(2) Abbreviations used are L-SAM, *S*-adenosyl-L-methionine; L-SAM-$^{14}CH_3$, *S*-adenosyl-L-methionine-*methyl-^{14}C*; L-SAH, *S*-adenosyl-L-homocysteine; STH, *S*-tubercidinyl-L-homocysteine; 8-aza-SAH, *S*-8-azaadenosyl-L-homocysteine; N^6-Me-SAH, *S*-N^6-methyladenosyl-L-homocysteine; N^6-Me_2-SAH, *S*-N^6-dimethyladenosyl-L-homocysteine; L-SAC, *S*-adenosyl-L-cysteine; D-SAH, *S*-adenosyl-D-homocysteine; D-SAH sulfoxide, *S*-adenosyl-D-homocysteine sulfoxide; N^{α}-Ac-D-SAH, *S*-adenosyl-D-*N*-acetylhomocysteine; COMT, catechol *O*-methyltransferase (E.C. 2.1.1.6); PNMT, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1.28); HMT, histamine *N*-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole *O*-methyltransferase (E.C. 2.1.1.4); INMT, indoleethylamine *N*-methyltransferase; K_{is}, inhibition constant for the slope.
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Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 5. Role of the Asymmetric Sulfonium Pole in the Enzymatic Binding of *S*-Adenosyl-L-methionine

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The configuration at the asymmetric sulfonium pole of *S*-adenosyl-L-methionine (SAM) necessary for optimal enzymatic binding and methyl donation has been elucidated in this study. For the transmethylations catalyzed by catechol *O*-methyltransferase, phenylethanolamine *N*-methyltransferase, histamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase, it was demonstrated that only the natural (−) enantiomer of SAM was active as a methyl donor. The corresponding (+)-SAM, which was prepared by enzymatic resolution of synthetic (±)-SAM, was shown to be inactive as a methyl donor in these enzymatic reactions. The (+)-SAM was found, however, to be a potent inhibitor of each of these enzyme-catalyzed transmethylations. These results suggest that the (+) enantiomer offers a nonproductive configuration for the methyl-transfer reaction itself; however, this configuration fails to hamper enzymatic binding. These results are discussed relative to the geometric requirements necessary for the methyl-transfer reaction and the requirements for enzymatic binding.

For numerous biological transmethylation reactions, the natural methyl donor is *S*-adenosylmethionine (SAM).[2] Many of the structural features of the amino acid, sugar, and base portions of SAM which are required to produce optimal enzymatic binding and maximal rates of methyl transfer have been elucidated in an accompanying paper in this series.[3] The functional group of fundamental importance in the transmethylation reaction itself is the sulfonium pole. Modifications of the sulfonium center of SAM have primarily involved the replacement of sulfur by selenium[4] and the replacement of the methyl group by an ethyl[4b,5] or by an *n*-propyl[6] group. In addition, earlier studies[7-11] have investigated the role of the configuration of the sulfonium pole in these enzyme-catalyzed transmethylations. Because the absolute configuration of the sulfonium center has not yet been determined, stereoisomers are designated (+) and (−) based on polarimetry. De La Haba et al.[7] have shown that SAM prepared enzymatically using adenosine triphosphate:L-methionine *S*-adenosyltransferase (E.C. 2.5.1.6) has the (−) sulfonium configuration and, therefore, is referred to as (−)-L-SAM. SAM chemically synthesized from the methylation of *S*-adenosyl-L-homocysteine[7] or by condensation of 5′-methylthioadenosine with 2-amino-4-bromobutyric acid[8] is racemic at the sulfonium pole and is referred to as (±)-L-SAM. SAM with the (+) sulfonium configuration [(+)-L-SAM] has been prepared by treatment of (±)-L-SAM with guanidinoacetate methyltransferase (E.C. 2.1.1.2), which selectively utilizes only the (−)-L-SAM as a substrate.[7,10] By using these purified stereoisomers of SAM [(−)-L-SAM, (+)-L-SAM, and (±)-L-SAM], it has been demonstrated that most methyltransferases show a high degree of specificity for the (−) sulfonium configuration in the methyl-transfer reaction itself; e.g., only the (−)-L-SAM was shown to be a substrate for histamine *N*-methyltransferase (HMT),[11] hydroxyindole *O*-methyltransferase (HIOMT),[11] catechol *O*-methyltransferase (COMT),[7] guanidinoacetate methyltransferase,[7,11] and *S*-adenosylmethionine decarboxylase.[11] With each of these enzymes the corresponding (+)-L-SAM was not a methyl

donor. The one interesting exception to this high specificity pattern is homocysteine S-methyltransferase, which is capable of utilizing both the (−)-L-SAM and (+)-L-SAM as substrates.[9,11]

Because our laboratory was investigating analogues of S-adenosyl-L-homocysteine (L-SAH)[12–17] and SAM[3] as inhibitors of SAM-dependent methyltransferases, we became interested in determining why (+)-L-SAM was not a substrate for these methyltransferases. Did the (+) configuration at the sulfonium center of SAM adversely affect enzymatic binding or was the geometry offered by this configuration undesirable for methyl transfer to the acceptor substrate? If the geometry offered by the (+) configuration was nonproductive for methyl transfer, but did not adversely affect enzymatic binding, this would offer a simple way to modify SAM, converting it from a biological methyl donor to an inhibitor of methyltransferases. This possibility has been explored using the transmethylations catalyzed by COMT, HMT, HIOMT, and phenylethanolamine N-methyltransferase (PNMT) and the results are reported in this paper.

Experimental Section

The general experimental techniques and equipment used in this study were described in a preceding paper in this series.[17] The following compounds are commercially available from the indicated sources: 3,4-dihydroxybenzoate (Aldrich); DL-β-phenylethanolamine, histamine dihydrochloride, N-acetylserotonin, (−)-SAM iodide (Sigma); (−)-S-adenosyl-L-methionine-*methyl*-^{14}C (SAM-$^{14}CH_3$, 55.0 mCi/mmol), (−)-S-adenosyl-L-methionine-*carboxyl*-^{14}C (SAM-$^{14}CO_2H$, 54 mCi/mmol) (New England Nuclear); $^{14}CH_3I$ (55.0 mCi/mmol) (Amersham/Searle). S-Adenosyl-L-homocysteine (L-SAH) was synthesized according to a previously described procedure.[18]

(±)-S-Adenosyl-L-methionine [(±)-L-SAM]. The (±)-L-SAM was prepared using a modification[3] of the procedure first described by Jamieson.[19] L-SAH (50 mg, 0.13 mmol) was dissolved in formic acid (2 ml) to which was added an excess of methyl iodide (1.0 ml). The reaction mixture was kept stoppered in the dark for 5 days after which ice-cold water (ca. 5 ml) was added and the unreacted methyl iodide extracted with cold Et_2O. The aqueous layer was lyophilized and the residue dissolved in pH 7.0, 0.01 M phosphate buffer. The buffer solution was applied to a column (2 × 8 cm) of Amberlite IRC-50 ion-exchange resin previously equilibrated with 0.01 M phosphate buffer, pH 7.0. After eluting the unreacted SAH with 100 ml of 0.01 M phosphate buffer, pH 7.0, and 50 ml of 0.25 N HOAc, the (±)-SAM was eluted with 50 ml of 4 N acetic acid. The eluate was lyophilized to yield (±)-L-SAM in 80% yield. The (±)-L-SAM was shown to be homogeneous and indistinguishable from commercially available (−)-L-SAM in four thin-layer chromatography systems. Degradation experiments similar to those described earlier by Zappia et al.[20] further confirmed the SAM structure. These experiments included hydrolysis of (±)-L-SAM using 0.1 N NaOH at 100° for 10 min resulting in the formation of adenine and methionine which could be identified by TLC.

(±)-S-Adenosyl-L-methionine-*methyl*-^{14}C [(±)-L-SAM-$^{14}CH_3$]. The (±)-L-SAM-$^{14}CH_3$ was prepared using a procedure similar to that described above for the unlabeled (±)-L-SAM. L-SAH (5.0 mg, 0.013 mmol) was dissolved in a mixture of HCOOH (0.5 ml) and glacial HOAc (0.05 ml) which contained 100 μCi of $^{14}CH_3I$ (specific activity 0.5 mCi/mmol). The reaction mixture was allowed to stand in the dark for 5 days after which time the desired (±)-L-SAM-$^{14}CH_3$ was isolated as described above for the unlabeled (±)-L-SAM. The specific activity of the isolated (±)-L-SAM-$^{14}CH_3$ varied slightly from batch to batch but generally was approximately 0.4 mCi/mmol (1000 dpm/nmol). The (±)-L-SAM-$^{14}CH_3$ was characterized by its thin-layer chromatographic properties and degradation experiments.[20]

(+)-S-Adenosyl-L-methionine [(+)-L-SAM]. The (±)-L-SAM was enzymatically resolved to yield pure (+)-L-SAM by taking advantage of the substrate specificity of COMT. This enzyme was shown in earlier studies[7] to use only (−)-L-SAM and not (+)-L-SAM as a methyl donor. The enzymatic resolution of (±)-L-SAM reported here is similar to that described earlier by Jamieson,[19] except for the use of COMT instead of guanidinoacetate methyltransferase.

A reaction mixture containing potassium phosphate buffer (pH 7.60, 1500 μmol), 3,4-dihydroxybenzoate (30 μmol), magnesium chloride (18.15 μmol), dithiothreitol (60 μmol), (±)-L-SAM (11.31 μmol), (−)-L-SAM-$^{14}CO_2H$ (0.25 μCi; specific activity 54 mCi/mmol), 12 ml of a COMT preparation (specific activity 24.48 nmol of product/mg of protein/min; protein concentration 9.27 mg/ml), and water to a total volume of 24.3 ml was incubated for 180 min at 37°. The reaction mixture was then immediately filtered through a Millipore filter (HAMK, 25 mm, pore size 0.45 μM). The filtrate was concentrated by lyophilization and the residue dissolved in 2 ml of 0.01 M phosphate buffer, pH 7.0. The phosphate buffer solution was applied to a column (1 × 15 cm) of Amberlite IRC-50 ion-exchange resin previously equilibrated with 0.01 M phosphate buffer, pH 7.0. The excess 3,4-dihydroxybenzoate, the methylated products, and S-adenosyl-L-homocysteine-*carboxyl*-^{14}C (L-SAH-$^{14}CO_2H$) were eluted with 100 ml of 0.01 M phosphate buffer, pH 7.0. An intermediate fraction was eluted with 50 ml of 0.25 N HOAc. The desired (+)-L-SAM was then eluted with about 50 ml of 4 N HOAc and the resulting eluate was lyophilized. The residue was dissolved in 1 ml of water and the concentration of (+)-L-SAM determined by the uv absorbance. The yield of pure (+)-L-SAM was generally 2.5–3.5 μmol (45–62%). The resolved (+)-L-SAM was shown to be homogenous and chromatographically indistinguishable from (±)-L-SAM or (−)-L-SAM in four thin-layer chromatography systems as well as paper chromatography. Degradation experiments similar to those described earlier by Zappia et al.[20] further confirmed the SAM structure of the isolated product.

The resolved (+)-L-SAM was not contaminated with any significant amount of L-SAH, since no L-SAH was observed by TLC or paper chromatography. By including (−)-L-SAM-$^{14}CO_2H$ in the incubation mixture, we were able to label the pool of (−)-L-SAM and also label the pool of SAH (L-SAH-$^{14}CO_2H$) formed during the reaction. This has provided a sensitive method to determine the extent of the reaction [i.e., all of the (−)-L-SAM was consumed] and that the isolated (+)-L-SAM was not contaminated with L-SAH. In the samples of purified (+)-L-SAM only trace amounts of radioactivity were detected indicating that at least 98% of the (−)-L-SAM was consumed and that the samples were free of L-SAH.

Enzyme Isolation and Assay Techniques. The enzymes used in this study were purified from the following sources according to previously described procedures: COMT,[12,21] rat liver (male, Sprague–Dawley, 180–200 g); PNMT,[12,22] bovine adrenal medulla (Pel-Freez Biologicals); HMT,[12,23] guinea pig brain (Pel-Freez Biologicals); and HIOMT,[12,24] bovine pineal glands (Pel-Freez Biologicals). COMT, PNMT, HMT, and HIOMT were assayed using radiochemical techniques measuring the transfer of methyl-^{14}C from (−)-L-SAM-$^{14}CH_3$ to the appropriate acceptor molecules as described in the preceding papers in this series.[12–17]

For each of the enzyme reactions studied the extent of methyl transfer from (−)-L-SAM-$^{14}CH_3$ or (±)-L-SAM-$^{14}CH_3$ to the appropriate acceptor molecules was determined. This was accomplished by prolonged incubation of the appropriate enzyme, the acceptor substrate (250 nmol), and (−)-L-SAM-$^{14}CH_3$ (12.5 nmol, 0.05 μCi) [or (±)-L-SAM-$^{14}CH_3$ (12.5 nmol, 0.005 μCi)] and monitoring the ^{14}C-labeled product formed by simple extraction of the product and counting for radioactivity.[12] In addition, the ^{14}C-labeled products were separated on paper chromatography to confirm the efficiency of the extraction procedures described above and the identity of the products (Figure 2).

Enzyme Kinetics. The (+)-L-SAM prepared in this study was tested as an inhibitor of transmethylations catalyzed by COMT, PNMT, HMT, and HIOMT from (−)-L-SAM-$^{14}CH_3$ to the appropriate acceptor molecules. The procedures used to determine the inhibition constants (K_{is}) are identical with those described earlier in our studies of SAH analogue.[12–17] Processing of the kinetic data was achieved as previously described.[12–17,25,26]

Results and Discussion

Preparation of (±)-L-SAM and (+)-L-SAM. The (±)-L-SAM and (±)-L-SAM-$^{14}CH_3$ were prepared by reaction of L-SAH with CH_3I or $^{14}CH_3I$ in formic acid. This

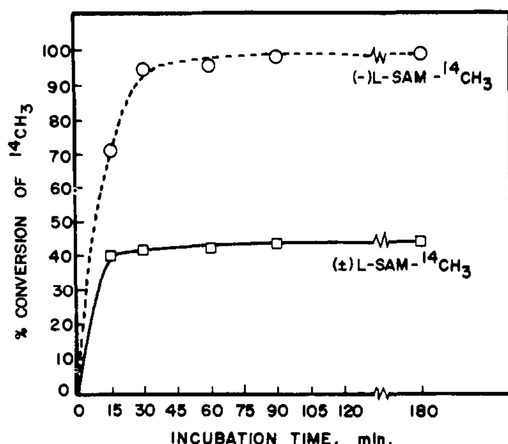


Figure 1. Percent methyl transfer from (−)-L-SAM-$^{14}CH_3$ and (±)-L-SAM-$^{14}CH_3$ to 3,4-dihydroxyacetophenone by COMT. Incubation mixtures were prepared containing 3,4-dihydroxyacetophenone (0.50 μmol), Mg^{2+} (0.30 μmol), dithiothreitol (1.0 μmol), COMT preparation (250 μg), phosphate buffer, pH 7.60 (25 μmol), and (−)-L-SAM-$^{14}CH_3$ (0.05 μCi, 0.025 μmol) [or (±)-L-SAM-$^{14}CH_3$ (0.005 μCi, 0.025 μmol)] in a total volume of 0.25 ml. Incubations were carried out for the indicated times at 37° after which the reactions were stopped with 0.10 ml of 1 N HCl. The assay mixtures were extracted with 10 ml of toluene–isoamyl alcohol (7:3), and after centrifugation a 5-ml aliquot of the organic phase was measured for radioactivity. The results were corrected using the appropriate blanks. Percent conversion of methyl-$^{14}CH_3$ was calculated based on the total labeled methyl donor available. Points represent averages of duplicate determinations.

method for preparing (±)-L-SAM was a modification of the procedure first described by Jamieson.[19] These chemically synthesized samples of SAM were racemic at the sulfonium center and the structures were confirmed by comparison with enzymatically prepared (−)-L-SAM with respect to their chromatographic properties, their NMR and uv spectral properties, and by comparison of the products obtained after hydrolysis under basic conditions.[20]

The (+)-L-SAM was prepared by a process of enzymatic resolution, where the strict substrate specificity of COMT was utilized. Shown in Figure 1 is a comparison of the ability of COMT to use (−)-L-SAM or (±)-L-SAM as methyl donors. When this transmethylation reaction was carried out using (−)-L-SAM-$^{14}CH_3$ as a substrate, complete transfer of the methyl-^{14}C from (−)-L-SAM to the product was observed. However, if (±)-L-SAM-$^{14}CH_3$ was used as a substrate, no more than 50% conversion of the methyl-^{14}C to the product was detected. These results are consistent with the earlier observations[7] that methyltransferases, in general, utilize only one of two possible isomers at the sulfonium center. The interpretation of the data for (±)-L-SAM shown in Figure 1 would be that COMT also preferentially utilizes one sulfonium isomer as a methyl donor. This is consistent with data previously reported by De La Haba et al.[7]

To further substantiate these findings, incubation mixtures containing COMT similar to those described in Figure 1 were prepared using either (−)-L-SAM-$^{14}CH_3$ or (±)-L-SAM-$^{14}CH_3$ as substrates and the products characterized by paper chromatography. Prior to incubation, samples were removed and chromatographed on paper to determine the identity of the radioactive material. With both the (−)-L-SAM-$^{14}CH_3$ and (±)-L-SAM-$^{14}CH_3$ incubation mixtures, the radioactivity had the same R_f values as SAM (Figure 2). These reaction mixtures were then incubated at 37° for 180 min after which time another paper chromatogram was run and the results are also

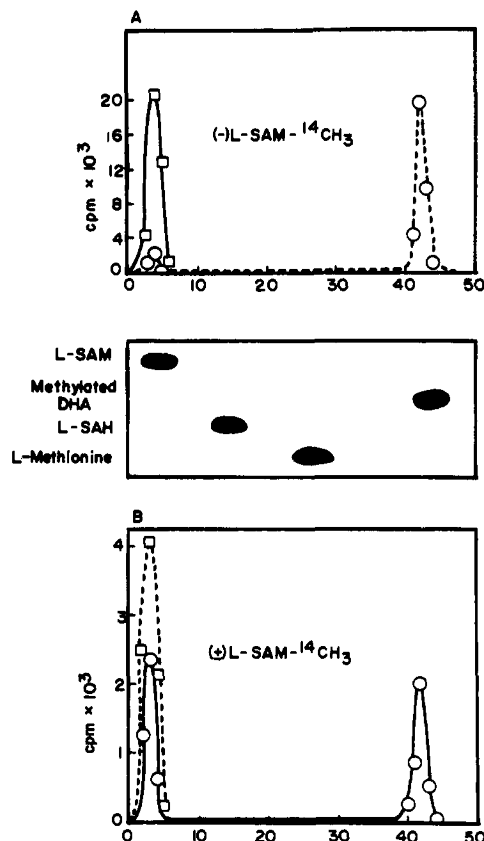


Figure 2. Chromatographic identification of labeled products from the incubation mixtures containing COMT and (−)-L-SAM-$^{14}CH_3$ [or (±)-L-SAM-$^{14}CH_3$]. Incubation mixtures were prepared in a manner identical with that described in Figure 1. Paper chromatography system: 1-butanol–acetic acid–H_2O (12:3:5). The center panel shows the respective chromatographic patterns for L-SAM, L-SAH, methylated DHA, and methionine. (A) Results using (−)-L-SAM-$^{14}CH_3$ as a methyl donor: □–□, aliquot (0.1 ml) removed at 0 time prior to incubation and chromatographed; ○–○, aliquot (0.1 ml) removed after incubation at 37° for 180 min and chromatographed. (B) Results using (±)-L-SAM-$^{14}CH_3$ as a methyl donor: □–□, aliquot (0.1 ml) removed at 0 time prior to incubation and chromatographed; ○–○, aliquot removed after incubation at 37° for 180 min and then chromatographed.

shown in Figure 2. As can be seen, when (−)-L-SAM-$^{14}CH_3$ was the substrate, all of the radioactivity chromatographed with the methylated products. However, when (±)-L-SAM-$^{14}CH_3$ was the substrate, about 50% of the radioactivity chromatographed with the methylated products and the other 50% with SAM. Further evidence that the radioactivity which chromatographed with SAM was indeed unreacted (+)-L-SAM-$^{14}CH_3$ was obtained by treating an aliquot of this incubation mixture with 0.1 N NaOH at 100° for 10 min and the resulting solution chromatographed on paper (under these conditions sulfonium nucleosides such as L-SAM hydrolyze to methionine and adenine[20]). After hydrolysis of this suspected sample of (+)-L-SAM-$^{14}CH_3$, the radioactivity chromatographed with L-methionine, consistent with the structural assignment.

All of the data described above are compatible with the idea that COMT preferentially utilizes only one isomer of (±)-L-SAM. Taking advantage of this substrate specificity, we have used the COMT-catalyzed reaction to prepare large quantities of the (+)-L-SAM in order to study its inhibitory properties. In these large-scale incubation mixtures, we routinely incorporated a small quantity of (−)-L-SAM-$^{14}CO_2H$, which provided us with a simple way of labeling the pool of (−)-L-SAM. In this way we could

Table I. Inhibition Constants for (+)-L-SAM and L-SAH toward COMT, PNMT, HMT, and HIOMT[a]

Enzyme	Inhibn constants, μM,[b] K_{is} ± SEM	
	(+)-L-SAM	L-SAH[c]
COMT	28.83 ± 3.65	36.3 ± 2.20
PNMT	32.16 ± 13.0	29.0 ± 2.84
HMT	7.35 ± 2.20	18.5 ± 2.19
HIOMT	28.98 ± 5.4	18.5 ± 1.9

[a] COMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section. (−)-L-SAM concentrations, 3.3-53.0 μM. [b] Each inhibitor showed linear competitive kinetics and the inhibition constants were calculated as previously described.[12-17] [c] Data taken from ref 12.

determine that (1) the reaction was completed and all of the (−)-L-SAM was consumed, and (2) the isolated (+)-L-SAM was not contaminated with L-SAH. In the purified (+)-L-SAM used in our inhibitory studies, only trace amounts of radioactivity were detectable. These trace levels of radioactivity appear to be attributable to unreacted (−)-L-SAM which amounted to no more than 1-2% of the (+)-L-SAM present in the sample.

Methyl Transfer from (±)-L-SAM Using PNMT, HMT, and HIOMT. We have obtained results for PNMT, HMT, and HIOMT similar to those shown in Figures 1 and 2 for COMT indicating these enzymes also preferentially utilize (−)-L-SAM as a methyl donor. With each of these enzymes no greater than 50% conversion of the labeled methyl group of (±)-L-SAM-$^{14}CH_3$ to the appropriate acceptor molecule could be detected. This was further confirmed by extensively incubating purified samples of (+)-L-SAM-$^{14}CH_3$ with these enzymes in an effort to detect any possible methyl donor properties. However, using (−)-L-SAM-$^{14}CH_3$ as a methyl donor, complete transfer (100%) of the labeled methyl group to the acceptor molecule was observed with each of the enzymes tested. Results similar to these had been reported earlier for HMT and HIOMT.[6]

Inhibitory Activity of (+)-L-SAM. Having available sufficient quantities of (+)-L-SAM, we were interested in determining whether this isomer was inactive as a methyl donor because it failed to bind to the enzymes or because it bound with an orientation of the methyl group that did not permit transfer to the acceptor substrate. Therefore, the inhibitory properties of (+)-L-SAM were studied using the COMT, PNMT, HMT, and HIOMT catalyzed reactions from (−)-L-SAM-$^{14}CH_3$ to the appropriate acceptor molecules. Preliminary experiments showed that (+)-L-SAM had potent inhibitory effects on these enzymatic transmethylations. Using reciprocal velocity vs. reciprocal (−)-L-SAM plots, the kinetic patterns for inhibition of COMT, PNMT, HMT, and HIOMT by (+)-L-SAM were determined and the resulting inhibition constants are listed in Table I. In all cases linear competitive patterns of inhibition were observed when (−)-L-SAM was the variable substrate. For example, in Figure 3 is shown the kinetic pattern for inhibition of PNMT by (+)-L-SAM. The linear competitive kinetic patterns suggest that the binding sites for (+)-L-SAM are identical with the (−)-L-SAM binding sites. For comparison, the inhibition constants (K_{is}) for L-SAH are also provided in Table I. It is extremely interesting to note that (+)-L-SAM shows inhibitory activities toward these four enzymes comparable to L-SAH. Therefore, from these results it is apparent that the lack of methyl donor compatibility of (+)-L-SAM resides in the misorientation of the methyl group at the sulfonium center in relationship to the nucleophile portion of the acceptor molecule at the enzyme surface. This "misoriented"

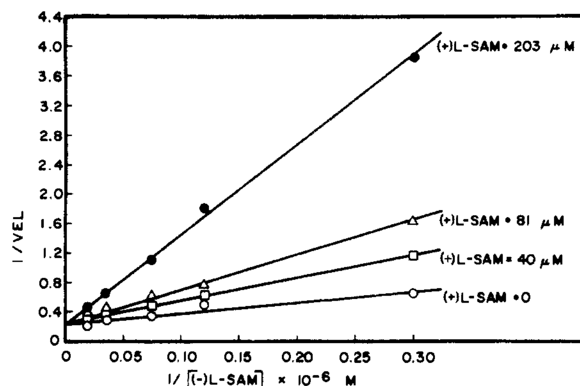
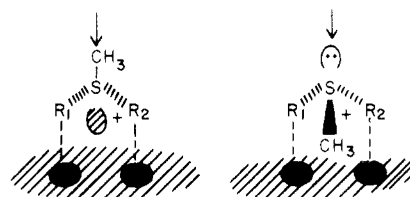


Figure 3. (+)-L-SAM inhibition of PNMT. Reciprocal plots with (−)-L-SAM as the variable substrate. Assay conditions were outlined in the Experimental Section except (−)-L-SAM concentration, 3.3-53.0 μM [(−)-L-SAM-$^{14}CH_3$ = 0.05 μCi]. DL-β-Phenylethanolamine concentration, 1.0 mM. Vel = nmol of product/mg of protein/min. Points represent averages of duplicate determinations.

Chart I. Possible Configurations of the Sulfonium Center of L-SAM[a]



[a] R_1 = $-CH_2CH_2CH(NH_2)CO_2H$ (L); R_2 = 5'-adenosyl. Absolute configurations of sulfonium center are unknown. Arrow denotes predetermined approach of an enzymatically bound nucleophile.

methyl group, however, does not appear to adversely effect enzymatic binding, since (+)-L-SAM is a potent inhibitor of these enzymes.

Conclusion

We have attempted in this study to determine the specificity of COMT, PNMT, HMT, and HIOMT for the configuration at the sulfonium center of the methyl donor L-SAM. Earlier studies[7-11] have shown that COMT, HMT, and HIOMT utilize only (−)-L-SAM as a methyl donor and not the corresponding (+)-L-SAM. In this study, we have confirmed these findings for COMT, HMT, and HIOMT and, in addition, have shown that PNMT exhibits a similar preference for the (−) isomer of L-SAM as a substrate. With PNMT, (+)-L-SAM showed no methyl-donating properties.

Of primary concern in this study was to determine if (+)-L-SAM was inactive as substrate because it failed to bind to the enzyme or if it was inactive because the geometry offered by this configuration was undesirable for methyl transfer to the acceptor substrate. To answer this question we prepared pure (+)-L-SAM by enzymatic resolution of (±)-L-SAM utilizing the substrate specificity of COMT. This pure (+)-L-SAM was found to be a potent inhibitor of COMT, PNMT, HMT, and HIOMT. These observations clearly demonstrate that the enzymatic sites have a high affinity for (+)-L-SAM indicating that the (+) configuration of the sulfonium center does not adversely affect enzymatic binding. However, the configuration of the sulfonium center of (+)-L-SAM must be nonproductive for methyl transfer, since this isomer shows no methyl-donating abilities. Shown in Chart I are the two possible orientations of the sulfonium center of L-SAM, where the

amino acid moiety is depicted as R_1 and the adenosyl moiety is depicted by R_2. In earlier studies from our laboratory we have shown that there are functional groups crucial for enzymatic binding on the amino acid, sugar, and base portions of L-SAH[12-17] and L-SAM.[3] Therefore, it could be expected that with functional groups on the amino acid (R_1) and adenosyl portion (R_2) tightly bound to the enzyme surface, the sulfonium center would not be capable of free rotation. In that case it is not unreasonable to find that only one of the two possible isomers at the sulfonium center serves as a methyl donor. The approach of the enzymatically bound nucleophile (denoted by arrows in Chart I) would be predetermined, so that only if the nucleophile and methyl group are properly aligned would methyl transfer occur. Since (+)-L-SAM is enzymatically bound, it could be concluded that there exists sufficient space at this binding site to accommodate the "misplaced" methyl group, but not sufficient flexibility in the enzyme–ligand complex to permit rotation of the sulfonium center into a configuration favorable for methyl transfer. In order to achieve a favorable configuration for methyl transfer in (+)-L-SAM, binding through functional groups in the amino acid (R_1) or adenosyl group (R_2) would have to be sacrificed.

The activity of (+)-L-SAM as an inhibitor of these enzymes, yet its lack of activity as a methyl donor, is of substantial interest, since it points out the fact that by a simple inversion of the configuration at the sulfonium center of L-SAM, the potential for methyl donation is completely lost, while at the same time little is sacrificed in the way of enzymatic binding. These observations demonstrate the strict geometric requirements for the methyl-transfer reaction but, in addition, suggest that there exists some vacant space at the sulfonium binding site to accommodate a "misoriented" methyl group.

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References and Notes

(1) Established Investigator of the American Heart Association.
(2) (a) Abbreviations used are (−)-L-SAM, (−)-S-adenosyl-L-methionine; (−)-SAM-$^{14}CH_3$, (−)-S-adenosyl-L-methionine-*methyl*-^{14}C; (−)-SAM-$^{14}CO_2H$, (−)-S-adenosyl-L-methionine-*carboxyl*-^{14}C; (±)-L-SAM, (±)-S-adenosyl-L-methionine; (±)-SAM-$^{14}CH_3$, (±)-S-adenosyl-L-methionine-*methyl*-^{14}C; (+)-L-SAM, (+)-S-adenosyl-L-methionine; L-SAH, S-adenosyl-L-homocysteine; COMT, catechol O-methyltransferase (E.C. 2.1.1.6); PNMT, phenylethanolamine N-methyltransferase (E.C. 2.1.1.28); HMT, histamine N-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole O-methyltransferase (E.C. 2.1.1.4); K_{is}, inhibition constant for the slope. (b) S. K. Shapiro and F. Schlenk, Ed., "Transmethylation and Methionine Biosynthesis", University of Chicago Press, Chicago, Ill., 1965. (c) E. Borek, Ed., "The Biochemistry of S-Adenosylmethionine", Columbia University Press, New York, N.Y., in press.
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