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INACTIVATION OF S-ADENOSYLHOMOCYSTEINE HYDROLASE BY 5'-DEOXY-5'-METHYLTHIOADENOSINE

Adolph J. Ferro[a], Arthur A. Vandenbark[b,c], and Margaret R. MacDonald[a]

[a]Department of Microbiology, Oregon State University, Corvallis, OR 97331

[b]Surgical Research Laboratory, Veterans Administration Medical Center, Portland, OR 97201, and [c]Department of Microbiology and Department of Neurology, University of Oregon Health Sciences Center, Portland, OR 97201

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Summary. S-Adenosylhomocysteine hydrolase from human red blood cells is inactivated by the naturally occurring metabolite, 5'-deoxy-5'-methylthioadenosine. Utilizing an 1896-fold purified enzyme preparation, the kinetics of inactivation by 5'-deoxy-5'-methylthioadenosine were examined and a K_I value of 36 μM determined. Neither 5-methylthioribose nor adenine, degradation products of 5'-deoxy-5'-methylthioadenosine, inactivated the hydrolase, while both S-adenosylhomocysteine and adenosine protected the enzyme from inactivation by 5'-deoxy-5'-methylthioadenosine. The inactivation of S-adenosylhomocysteine hydrolase by 5'-deoxy-5'-methylthioadenosine may explain the cell growth inhibitory properties of this nucleoside.

5'-Deoxy-5'-methylthioadenosine (MTA) is synthesized from S-adenosylmethionine (SAM) in mammalian cells by several biosynthetic routes, most notably during the synthesis of the polyamines spermidine and spermine (1). This sulfur-containing nucleoside, however, does not appear to normally accumulate, but rather is either excreted from cells (2) or is rapidly degraded. MTA phosphorylase is the only known enzyme in mammalian cells capable of degrading MTA and has now been identified in a variety of normal and transformed mammalian tissues (3-9). The products of the phosphorolytic cleavage of MTA are 5-methylthioribose-1-phosphate and adenine (3,6).

Abbreviations: MTA, 5'-deoxy-5'-methylthioadenosine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

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Vandenbark et al. (10) first described the cytostatic action of MTA to human peripheral lymphocyte cultures stimulated with mitogens, antigens, or allogeneic cells. Recently, Pegg et al. (11) also demonstrated the growth inhibitory effects of this nucleoside on SV-3T3 fibroblast cells.

Adenosine and several of its structural analogs are inhibitory to a variety of physiological and biochemical processes. One hypothesis explaining the cytotoxic action of these nucleosides suggests that toxicity is mediated through inhibition of S-adenosylhomocysteine (SAH) hydrolase (12). SAH hydrolase is responsible for the catabolism of SAH to adenosine and homocysteine in mammalian cells (13,14). Since the equilibrium constant for SAH hydrolase greatly favors the synthesis of SAH over its degradation (13), the exposure of cells to adenosine and homocysteine results in the accumulation of higher than normal levels of SAH (12). In addition, nucleosides such as 2'-deoxyadenosine have been shown to irreversibly inactivate SAH hydrolase (15). Increased SAH levels have been shown to have two main effects: 1) inhibition of transmethylation reactions (12,16), and 2) enhancement of the cells response to several adenylate cyclase activators (17). Either or both of these mechanisms may be responsible for the toxicity observed with adenosine.

Here it will be shown that SAH hydrolase activity is inhibited by MTA. In addition, the inhibitory effect of MTA on SAH hydrolase is compared to 2'-deoxyadenosine, and our results suggest that the inactivation of the enzyme by MTA may be responsible for the inhibitory action of this nucleoside on cellular proliferation.

EXPERIMENTAL PROCEDURES

Labeled MTA was prepared from S-adenosyl-L-[methyl-^{14}C]-methionine (Amersham, 59 mCi/nmol) by the procedure of Schlenk and Ehniger (18). SAH hydrolase activity was assayed in the direction of synthesis. Cell extract was incubated in a reaction mixture (0.06 ml final volume) containing 3.3 mM L-homocysteine, 16.7 μM EHNA (erythro-9-(2-hydroxy-3-

nonyl)adenine), 20 mM K_2HPO_4 (pH 7.6), 0.8 mM DTT, 0.8 mM EDTA, 0.003% sodium azide and 0.17 mM [U-^{14}C]adenosine (2.2 x 10^7 cpm/μmol, Amersham). Following incubation for various times at 37°C, assays were stopped by removing a 0.05 ml aliquot and adding it to 0.015 ml of 1.42 M perchloric acid on ice. After 5 min, 0.025 ml of 0.72 M KOH containing 0.6 M $KHCO_3$ was added and the tubes left on ice 30 min (19). Cold standards containing hypoxanthine, inosine, SAH, and adenosine were added to the neutralized reaction mixture, the resulting precipitate removed by centrifugation, aliquots spotted on cellulose F_{254} thin layer sheets, and developed in n-butanol/acetone/acetic acid/water (35:35:10:20 v/v). The ultraviolet absorbing spots corresponding to SAH and adenosine were scraped and their radioactivities measured in a liquid scintillation spectrometer. MTA phosphorylase activity was assayed by measuring the conversion of [$^{14}CH_3$]MTA to 5-[$^{14}CH_3$]methylthioribose-1-phosphate (20).

Human red blood cells were separated from whole blood and homogenized 5 min in two volumes of 0.01 N acetic acid. After spinning 20 min at 14,000 xg the supernatant fluid was filtered through glass wool and adjusted to pH 7.6 with 2 N NaOH. Dithiothreitol (DTT) and EDTA were added to 2 mM and 1 mM, respectively. Powdered $(NH_4)_2SO_4$ was added to 40% saturation; the pH was continually adjusted to 7.6. The precipitate obtained after centrifugation (14,000xg) was resuspended in 10 mM potassium phosphate (pH 7.6) containing 2 mM DTT and 1 mM EDTA and dialyzed against the same buffer overnight. The enzyme was then stirred 15 min on ice with 10 ml of settled DEAE which had been equilibrated with the dialysis buffer. The DEAE was washed with 20 mM potassium phosphate (pH 7.6), containing 2 mM DTT and 1 mM EDTA, and eluted with 40 ml of the same buffer containing, in addition, 60 mM $(NH_4)_2SO_4$. $(NH_4)_2SO_4$ was added to saturation, and the precipitate resulting from centrifugation (14,000xg) was suspended in 10 ml 8 mM potassium phosphate (pH 7.6 containing 5 mM DTT and 1 mM EDTA and dialyzed overnight against the same buffer $(NH_4)_2SO_4$ was then added to 40, 50, 60, and 70% saturation, the reseptive precipitates collected by centrifugation, (15,000xg) and resuspended in 5 ml 0.01 M potassium phosphate (pH 7.6) containing 5 mM DTT and 1 mM EDTA. After dialysis overnight against this same buffer, the activity was determined to be in the 50% $(NH_4)_2SO_4$ cut.

RESULTS

The effect of MTA and 2'-deoxyadenosine on human red blood cell SAH hydrolase is shown in Table 1. In preliminary experimenta, preincubation of the cell extract with 600 μM MTA for 10 min inhibited SAH hydrolase 71%, while under the same conditions, the presence of 600 μM 2'-deoxyadenosine resulted in a 74% inactivation. Prolonged dialysis of the inactivated enzyme preparations did not significantly restore activity to either the MTA or 2'-deoxyadenosine inactivated enzyme.

To establish whether the inactivation of the hydrolase observed with MTA was due directly to MTA or to products from the metabolism of the

Table 1

Effect of MTA and 2'-Deoxyadenosine on Human Red Blood Cell S-Adenosylhomocysteine Hydrolase

Addition	SAH Hydrolase Activity ($pmol \cdot mg^{-1} \cdot min^{-1}$)	
	before dialysis	after dialysis
none	26.2	23.2
600 μM MTA	7.7	11.2
600 μM 2'-deoxyadenosine	6.9	9.5

Crude enzyme was preincubated with 600 μM MTA or 2'-deoxyadenosine for 10 min at 37° and then assayed for SAH Hydrolase activity. After dialysis of the treated extracts for 48 h against 0.01 M potassium phosphate (pH 7.6) containing 5 mM dithiothreitol and 1 mM EDTA, enzyme activity was reassayed.

nucleoside by the crude extract, the SAH hydrolase was purified over 1800-fold by ammonium sulfate fractionation and DEAE chromatography (Table 2). The resulting 50% pellet from the second ammonium sulfate fractionation contained the SAH hydrolase activity, while MTA phosphorylase activity (data not shown) was found mainly in the 70% pellet. Only 1.3% of the original phosphorylase activity was found in the 50% sulfate fraction.

The effect of MTA on the 1896-fold purified enzyme preparation is shown in Fig. 1. The enzyme was preincubated with MTA (0-600 μM) for 0, 10, 20, and 30 min, then assayed for SAH hydrolase activity. The kinetics of inactivation by MTA are similar to those observed for 2'-deoxyadenosine (15), and from a reciprocal plot of first order rate

Table 2

Purification of Human Red Blood Cell S-Adenosylhomocysteine Hydrolase

Step	Total Protein ($mg \cdot ml^{-1}$)	Total Activity ($nmol \cdot min^{-1}$)	Specific Activity ($pmol \cdot mg^{-1} \cdot min^{-1}$)	Purification (-Fold)
Crude extract	105.7	648	34	1
$(NH_4)_2SO_4$ (40%)	17.6	235	1,027	30
DEAE	0.74	228	23,381	686
$(NH_4)_2SO_4$ (50%)	0.745	226	64,660	1,896

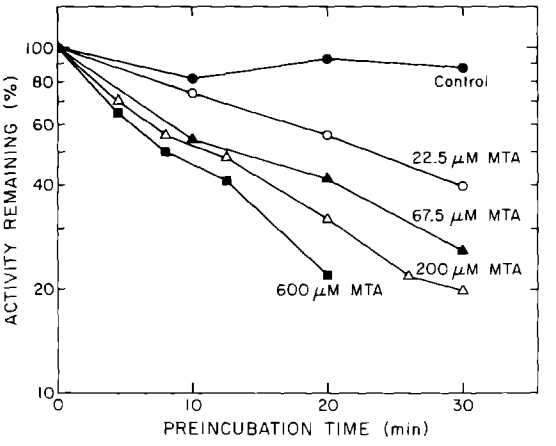

Fig. 1. The kinetics of inactivation of human red blood cell SAH hydrolase by MTA. The enzyme was preincubated for the designated times with the following concentrations of MTA: (●), none; (○), 22.5 μM; (▲), 67.5 μM; (△) 200 μM MTA; (■), 600 μM MTA.

constants versus MTA concentrations, a K_I value (an index of the equilibrium constant for the MTA-enzyme complex) of 36 μM was determined.

To further ensure that inactivation of SAH hydrolase by MTA was not due to degradation products of MTA, the effect of 5-methylthioribose and adenine was also determined (Fig. 2). Both MTA (67.5 μM) and 2'-deoxy-

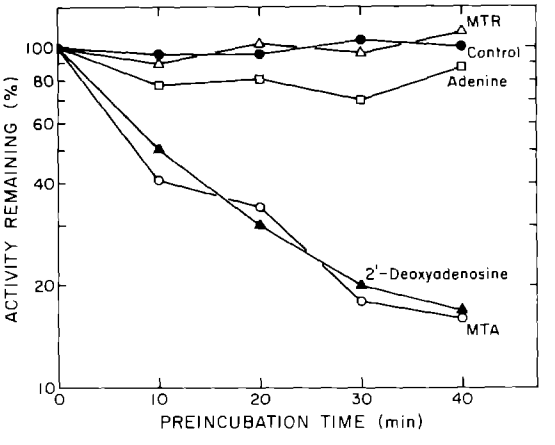

Fig. 2. The kinetics of inactivation of human red blood cell SAH hydrolase by MTA and 2'-deoxyadenosine. The enzyme was preincubated for the designated times under the following conditions: (●), no supplement; (△), plus 67.5 μM 5-methylthioribose (MTR); (□), plus 67.5 μM adenine; (▲), plus 67.5 μM 2'-deoxyadenosine; (○), plus 67.5 μM MTA.

Table 3

Protection from MTA Inactivation of
S-Adenosylhomocysteine Hydrolase by Substrates

Addition	% Activity Remaining	
	Preincubation time	
	10 min	30 min
67.5 μM MTA	46	21
67.5 μM MTA + 20 μM SAH	62	41
67.5 μM MTA + 20 μM Adenosine	60	39
67.5 μM MTA + 1.25 mM Homocysteine	40	21
67.5 μM MTA + 20 μM SAM	45	21

adenosine (67.5 μM) inactivated the enzyme at about the same rate, whereas neither adenine nor 5-methylthioribose were effective at these same concentrations. Under these same conditions, adenine at concentrations up to 600 μM was unable to inactivate the hydrolase to any significant extent (data not shown).

SAH hydrolase is protected against inactivation by 2'-deoxyadenosine by the presence of the purine-containing substrates or products (SAH or adenosine), but not by homocysteine (15). To determine whether inactivation of SAH hydrolase by MTA could also be protected or slowed by these same compounds, SAH hydrolase activity was measured after preincubation of the enzyme preparation for 10 and 30 min in the presence of 67.5 μM MTA and MTA plus either 20 μM adenosine, 20 μM SAH, 1.25 mM homocysteine, or 20 μM SAM (Table 3). SAH and adenosine slowed the rate of inactivation by MTA, whereas, SAM or even high concentrations of homocysteine were without effect.

DISCUSSION

MTA is a naturally occurring metabolite of SAM recently found to inhibit human lymphocyte blastogenesis (10) and the growth of SV-40-virus-transformed 3T3 mouse embryo fibroblasts (11). The observations that MTA is a potent *in vitro* inhibitor of spermidine and spermine

synthases (21-22), suggest that this nucleoside may be exerting its inhibitory effect via the inhibition of polyamine biosynthesis. Pegg et al (11), however, showed that although exogenous MTA inhibited cell growth and decreased the endogenous spermidine content, cell growth could not be restored by the addition of spermidine, suggesting that MTA may have another inhibitory action toward cellular proliferation.

The present work suggests that this alternative site of action may be SAH hydrolase. Several analogs of adenosine are inhibitors of SAH hydrolase and some have been shown to create elevated intracellular SAH levels (23,24). Recently it has been demonstrated that 2'-deoxyadenosine irreversibly inactivates the hydrolase with characteristics similar to suicide inactivation (15), while this paper presents data which shows that MTA inactivates SAH hydrolase activity, and is in agreement with preliminary observations discussed earlier (15). Abeles et al (25) have proposed that during inactivation of SAH hydrolase by 2'-deoxyadenosine, the 3'-position of 2'-deoxyadenosine is oxidized by enzyme bound NAD and that the keto group formed at C-3' activates the hydrogen at C-2' and elimination of adenine occurs. Since this trans-elimination can occur with 2'-deoxyadenosine and ara-adenosine, but not with adenosine (25), it would also not be expected to occur with MTA. Thus, although MTA and 2'-deoxyadenosine both inactivate SAH hydrolase, the mechanisms by which they do so probably are different.

Both studies demonstrating the growth inhibitory effects of MTA (10,11) suggest that MTA phosphorylase may play a critical role in maintaining low intracellular levels of MTA and that this nucleoside itself is exerting the inhibitory action. The present study demonstrates that neither 5-methylthioribose nor adenine, both degradation products of MTA, inactivate the red blood cell SAH hydrolase, supporting this proposal. That adenine does inhibit the rat brain enzyme (26), but not the purified red blood cell preparation used here, may be due to the

source of cells utilized, purity of the extract or concentrations of adenine used. Studies investigating the possibility that exogenously supplied MTA may create an increased intracellular level of SAH are now in progress.

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REFERENCES

1. Pegg, A. E., and Williams-Ashman, H. G. (1969) J. Biol. Chem. 244, 682-693.
2. Kamatani, N., and Carson, D. A. (1980) Cancer Res. 40, 4178-4182.
3. Pegg, A. E., and Williams-Ashman, H. G. (1969) Biochem. J. 115, 241-247.
4. Toohey, J. I. (1978) Biochem. Biophys. Res. Commun. 83, 27-35.
5. Zappia, V., Oliva, A., Galletti, P., Mignucci, G., and Carteni-Farina, M. (1978) Biochem. J. 175, 1043-1050.
6. Ferro, A. J., Wrobel, N. C., and Nicolette, J. A. (1979) Biochim. Biophys. Acta 570, 65-73.
7. Ferro, A. J., Vandenbark, A. A., and Marchitto, K. (1979) Biochim. Biophys. Acta 588, 294-301.
8. Garbers, D. L. (1980) Biochim. Biophys. Acta 523, 82-93.
9. Savarese, T., Crabtree, G. W., and Parks, R. E. (1979) Biochem. Pharmacol. 28, 2227-2230.
10. Vandenbark, A. A., Ferro, A. J., and Barney, C. L. (1980) Cell Immunol. 49, 26-33.
11. Pegg, A. E., Borchardt, R. T., and Coward, J. K. (1981) Biochem. J. 194, 79-89.
12. Kredich, N. M., and Martin, D. W., Jr. (1977) Cell 12, 931-938.
13. DeLaHaba, G. and Cantoni, G. L. (1959) J. Biol. Chem. 234, 603-608.
14. Walker, R. D. and Duerre, J. A. (1975) Can. J. Biochem.
15. Hershfield, M. S. (1979) J. Biol. Chem. 254, 22-25.
16. Hirata, F., Toxorshima, S., Axelrod, J. and Waxdal, M. J. (1980) Proc. Nat. Acad. Sci. 77, 862-865.
17. Zimmerman, T. P., Schmitges, C. J., Wolberg, G., Deeprose, R. D., Duncan, G. S., Cuatrecasas, P., and Elion, G. B. (1980) Proc. Nat. Acad. Sci. 77, 5639-5643.
18. Schlenk, F., and Ehninger, D. J. (1964) Arch. Biochem. Biophys. 106, 95-100.
19. Uelandm P. M., and Saebø, J. (1979) Biochem. Biophys. Acta. 585, 512-526.
20. Ferro, A. J., Barrett, A., and Shapiro, S. K. (1976) Biochim. Biophys. Acta 438, 487-494.
21. Pajula, R.-L., and Raina, A. (1979) FEBS Lett. 99, 343-345.
22. Hibasami, H., Borchardt, R. T., Chen, S. Y., Coward, J. K., and Pegg, A. E. (1980) Biochem. J. 187, 419-428.
23. Chiang, P. K., Richards, H. H. and Cantoni, G. L. (1977) Mol. Pharmacol. 13, 939-947.

24. Zimmerman, T. P., Wolberg, G., Duncan, G. S. and Elion, G. B. (1980) Biochemistry 19, 2252-2259.
25. Abeles, R. H., Tashjian, A. H., Jr., and Fish, S. (1980) Biochem. Biophys. Res. Commun. 95, 612-617.
26. Schatz, R. A., Vunnam, R. A., and Sellinger, O. Z. (1979) in: Conference on Transmethylation, (Borchardt, R. T., Creveling, C. R., and Usdin, E., eds.) Elsevier North Holland, Inc., New York, pp. 143-153.