INHIBITION OF INDOLETHYLAMINE-N-METHYLTRANSFERASE BY S-ADENOSYLHOMOCYSTEINE

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SUMMARY

S-Adenosylhomocysteine (SAH) is a potent product inhibitor for indoleamine-N-methyltransferase (INMT) from rabbit lung. The kinetic studies showed that this inhibition was competitive with respect to S-adenosylmethionine (SAM) and noncompetitive with respect to N-methylserotonin (NMS). The K_i value of 1.0×10^{-5}M indicated that SAH had a higher affinity than SAM or NMS for the enzyme. SAH seems to form a reversible complex with the enzyme.

INTRODUCTION

In a series of studies reported from our laboratories (1,2,3,4), N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine and 5-hydroxy-N,N-dimethyltryptamine (bufotenin) have been identified in urine and blood of patients with a diagnosis of schizophrenia. The presence of indoleamine-N-methyltransferase (INMT), the enzyme which catalyzes the formation of these psychotogenic compounds, has been demonstrated in lung of rabbit (5,6,7) and human (8), in brain of rat (9), chick (10) sheep and human (11), and in human serum (12).

As in the case of other methylating enzymes, INMT requires S-adenosylmethionine (SAM) as the methyl donor. The enzymatic reaction product, S-adenosylhomocysteine (SAH), has been reported to inhibit the activity of many methylating enzymes, such as phenylethanolamine-N-methyltransferase (13), acetylserotonin methyltransferase EC 2.1.1.4 (13), catechol methyltransferase EC 2.1.1.6 (13,14,15), histamine methyltransferase EC 2.1.1.8 (16), tRNA methyltransferase (17) and S-adenosylmethionine:glycine N-methyltransferase (17). The present communication reports an inhibitory action of SAH on the activity of INMT from rabbit lung.

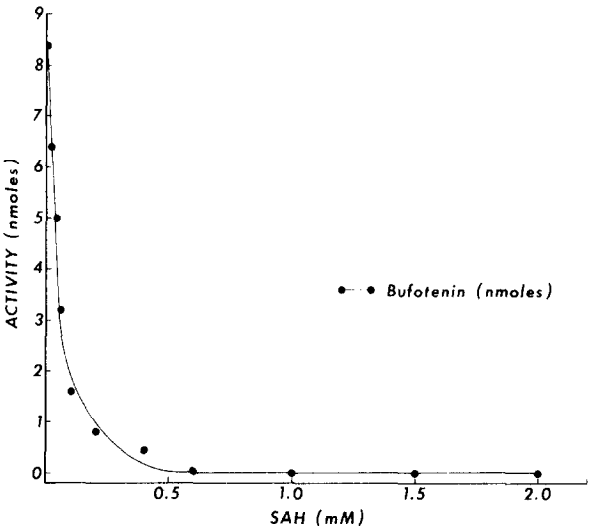

Fig. 1 The effects of changes in the concentration SAH on the activity of INMT.

Enzyme activity was measured under standard assay conditions (see Experimental) with increasing concentrations of SAH.

EXPERIMENTAL

Preparation of INMT

Lung tissue of young adult rabbit was homogenized in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.6) and centrifuged at 100,000 x g for 30 min. Ammonium sulfate was added to the supernatant solution to bring to 40% saturation followed by centrifugation at 10,000 x g for 10 min. The precipitate was discarded and the supernatant solution was brought to 60% saturation of ammonium sulfate. After centrifugation, the final precipitate was dissolved into an amount of 0.1 M potassium phosphate buffer (pH 7.6) equal to one-tenth of the volume of the original supernatant solution. Two ml of this fraction was further purified by passage through a Sephadex G-200 column (2 x 100 cm). The active fractions were pooled and concentrated and used as the source of INMT.

All preparative procedures were carried out at 0-4°C. The protein content of each preparation was determined by the method of Lowry et al. (18).

Assay of INMT

The activity of INMT was measured by methods recently developed in our

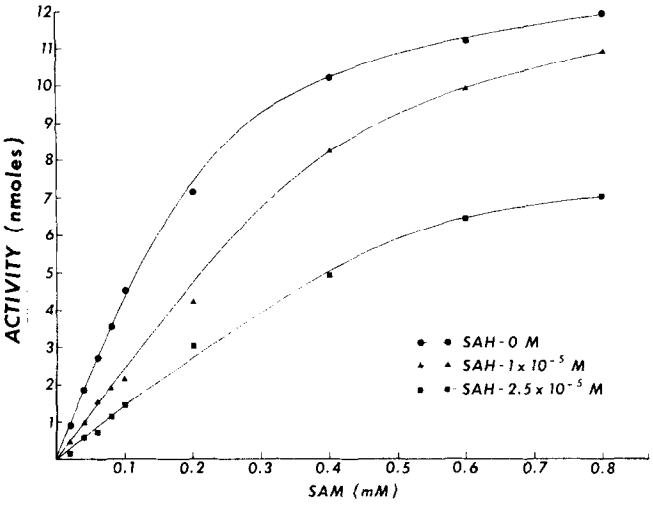

Fig. 2 INMT activity as a function of the concentration of SAM. Enzyme activity was determined under standard assay conditions except for the use of varied concentrations of SAM in the absence and in the presence of SAH as indicated.

laboratories. The validity and detailed descriptions of the methods are the subject of another communication (19). In brief, the assay of INMT was based on the conversion of N-methylserotonin (NMS) to bufotenin, which can be fluorometrically quantitated at the 50 ng level after thin-layer chromatography (TLC) and o-phthalaldehyde (OPT) reaction (20).

Unless otherwise stated, the standard assay mixture contained 100 mM potassium phosphate buffer (pH 7.6), 1 mM NMS, 0.2 mM SAM and 100 µg enzyme protein in a total volume of 0.5 ml. After incubation at 37°C for 90 min, the reaction was stopped by the addition of 0.1 ml of 5% ammonium hydroxide and the mixture was extracted into 12 ml of ethyl acetate on a mechanical shaker for 15 min. After centrifugation, 10 ml of the ethyl acetate fraction was dried under vacuum. The residue was redissolved into 100 µl of ethyl acetate and duplicate samples of 10 µl each were used for one-dimensional TLC with isopropanol, NH_4OH and H_2O (85:15:5) as the solvent system. The TLC plate was sprayed with OPT and the bufotenin spot was eluted with 6 N HCl and read on an Aminco Bowman spectrofluorometer at activation 360 nm and fluorescence 480 nm. A reference standard of 0.5 µg bufotenin was used in duplicate on each TLC plate.

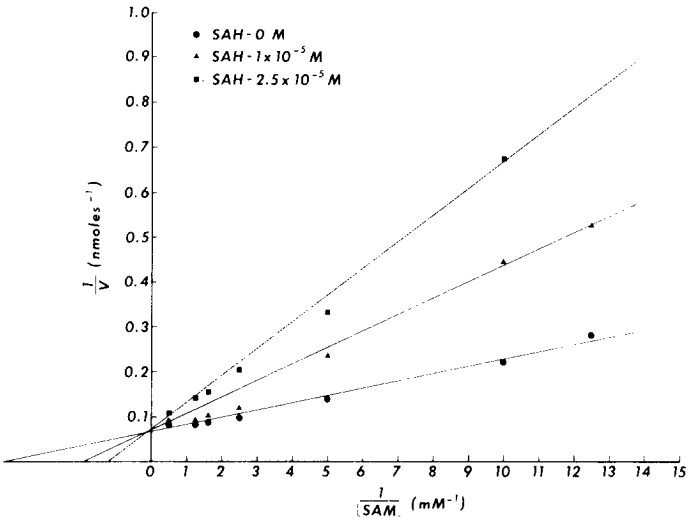

Fig. 3 Inhibition of INMT by SAH: Double-reciprocal plot with SAM as the variable substrate (see Fig. 2).

RESULTS

Inhibition of INMT by SAH

When the INMT preparation was assayed under standard conditions with increasing concentrations of SAH up to 2 mM, the concentration of SAH required to obtain a 50% inhibition of the reaction (I/50) was 5.0 x 10^{-5}M (Fig. 1). At equimolar concentrations of SAH and SAM, a greater than 90% inhibition of the reaction was observed.

Kinetics of Inhibition by SAH

In the study of the kinetics of the inhibition by SAH, two fixed concentrations of SAH were used with increasing concentrations of either SAM or NMS. The results and their corresponding Lineweaver-Burk (21) plots are shown in Figs. 2-5. The double-reciprocal plot indicated that the inhibition by SAH was competitive with respect to SAM and noncompetitive with respect to NMS. The kinetic constants calculated from the data are as follows: the K_i value was 1.0 x 10^{-5}M for SAH, which, when compared to the K_m value of 2.5 x 10^{-4}M for SAM, indicated the high affinity of SAH for INMT. When the concentration of NMS was varied, the K_m value was 1.0 x 10^{-3}M for NMS and the K_i value was 1.2 x 10^{-5}M for SAH.

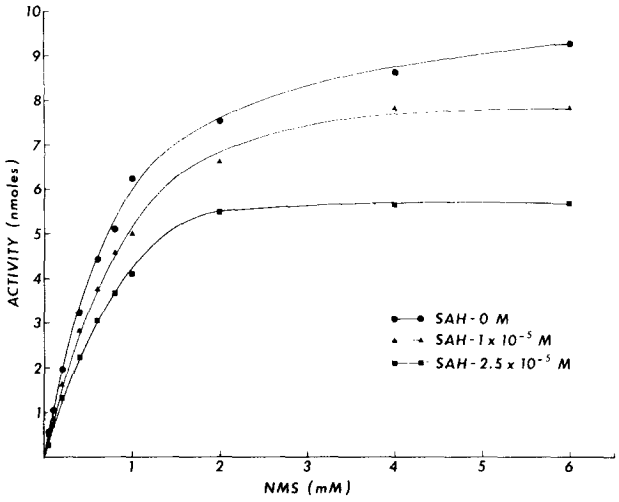

Fig. 4 INMT activity as a function of the concentration of NMS. Enzyme activity was measured under standard assay condition except for the use of varied concentrations of NMS in the absence and in the presence of SAH as indicated.

Preincubation Experiments

Different mixtures (see Table 1) were preincubated at 37°C for 20 min. Incubation was performed under standard assay conditions with or without SAH (10^{-4}M). The results are shown in Table 1. Preincubation of the enzyme with SAH did not enhance, but did slightly release, the inhibition. The strongest inhibition was observed when the enzyme was preincubated with SAM and SAH. That the activity of the enzyme was relatively increased only by preincubation with SAM is of interest, for this indicates that SAM could form a complex with the enzyme before transferring the methyl group. In the other cases, preincubation did not increase the enzyme activity and had no effect on the inhibition by SAH.

In order to determine the effect of dialysis on SAH-inhibited INMT, the purified enzyme was preincubated with SAH (10^{-4}M) at 37°C for 20 min and then dialyzed against 1000 volumes of 0.1 M potassium phosphate buffer (pH 7.6) for 16 hr. The activity of both the dialyzed and nondialyzed enzyme was measured and compared with the activity of enzyme treated in the same way but without SAH, and of nontreated enzyme. The results are shown in Table 2. The fact

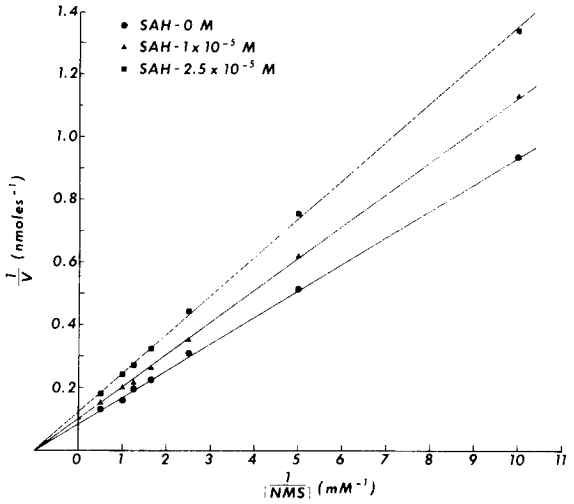

Fig. 5 Inhibition of INMT by SAH: Double-reciprocal plot with NMS as the variable substrate (see Fig. 4).

that the activity of the enzyme was essentially restored by dialysis suggests that the inhibitor may form a reversible complex with the enzyme.

DISCUSSION

The results presented in this communication clearly show that SAH, the product formed during many transmethylations including methylation by INMT, is a potent inhibitor *in vitro* of INMT from rabbit lung. Such an inhibitory action for INMT is first reported here. The concentration of SAH required to obtain a 50% inhibition (I/50) at a 0.2 mM concentration of SAM is about 5.0×10^{-5}M. Since the kinetic study showed that SAH is a competitive inhibitor with respect to SAM, the I/50 depends on the concentration of SAM used in the assay. At equimolar concentrations of SAH and SAM, a greater than 90% inhibition of INMT was observed.

For the INMT used in this study, the K_m for SAM ix 2.5×10^{-4}M and for NMS is 1.0×10^{-3}M. These values are comparable to those previously reported for INMT (6,17,22). That the K_i for SAH is 1.0×10^{-5}M for this INMT indicates a much higher affinity for the enzyme of the product, SAH, than of the substrate, SAM. Such an observation has also been reported for other methyltransferases (13,14,15,16,17).

TABLE 1

Preincubation Experiments with INMT

Preincubation	Incubation	SAH added to Preincubation	SAH added to Incubation	INMT Activity (nmoles/assay)	% of Inhibition
INMT	SAM + NMS	-	-	6.46	--
		+	-	4.07	37
		-	+	3.23	50
INMT + SAM	NMS	-	-	9.77	--
		+	-	3.32	66
		-	+	5.04	48
INMT + NMS	SAM	-	-	6.39	--
		+	-	3.30	48
		-	+	3.15	50
SAM + NMS	INMT	-	-	6.03	--
		+	-	3.33	45
		-	+	3.46	43
SAM	INMT + NMS	-	-	5.91	--
		+	-	3.14	47
		-	+	3.18	47
NMS	INMT + SAM	-	-	6.14	--
		+	-	3.30	46
		-	+	2.88	53

The different preincubations were carried out at 37°C for 20 min.

The incubation was performed under standard assay conditions.

TABLE 2

Effect of Dialysis on SAH-inhibited INMT

Source	INMT Activity (nmoles/assay)
INMT (No preincubation)	6.87
INMT + SAH (No preincubation)	3.55
INMT + SAH (Preincubated)	3.82
INMT + SAH (Preincubated and dialyzed)	6.79

The preincubation was carried out with 10^{-4}M SAH at 37°C for 20 min.

Dialysis was performed against 1000 volumes of 0.1 M potassium phosphate buffer (pH 7.6) at 4°C for 16 hr. Enzyme activity was measured under standard assay conditions.

The preincubation experiments show that when SAM is preincubated with INMT, the activity of the enzyme is enhanced. In addition, the kinetics of product inhibition by SAH indicate that SAH is a competitive inhibitor with respect to SAM and noncompetitive with respect to NMS. These results suggest that the mechanism of this methylation process is probably of the "Ordered Bi Bi" type of Cleland's (23) classification. However, more detailed kinetic studies of the enzyme and the product inhibitions and isotope exchange studies will be required to clarify the true mechanism of the reaction.

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