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Biochemical Pharmacology, Vol. 24, pp. 1542–1544. Pergamon Press. 1975 Printed in Great Britain.

Inhibition of indolethylamine-*N*-methyltransferase by analogs of *S*-adenosylhomocysteine

(*Received* 8 *October* 1974; *accepted* 20 *December* 1974)

Indolethylamine-*N*-methyltransferase (INMT), which was first isolated from rabbit lung [1, 2], catalyzes the transfer of the methyl group from *S*-adenosylmethionine (SAM) to the amino group of a variety of indoleamines. Recently, a similar enzyme has been isolated from human brain [3] and serum [4] and has been implicated in certain mental disorders [3–5]. Because of the recent observations by Lin *et al.* [6] that *S*-adenosylhomocysteine (SAH), a general product of all SAM-dependent methyltransferases, inhibited INMT *in vitro*, we decided to explore the specificity of this inhibition using various analogs of SAH, which had been previously prepared in our laboratory [7–9.*].

INMT was isolated from rabbit lung (Pel-Freez Biologicals) according to the procedure of Mandel *et al.* [10] and purified through the Sephadex G-150 step, which resulted in a 28-fold purification of the enzyme with a sp. act. of 16·8 nmoles product/mg of protein/hr. For the INMT assay, $^{14}CH_3$-SAM (New England Nuclear, 55·0 mCi/m-moles) was diluted to a concentration of 10 μCi/ml and stored at −20° F. SAM iodide (Sigma) and *N*-methyltryptamine (Aldrich) were each stored as 0·01 M aqueous stock solutions. Enzyme activity was measured by a previously described radiochemical assay [1] and a normal incubation mixture consisted of the following components (in μmoles): water, so that the final vol was 0·25 ml; *N*-methyltryptamine (0·25); SAM (variable); inhibitor (variable); 0·05 μCi $^{14}CH_3$-SAM; phosphate buffer, pH 7·9 (25); and the enzyme preparation. Assay mixtures were incubated for 60 min at 37° and the reaction was terminated by addition of 0·25 ml of 0·5 M borate buffer, pH 10·0. The aqueous layer was extracted with 10 ml isoamyl alcohol (water saturated) and an aliquot (5 ml) of the organic phase was checked for radioactivity. The enzyme activity was corrected using a *N*-methyltryptamine blank. In the kinetic experiments, inhibition constants were calculated according to the method of Cleland [11] using a Hewlett–Packard 2100A digital computer and a Fortran IV program [12–15]. The synthesis of the SAH analogs used in this study has been previously reported [7–9.*]. SAH analogs were each stored as 0·01 M aqueous stock solutions.

Table 1 shows the degree of inhibition of INMT activity produced by the various structural analogs of SAH. In discussing their inhibitory activities, the analogs have been divided into three general classes: (a) amino acid-modified derivatives; (b) base-modified derivatives; and (c) sugar-modified derivatives. From the low inhibitory activities exhibited by the various amino acid derivatives, it is apparent that INMT shows a fairly high specificity for the structural features of the homocysteine portion of L-SAH. The structural features of primary importance in binding appear to be: (1) the chirality of the amino acid asymmetric carbon; (2) the terminal amino group; (3) the terminal carboxyl group; (4) the sulfur atom; and (5) the 3-carbon distance between the sulfur atom and the terminal amino and carboxyl groups. This high specificity for the homocysteine portion of SAH appears to be a general characteristic of most methyltransferases which have been studied [7].

From the inhibitory activities of the various base-modified derivatives shown in Table 1, it can be concluded that there exists a very strict requirement for the adenine moiety of SAH, particularly the 6-amino group in binding to this enzyme. This conclusion was derived from the fact that substitution of guanine (SGH), hypoxanthine (SIH), uracil (SUH) or cytosine (SCH) in place of adenine in SAH resulted in almost complete loss of inhibitory activity. Interestingly, replacement of the adenine moiety with 3-deaza-adenine (3-deaza-SAH, compound 7, Table 1) produced a potent INMT inhibitor. Therefore, it appears that the nitrogen in the 3-position of adenine is not an absolute requirement for the binding of SAH to INMT. In fact, there may exist a general lack of importance of the adenine ring nitrogens in binding, since Coward† has observed recently that the tubercidin analog of SAH is a potent inhibitor of INMT. Mono-methylation of the 6-amino group of 3-deaza-SAH had little effect on binding, since N^6-methyl-3-deaza-SAH (compound 8) produced strong inhibition of INMT. However, the dimethylamino analog (compound 9) was completely inactive as an inhibitor of INMT. This reduction in activity is probably the result of increased steric bulk at the 6-position rather than an electronic effect [8, 9].

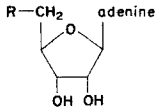
The sugar-modified derivatives of SAH have exhibited a very interesting inhibitory profile. The 2′-hydroxy group of SAH appears to be important in binding to INMT, since 2′-deoxy-SAH (compound 10) was completely inactive as an inhibitor, whereas the arabinose derivative (compound 12) produced strong inhibition of INMT, but less than that for SAH itself. Whether the 2′-hydroxyl group is involved directly in binding or is important in maintaining the proper relative conformation between the base and amino acid portion of SAH has yet to be resolved. 3′-Deoxy-SAH (compound 11) was also found to be a potent inhibitor of INMT. Of the methyltransferases studied in our laboratory, strong inhibition by 3′-deoxy-SAH has

* R. T. Borchardt and Y. S. Wu, *J. med. chem.* **18**, 300 (1975).

† James Coward, Yale University School of Medicine, personal communication.

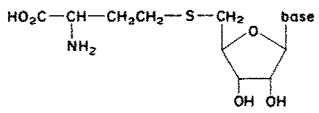
Table 1. Inhibition of INMT by analogs of *S*-adenosylhomocysteine*

Amino acid modifications



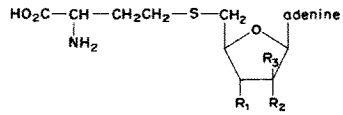
Compound	R	% Inhibition† I = 0·2 mM	I = 2·0 mM
L-SAH	—S—$CH_2CH_2CH(NH_2)CO_2H$(L)	65	95
D-SAH	—S—$CH_2CH_2CH(NH_2)CO_2H$(D)	0	8
SAHO	—S(→O)—$CH_2CH_2CH(NH_2)CO_2H$	0	11
$SAHO_2$	—S(→O)(→O)—$CH_2CH_2CH(NH_2)CO_2H$	0	0
1	—S—$CH_2CH_2CH_2NH_2$	11	19
2	—S—$CH_2CH_2CH_2NHAc$	0	3
3	—S—$CH_2CH_2CH_2CO_2H$	0	14
4	—S—$CH_2CH_2CH_2CO_2CH_3$	0	0
5	—S—$CH_2CH_2CH(NAc)CO_2H$	0	0
6	—S—$CH_2CH(NH_2)CO_2H$	0	0

Base modifications



Compound	Base	% Inhibition I = 0·2 mM	I = 2·0 mM
SGH	Guanine	0	6
SIH	Hypoxanthine	4	6
SUH	Uracil	0	6
SCH	Cytosine	0	6
7	3-Deaza-adenine	42	84
8	N^6-methyl-3-deaza-adenine	26	79
9	N^6-dimethyl-3-deaza-adenine	4	16

Sugar modifications



Compound	R_1	R_2	R_3	% Inhibition I = 0·2 mM	I = 2·0 mM
10	OH	H	H	8	15
11	H	OH	H	35	86
12	OH	H	OH	14	59

* INMT was purified and assayed as described in the text. Assay conditions were: SAM concentration, 1·0 mM; $^{14}CH_3$-SAM, 0·05 μCi; *N*-methyltryptamine concentration, 1·0 mM; phosphate buffer, pH 7·9; inhibitor concentration (I), 0·2 or 2·0 mM. Incubations were carried out at 37° for 60 min and the reactions stopped with 0·25 ml of 0·5 M borate buffer, pH 10·0. The aqueous layers were extracted with 10 ml isoamyl alcohol as described in the text.

† Expressed as per cent inhibition of the *N*-methylation of *N*-methyltryptamine. Data represent averages of duplicate runs.

been observed with INMT, phenylethanolamine-*N*-methyltransferase (PNMT)* and catechol-O-methyltransferase (COMT).*

The kinetic patterns for inhibition of INMT by SAH, and several of the active SAH analogs were determined and in all cases found to be competitive with varying SAM (Table 2). The high affinity of INMT for SAH is indicated by the K_i values 8·65 ± 0·71 μM for SAH inhibition compared to a K_m 54·3 ± 5·7 μM for SAM. From the data shown in Table 2, it is apparent that 3-deaza-SAH, N^6-methyl-3-deaza-SAH, 3′-deoxy-SAH and the arabinose derivative are nearly as potent as SAH itself in inhibiting

Table 2. Comparison of the inhibition constants for SAH analogs toward INMT, COMT, PNMT, HMT and HIOMT

Inhibitor	Inhibition constants*† ($K_i \pm$ S. E. M., μM)				
	INMT‡	COMT§	PNMT§	HMT§	HIOMT§
SAH	8·65 ± 0·71	36·3 ± 2·2	29·0 ± 2·8	18·1 ± 2·2	18·5 ± 1·9
3-Deaza-SAH	26·6 ± 1·2	80·6 ± 5·0	81·1 ± 14·9	59·2 ± 6·1	229 ± 11
N^6-methyl-3-deaza-SAH	70·2 ± 6·6		1243 ± 141		
3′-Deoxy-SAH	51·9 ± 7·8	138 ± 31	42·7 ± 2·5	2070 ± 864	
Arabinose derivative	85 ± 8·7		206 ± 27		

* When low inhibitory activity was observed from preliminary studies, no extensive studies were done to determine the inhibition constants.

† Each inhibitor showed linear competitive kinetics when plots of the reciprocal velocities vs reciprocal SAM concentrations were made. The inhibition constants were calculated as previously described [11–15].

‡ INMT was purified and assayed as described in the text and Table 1. SAM concentration = 24–210 μM; *N*-methyltryptamine concentration = 1·0 mM; inhibitor concentrations = 20–400 μM. In general, at least three concentrations of each inhibitor were used in determining the inhibition constants.

§ Data taken from Ref. 8 and from R. T. Borchardt and Y. S. Wu, *J. med. chem.* **18**, 300 (1975).

INMT. For the sake of comparison, the inhibition constants for these analogs toward PNMT, COMT, histamine-*N*-methyltransferase (HMT) and hydroxyindole-*O*-methyltransferase (HIOMT) are also listed in Table 2 [8,*]. The relatively strong inhibitory activity of N^6-methyl-3-deaza-SAH is of particular interest, because INMT appears to be the only methyltransferase investigated in our laboratory [8] which is strongly inhibited by this analog. A related compound, *N*-methyl-SAH was previously reported to inhibit tRNA methylase [16] and PNMT [8]. Another interesting difference in the inhibition profile for INMT is the strong inhibition produced by 3′-deoxy-SAH and the arabinose derivative. The only other enzyme showing a similar profile is PNMT [8].

From the inhibitory activities observed for the various analogs used in this study, it can be generally concluded that INMT shows a fairly strict specificity for the structural features of SAH, which appears to be common for most of the methyltransferases studied [9]. However, several unique characteristics of the binding site on INMT have been observed and several analogs of SAH have shown potent inhibitory activity toward INMT (3-deaza-SAH, N^6-methyl-3-deaza-SAH and 3′-deoxy-SAH). These observations suggest that analogs of SAH could be useful for the inhibition *in vivo* of this enzyme. This possibility is presently being explored in our laboratory.

Acknowledgements—The author gratefully acknowledges support of this project by a Research Grant from the National Institutes of Neurological Diseases and Stroke (NS-10198) and a Grant-in-Aid from the American Heart Association with funds contributed in part by the Kansas Heart Association. This work was done during the tenure of an Established Investigatorship of the American Heart Association. The excellent technical assistance of Sharon Elrod and the synthetic capabilities of Yih Shiong Wu and Joan A. Huber are gratefully acknowledged.

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Biochemical Pharmacology, Vol 24, pp 1544–1547. Pergamon Press, 1975 Printed in Great Britain.

Repression of dimethylnitrosamine-demethylase by typical inducers of microsomal mixed-function oxidases

(*Received* 7 *October* 1974; *accepted* 17 *January* 1975)

Previous investigations in this laboratory have shown that the synthesis of the microsomal mixed-function oxidase, which *N*-demethylates the carcinogen, dimethylnitrosamine (DMN), is inhibited by pretreatment of the animals with the typical enzyme inducers, 3-methylcholanthrene (MC) [1–3] as well as other polycyclic hydrocarbons [4]