The Enzymatic Decomposition of S-Adenosyl-L-Methionine*

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The role of S-adenosyl-L-methionine as a methyl donor has been investigated in mammalian systems (1) and in microbial metabolism (2-4). In addition, the compound is involved in the formation of spermidine from putrescine (5). Information concerning the stability of S-adenosylmethionine in various enzymatic systems is a necessary prerequisite for studies concerning its role in group transfer. In the course of studies with S-adenosyl-L-methionine as a methyl donor for the adenosylmethionine-homocysteine transmethylase system of bacterial and yeast cells (2), it was apparent that the methyl donor is decomposed enzymatically. Preliminary observations concerning this enzymatic decomposition of S-adenosylmethionine have been reported previously from this laboratory (6-8). In all the systems that have been investigated, the pattern of enzymatic destruction appears to be similar, but the speed of the reaction varies greatly. Cell-free extracts of *Aerobacter aerogenes* were selected for this study, because the enzymes degrading S-adenosylmethionine are particularly active.

**METHODS**

**Preparation of Enzymes—** *A. aerogenes*, NRRL No. 199, was grown in a medium consisting of 1.5 per cent peptonized milk (Difco) and 0.75 per cent K₂HPO₄. The phosphate was adjusted to pH 7.0 and autoclaved separately from the peptonized milk. The cells were transferred successively into 10 ml., 100 ml., 1 liter, and 10 liters of this medium. The cultures were vigorously aerated and maintained at 35°. After 6 hours' growth, the final 10 liter culture was harvested in a refrigerated Sharples centrifuge. The cells were washed twice with 0.1

**Compounds—** S-Adenosyl-L-methionine, S-adenosyl-L-methionine, and methylthioadenosine were prepared according to the methods developed in this laboratory (10-12). Methylthioribose was prepared by the hydrolysis of methylthioadenosine with 0.1 N H₂SO₄ at 100° for 5 hours. A sample of α-amino-β-butyrolactone was generously supplied by Dr. M. D. Armstrong. All other compounds were obtained from commercial sources.

**Analytical Methods—** The reaction mixtures contained 5 to 10 mg. of protein and 2 to 10 μmoles of substrate per ml. in 0.1 M phosphate buffer, pH 6.8. Unless stated otherwise, all reactions were carried out at 35° for 3 hours. Under these conditions there is virtually no chemical decomposition of S-adenosylmethionine (13). The reactions were stopped by the addition of 0.1 ml. of a 50 per cent acetic acid solution per ml. of reaction mixture. For controls, substrates and enzymes were incubated separately until the end of the experiment, then mixed and acidified. After centrifugation, aliquots of the supernatant fluid were placed on Whatman No. 1 or No. 3 papers and analyzed by paper chromatography with the solvent system butanol-acetic acid-water (80:15:25, volume for volume), which separates all the reaction products under investigation. The purine compounds were detected by scanning with an ultraviolet lamp (Mineralite, No. SL-2537), and amino acids were located with a solution of 1.0 per cent ninhydrin in water-saturated n-butanol. The sulfur-containing compounds were detected with a platinoic iodide spary (14). After location with ultraviolet light, the purine compounds may be eluted with water and measured quantitatively. For greater accuracy and convenience, the procedure of Schlenk and DeFainn (10) was modified as follows: Dowex 50 is equilibrated with 0.1 N H₂SO₄, and 2.0 ml. of the supernatant fluid from the acidified reaction mixture is placed on a 10 by 30 mm. column. Interfering compounds are eluted with 0.1 N, followed by 0.5 N, H₂SO₄. Methylthioadenosine, adenine, and S-adenosylmethionine are eluted successively with 1.0, 2.0, and 4.0 N H₂SO₄. The concentration of the compounds in the eluates is determined with the Beckman DU spectrophotometer.

Homoserine and α-amino-γ-butyrolactone were eluted from paper chromatograms (15) and measured quantitatively (16). The latter compound is readily identified because it forms a characteristic yellowish brown area on paper chromatograms that have been sprayed with ninhydrin. Methylthioribose may be measured directly in the reaction mixtures as follows. To 1 ml. of reaction mixture are added in order 0.6 ml. of 20 per cent phosphotungstic acid, 0.2 ml. of perchloric acid, and 0.2 ml. of water. The phosphotungstic acid precipitates adenosylmethionine (11) and methylthioadenosine (17), whereas methylthioribose remains in solution and is measured by the nitroprusside test.

**RESULTS AND DISCUSSION**

After incubation of S-adenosylmethionine with cell-free extracts of *A. aerogenes*, aliquots of the reaction mixtures were placed on paper chromatograms for analysis of the decomposition products. Adenine and much smaller amounts of hypoxanthine were the only free purine compounds formed. The only sulfur-containing product was methylthioribose. Homoserine

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and α-amino-γ-butyrolactone (homoserine lactone) were the only amino acid decomposition products.

Formation of α-Amino-γ-butyrolactone—The isolation and identification of α-amino-γ-butyrolactone as a product of the chemical hydrolysis of S-adenosyl-L-methionine has been observed by Parks and Schlenk (18). α-Amino-γ-butyrolactone is formed from S-adenosyl-L-methionine by extracts of A. aerogenes.

FORMATION OF α-AMINO-γ-BUTYROLACTONE—The isolation and identification of α-amino-γ-butyrolactone as a product of the chemical hydrolysis of S-adenosyl-L-methionine has been observed by Parks and Schlenk (18). α-Amino-γ-butyrolactone is formed from S-adenosyl-L-methionine by extracts of A. aerogenes.

![Graph](image-url)

**FIG. 1.** The formation of methylthioribose from S-adenosyl-L-methionine (○) and methylthioadenosine (△) as a function of time. 10 ml. of each reaction mixture contained 80 μmoles of substrate and 50 mg. of protein of Aerobacter aerogenes extract, and were incubated at 35°C in 0.1 M phosphate buffer, pH 6.8. 1 ml. samples were removed every 30 minutes for the determination of methylthioribose.

**TABLE I**

<table>
<thead>
<tr>
<th>Enzymatic decomposition of S-adenosylmethionine and methylthioadenosine*</th>
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<tbody>
<tr>
<td>S-Adenosyl-L-methionine</td>
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<td>9.4</td>
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<td>9.3</td>
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* All quantities are expressed as μmoles per 2 ml. of reaction mixture. The mixtures contained 10 mg. protein of Aerobacter aerogenes extract and were incubated for 3 hours at 35°C in 0.1 M phosphate buffer, pH 6.8.
† Not determined; see text.

Identical reaction mixtures with homoserine as substrate failed to yield detectable amounts of the lactone during a period of 5 hours. The identity of this enzymatic product was determined by comparing it with a sample of α-amino-γ-butyrolactone. It seems that the enzymatic formation of the latter compound precedes the formation of homoserine. Furthermore, pure α-amino-γ-butyrolactone is converted to homoserine at virtually the same rate with or without the presence of a crude enzyme mixture. Consequently, the actual enzymatic product resulting from the 4-carbon amino acid moiety of S-adenosyl-L-methionine is apparently α-amino-γ-butyrolactone, which is converted to homoserine by a nonenzymatic process.

Decomposition of Methylthioadenosine—Along with the formation of α-amino-γ-butyrolactone, one would expect methylthioadenosine. However, only traces of this compound are found during the enzymatic decomposition of S-adenosyl-L-methionine, but adenine and methylthioribose accumulate. A comparison of the rate of formation of methylthioribose from S-adenosyl-L-methionine and methylthioadenosine is illustrated in Fig. 1. It is obvious that methylthioadenosine is decomposed much more rapidly than is S-adenosyl-L-methionine (about 6 times faster). Under identical conditions methylthioadenosine is converted quantitatively to methylthioribose in 2.5 hours, whereas only 25 per cent of S-adenosyl-L-methionine is decomposed to the same product. Thus it is clear that methylthioadenosine is decomposed as rapidly as it is formed from S-adenosyl-L-methionine.

Comparison of Reactions—A comparison of the reaction products formed from S-adenosylmethionine, methylthioadenosine and adenosine is given in Table I. For each substrate a control was prepared as described earlier. The homoserine figure represents the value obtained from the combined eluates of α-amino-γ-butyrolactone and homoserine. It is interesting to note that methylthioadenosine is more rapidly decomposed than adenosine, suggesting that different nucleosidases are involved. It is again clear that methylthioadenosine is much more rapidly decomposed than S-adenosyl-L-methionine. Thus the enzymatic decomposition of S-adenosyl-L-methionine may be described as illustrated in Scheme 1.

Under these conditions, homoserine, adenine, and methylthioribose are not metabolized and accumulate as end products of the reaction. With fresh preparations of enzyme, adenine is deaminated to hypoxanthine. Since Reaction II proceeds much faster than Reaction I, the concentration of methylthioribose may be used as a convenient measure of the rate of decomposition of S-adenosyl-L-methionine.

The reaction is stereospecific in that only S-adenosyl-L-methionine serves as a substrate. The amount of methylthioribose
produced from S-adenosyl-\(\text{L}\)-methionine (Table I) is equivalent to the amount of \(\text{L}\)-isomer present in the preparations of S-adenosyl-\(\text{L}\)-methionine (10).

Extracts of \(\text{A. aerogenes}\) that have been dialyzed for 24 hours against 0.01 M phosphate buffer pH 6.8, retain full activity in the enzymatic decomposition of S-adenosyl-\(\text{L}\)-methionine and methylthioadenosine. Heating the crude extract for 5 minutes at 80\(^\circ\) results in inactivation of both enzymes. However, when this extract is heated at 70\(^\circ\) for 2 minutes, Reaction II is decreased sufficiently so that methylthioadenosine can be identified qualitatively as a product of Reaction I.

Cell-free extracts of \(\text{Escherichia coli K}_{12}\) and \(\text{Saccharomyces cerevisiae}\) have been shown to possess adenosylmethionine-homocysteine transmethylases (2). These extracts also show activity in the decomposition of the methyl donor. However, less than 10 per cent of S-adenosyl-\(\text{L}\)-methionine is decomposed under these conditions, whereas with extracts of \(\text{A. aerogenes}\) at least 25 per cent of the compound is destroyed in 3 hours' incubation at 35\(^\circ\) in the presence of 8 \(\mu\)moles of the substrate.

**SUMMARY**

The enzymatic decomposition of S-adenosyl-\(\text{L}\)-methionine by cell-free extracts of \(\text{Aerobacter aerogenes}\) involves at least two enzymatic reactions. The first step is the formation of methylthioadenosine and \(\alpha\)-amino-\(\gamma\)-butyrolactone. The second step is the rapid decomposition of methylthioadenosine to adenine and methylthioribose.

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