THE STABILITY AND HYDROLYSIS OF S-ADENOSYL-
METHIONINE; ISOLATION OF
S-RIBOSYLMETHIONINE*

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Recent improvements in the methods for biosynthesis and isolation of
S-adenosylmethionine (1, 2) have stimulated extensive experimentation
with this compound. Detailed information upon its stability under various
conditions is a prerequisite to microbiological, nutritional, and enzymatic
research involving this substance. Not only is it desirable to have an
estimate of the residual amount of S-adenosylmethionine at any given time
during an experiment, but in many instances it is necessary also to know
what fragments may accrue. Erroneous results may be surmised,
especially in microbiological studies with mutants, if the response to hy-
drolytic products is mistaken for an effect of the parent substance. Ex-
periments were carried out, therefore, on the stability of S-adenosyl-
methionine under the conditions of biological experimentation. Our
study soon revealed that several hydrolytic processes occur simultaneously.
To gain insight into the conditions which favor individual reactions, ex-
tremes of pH and temperature were included in this investigation. The
latter phase of our work revealed conditions for splitting methionine from
the molecule and for hydrolysis to adenine and a hitherto unknown frag-
ment, S-ribosylmethionine. Directions for preparation and a description
of some of the properties of this new compound are given.

Our data extend the earlier observations of Cantoni, (3) and of Baddiley,
Cantoni, and Jamieson (3, 4) on the hydrolysis of S-adenosylmethionine;
they observed the formation of adenine by acid hydrolysis and of methyl-
thioadenosine and homoserine by heating in neutral solution.

Materials and Methods

The preparation of S-adenosylmethionine from yeast, as well as suitable
analytical techniques, has been described in our earlier reports (1, 2).
Minor details and modifications will be noted in connection with the ex-
periments.

Dimethyladenosylthetin (5'-Dimethylthioadenosine)—This material was

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Commission.
obtained by the reaction of 5'-methylthioadenosine (5) with methyl iodide. It was purified by ion exchange chromatography with Dowex 50 resin (2). The absorption spectrum of the resulting material was identical with that of S-adenosylmethionine. Chromatography on Whatman No. 1 paper showed $R_F$ 0.3 in n-butanol-acetic acid-water (60:25:15, v/v), and $R_F$ 0.6 in ethanol-water-acetic acid (65:34:1, v/v). The ratio of adenine to pentose was 1.0. A more detailed description of this compound will be published shortly.

5'-Methylthioribose—Reference material for the colorimetric determination of 5'-methylthioribose was obtained by the treatment of 5'-methylthioadenosine with 0.1 N H$_2$SO$_4$ at 100° for 5 hours. Paper chromatography showed that the hydrolysis is complete under these circumstances and that no further decomposition of 5'-methylthioribose has taken place. The neutralized solution was used as reference material. The presence of adenine does not interfere with the nitroprusside test.

Results

Stability under Physiological Conditions—The decline in the concentration of S-adenosylmethionine under conditions resembling those of biochemical experimentation was studied by incubation in 0.05 M phosphate buffer at 30° in the presence of a small amount of toluene as a preservative. The concentration of the sulfonium compound was 6 μmoles per ml.; samples were withdrawn at intervals for the determination of residual S-adenosylmethionine by means of a Dowex 50 column (1). Fig. 1 shows the results and in it the initial pH values are specified. The gradual abolition of the sulfonium configuration, according to $R_1(R_2)S^+R_3 + H_2O \rightarrow R_3SR_2 + R_3OH + H^+$, gave an increase in acidity to pH 8.2, 7.0, and 6.0, respectively. Simultaneous samples for paper chromatographic survey revealed the identity and approximate concentration of split products. The results of ultraviolet scanning and ninhydrin tests are listed in Table I.

Observations of the material incubated at weakly alkaline reaction indicated a novel hydrolytic mechanism leading to methionine; the other product expected from such a split would be adenosine; this, however, could not be detected. The principal purine product was adenine, indicating that hydrolysis of the glycosidic bond and of the linkage between carbon atom 5 of the ribose and the sulfur atom occurred, either simultaneously or in sequence. The lability of the bond between adenine and ribose in S-adenosylmethionine is not shared by adenosine and methylthioadenosine. To gain information on this point, an extension of the hydrolytic studies into the acid and alkaline range and a comparison with related compounds appeared desirable.

Acid Hydrolysis—S-Adenosylmethionine is rather stable in strong acids
at low temperature (2). A comparison of the stability of its glycosidic bond with those in adenosine and 5'-methylthioadenosine is shown in Fig. 2, which represents the release of adenine in 0.1 N HCl in a boiling water bath. The initial concentration of the compounds examined was 2 μmoles per ml. Samples withdrawn at the times specified were analyzed for adenine by ion exchange chromatography (1). No striking differences in the rates of acid hydrolysis are apparent.

**FIG. 1.** The destruction of S-adenosylmethionine in 0.05 M phosphate buffer at 30°.

**TABLE I**

Hydrolytic Products of S-Adenosylmethionine

<table>
<thead>
<tr>
<th>Products obtained</th>
<th>pH 6.0*</th>
<th>pH 7.0*</th>
<th>pH 8.2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Adenosylmethionine</td>
<td>++</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5'-Methylthioadenosine</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Adenine</td>
<td>±</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Homoserine</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Methionine</td>
<td>±</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

The experimental conditions are cited in the text.

* Incubation at 30° for 96 hours.
† Incubation at 30° for 48 hours.
**Alkaline Hydrolysis**—Paper chromatography of hydrolysates of S-adenosylmethionine obtained with 0.1 N NaOH at the temperature of a boiling water bath revealed adenine and methionine as the main products; these were accompanied by smaller amounts of homoserine, methylthioadenosine, methylthioribose, and presumably some other split products.

![Graph](http://www.jbc.org)

**FIG. 2.** The splitting of adenine from S-adenosylmethionine, adenosine, and 5’-methylthioadenosine by 0.1 N HCl in a boiling water bath.

**FIG. 3.** Absorption spectra of S-adenosylmethionine (○) and adenine (●). The measurement of S-adenosylmethionine was carried out at pH 9.7 and 7.0 with identical results; measurement in 0.1 N NaOH is precluded because of the instability of the compound. Adenine was measured in 0.1 N NaOH. The concentration of each compound was 0.062 μmole per ml. Maximal decrease in optical density during the reaction, S-adenosylmethionine → adenine + S-ribosylmethionine, occurs at 250 μμ.

Since the destruction of S-adenosylmethionine was completed under these circumstances in less than 3 minutes, and since the hydrolysis obviously was multiple in character, the procedure was carried out at 25°. Under these circumstances no S-adenosylmethionine was left after 10 minutes. Paper chromatograms showed only traces of methionine and homoserine. A heavy adenine spot and an intensely ninhydrin-responsive, ultraviolet-negative area were found; the latter was located in a position similar to that of the parent substance, S-adenosylmethionine. The identification of adenine and the presence of only one other major split product suggested
that the latter might be S-ribosylmethionine. This was confirmed by the analysis of eluates from large scale paper chromatograms.

The difference between the absorption spectra of S-adenosylmethionine and adenine in alkaline solution (Fig. 3) permits spectrophotometric observation of the reaction, S-adenosylmethionine → adenine + S-ribosylmethionine, taking advantage of the drop in optical density at 250 m\(\mu\). The course of the hydrolysis is seen in Fig. 4; spectrophotometry of adenosine and 5'-methylthioadenosine in 0.1 N NaOH confirmed their stability in alkali (6, 7). Because of the sensitivity of S-adenosylmethionine to alkali, it was not possible to obtain an absorption spectrum in 0.1 N NaOH; nor could a zero time value for the alkaline hydrolysis be measured. The

![Graph](image)

**Fig. 4.** The effect of 0.1 N NaOH at 25° on S-adenosylmethionine (X), dimethyladenosylthetin (O), adenosine (●), and 5'-methylthioadenosine (△). The optical density at 250 m\(\mu\) was recorded; the decrease to 55 per cent of the original density value indicates complete hydrolysis of the glycosidic bond.

initial value given in Fig. 4 was obtained before the addition of sodium hydroxide; it is corrected for the dilution. The absorption spectra at pH 9.7 and pH 7.0 were identical. Likewise the absorption spectra of adenosine and 5'-methylthioadenosine in 0.1 N NaOH were found to be identical with those taken at pH 7.0, except for a minute leveling of the minimum near 230 m\(\mu\).

**Comparison with Dimethyladenosylthetin**—The extreme lability of the glycosidic bond in S-adenosylmethionine toward alkali appeared to be a special effect of the sulfonium configuration in the molecule. In view of the novelty of this observation, it was desirable to amplify the information on this point. A related compound, dimethyladenosylthetin, was available for this purpose. It has been obtained for transmethylation studies from 5'-methylthioadenosine and methyl iodide by a procedure patterned after the method of Toennies and Kolb for the synthesis of sulfonium compounds (8). This compound showed sensitivity to alkali in a
fashion similar to S-adenosylmethionine (see Fig. 4). Adenine, which resulted from the hydrolysis, was readily identified by paper chromatography.

![Chemical structure of Dimethyladenosylthetin](attachment:image)

(Dimethyladenosylthetin)

**Preparation of S-Ribosylmethionine**—It has been found advantageous to minimize side reactions in the hydrolysis of adenine from S-adenosylmethionine by carrying out the hydrolysis in an ice bath; at least 3 hours are required to complete the process under these circumstances. S-Ribosylmethionine migrates on Dowex 50 columns in a fashion similar to S-methylmethionine (1); however, in contrast to S-adenosylmethionine, its precipitation by phosphotungstic acid from the acid eluates is not satisfactory. Therefore, adenine was removed from the hydrolysate by precipitation. The following procedure is recommended.

A water solution of highly purified S-adenosylmethionine, containing 10 to 20 μmoles per ml., is cooled in an ice bath and adjusted with cold 1.0 N NaOH to 0.1 N alkali content. After 5 hours, twice the calculated amount of 1 N H₂SO₄ is added to attain an acid concentration of 0.1 N. Adenine is removed by precipitation with Ag₂SO₄; for each 100 μmoles 4.0 ml. of 0.5 per cent solution are used. After 1 hour at low temperature, the precipitate is removed by centrifugation and washed twice with a small amount of water. The combined solutions are treated with hydrogen sulfide, followed by nitrogen to remove the excess of the precipitant gas. Silver sulfide is now removed by filtration or centrifugation, and the precipitate is washed twice. The treatment with nitrogen is continued for several hours to minimize the hydrogen sulfide content of the solution. Freshly prepared barium carbonate suspension or Dowex 2-HCO₃ (2) is used for the removal of excessive H₂SO₄. Centrifugation and washing of the precipitate lead to a clear solution which may be concentrated by lyophilization; complete dehydration should be avoided since S-ribosylmethionine, like S-adenosylmethionine, shows very limited stability in the dry form. Solutions may be stored at or below pH 7.5 for prolonged periods in the frozen state.

**Analysis and Properties of S-Ribosylmethionine**—No traces of adenine or adenine derivatives can be found in the preparations by paper chroma-
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S-Ribosylmethionine is readily detected on paper chromatograms by the ninhydrin spray although the platinum iodide reagent (9) is less sensitive. In butanol-water-acetic acid (60:25:15, v/v) $R_F$ is 0.1; in ethanol-water-acetic acid (65:34:1, v/v) $R_F$ is 0.35. Application of graded amounts, up to 0.3 μmole per spot, permits the identification and estimation of impurities. Methionine and homoserine, not in excess of a few per cent, are sometimes observed. Paper chromatography and elution yield preparations of high purity.

\[
\begin{align*}
\text{CHO} & \quad \text{HCOH} & \quad \text{COOH} \\
\text{HCOH} & \quad \text{H}_2\text{NCH} \\
\text{HCOH} + \text{CH}_2 & \quad \text{CH}_2 \quad \text{S} \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_3 \\
\end{align*}
\]

$S$-Ribosylmethionine

The structure of $S$-ribosylmethionine is inferred mainly from the composition and configuration of its parent substance, $S$-adenosylmethionine, and from the observation that under the hydrolytic conditions specified here adenine is the only other principal fragment. Unfortunately, $S$-ribosylmethionine proved refractory to isolation in the dry or crystallized state. Thus it became necessary to establish a measure of the concentration of the compound in solution by a suitable analytical test. Ultraviolet spectrophotometry, which serves for this purpose in experimentation with $S$-adenosylmethionine, cannot be used for lack of a specific light-absorbing group in the molecule of $S$-ribosylmethionine. Pentose determination by the orcinol reagent was attempted, therefore. For this, it had to be ascertained that the response of the ribose bound in $S$-ribosylmethionine is equivalent to that of the free ribose used as a standard; this prerequisite is satisfied by $S$-adenosylmethionine (1). Measured amounts of three $S$-adenosylmethionine preparations were hydrolyzed by alkali to $S$-ribosylmethionine and adenine, and the hydrolysates were tested with the orcinol reagent. The presence of adenine in the solution has no influence on the color intensity; experiments with ribose and admixed adenine showed no effect of equimolar amounts nor even a 5-fold excess of the base. The results listed in Table II indicate that with a 10 per cent limit of accuracy pentose determination may serve as a measure of the concentration of $S$-ribosylmethionine, provided that the identity and uniformity of the product are ascertained by concomitant paper chromatography.

A preparation of $S$-ribosylmethionine from which adenine had been removed contained 7.8 μmoles per ml. as determined by the orcinol reaction. Hydrolysis at pH 5.3 for 30 minutes at 100° yielded methylthioribose (7.2 μmoles per ml.) and homoserine (8.5 μmoles per ml.). The former
was determined by the nitroprusside reaction (11) and the homoserine by the ninhydrin test (12). Experiments with L-amino acid oxidase (1) showed 11.0 μmoles of the preparation inert as a substrate although methionine was oxidized almost completely. Heating in 0.1 N NaOH destroyed the sulfonium group and produced methionine in 65 per cent yield as determined by the amino acid oxidase technique (1).

**TABLE II**

*Response of S-Adenosylmethionine and S-Ribosylmethionine to Orcinol Pentose Reagent*

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>Concentration of S-adenosylmethionine*</th>
<th>Concentration of pentose†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles per ml.</td>
<td>μmoles per ml.</td>
</tr>
<tr>
<td>1</td>
<td>23.8</td>
<td>24.4</td>
</tr>
<tr>
<td>2</td>
<td>13.2</td>
<td>13.3</td>
</tr>
<tr>
<td>3</td>
<td>11.3</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* The concentration of S-adenosylmethionine was determined by spectrophotometry.
† The orcinol reaction was used with slight modifications (10). D-Ribose served as a reference standard. The data were obtained (A) before hydrolysis; (B) after hydrolysis in 0.1 N NaOH at 0° for 5 hours; (C) after hydrolysis in 0.1 N NaOH at 25° for 10 minutes. All the samples were diluted 100- to 300-fold for the analytical tests.

**DISCUSSION**

It may be assumed from our data that the loss of S-adenosylmethionine caused by its chemical lability remains small during the short periods of incubation in enzymatic studies. In nutritional and microbiological investigations the lability may be a complicating factor, and in all types of experiments involving intact cells, tissue homogenates, or crude enzyme preparations the additional rapid enzymatic destruction has to be considered.

The experiments on hydrolysis are illustrated in Fig. 5, in which various modes of splitting are indicated. The earlier observations (3, 4) on the formation of methylthioadenosine and homoserine (site A, Fig. 5) have been confirmed; heating at pH 4 or in neutral solution for 30 minutes gives nearly quantitative results. More rigorous treatment with acid leads to further degradation of methylthioadenosine (site B, Fig. 5), and results in adenine and methylthioribose.

Methionine can be obtained by brief heating in alkali (site C, Fig. 5); we have not been able to detect adenosine, which would be the other frag-
ment expected from this split. Control experiments with adenosine, heated with alkali under the same conditions, proved its stability. Therefore, adenosine could not be considered as a product or as an intermediate compound in the alkaline hydrolysis of \( S \)-adenosylmethionine. The quantitative formation of adenine by alkali suggested that the split at site B precedes that at site C. This has been confirmed by alkaline hydrolysis at low temperature. Under these conditions, the split at site C becomes negligible compared with that at B; adenine and \( S \)-ribosylmethionine are formed. The designation of the latter product as \( S \)-ribosylmethionine is based on the following observations: precipitability and chromatographic behavior as an entity, the orcinol response characteristic of pentoses, its alkaline hydrolysis leading to methionine with destruction of the pentose, splitting by weak acid to methylthioribose and homoserine, and the identification of these compounds by paper chromatography. The sulfonium character is implied from the structure of the parent compound whose characteristics of stability and hydrolytic behavior are retained in the molecule. Like other sulfonium compounds, \( S \)-ribosylmethionine is resistant to amino acid oxidase. Splitting of \( S \) adenosylmethionine by enzyme action at sites D and E (3, 13) has been observed (see Fig. 5). No analogous chemical hydrolyses have been detected in our studies.

The extreme lability of the glycosidic bond in \( S \)-adenosylmethionine has no analogy in ribose nucleoside and nucleotide chemistry with the exception of the nicotinamide ribose linkage in the nicotinamide nucleotides (14); here, the pyridinium nitrogen changes to the trivalent state when nicotinamide is liberated by alkali. It was surmised at first that by an intramolecular rearrangement of \( S \)-adenosylmethionine the methyl group might be shifted from the sulfur atom to nitrogen atom 9 of the adenine with resultant labilization of the glycosidic bond. However, 9-methyladenine and \( S \)-ribosylhomocysteine would then be expected as fragments;

![Diagram of various hydrolyses of \( S \)-adenosylmethionine.](http://www.jbc.org/)

Fig. 5. Various hydrolyses of \( S \)-adenosylmethionine. A discussion is given in the text.
they were not found in our hydrolysates. In view of the stability of adeno-
sine and methylthioadenosine toward alkali, it became clear that the
presence of the sulfonium group in S-adenosylmethionine must be responsi-
bile for the lability of the adenine ribose linkage in alkaline medium. Con-
fidence in this concept was gained by the observation of almost identical
behavior of the related sulfonium compound, dimethyladenosylthetin.

Labilizing effects exerted by sulfonium groups are well known in organic
chemistry (15, 16). Hydrolytic cleavage of bonds is usually observed, or
desaturation at a neighboring site may occur; the sulfonium group is rarely
preserved in these processes (17–19). The relative lability of the sulfur
carbon bonds A and C in S-adenosylmethionine (see Fig. 5) compared with
the stability of these linkages in the corresponding thio ethers is in accord
with previous experience concerning the effects of sulfonium groups. The
easy splitting of the adenine ribose linkage, however, is novel in that it is
removed from the activating sulfonium group by five interatomic bonds.
Furthermore, the split occurs at a carbon nitrogen bond, and the sulfonium
group of the molecule remains intact. The biochemical significance of
this lability is under investigation.

The authors wish to acknowledge the technical assistance of Mrs. S.
Stanford.

SUMMARY

The stability of S-adenosylmethionine under conditions of biochemical
experimentation has been examined. Non-enzymatic decomposition de-
creases the concentration significantly on prolonged incubation. Alkaline
hydrolysis with heating leads to methionine; alkali at low temperature
yields adenine and S-ribosylmethionine. The reasons for the instability
of the glycosidic bond in S-adenosylmethionine are described. Experiments
with a related compound, dimethyladenosylthetin (5′-dimethylthioadeno-
sine), revealed sensitivity of its glycosidic bond to alkali similar to that
observed with S-adenosylmethionine.

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