of SAH (6-10). The noncompetitive pat-

Table 1

Effect of SAH, SAH Dialdehyde (1) and 2',3'-Acyclic SAH (2) on HMT Activity[a]

Compound	Concn mM	Inhibition %
SAH	0.2	40
SAH	2.0	89
SAH dialdehyde (1)	0.2	29
SAH dialdehyde (1)	2.0	90
2',3'-acyclic SAH (2)	0.2	0
2',3'-acyclic SAH (2)	2.0	19

[a]The standard assay mixture contained the following components (in μmol) added in this sequence: water, so that the final volume was 0.25 ml; histamine (0.5); inhibitor (variable); SAM (0.25); 0.05 μCi of SAM-$^{14}CH_3$; phosphate buffer, pH 7.40 (10); and the enzyme preparation. Incubation was carried out for 60 min at 37° after which the reaction was stopped by addition of 0.25 ml of 0.5 M borate buffer (pH 10) and the N-methylated product isolated as described in the text.

tern observed for SAH dialdehyde suggested a possible irreversible inactivation of the enzyme. When reaction mixtures containing the enzyme, buffer and SAH dialdehyde were preincubated for varying time periods prior to assaying for enzyme activity, a time dependent loss of HMT activity was observed. The time course for inactivation of HMT by various concentrations of SAH dialdehyde is presented in Figure 1. This inactivation of HMT follows pseudo-first order kinetics and the rate of inactivation is dependent upon the concentration of SAH dialdehyde. The enzyme activity cannot be recovered after dialysis or gel filtration on Sephadex G-25 indicating the inhibition is completely irreversible. In contrast, when similar preincubation experiments were carried out

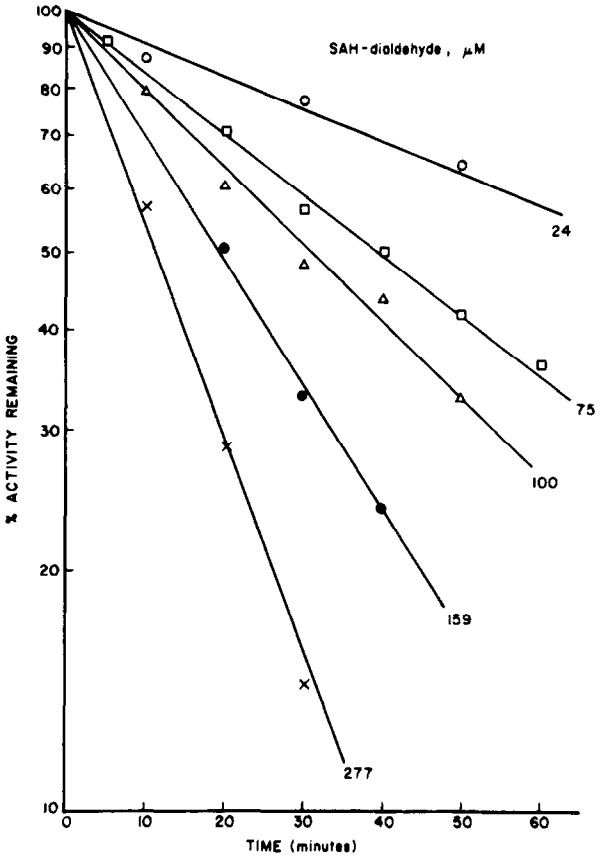


Figure 1: Effect of SAH dialdehyde (1) concentration on the rate of inactivation of HMT. Activity remaining after the appropriate preincubation time was determined as outlined in the text and in Table 2. The pseudo-first order rate constants of inactivation, K_{app}, were calculated from the slopes for each concentration of inhibitor.

with SAH or 2',3'-acyclic SAH, no irreversible inactivation of HMT was observed. These results suggest a crucial role for the aldehyde functionalities of SAH dialdehyde in this inactivation process

To establish whether the inactivation of HMT by SAH dialdehyde proceeds via a unimolecular reaction within a dissociable complex rather than via a non-specific bimolecular reaction, the rate of enzyme inactivation as a function of inhibitor concentration was determined. The model for a mechanism involving a dissociable

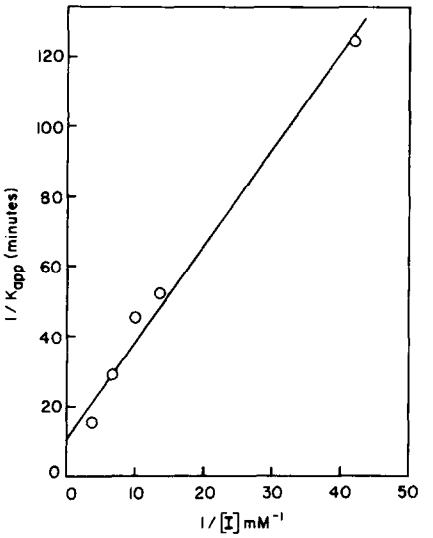


Figure 2: Double reciprocal plot of the pseudo-first order rate constant of inactivation, K_{app}, vs. SAH dialdehyde concentration. k_2 and K_I were calculated from the y-intercept and the slope, respectively, using the least squares method.

complex as described by Kitz and Wilson (23) is shown in eq. 1 and eq. 2, where E•I is the reversible complex, E-I the inactive enzyme, K_I the steady state constant of inactivation and k_2 the first order rate constant.

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\bullet I \xrightarrow{k_2} E\text{-}I \qquad (1)$$

$$K_I = \frac{[E][I]}{[E\bullet I]} \qquad (2)$$

The variation of the rate of HMT inactivation as a function of the concentration of SAH dialdehyde is shown in Figure 1. As predicted for each concentration of SAH dialdehyde, pseudo-first order kinetics were observed and apparent pseudo-first order rate constants (K_{app}) were calculated. As shown in Figure 2, a recip-

Table 2

Substrate Protection of HMT from Inactivation

Additions[a]	Concn mM	Residual activity after 60 min, 37°
none	--	7
SAM	1	53
histamine	1	7

[a]The standard preincubation mixture consisted of SAH dialdehyde (0.1 mM); phosphate buffer, pH 7.6 (40 mM) and purified enzyme preparation (65 µg) in a total volume of 0.25 ml. The preincubation was carried out for 60 min at 37° after which the samples were assayed as described in the text.

rocal plot of the pseudo-first order rate constants ($1/K_{app}$) vs. the reciprocal of the inhibitor concentrations (1/[I]), according to eq. 3, gave a positive intercept on the abscissa. This indicates saturation of HMT by SAH dialdehyde with a limiting rate constant of inactivation, $k_2 = 0.079 \pm 0.006$ min^{-1} and a steady-state constant of inactivation, $K_I = 216 \pm 16$ µM. The linearity and positive intercept on the abscissa observed in this reciproca plot provide evidence for the formation of a dissociable enzyme-inhibitor complex prior to enzyme inactivation.

$$\frac{1}{K_{app}} = \frac{K_I}{k_2[I]} + \frac{1}{k_2} \qquad (3)$$

Further evidence to support the formation of a dissociable complex prior to the irreversible inactivation of HMT comes from the results of substrate protection experiments shown in Table 2. The enzyme can be partially protected from inactivation by inclusion of SAM in the preincubation mixture. However, addition of

histamine during the preincubation did not provide protection from inactivation by this inhibitor.

These results are consistent with SAH-dialdehyde first binding to the SAM site on HMT via a dissociable complex, followed by reaction of the aldehyde functionalities of SAH dialdehyde (2) with an amino acid residue in an irreversible step. Therefore, it can be concluded that SAH dialdehyde (2) is an effective affinity labeling agent for HMT. Further studies to identify the modified amino acid residue on HMT are in progress.

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