Enzymatic Cleavage of S-Adenosylmethionine*

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S-adenosylmethionine, known for some time to be the biological methyl donor in mammalian enzymatic transmethylation reactions (1, 2), has more recently been implicated in other transalkylation reactions in bacteria and yeast (3-6). The enzyme system catalyzing the condensation of ATP and methionine to form AMe has been found in a variety of tissues and organisms, and has been purified from two sources and investigated in detail (7, 8). The broad distribution of these enzymes suggests that AMe likewise has widespread distribution and lends importance to a study of its further metabolism.

The present paper reports the presence of an enzyme in baker's yeast which decomposes AMe to 5'-methylthioadenosine and α-aminobutyrolactone (Equation 1; A = adenine, R = ribose).

\[
\begin{align*}
\text{AR-S} & \quad \text{CH}_2\text{CH}_2\text{CHCOO}^- \\
\text{NH}_3 & \\
& \downarrow \\
\text{AR-S + CH}_2\text{CH}_2\text{CHCO} & \quad \text{NH}_3 \\
& \downarrow \\
\text{H}_2\text{O} & \\
\text{HOCH}_2\text{CH}_2\text{CHCOO}^- & + \text{H}^+ \\
\text{NH}_3 & \\
\end{align*}
\]

The partial purification of this system and some of its properties are discussed. A preliminary report of this work has been published (9). Recent independent results from another laboratory indicate the presence of a similar enzyme in bacterial extracts (10).

EXPERIMENTAL

Materials—AMe and S-adenosylmethionine were synthesized enzymatically with the methionine activating enzyme of rabbit liver (11). Methionine methylsulfonium bromide was prepared by the method of Floyd and Lavine (12). S-ribosyl-L-methionine was made by the alkaline decomposition of AMe (13). Dimethyladenosylsulfonium acetate was prepared by the methylation of MTA under conditions such as were used by de la Haba and Cantoni for methylation of S-adenosylhomocysteine, followed by isolation on a column of Amberlite IRC-50 (XE-64) resin (see below).

MTA was prepared from yeast (14). S-adenosyl-L-homocysteine was prepared enzymatically. 5'-Ethylthioadenosine was prepared by the decomposition of S-adenosylmethionine at 100° at pH 5.6.

5'-O-methyladenosine was prepared from 2',3'-O-isopropylidenadenosine synthesized according to Baddiley (15). The method used was a modification of that developed by Baddiley and Jamieson for the synthesis of MTA (16). For the present synthesis the potassium methylmercaptide used by these authors was replaced by sodium methoxide in dimethylformamide. The material was purified by the method of Baddiley and Jamieson (16), with the use of all steps through adsorption on and elution from a Dowex 50-H⁺ column. The compound at this point was contaminated with adenosine and yellow-colored material. It was further purified by preparative paper chromatography with the use of isopropanol, ammonia, and water (85:0.3:15) as solvent. Because of the low yield, the compound was not crystallized, but in addition to the method of synthesis the following criteria helped to establish its identity: (a) The compound migrates as a single ultraviolet absorbing spot in several solvent systems with \( R_f \)'s similar to, but not identical with, the \( R_f \)'s of MTA. (b) Its ultraviolet absorption spectrum is indistinguishable from that of adenosine. Moreover, after hydrolysis under conditions known to cleave the glycosidic bond of adenine ribosides (1 N sulfuric acid for 30 minutes at 100°) the ultraviolet absorption spectra at pH 2 and 11 were identical to those of adenine. These findings establish the identity of the base portion of the compound. (c) Periodate titration (17) showed uptake of 1 mole of periodate for each mole of compound as measured by ultraviolet absorption. (Adenosine was used as a standard in each case.) (d) The compound was completely inert to treatment with adenosine deaminase. Taken together, these data firmly support the proposed structure.

α-Aminobutyrolactone was prepared by incubating α-homoserine in 6 N hydrochloric acid for 1 hour at room temperature. The equilibrium mixture of lactone (approximately 60 per cent) and homoserine (approximately 40 per cent) (18) was satisfactory for paper chromatography. To prepare homoserine hydroxamate, an aliquot of this mixture was combined with hydroxylamine hydrochloride and the solution brought approximately

* The 13th paper in a series on enzymatic mechanisms in transmethylation.

1 The abbreviations used are: AMe, S-adenosyl-L-methionine, where L refers to the configuration of the α-amino acid part of the molecule; MTA, 5'-methylthioadenosine; Tris, tris(hydroxymethyl)aminomethane.

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The final concentration of hydroxylamine was 0.5 M. Incubation for 30 minutes at 37° produced a good yield of homoserine hydroxamate as judged by the formation of material absorbing at 259 nm upon acidification and treatment with FeCl₃ reagent as given in Davie et al. (19). Quantitative information upon the color equivalent of this material was not obtained.

2-Chloro-N-L-homoserine hydroxamate was prepared with 2-Chloro-N-methionine as the starting material. The latter was enzymatically converted to AMe, which was isolated (11), and decomposed by heating to 100° for 15 minutes at pH 5.6. The resulting solution of L-homoserine, L-amino-3-butyrolactone, and MTA was loaded on an XE-64 column prepared as described below, but buffered with sodium acetate at pH 5.5. The amino acids were eluted by washing with sodium acetate, 0.01 M, pH 5.5. At this pH the MTA adheres to the column. The eluate was concentrated by lyophilization, dissolved in a small volume of 6 N HCl and incubated for 50 minutes at room temperature. Before use in enzymatic reactions this equilibrium mixture was diluted and carefully brought to pH 5 with ice-cold KOH while being stirred mechanically in an ice bath.

Methods—Many of the sulfonium compounds were purified before use by adsorption on and elution from XE-64 washed and eluted through the sodium and acid forms as described by Hirs et al. (20). A column (6 by 1 cm.) of the purified resin was prepared by treating with 50 ml. of 0.25 M potassium phosphate, pH 7.0, followed by 15 ml. of a 1:25 dilution of the same buffer. After adsorption of the compounds, impurities were removed by washing with the dilute buffer. S-Ribosyl-L-methionine and methionine methylsulphonium could be eluted with 0.25 N acetic acid. AMe was eluted with 4.0 N acetic acid.

All paper chromatography was performed with acid-washed Whatman No. 1 paper by the ascending technique. If an enzymatic reaction mixture was to be subjected to paper chromatography, the protein would be precipitated with trichloroacetic acid. The deproteinized solution was extracted three times with several volumes of ether and aliquots of the aqueous phase were used for chromatography.

Proteins were determined by the method of Warburg and Christian (21) or Sutherland et al. (22).

RESULTS

The amount of AMe in a reaction mixture can be followed readily by its isolation with the use of a buffered XE-64 column and determination of the compound by its ultraviolet absorption.

Preliminary experiments showed that incubation of AMe in the presence of dialyzed extracts of air-dried yeast resulted in the disappearance of AMe. In addition, with these crude extracts a net loss of material absorbing at 259 nm occurred, indicating a further decomposition of the adenosine moiety. However, after purification, the disappearance of AMe was balanced by the appearance of a new ultraviolet absorbing material which did not adhere to the buffered XE-64 column. At this stage, the enzyme may be assayed by the appearance of this material.

Enzyme Assay and Definition of Unit—The assay as finally developed was as follows (all amounts in μmoles): potassium acetate, pH 5.6, 60; potassium phosphate, pH 5.6, 20; AMe, 1.5; and enzyme in 0.5 ml. of final volume were incubated for 2 hours at 37°. The mixture was cooled, deproteinized with 0.05 ml. of 30 per cent perchloric acid, and centrifuged. An aliquot of the supernatant fluid was carefully brought to pH 6.7 to 7.0 by the addition of an ice-cold mixture of potassium hydroxide and dilute potassium phosphate. Potassium perchlorate, which precipitated upon neutralization, was removed by centrifugation and an aliquot of the supernatant fluid was placed upon a buffered XE-64 column prepared as described above. 10 to 20 ml. of 0.01 M potassium phosphate, pH 7.0, were used to wash the MTA through the column. AMe was eluted with 10 to 20 ml. of 4 N acetic acid. Controls were run with enzyme and AMe incubated separately, the missing component being added after the perchloric acid. There was no appreciable breakdown of AMe in the absence of enzyme. A unit of enzyme was defined as the amount catalyzing the decomposition of 1 μ mole of AMe in 1 hour under the standard conditions.

Under these conditions the reaction rate diminished with increase in enzyme and with time (Fig. 1). This was probably caused by inhibition of the reaction by MTA (see below). Therefore, for the standard assay it was best to keep the final concentration of MTA in the assay mixture below 0.7 × 10⁻³ M. The activity was little altered by change in pH from 5.6 to 7.4. Acetate or phosphate buffer could be used. The affinity for AMe was difficult to measure because the enzymatic reaction proceeded at levels of substrate that were low relative to the amount which must react to obtain a reliable assay (0.2 μmoles). However, the data available allow one to estimate that a maximum value for the Kₘ (Michaelis) is 3 × 10⁻⁴ M.

Purification of Enzyme—The enzyme was extracted from air-dried baker’s yeast by autolysis for 4 hours at 37° (33% per cent weight per volume yeast in 0.067 M KH₂PO₄). Precipitation between 33 per cent and 50 per cent acetone, volume for volume, at -8°, differential heat inactivation (91° for 12 minutes at pH 6.3) and precipitation by ammonium sulfate between 300 and 400 gm. per l. at 2° were subsequently used. The result was an increase in specific activity from 0.022 unit per mg. of protein in the initial extract to 0.41 in the final fraction with a yield of 60 per cent. Almost all of the studies in this paper were carried out on such a partially purified preparation. Several preparations were made as outlined above, always starting from one large batch of air-dried yeast. When it was subsequently attempted to prepare enzyme from other batches of yeast, the yield was much lower and less reproducible. The reasons for this are not clear, but it seems that air drying is detrimental to the enzyme. Therefore, another method of extraction was developed. The fresh yeast was frozen in liquid nitrogen and subsequently extracted as above. This procedure gave a higher yield of enzyme per gm. of yeast than the best air-dried preparation. The enzyme has been partially purified from these extracts by heat treatment (12 minutes, 53°, pH 6.3) and am-
butyrolactone is an intermediate in the formation of homoserine.

hydroxamic acid upon chromatography in Solvents a (RF 0.38) and b (RF 0.08). These facts complete the proof that cr-amino-

performing the enzymatic reaction in the presence of 0.5

ammonium hydroxide, 0.6 ml., water, 40 ml. (RF 0.51); (f) assay the lactone hydrolyzes to homoserine. Furthermore, by

homoserine with the yeast enzyme. Evidence was obtained

discovery of a bacterial enzyme which cleaves AMe to products

of AMe at slightly acid pH is oc-aminobutyrolactone and the

absorb ultraviolet light, and gave a negative test with the

of these systems separated the product from threonine, methio-

yellow color when sprayed with ninhydrin, as did the authentic

material. The relative quantities of homoserine and cr-amino-

butyrolactone, 3 it became apparent that this compound may be an intermediate in the formation of

the catalytic properties of the enzyme so extracted are identical to those of the enzyme prepared from air-dried yeast.

Identification of Products—MTA was identified by its ultra-

violet absorption spectrum and by chromatography in the

following solvent systems (all given in volumes): (a) isopropanol,

70, formic acid, 10, water, 20 (RF 0.62); (b) sec-butanol, 85,

formic acid, 5, water, 10 (RF 0.27); (c) ethylene glycol mono-

methyl ether, 90, water, 10 (RF 0.67); (d) ethanol, 80, acetic acid,

0.8, water, 20 (RF 0.56). The product had Rf's not significantly
different from standard MTA and the compounds chromatographed together always gave only one spot. In one or more of these systems the product was clearly separated from adenine, adenosine, S-adenosylhomocysteine, and AMe. The product after chromatography was visualized as an ultraviolet absorbing spot which gave a positive test for sulfur when sprayed with a modification of the potassium iodoplatinate solution of Winegard and Toennies (23) but did not stain with ninhydrin or with the aniline-phthalate spray for reducing sugars (24).

Homoserine was identified as a product by paper chromatography in Solvent e to d, above (RF's, in order, 0.54, 0.11, 0.23,

0.30), and in the following solvents: (e) phenol crystals, 160 gm.,

ammonium hydroxide, 0.6 ml., water, 40 ml. (RF 0.51); (f) n-propanol, 85, water, 15, diethylamine, 4 (RF 0.51); (g) n-

butanol, 60, acetic acid, 15, water, 25 (RF 0.20). One or more of these systems separated the product from threonine, methio-

nine, methionine sulfoxide, 3-aminopropanol, and serine. The product was detected as a ninhydrin staining spot, that did not

absorb ultraviolet light, and gave a negative test with the

iodoplatinate and the aniline-phthalate sprays.

With the finding that the product of the chemical breakdown of AMe at slightly acid pH is α-amino-β-butyrrolactone and the discovery of a bacterial enzyme which cleaves AMe to products which include α-amino-β-butyrrolactone,3 it became apparent that this compound may be an intermediate in the formation of homoserine with the yeast enzyme. Evidence was obtained that this is indeed the case by the finding that during the enzymatic reaction a product is formed which (a) could be separated from homoserine in Solvent g, (b) traveled with authentic α-amino-β-butyrrolactone in this solvent (RF 0.31), and (c) gave a yellow color when sprayed with ninhydrin, as did the authentic material. The relative quantities of homoserine and α-amino-

β-butyrrolactone found are variable because during the enzyme assay the lactone hydrolyzes to homoserine. Furthermore, by

performing the enzymatic reaction in the presence of 0.5 M hydroxylamine at pH 6.0 to 6.5 it was possible to demonstrate the formation of hydroxamic acid, dependent upon the presence of both enzyme and AMe. 1-Homoserine incubated with or without enzyme under the same conditions did not form detectable hydroxamic acid. The enzymatically formed hydroxamate exhibited behavior identical to that of authentic homoserine hydroxamic acid upon chromatography in Solvents e (RF 0.38) and b (RF 0.08). These facts complete the proof that α-amino-

β-butyrrolactone is an intermediate in the formation of homoserine.

F. Schlenk, personal communication.
composed more rapidly and indeed inhibited the reaction with AMe as substrate.

Sulfonium compounds which contain three different substituents at the sulfur atom have been shown to be optically active about this center (26, 27). Since AMe falls in this category, it was of interest to explore the specificity of the cleaving enzyme for the diastereoisomers of AMe differing in configuration at the sulfur atom. This became possible with the availability of S-adenosyl-L-homocysteine racemic about the sulfur atom, synthesized by Dr. de la Haba by the action of methyliodide on S-adenosyl-L-homocysteine. AMe formed enzymatically by the methionine activating enzyme of liver and AMe synthesized chemically as above were decomposed by the cleaving enzyme at greatly differing rates. More detailed studies show that indeed the enzyme exhibits a high degree of specificity for one of these diastereoisomers of AMe. Furthermore, allo-S-adenosyl-L-methionine, the name given to the diastereoisomer which is not attacked by this enzyme, inhibits the enzymatic decomposition of the natural substrate, AMe. (See below and Fig. 2.)

In addition to the compounds mentioned above, dimethyladenosylsulfonium was tested. This contains the MTA moiety of AMe, but the sulfur atom has an altered third substituent. This compound was completely inactive as a substrate.

Methionine and S-adenosyl-L-homocysteine, the others which contain the 4-carbon side chain of AMe, were not decomposed by the enzyme.

**Inhibition by Structural Analogues of AMe**—It was pointed out earlier that one of the products of the reaction, MTA, inhibits

*To be published in collaboration with Dr. G. de la Haba and Dr. G. A. Jamieson. Recent optical rotation measurements have demonstrated that AMe formed by liver or yeast enzymes with L-methionine as substrate is correctly described as (−)-S-adenosyl-L-methionine, where the (−) refers to the contribution of the sulfonium center to the overall optical rotation of the molecule. The term allo-S-adenosyl-L-methionine is therefore superseded by (+)-S-adenosyl-L-methionine.*
the rate of cleavage of AMe (Fig. 2). The possibility was considered that this inhibition might be due to a reversal of the forward reaction, but this was apparently ruled out by finding that labeled MTA was not incorporated into AMe during incubation with AMe and enzyme. It was further shown that the inhibition could be overcome by raising the amount of AMe present, and it was concluded that the inhibition was most probably because of competition for the active site on the enzyme.

In order to study the factors governing binding at this site a series of structurally related compounds was investigated as inhibitors. The compounds were usually tested at $4 \times 10^{-3}$ M with AMe present at $1.5 \times 10^{-3}$ M. The results are shown in Fig. 2. Here, the compounds are classified in one of three categories according to their inhibitory power. In all cases the available data allow unequivocal assignment to one of these classes, although there is some variation in the relative potency of any one inhibitor if repeated tests are performed.

**DISCUSSION**

MTA was first isolated from yeast in 1907. It has since been the subject of much chemical work which culminated in the synthesis of the compound independently in three laboratories in 1951 (15, 28, 29). The biological origin of MTA remained obscure, however, until its structural relationship to AMe became clear. The present paper describes an enzyme which leads to the formation of MTA. A second pathway leading from AMe to MTA is suggested by the work of Tabor et al. (30). These authors have studied a transalkylation reaction in which decarboxylated AMe transfers its aliphatic side chain to putrescine. The expected nucleoside product is MTA (30), but this material has not yet been identified, perhaps because of the presence of enzymes which further metabolize MTA.

The enzymatic reactions in which AMe and other sulfonium compounds participate are readily understood in terms of the well known chemical properties of such compounds (31). Thus, transalkylation reactions may be viewed as nucleophilic substitutions in which there is attack upon one of the carbons immediately adjacent to the positively charged sulfur. The overall result is the formation of a-aminobutyrolactone in a reaction similar to the reported lactonization of cis,cis-muconic acid (39). At present there is no critical evidence available to decide between the two pathways.5

As noted above, an attempt has been made to study the enzyme-substrate binding by the use of structural analogues of AMe as inhibitors. It may be pointed out that the relatively simple geometry of this system may offer some advantages for study. Thus, if the substrate be regarded as a sulfur atom bonded to three variously substituted carbon atoms, there is available a class of analogues, the thio ethers, each consisting of a sulfur atom bonded to any two of these carbons. In passing from substrate to analogue the spatial relationship of the sulfur to these carbons is little altered in the sense that the three atoms continue to lie, of course, in a single plane. Moreover, the carbon-sulfur-carbon bond angle which is known to be $106 \pm 4^\circ$ in the thio ether (40) may be estimated to be $110 \pm 5^\circ$ in the sulfonium substrate. (The second figure in the absence of accurate measurements has been arrived at by analogy with the corresponding angle in sulfoxide compounds (40, 41).) These materials then may comprise a type of analogue not available when studying a more complicated 4-bonded carbon substrate.

The above description must be regarded as only a summary of the over-all results. The final analysis of any of these reactions must, of course, account for the catalytic role of the enzyme involved, but so little information is currently at hand in this area that further discussion seems unwarranted.

The reaction described in this paper can be formulated also according to this general mechanism. Here, a carboxyl oxygen of AMe itself attacks the γ-carbon atom adjacent to the sulfur, leading to displacement of the thio ether MTA and to formation of α-aminobutyrolactone.

An alternative mechanism is suggested by consideration of the known tendency of sulfonium compounds to undergo elimination reactions (37). Such a reaction in the present case would result in the formation of MTA and 2-amino-3-butenolic acid. This would be analogous to the known enzymatic formation of dimethyl sulfoxide and acrylic acid from dimethyl propiothetin (38). The unsaturated compound might form α-aminobutyrolactone in a reaction similar to the reported lactonization of cis,cis-muconic acid (39). At present there is no critical evidence available to decide between the two pathways.5

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These relationships are shown diagrammatically in Fig. 2. Here the compounds are drawn as projected upon the plane of the paper. In reality, the sulfonium compounds are pyramidal with the sulfur being in front of or behind the plane of the paper. The role of the various portions of the AMe molecule in binding to the enzyme may be postulated from the available results. The adenine portion of AMe seems essential since all the compounds which inhibit to a significant extent contain this moiety and compounds in which it is missing (Nos. 8-10, 14) are almost inactive. However, the presence of the adenine or adenosine portion is not sufficient for a compound to be inhibitory. Compounds 7, 11, 12, and 13 illustrate this point. In all active compounds the adenosine has a 5'-thio-substituent. A change to a 5'-oxygen-substituent results in complete loss of activity (Compound 11). Of special interest is a comparison between Compounds 2 and 7. The over-all architecture of Compound 7 would appear to approximate that of AMe at least as well as does MTA and yet Compound 7 has lost most of its inhibitory power. The inference is drawn that the positive charge on the sulfur atom has decreased the affinity of the compound for the enzyme.

**SUMMARY**

1. An enzyme which catalyzes the conversion of S-adenosyl-l-methionine to 5'-methylthioadenosine and α-aminobutyrolactone has been extracted from baker’s yeast and partially purified.

2. The properties of the enzyme in regard to pH optimum, reversibility, substrate affinity, and substrate specificity are presented.

3. The factors contributing to enzyme-substrate binding have been studied by the use of structural analogues of S-adenosyl-l-methionine as inhibitors of the enzyme.

4. A brief discussion of the possible reaction mechanism is given.

Recently 2-amino-3-butenolic acid has been experimentally excluded as an intermediate by the demonstration that homoserine formed enzymatically from AMe in the presence of tritium-labeled water does not contain nonexchangeable tritium. Details of this experiment will be published separately.
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