MOLECULAR PHARMACOLOGY, 13, 939–947

S-Adenosyl-L-homocysteine Hydrolase: Analogues of *S*-Adenosyl-L-homocysteine as Potential Inhibitors

PETER K. CHIANG, HENRY H. RICHARDS, AND GIULIO L. CANTONI

Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20014

(Received February 15, 1977)
(Accepted May 19, 1977)

SUMMARY

CHIANG, PETER K., RICHARDS, HENRY, H. & CANTONI, GIULIO L. (1977) *S*-Adenosyl-L-homocysteine hydrolase: analogues of *S*-adenysyl-L-homocysteine as potential inhibitors. *Mol. Pharmacol.*, **13**, 939–947.

Structural analogues of *S*-adenosyl-L-homocysteine with modifications in the purine, sugar, 5′-thioether linkage, and/or amino acid portion were tested as substrates and/or inhibitors of *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1). It was observed that *S*-3-deazaadenosyl-L-homocysteine was the only analogue that could serve as a substrate for the enzyme as well as *S*-adenosyl-L-homocysteine itself. When tested in the direction of *S*-adenosyl-L-homocysteine hydrolysis coupled with calf intestinal adenosine deaminase, the following were found to be the more potent inhibitory analogues: 5′-deoxy-5′-butylthioadenosine, N^{γ}-adenosyl-α,γ-diaminobutyric acid, 9-(5′-deoxy-5′-methylthio-α-D-arbinofuranosyl)adenine, 5′-deoxy-5′-aminoethylthio-2-chloroadenosine, 3-deazaadenosine, and *S*-3-deazaadenosyl-L-homocysteine. The inhibitory ability of 3-deazaadenosyl-L-homocysteine is probably related to its ability to serve as substrate, since its hydrolysis generates 3-deazaadenosine, which is the most potent inhibitor of *S*-adenosyl-L-homocysteine hydrolase as well as being resistant to adenosine deaminase. When tested *in vitro* in isolated rat hepatocytes, 3-deazaadenosine caused a drastic increase in the levels of *S*-adenosyl-L-homocysteine and *S*-adenosyl-L-methionine, with the formation of *S*-3-deazaadenosyl-L-homocysteine. 3-Deazaadenosine is thus an interesting analogue with biological potential.

INTRODUCTION

The enzymatic transfer of the methyl group of *S*-adenosyl-L-methionine yields *S*-adenosyl-L-homocysteine as one of the products of the reaction (1). In eukaryotes, as far as is known, the principal pathway for the metabolism of adenosylhomocysteine is its hydrolysis to L-homocysteine and adenosine by the action of an enzyme, adenosylhomocysteine hydrolase (EC 3.3.1.1), first described in rat liver by de la Haba and Cantoni (2). Although the reaction is reversible, the equilibrium lies in the direction of synthesis of adenosylhomocysteine. The reaction catalyzed by adenosylhomocysteine hydrolase is described in Eq. 1 (Fig. 1).

$$\text{S-Adenosyl-L-homocysteine} \xrightleftharpoons[\text{H}_2\text{O}]{E} \text{adenosine} + \text{L-homocysteine} \qquad (1)$$

The details of the mechanism of adenosylhomocysteine hydrolase reaction have recently been proposed (3).

Adenosylhomocysteine has been found to inhibit competitively most of the methyltransferases which utilize adenosylmethionine as the methyl donor, but with K_i values that vary over a wide range (4–10).

ISSN 0026-895X

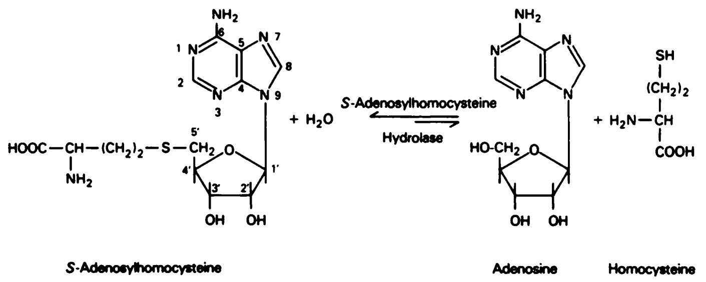

FIG. 1. *Structural formula for Eq. 1*

Because of the different sensitivities of various methyltransferases toward adenosylhomocysteine, the regulation of adenosylhomocysteine hydrolase by various effectors can be of physiological importance in controlling biological methylations. The observation that the level of adenosylhomocysteine hydrolase in tissues is affected by nutritional and hormonal factors (11) suggests a regulatory role for this enzyme. Finally, adenosylhomocysteine, by virtue of its ability to inhibit betaine-homocysteine methyltransferase and its ability to activate cystathionine synthase (12), may play a role in liver in determining whether homocysteine will be remethylated to methionine or utilized for the synthesis of cystathionine (12).

It is possible to synthesize adenosylhomocysteine analogues that differ from the parent compound in the purine, sugar, sulfur atom, or amino acid (homocysteinyl) portion of the molecule. Recently a number of these analogues have been synthesized and studied as potential inhibitors of enzymatic transmethylation reactions, and some interesting differences in the biological activity of these analogues have been reported (13–21).

We report here the results of studies on the interaction between adenosylhomocysteine analogues and purified adenosylhomocysteine hydrolase from beef liver. These analogues have been surveyed for their abilities to serve as substrates and/or inhibitors of this enzyme. The interaction of adenosylhomocysteine analogues with adenosylhomocysteine hydrolase can be measured by three different ways. (a) The ability of adenosylhomocysteine analogue to inhibit adenosylhomocysteine hydrolysis can be determined when coupled with excess adenosine deaminase. (b) The ability of adenosine or homocysteine analogues to support the synthesis of adenosylhomocysteine-like compounds can be measured directly as shown in Eq. 1. (c) Finally, the ability of adenosylhomocysteine analogues to act as substrates can be assayed by determining their ability to substitute for adenosylhomocysteine in the homocysteine exchange reaction, in which the incorporation of ^{35}S from [^{35}S]homocysteine ([^{35}S]Hcy) into adenosylhomocysteine can be measured (Eq. 2).

$$[^{35}\mathrm{S}]\mathrm{Hcy} + \text{adenosylhomocysteine} \underset{\mathrm{H_2O}}{\overset{E}{\rightleftharpoons}} [^{35}\mathrm{S}]\text{adenosylhomocysteine} + \mathrm{Hcy} \qquad (2)$$

MATERIALS AND METHODS

Purified adenosylhomocysteine hydrolase from beef liver was prepared by a method devised in our laboratory.[1] [^{35}S]Adenosylhomocysteine or [^{14}C]adenosylhomocysteine was prepared according to de la Haba and Cantoni (2). [^{14}C]-Adenosylhomocysteine was labeled uniformly in the adenosine moiety. L-[^{35}S]Homocysteine was prepared by incubation of L-[^{35}S]homocysteine (Amersham/

---

[1] H. H. Richards, P. K. Chiang, and G. L. Cantoni, manuscript in preparation. The specific activity of the purified adenosylhomocysteine hydrolase varied between 0.9 and 1.5 units/mg of protein, 1 unit being defined as 1 μmole of adenosylhomocysteine hydrolyzed in 1 min at 37° in the presence of excess intestinal adenosine deaminase. The purification of the adenosylhomocysteine hydrolase involved conventional procedures, such as chromatography on DEAE-cellulose, hydroxylapatite, and Sephadex G-100, and ammonium sulfate fractionation.

Searle) in 10 mM Tris-HCl, pH 8.0, and a 6-fold excess of dithiothreitol for 30 min at 37°. Adenosine deaminase from calf intestinal mucosa was obtained from Sigma Chemical Company;[2] the chemical analogues used here were synthesized and supplied by Dr. R. T. Borchardt (16–19), University of Kansas, Lawrence; Dr. J. A. Montgomery (Tables 4 and 5), Southern Research Institute, Birmingham, Al.; Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, France (13–15); and Dr. J. Coward (9, 20, 21), Yale University.

A sensitive assay of adenosylhomocysteine hydrolase, developed in this laboratory, is based on the conversion of [^{14}C]-adenosine, a product of the reaction, to [^{14}C]inosine in the presence of excess adenosine deaminase in the assay medium. [^{14}C]Adenosylhomocysteine and [^{14}C]inosine can be separated by a small column of a cation exchanger, SP-Sephadex C-25 (22); [^{14}C]inosine is eluted from the column with 0.1 N formic acid, whereas [^{14}C]adenosylhomocysteine is eluted only with 0.5 N NaOH. To start the assay, adenosylhomocysteine hydrolase was added to a reaction mixture of 0.5 ml containing 4 units of adenosine deaminase, 150 mM potassium phosphate, 1.0 mM EDTA, and 0.1 mM [^{14}C]adenosylhomocysteine (K_m = 0.06 mM), pH 7.6, at 37°. After incubation for 10 min, during which the reaction was linear, 100 μl of 5 N formic acid were added, and the reaction mixture was then poured onto a 0.8 × 2.5 cm column of SP-Sephadex C-25, equilibrated in 0.1 N formic acid. Each test tube was rinsed with 0.5 ml of 0.1 N formic acid. [^{14}C]Inosine formed was then eluted by the addition of 3.5 ml of 0.1 N formic acid. The eluate was collected in a scintillation vial, and the radioactivity was determined after the addition of 10 ml of Aquasol (New England Nuclear).

The ability of adenosylhomocysteine analogues to inhibit adenosylhomocysteine hydrolase was determined by adding them to the reaction mixture as described above.

The ability of adenosylhomocysteine analogues to act as substrates was measured by utilizing an exchange reaction in which the incorporation of ^{35}S from [^{35}S]-homocysteine into these adenosylhomocysteine analogues was measured (Eqs. 1 and 2). The assay mixture, in 1.0 ml, contained 150 mM potassium phosphate, 2.0 mM dithiothreitol, 1.0 mM EDTA, 0.1 mM L-[^{35}S]homocysteine, 1.0 mM adenosylhomocysteine, or an adenosylhomocysteine analogue, at pH 7.6 and 37°. The reaction was started by the addition of adenosylhomocysteine hydrolase, and after 30 min of incubation, 200 μl of 5 N formic acid were added. A control was performed by adding adenosylhomocysteine hydrolase to the same reaction mixture containing 200 μl of 5 N formic acid. The reaction mixture was poured onto a 0.8 × 2.5 cm column of SP-Sephadex C-25 equilibrated in 0.1 N formic acid, the test tube was rinsed with 1 ml, and the column was washed with 5 ml of 0.1 N formic acid to remove unreacted [^{35}S]homocysteine. The [^{35}S]adenosylhomocysteine formed was subsequently eluted with 5.0 ml of 0.5 N NaOH directly into a scintillation vial, and the radioactivity was determined after addition of 10 ml of Aquasol.

Male Wistar rats (Carworth Farms) were fed ad libitum. Rat hepatocytes were isolated by the method of Krebs *et al.* (23). Hepatocytes, approximately 1 × 10^8 cells, were incubated in 5 ml of Krebs-Henseleit-NaCl containing 2% dialyzed albumin in the presence of 95% O_2–5% CO_2. After incubation for 60 min at 30° in a shaking water bath, 0.52 ml of 50% sulfosalicylic acid was added to stop the reaction. The entire suspension was then homogenized by a glass homogenizer with a motor-driven Teflon pestle. After centrifugation at 20,000 × *g* for 10 min, the supernatant was used for the quantitative determination of adenosylhomocysteine and adenosylmethionine by a VYDAC cation exchange column as described by Hoffman (24). Column effluents were monitored at 254 nm by a Pharmacia DuoMonitor. Protein content of the centrifuged pellet was estimated by the biuret method (25), with adjustment for the percentage of albumin present.

---

[2] Calf intestinal adenosine deaminase had a specific activity of 200 units/mg; 1 unit will deaminate 1.0 μmole of adenosine to inosine per minute, as defined by the supplier.

RESULTS

Tables 1-5 summarize the effects of the chemical analogues on adenosylhomocysteine hydrolase. I_{50} values, concentrations required for 50% inhibition of the enzyme, were not determined for those analogues that were unable to inhibit the adenosylhomocysteine hydrolase activity by 50% at 1 mM. Most of the analogues with a modified amino acid position exerted moderate inhibition on adenosylhomocysteine hydrolase (Table 1). Two butyl analogues (compounds 3 and 4), 5′-deoxy-5′-butylthioadenosine and 5′-deoxy-5′-isobutylthioadenosine, were the more potent compounds in this group. The former had an I_{50} of 0.68 mM, while neither was a substrate for adenosylhomocysteine hydrolase since neither acted as a substrate in the exchange reaction described above.

The sulfur atom of adenosylhomocysteine can be modified or substituted, and the resulting analogues possess inhibitory activity (Table 2). Compound 18, N^{γ}-adenosyl-α,γ-diaminobutyric acid, synthesized by Chang and Coward (21) with the expectation that it might be resistant to adenosylhomocysteine hydrolase, is the second most powerful inhibitor of adenosylhomocysteine hydrolase tested so far, with an I_{50} of 0.14 mM. The sulfoxide and sulfone analogues of adenosylhomocysteine (compounds 16 and 17) were moderate inhibitors. Adenosylhomocysteine sulfoxide can be formed easily from adenosylhomocysteine by the action of H_2O_2, but nothing is known about its natural occurrence. No inhibitory effect could be observed with adenosylmethionine (compound 15).

Of the compounds with a modified adenosine portion, the most interesting is 3-deazaadenosylhomocysteine (compound 19) (Table 3). In the absence of adenosine deaminase, this compound can function as a substrate for the exchange reaction as well as adenosylhomocysteine itself; yet, when tested as an inhibitor of adenosylho-

TABLE 1

Effects of S-adenosyl-L-homocysteine analogues with modifications in amino acid portions on activity of S-adenosyl-L-homocysteine hydrolase

Compound	Amino acid substitution	Inhibition at 1 mM[a]	I_{50}[b]	As substrate in exchange reaction[c]
		%	*mM*	*% adenosylhomocysteine*
1	(D) $-CH_2CH_2CH(NH_2)CO_2H$	29	–[d]	10
2	$-CH_2CH_2CH_2CO_2H$	0	–	0
3	$-CH_2CH_2CH_2CH_3$	60	0.68	7
4	$-CH_2CH(CH_3)CH_3$[e]	47	–	0
5	$-CH_2CH_2CH_2NHC(=0)CH_3$	30	–	7
6	$-CH_2CH_2CH_2CO_2CH_3$	30	–	5
7	$-CH(CH_3)CH_3$	28	–	0
8	$-CH_2CH_2CH(CO_2H)NHC(=0)CH_3$	16	–	0
9	$-CH_2CH(OH)CH_2OH$	33	–	0
10	$-CH_2CH_2CO_2H$	11	–	0
11	$-CH_2CH_2OH$	43	–	0
12	$-CH_2CH_2NH_2$	28	–	0
13	$-CH_2CH_2CH_2NH_3^{\oplus}HSO_4^{\ominus}$	25	–	0
14	$-CH_3$	36	–	0

[a] Percentage inhibition was determined as described in MATERIALS AND METHODS at 0.1 mM adenosylhomocysteine and 1 mM analogue in the presence of adenosine deaminase.

[b] Concentration of an analogue required for 50% inhibition (I_{50}) was determined at 0.1 mM adenosylhomocysteine, and at varying concentrations of the analogue, in the presence of adenosine deaminase.

[c] The ability of an analogue to function as a substrate in the exchange reaction was determined as described in MATERIALS AND METHODS at 1 mM adenosylhomocysteine and 0.1 mM analogue in the absence of adenosine deaminase.

[d] Not determined.

[e] 5′-Deoxy-5′-isobutylthioadenosine.

mocysteine hydrolysis, i.e., in the presence of adenosine deaminase, it is a good inhibitor, with an I_{50} of 0.18 mM. The difference between these two experiments lies in the fact that adenosine deaminase was absent from the exchange reaction, and was added in excess in the assay of adenosylhomocysteine hydrolysis. As was originally reported by de la Haba and Cantoni (2), adenosine is a powerful inhibitor of adenosylhomocysteine hydrolase. 3-Deazaadenosine (compound 26) was found to be an equally powerful inhibitor in the presence and absence of adenosine deaminase. We observed that 3-deazaadenosine was not deaminated by the calf intestinal adenosine deaminase used in the present assay system. Thus the hydrolysis of 3-deazaadenosylhomocysteine yields 3-deazaadenosine as one of the hydrolysis products. 3-Deazaadenosine had an I_{50} of 8 μM, about 20 times smaller than that of 3-deazaadenosylhomocysteine. Quantitatively, therefore, the formation of 3-deazaadenosine by hydrolysis of less than 5% of 3-deazaadenosylhomocysteine at the I_{50} concentration would be sufficient to account for the inhibition observed.

TABLE 2

Effects of S-adenosyl-L-homocysteine analogues with modifications or substitutions in the sulfur moiety on activity of S-adenosyl-L-homocysteine hydrolase

Conditions for determining percentage inhibition, I_{50}, and exchange reaction were the same as in Table 1.

Compound	Modification	Inhibition at 1 mM	I_{50}	As substrate in exchange reaction
		%	*mM*	*% adenosylhomocysteine*
15	$-\overset{\oplus}{\mathrm{S}}(\mathrm{CH}_3)-\mathrm{AA}$[a, b]	0	–[c]	–
16	$-\underset{\mathrm{O}}{\underset{\downarrow}{\mathrm{S}}}-\mathrm{AA}$	39	–	15
17	$-\underset{\mathrm{O\,O}}{\underset{\swarrow\searrow}{\mathrm{S}}}-\mathrm{AA}$	39	–	0
18	$-\mathrm{NH}-\mathrm{AA}$[d]	81	0.14	15

[a] AA = amino acid moiety.

[b] S-Adenosyl-L-methionine.

[c] Not determined.

[d] N^{γ}-Adenosyl-α,γ-diaminobutyric acid.

In contrast to the β-pentosyl analogues of the parent compound, adenosylhomocysteine, the α-pentosyl analogues (compounds 33 and 35) were completely without inhibitory effect (Table 4). It is notable that compound 32, 9-(5′deoxy-5′-methylthio-β-D-arabinofuranosyl)adenine, was found to be a potent inhibitor of adenosyl-

TABLE 3

Effects of S-adenosyl-L-homocysteine or adenosine analogues with modifications in the adenine portions on activity of S-adenosyl-L-homocysteine hydrolase

Conditions for determining percentage inhibition, I_{50}, and exchange reaction were the same as in Table 1.

Compound	Inhibition at 1 mM	I_{50}	As substrate in exchange reaction
	%	*mM*	*% adenosylhomocysteine*
19. S-3-Deazaadenosyl-L-homocysteine	84	0.18	102
20. N^6-Methyl-3-deazaadenosyl-L-homocysteine	20	–[a]	12
21. N^6-Dimethyl-3-deazaadenosyl-L-homocysteine	0	–	0
22. S-7-Deazaadenosyl-L-homocysteine (S-tubercidinyl-L-homocysteine)	0	–	0
23. S-Cytidyl-L-homocysteine	0	–	0
24. S-Inosyl-L-homocysteine	0	–	0
25. S-Guanyl-L-homocysteine	0	–	0
26. 3-Deazaadenosine	94	0.008	–
27. 1,N^6-Ethenoadenosine	0	–	–
28. 7-Deazaadenosine (tubercidin)	13	–	0
29. 8-Aza-9-deazaadenosine (formycin)	0	–	–

[a] Not determined.

homocysteine hydrolase. Neither the 2′-deoxy nor the 3′-deoxy analogues of adenosylhomocysteine (compounds 30 and 31) exhibited significant inhibition.

The addition of a fluorine or chlorine atom at position 2 of adenine produced effective inhibitors, 5′-deoxy-5′-ethylthio-2-fluoroadenosine (compound 38), 5′-deoxy-5′-butylthio-2-chloroadenosine (compound 40), and 5′-deoxy-5′-aminoethylthio-2-chloroadenosine (compound 39), the last being the most potent in this series (Table 5). However, the 2-hydroxyl analogue (compound 42) was virtually ineffective.

Since adenosine deaminase was required by the present assay method for adenosylhomocysteine hydrolase, analogues exhibiting inhibitory properties were tested for their effect on adenosine deaminase. None of these compounds was found to inhibit adenosine deaminase under the assay conditions described in MATERIALS AND METHODS.

Because 3-deazaadenosine was the most potent inhibitor found for adenosylhomocysteine hydrolase, it was of interest to test the ability of 3-deazaadenosine to affect the levels of adenosylhomocysteine and adenosylmethionine in isolated rat hepatocytes (Table 6). In the absence of exogenous L-methionine in the incubation medium, 0.1 mM 3-deazaadenosine caused a 10-fold increase in the concentration of adenosylhomocysteine, and a 7-fold in-

TABLE 4

Effects S-adenosyl-L-homocysteine analogues with modifications in the adenine, ribose, and amino acid groups on activity of S-adenosyl-L-homocysteine hydrolase

Conditions for determining percentage inhibition, I_{50}, and exchange reaction were the same as in Table 1.

Compound	Inhibition at 1 mM	I_{50}	As substrate in exchange reaction
	%	*mM*	*% adenosylhomocysteine*
30. S-2′-Deoxyadenosyl-L-homocysteine	13	–[a]	0
31. S-3′-Deoxyadenosyl-L-homocysteine	10	–	–
32. 9-(5′-Deoxy-5′-methylthio-β-D-arabinofuranosyl)adenine	65	0.43	5
33. 9-(5′-Deoxy-5′-methylthio-α-D-xylofuranosyl)adenine	0	–	–
34. 9-(5′-Deoxy-5′-methylthio-β-D-xylofuranosyl)adenine	44	–	–
35. 2-Chloro-9-(5′-deoxy-5′-methylthio-α-D-xylofuranosyl)adenine	0	–	–
36. 2-Chloro-9-(5′-deoxy-5′-methylthio-β-D-xylofuranosyl)adenine	14	–	–

[a] Not determined.

TABLE 5

Effects of S-adenosyl-L-homocysteine analogues with modifications in the adenine and amino acid groups on activity of S-adenosyl-L-homocysteine hydrolase

Conditions for determining percentage inhibition, I_{50}, and exchange reaction were the same as in Table 1.

Compound	Inhibition at 1 mM	I_{50}	As substrate in exchange reaction
	%	*mM*	*% adenosylhomocysteine*
37. 5′-Deoxy-5′-ethylthio-2-aminoadenosine	27	–[a]	–
38. 5′-Deoxy-5′-ethylthio-2-fluoroadenosine	40	–	–
39. 5′-Deoxy-5′-aminoethylthio-2-chloroadenosine	37	0.32	0
40. 5′-Deoxy-5′-butylthio-2-chloroadenosine	57	0.56	0
41. 5′-Deoxy-5′-ethylthio-2-chloroadenosine	11	–	–
42. 5′-Deoxy-5′-ethylthio-2-hydroxyadenosine	0	–	–
43. 4-Amino-7-(5′-butylthio-5′-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine	10	–	–

[a] Not determined.

TABLE 6

Effect of 3-deazaadenosine on levels of S-adenosyl-L-homocysteine, S-adenosyl-L-methionine, and S-3-deazaadenosyl-L-homocysteine in isolated rat hepatocytes

Hepatocytes were incubated for 1 hr at 30° in Krebs-Henseleit-NaCl plus 2% dialyzed albumin.

Additions	Adenosylhomocysteine	3-Deazaadenosylhomocysteine	Adenosylmethionine
		nmoles/g protein	
Experiment A			
None	65	0	374
0.1 mM 3-deazaadenosine	692	353	2589
Experiment B			
0.5 mM L-methionine	65	0	776
0.5 mM L-methionine + 0.1 mM 3-deazaadenosine	561	654	4131
0.5 mM L-methionine + 0.3 mM 3-deazaadenosine	1907	602	4972

crease in the level of adenosylmethionine. When the hepatocytes were incubated with 0.5 mM L-methionine alone, the level of adenosylmethionine increased about 2-fold, whereas the concentration of adenosylhomocysteine remained unchanged. With the addition of 0.5 mM L-methionine to the incubation medium, 3-deazaadenosine caused an increase in the adenosylhomocysteine concentration by 9- and 29-fold at 0.1 and 0.3 mM, respectively. The level of adenosylmethionine increased correspondingly, by about 5- and 6-fold. The formation of S-3-deazaadenosyl-L-homocysteine in rat hepatocytes was observed upon incubation with 3-deazaadenosine.

DISCUSSION

The results described above begin to provide information on the interaction between adenosylhomocysteine and adenosylhomocysteine hydrolase. It appears that the 6-amino group of adenosine and N-7 are required for binding of adenosylhomocysteine to adenosylhomocysteine hydrolase, since compounds lacking the 6-amino group or the N-7 atom, such as the inosyl, uridyl, cytidyl, guanyl, and tubercidinyl analogues of adenosylhomocysteine, do not interact with adenosylhomocysteine hydrolase. Replacement of the adenosine with 3-deazaadenosine yields a compound that is a powerful inhibitor of adenosylhomocysteine hydrolysis, as well as being resistant to adenosine deaminase. 3-Deazaadenosine can be utilized as a substrate for the synthesis of 3-deazaadenosylhomocysteine. It therefore is clear that the presence of a nitrogen atom at position 3 of the adenine ring is not required for interaction with the enzyme. However, the corresponding N^6-dimethyl analogue (compound 21) of 3-deazaadenosylhomocysteine was completely devoid of inhibitory activity toward adenosylhomocysteine hydrolase, and this is probably the result of increased steric bulk at position 6. The enzyme is stereospecific for the β-pentose configuration at N-9. It is noteworthy that α-adenosine is neither a substrate nor an inhibitor for adenosylhomocysteine hydrolase (not shown). The 5′-thioether linkage appears essential for interaction with adenosylhomocysteine hydrolase. Its modification by oxidation to the sulfoxide or sulfone leads to compounds with some inhibitory activity, whereas substitution of the 5′-sulfur atom by an amine group results in a powerful analogue, N^γ-adenosyl-α,γ-diaminobutyric acid. The amino acid (homocysteinyl) moiety seems less important and can be modified extensively. The 2′- and 3′-hydroxyl groups of the ribose are apparently crucial for the ability of adenosylhomocysteine analogues to bind to the enzyme, as shown by the negligible inhibition observed for S-2′- and S-3′-deoxyadenosylhomocysteine. Substitution of ribose with β-arabinofuranose results in an analogue, compound 32, with marked inhibitory properties. The insertion of a halogen atom in position 2 of adenine results in a series of analogues with good inhibitory potential, compounds 38–40.

The remarkable effect of 3-deazaadenosine on the levels of adenosylhomocysteine and adenosylmethionine, and the appearance of *S*-3-deazaadenosylhomocysteine in isolated rat hepatocytes, demonstrates the feasibility of correlating information derived from enzyme-analogue interaction with the pharmacological effects of the analogue on a biological system. The increased levels of adenosylhomocysteine in rat hepatocytes are consistent with the inhibition of adenosylhomocysteine hydrolase by 3-deazaadenosine observed *in vitro*. It can be postulated that the increase in the concentrations of adenosylmethionine was due to the inhibition of biological transmethylations by the elevated levels of adenosylhomocysteine and probably *S*-3-deazaadenosylhomocysteine. However, other possibilities have not been excluded. Further experiments on the effects of 3-deazaadenosine on cell systems are being explored in this laboratory.

Some of the analogues tested in this study have also been studied in other laboratories as potential inhibitors of some methyltransferases catalyzing the methylation of catechols, indoleamines, and tRNA (14–21). It is noteworthy, however, that the activity of some of these analogues as methyltransferase inhibitors does not correspond to their ability to inhibit adenosylhomocysteine hydrolase; for example, *S*-tubercidinylhomocysteine and *S*-2′-deoxyadenosylhomocysteine are effective inhibitors of several methyltransferases, but are not active as inhibitors of adenosylhomocysteine hydrolase. By contrast, 5′-deoxy-5′-isobutylthioadenosine, which is about 1000 times less effective than adenosylhomocysteine as an inhibitor of tRNA methyltransferases *in vitro* (14), was found, as demonstrated by the present investigation, to be an inhibitor of adenosylhomocysteine hydrolase.

It is of interest that both 5′-deoxy-5′-isobutylthioadenosine and 5′-deoxy-5′-butylthioadenosine were found to be effective inhibitors of virus-induced transformation of chick embryo fibroblasts *in vitro* (14, 15). We will report elsewhere the results of experiments that demonstrate that 0.1 mM 3-deazaadenosine is also capable of inhibiting both cell transformation induced by Rous sarcoma virus *in vitro* and the production of virus by these transformed cells.[3]

ACKNOWLEDGMENTS

The collaboration of Dr. N. W. Cornell in the experiments with rat hepatocytes is acknowledged. We also thank Drs. R. T. Borchardt, J. A. Montgomery, and J. Coward for their gifts of analogues, and Drug Research and Development, Chemotherapy, National Cancer Institute, for providing the majority of the analogues.

REFERENCES

1. Cantoni, G. L. & Scarano, E. (1954) *J. Am. Chem. Soc.*, 76, 4744.
2. de la Haba, G. & Cantoni, G. L. (1959) *J. Biol. Chem.*, 234, 603–608.
3. Palmer, J. L. & Abeles, R. H. (1976) *J. Biol. Chem.*, 251, 5817–5819.
4. Zappia, V., Zydek-Cwik, C. R. & Schlenk, F. (1969) *J. Biol. Chem.*, 244, 4499–4509.
5. Hurwitz, J., Gold, M. & Anders, M. (1964) *J. Biol. Chem.*, 239, 3474–3482.
6. Deguchi, T. & Barchas, J. (1971) *J. Biol. Chem.*, 246, 3175–3181.
7. Kerr, S. J. (1972) *J. Biol. Chem.*, 247, 4248–4252.
8. Coward, J. K., Slisz, E. P. & Wu, F. Y.-H. (1973) *Biochemistry*, 12, 2291–2297.
9. Coward, J. K., Bussolotti, D. L. & Chang, C.-D. (1974) *J. Med. Chem.*, 17, 1286–1289.
10. Borchardt, R. T. (1977) in *The Biochemistry of S-Adenosylmethionine* (Salvatore, F. & Borek, E., eds.), Columbia University Press, New York, in press.
11. Finkelstein, J. D. & Harris, B. J. (1973) *Arch. Biochem. Biophys.*, 159, 160–165.
12. Finkelstein, J. D., Kyle, W. E. & Harris, B. J. (1974) *Arch. Biochem. Biophys.*, 165, 774–779.
13. Hildesheim, J., Hildesheim, R. & Lederer, E. (1972) *Biochimie*, 54, 431–437.
14. Robert-Gero, M., Lawrence, F., Farrugia, G., Berneman, A., Blanchard, P., Vigier, P. & Lederer, E. (1975) *Biochem. Biophys. Res. Commun.*, 65, 1242–1249.
15. Bona, C., Robert-Gero, M. & Lederer, E. (1976) *Biochem. Biophys. Res. Commun.*, 70, 622–629.
16. Borchardt, R. T. & Wu, Y. S. (1974) *J. Med. Chem.*, 17, 862–868.
17. Borchardt, R. T., Huber, J. A. & Wu, Y. S. (1974) *J. Med. Chem.*, 17, 868–873.
18. Borchardt, R. T. (1975) *Biochem. Pharmacol.*,

---

[3] J. Bader, P. K. Chiang, and G. L. Cantoni, manuscript in preparation.

24, 1542–1544.

19. Borchardt, R. T., Huber, J. A. & Wu, Y. S. (1976) *J. Med. Chem.*, 19, 1094–1099.
20. Chang, C.-D. & Coward, J. K. (1975) *Mol. Pharmacol.*, 11, 701–707.
21. Chang, C.-D. & Coward, J. K. (1976) *J. Med. Chem.*, 19, 684–691.
22. Gustin, N. C. & Kemp, R. G. (1976) *Anal. Biochem.*, 71, 527–532.
23. Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) in *Alfred Benzon Symposium VI*, pp. 718–743, Munksgaard, Copenhagen.
24. Hoffman, J. (1975) *Anal. Biochem.*, 68, 522–530.
25. Layne, E. (1957) *Methods Enzymol.*, 3, 450–454.