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The specificity of *S*-adenosyl-L-methionine sulfonium stereoisomers in some enzyme systems

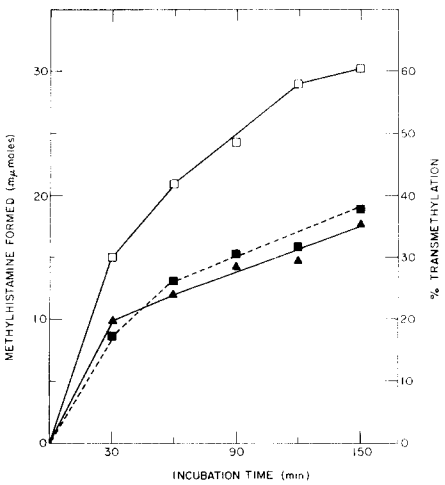
There is, as yet, little information on the biological specificity of stereoisomers of the methyl donor, *S*-adenosylmethionine. This compound contains two centers of asymmetry in addition to those in the ribose moiety: C-2 of the methionine part, and the sulfonium pole. The present report is concerned with the sulfonium stereoisomers; the L-methionine derivative was used in all instances. The designations (+) and (−), based on polarimetry, are used for the sulfonium stereoisomers because the absolute configuration of the sulfonium pole has not yet been determined.

DE LA HABA *et al.*[1] have shown that enzymatically produced *S*-adenosyl-L-methionine has the (−) sulfonium configuration. The racemic compound can be obtained by methylation of *S*-adenosyl-L-homocysteine[1] or by condensation of 5'-methylthioadenosine with 2-amino-4-bromobutyric acid[2]. A high degree of specificity of the sulfonium stereoisomers was observed[1] in several enzymatic systems: only the (−) configuration was active as substrate. On the other hand, DURELL *et al.*[3] showed that both diastereoisomers of methylethylacetothetin are active as methyl donors in the thetin:homocysteine methyltransferase system (EC 2.1.1.3).

To explore further the relationship between the steric configuration of the sulfonium pole and the biological activity of *S*-adenosyl-L-methionine, the activities of the (−) and (±) stereoisomers were compared with 4 enzymes that had not been previously investigated. Three methyltransferases and a decarboxylase were selected for this investigation: imidazole *N*-methyltransferase (EC 2.1.1.8), which forms methylhistamine, hydroxyindole *O*-methyltransferase (EC 2.1.1.4), which yields melatonin, homocysteine *S*-methyltransferase (EC 2.1.1.10), and *S*-adenosylmethionine decarboxylase.

(−)*S*-Adenosyl-L-[*Me*-^{14}C]methionine and (−)*S*-adenosyl-L-[*carboxy*-^{14}C]-methionine were prepared from labeled methionine by biosynthesis with yeast[4] and isolated according to SHAPIRO AND EHNINGER[5]. *S*-Adenosyl-L-homocysteine was prepared by enzymatic synthesis from adenosine and L-homocysteine[6] and isolated by ion-exchange chromatography[5,7]. (±)*S*-Adenosyl-L-[*Me*-^{14}C]methionine was prepared by methylation of *S*-adenosyl-L-homocysteine with labeled methyl iodide[8]; (±)*S*-adenosyl-L-[*carboxy*-^{14}C]methionine was obtained by methylation of *S*-adenosyl-L-[*carboxy*-^{14}C]homocysteine[8]. Imidazole *N*-methyltransferase was purified from guinea-pig brain according to BROWN *et al.*[9], and the enzymatic assay was performed according to SNYDER *et al.*[10]. Hydroxyindole *O*-methyltransferase was purified from pineal glands and assayed according to AXELROD AND WEISSBACH[11]. Homocysteine *S*-methyltransferase, purified from *Saccharomyces cerevisiae*[12], was a generous gift from Dr. S. K. Shapiro. *S*-Adenosylmethionine decarboxylase was purified from *Escherichia coli* according to TABOR[13]. The activity was measured by the release of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxy*-^{14}C]methionine.

The activity of (−)*S*-adenosyl-L-methionine in the methylation of histamine compared with that of the racemic compound, used at the same concentration, is shown in Fig. 1. The activity of the racemic compound is one half that of the (−) isomer. In addition, it may be seen that the rate of the reaction with the racemic



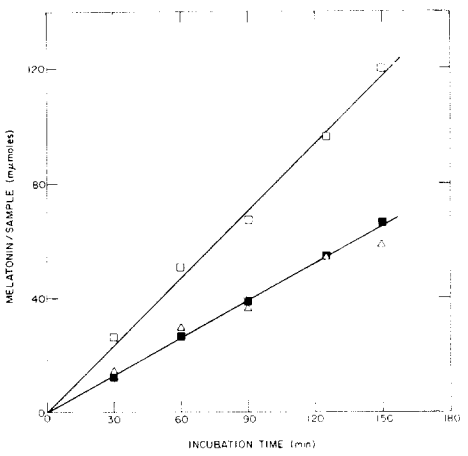


Fig. 1. Sulfonium stereoisomers of *S*-adenosyl-L-methionine as methyl donors in the methylation of histamine. Purified imidazole *N*-methyltransferase, 2.5 mg of protein, was incubated at 37° in the presence of 0.2 μmole of histamine, 50 μmoles sodium phosphate buffer (pH 7.5), and 50 (□- -□) or 25 (■—■) mμmoles of (- -)*S*-adenosyl-L-[*Me*-^{14}C]methionine, or 50 mμmoles (▲—▲) of (±)*S*-adenosyl-L-[*Me*-^{14}C]methionine, in a final volume of 0.5 ml. The specific activity of the the labeled methyl donors was $4.8 \cdot 10^5$ counts/min per μmole.

Fig. 2. Sulfonium stereoisomers of *S*-adenosyl-L-methionine as methyl donors in the hydroxyindole *O*-methyltransferase system. Purified hydroxyindole *O*-methyltransferase, 0.5 mg of protein, was incubated at 37° in the presence of 1.0 μmole of *N*-acetylserotonin, 50 μmoles of potassium phosphate buffer (pH 7.4), and 200 (□—□) or 100 (■—■) mμmoles of (- -)*S*-adenosyl-L-[*Me*-^{14}C]-methionine, or 200 mμmoles (△—△) of (±)*S*-adenosyl-L-[*Me*-^{14}C]methionine, in a final volume of 0.8 ml. The specific activity of the methyl donors was $1.1 \cdot 10^5$ counts/min per μmole.

compound is the same as that observed with half the concentration of the (- -) form. Corresponding results were obtained with hydroxyindole *O*-methyltransferase, as indicated in Fig. 2. The sulfonium racemate showed only half the activity compared to the (—) stereoisomer. In contrast, the data reported in Fig. 3 demonstrate that both stereoisomers are effective as methyl donors in the methylation of homocysteine. The (±) form is utilized in this system more rapidly than the (—) stereoisomer. Using a large excess of enzyme it was possible to obtain quantitative transmethylation with both stereoisomers.

In addition to these transmethylating enzymes, *S*-adenosyl-L-methionine decarboxylase was examined. Fig. 4 shows that the (—) sulfonium compound is about twice as reactive as the (±) form. This suggests that only the (—) sulfonium stereoisomer is decarboxylated.

The data reported here confirm that the (- -) sulfonium stereoisomer of *S*-adenosylmethionine is the biologically active form in most instances[1]. However, the activity of both stereoisomers in the methylation of L-homocysteine shows that a generalization regarding steric configuration and activity is not possible. The utilization of (+)*S*-adenosylmethionine by L-homocysteine *S*-methyltransferase may be related to the limited specificity of this enzyme which transmethylates also with

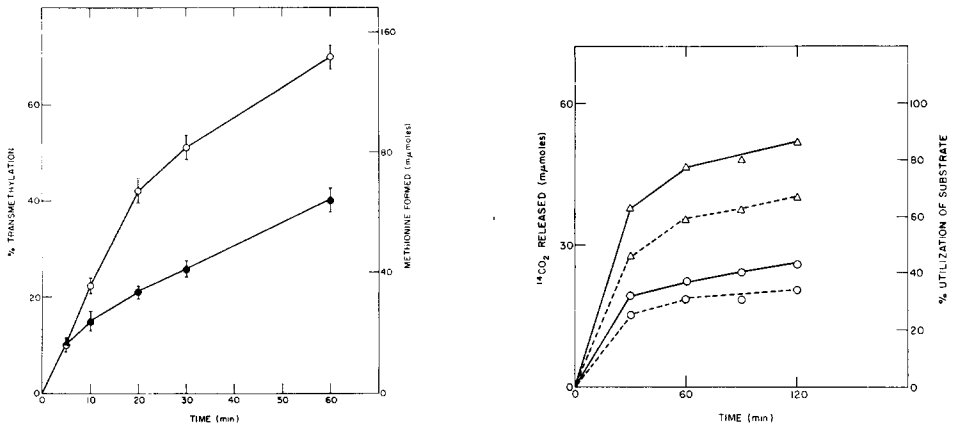


Fig. 3. Sulfonium stereoisomers of *S*-adenosyl-L-methionine as methyl donors in the homocysteine *S*-methyltransferase system. Purified enzyme, 1.3 mg of protein, was incubated with 2.0 mμmoles of Zn^{2+}, 10 μmoles sodium phosphate buffer (pH 7.2), 0.5 μmole L-homocysteine. 200 mμmoles (−)*S*-adenosyl-L-[*Me*-^{14}C]methionine (●—●) or (±)*S*-adenosyl-L-[*Me*-^{14}C]methionine (○—○), specific activity $4.1 \cdot 10^5$ counts/min per μmole, in a final volume of 0.2 ml. The enzyme was assayed by ion-exchange chromatography[12], and the standard deviations are indicated.

Fig. 4. The specificity of sulfonium stereoisomers in the *S*-adenosyl-L-methionine decarboxylase system. Purified enzyme, 8 mg (———) or 4 mg (— — — —) of protein, was incubated with 60 mμmoles of (−)*S*-adenosyl-L-[*carboxy*-^{14}C]methionine (△) or (±)*S*-adenosyl-L-[*carboxy*-^{14}C]-methionine (○), 50 mμmoles of Mg^{2+}, 50 μmoles of Tris–HCl buffer (pH 7.2), in a final volume of 0.4 ml. The reaction was carried out at 38° in Warburg vessels. The $^{14}CO_2$, released from the incubation mixture by the addition of 0.4 ml of 1.5 M perchloric acid from the side arm, was trapped by 0.1 ml of hyamine hydroxide in the central well, and the radioactivity was counted in a scintillation spectrometer. The specific radioactivity of the sulfonium compounds was $4.1 \cdot 10^5$ counts/min per μmole.

S-methylmethionine as the methyl donor. The latter compound does not have an asymmetric sulfonium center.

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