THE PURIFICATION AND CHARACTERIZATION OF AN ENZYME FROM YEAST THAT PRODUCES S-ADENOSYLHOMOCYSTEINE FROM ADENOSINE AND HOMOCYSTEINE

by

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A Dissertation Submitted to the Faculty of the

DEPARTMENT OF MICROBIOLOGY AND MEDICAL TECHNOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN MICROBIOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Richard Carl Knudsen

entitled The Purification and Characterization of an Enzyme from Yeast that Produces S-Adenosylhomocysteine from Adenosine and Homocysteine.

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

Dissertation Director — June 22, 1971 Date

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ACKNOWLEDGMENTS

I would like to thank the very many people who have helped, encouraged, and taught me during my studies at The University of Arizona. Most particularly I would like to thank Dr. Irving Yall, my major advisor, and my mother and family, whose support and encouragement made this possible.

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ABSTRACT

S-Adenosylhomocysteine (SAH) hydrolase was purified 25 fold from Baker's yeast by chemical methods and column chromatography. The purified enzyme could readily synthesize SAH from adenosine and homocysteine, but could hydrolyze only negligible amounts of SAH. The purified enzyme showed no activity towards S-Adenosylmethionine, Methylthioadenosine, or adenosine. Several nucleotides, sulfhydryl compounds, and ribose could not substitute for either adenosine or homocysteine in the reaction mixtures. S-Adenosylhomocysteine could be hydrolyzed by SAH hydrolase if adenosine deaminase was included in the reaction mixture. Under these conditions L-homocysteine could act as a product inhibitor. The activity of the enzyme in yeast cell ghosts paralleled the activity of the enzyme in vitro.

INTRODUCTION

The concept of transmethylation had its origin with the discovery by DuVigneaud et al. (11) and DuVigneaud, Dyer, and Kies (14) that a metabolic relationship existed between choline, homocysteine, and methionine. In a nutritional study using rats, they found that choline and homocysteine could replace methionine in the diet. The results suggested that a methyl group could be donated from choline to homocysteine forming methionine, and it was suggested that the reaction could be reversible.

When rats were fed methionine labled with deuterium in the methyl position (12, 13), the methyl group of methionine could be found in choline recovered from the tissues. The pattern of the deuterium label recovered from the methyl groups of choline demonstrated that the methyl group of methionine was transferred in toto, rather than passing through an intermediate such as formaldehyde.

Cantoni (1, 2) in his research on "active methionine" found the key to transmethylation with his discovery that the methyl donor in transmethlylation was methionine in the form of S-adenosylmethionine (SAM).

Since that time studies on SAM and its product after donating its methyl group, S-adenosylhomocysteine (SAH), have shown that these compounds are integrally related to

many areas of cell metabolism. Their biosynthesis involves carbohydrate, amino acid, and purine biosynthetic pathways, and they are involved in the biosynthesis of compounds as diverse as nucleic acids, spermine, adrenalin, and acetylcholine. S-Adenosylmethionine and SAH are known to occur in bacteria, plants, yeast, and mammalian cells.

A diagram of the known steps in the pathway of biosynthesis and utilization of SAM and SAH in yeast is shown in Figure 1. A similar, though not identical pathway occurs in bacteria, and the pathway in mammalian cells is probably more analogous to that of yeast.

As shown in Figure 1, homocysteine can be formed from hydrogen sulfide and O-acetylhomoserine by homocysteine synthetase (21, 4). Homocysteine can then be methylated to form methionine by either of two enzymes. One of these, SAM : homocysteine methyltransferase, requires SAM and homocysteine as substrates, and transfers the methyl group of SAM to homocysteine, forming methionine and SAH (37). The other enzyme has been demonstrated only in crude extracts of yeast (8) and requires folic acid, a methyl donor such as serine, a reducing system, and catalytic amounts of SAM.

Once methionine is formed it can be condensed with adenosine triphosphate by SAM synthetase (methionine activating enzyme) to form SAM, pyrophosphate, and orthophosphate (28). Pigg, Sorsoli, and Parks (29) demonstrated

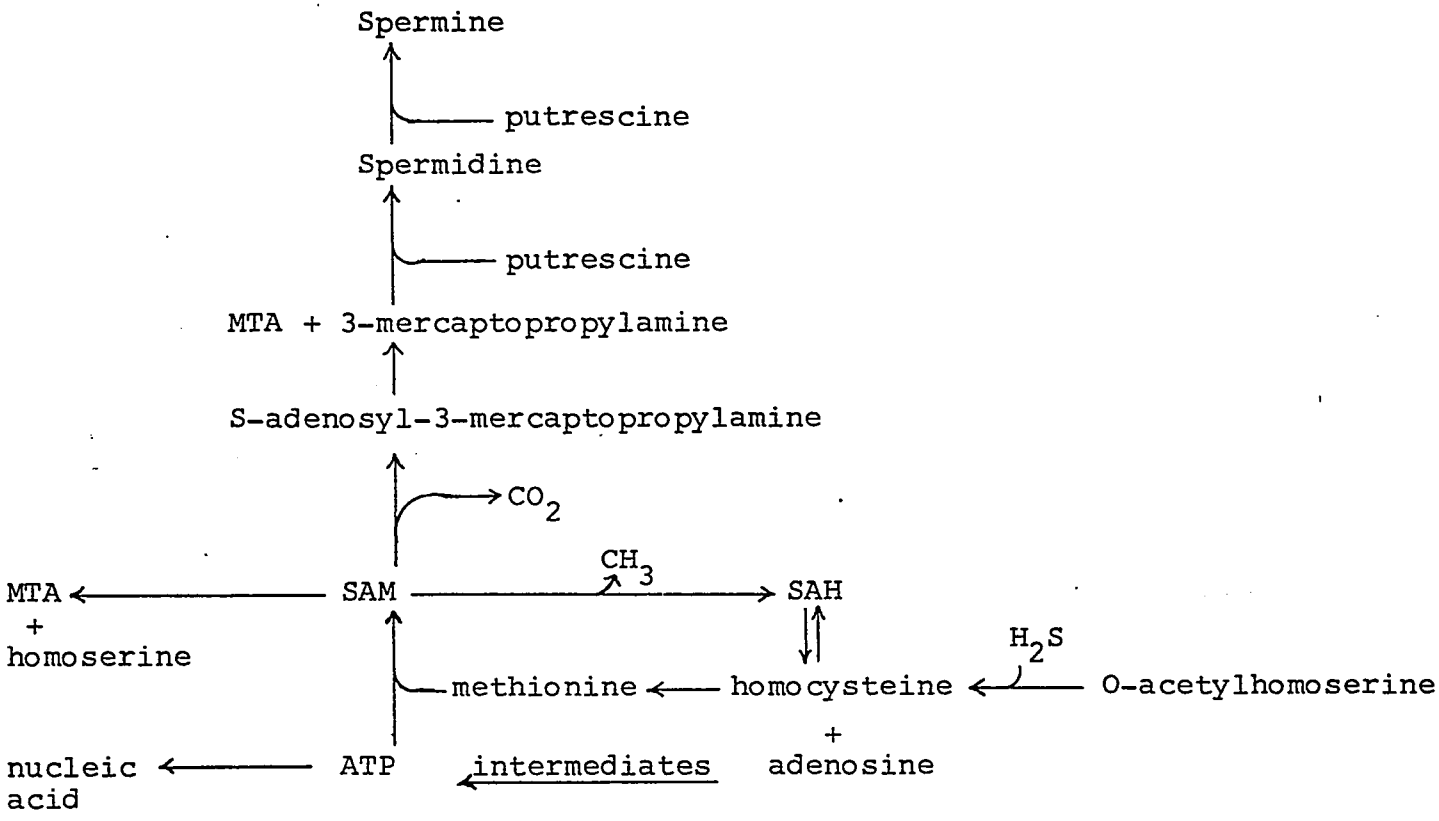

Figure 1. Pathway of biosynthesis and degradation of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in yeast -- Abbreviations: ATP, adenosine triphosphate; MTA, methylthioadenosine.

that this enzyme was inducible by methionine in yeast. The SAM formed can have four different fates.

In high concentrations of exogenous methionine SAM can accumulate in high concentrations intracellularly (31, 44), most of the accumulated SAM probably being compartmentalized in the vacuole (40). In the vacuole SAM can apparently not be used, and any other significance of this phenomenon is not known.

In bacteria SAM is an obligate intermediate in the biosynthesis of spermine and spermidine (41). In this pathway SAM is first decarboxylated to yield S-adenosyl-3-mercaptopropylamine. The propylamine portion is donated to putrescine forming spermidine and methylthioadenosine (MTA). An additional propylamine group can be donated to spermidine forming spermine. Methylthioadenosine is presumably cleaved to yield adenine and methylthioribose by a pathway similar to one demonstrated in bacteria (7). The biosynthesis of spermine and spermidine has not yet been demonstrated in yeast, but since spermine can be found in yeast (15), and no other pathway of spermine synthesis has been demonstrated in microorganisms, it is likely that the biosynthetic pathway in yeast is similar to the one in bacteria.

A third fate is the direct cleavage of SAM to yield α aminobutyrolactone and MTA (27). Alpha aminobutyrolactone spontaneously transforms into homoserine, and MTA is probably cleaved as mentioned above. Yall et al. (45) have

indicated that this pathway occurs in cells grown in high concentrations of methionine.

The fourth fate of SAM is to serve as a methyl donor in a wide variety of methyltransferase reactions. In a variety of cells, it can methylate C, O, N, S, atoms of various acceptor compounds (36). In yeast it is used in the biosynthesis of ergosterol (39), methionine (37), and it is the methyl donor in the methylation of transfer ribonucleic acid (tRNA) (23, 24), ribosomal ribonucleic acid (rRNA) (20, 24), and in bacteria, deoxyribonucleic acid (17). These macromolecules are all methylated at the macromolecular level.

After SAM donates its methyl group, SAH is formed. In vivo radiotracer experiments by Knudsen, Moore, and Yall (24), using an adenine requiring mutant of Saccharomyces cerevisiae, demonstrated that exogenously supplied SAM, upon being taken up by the yeast cells, could methylate tRNA and rRNA, forming SAH. Because the purine portion of the exogenously supplied SAM or SAH could be found in the nucleic acids, it was concluded that the SAH was cleaved to adenosine and homocysteine or adenine and S-ribosylhomocysteine. Crude extracts of this yeast revealed an enzyme that could condense adenosine and homocysteine to form SAH. A similar study on the in vivo and in vitro fate of SAH led Duerre (8) to similar conclusions.

A similar enzyme has been purified sixty fold from rat liver by DeLaHaba and Cantoni (5). This enzyme could form only small amounts of homocysteine and adenosine when SAH was the substrate. However, if adenosine and homocysteine were the substrates almost total conversion to SAH was achieved (equation 1).

$$H_2O + SAH \rightleftharpoons \text{adenosine} + \text{homocysteine} \qquad (1)$$

These authors demonstrated that SAH could be almost totally hydrolyzed to adenosine and homocysteine by including enzymatic systems in the reaction mixture which could convert adenosine and homocysteine to other compounds (inosine and methionine). By removing the end products of SAH hydrolysis as they are formed, the equilibrium of SAH hydrolysis is shifted from one favoring synthesis (equation 1) to one favoring hydrolysis (equation 2).

$$H_2O + SAH \longrightarrow \underset{\downarrow -NH_2}{\text{adenosine}} + \underset{\downarrow -CH_3}{\text{homocysteine}} \qquad (2)$$

$$\text{inosine} \qquad \text{methionine}$$

In bacteria SAH is degreaded to adenine and S-ribosylhomocysteine (7). The S-ribosylhomocysteine is then subsequently degraded to ribose and homocysteine (10). There has been no report of these enzymes occurring in eucaryotic cells.

S-Adenosylhomocysteine may have additional intracellular functions. It has been found to be a potent competitive inhibitor of Escherichia coli (19) and Saccharomyces cerevisiae (22) tRNA methylases in vitro, and quite possibly in yeast in vivo (22). It is possible that the degree of methylation of tRNA is dependent on the ratios of SAM to SAH located within the various compartments of the cell (22).

If SAH is a competitive inhibitor of tRNA methylases it may also be a competitive inhibitor of other methylases. High or low intracellular concentrations may have interesting regulatory functions. The enzyme responsible for degrading SAH, SAH hydrolase, seems to have its equilibrium, as demonstrated in crude extracts of yeast (8), in the direction of synthesis of SAH from adenosine and homocysteine. It was felt that a study of this enzyme, SAH hydrolase, under more carefully controlled conditions than available in crude extracts, may provide further information on the intracellular function and utilization of SAH. The objectives of the present study were twofold. First, to purify or partially purify the enzyme, SAH hydrolase, and secondly, to examine the catalytic properties of the purified enzyme.

MATERIALS AND METHODS

Microorganism

The microorganism used throughout this study was Saccharomyces cerevisiae obtained as a clonal isolate of commercial Baker's yeast (Fleischmann's).

Chemicals

DL-Homocysteine thiolactone and L-homocysteine thiolactone, adenosine, adenosine-8-^{14}C, were obtained from CalBioChem, Los Angeles, California. L-Homocysteine and DL-homocysteine were prepared from the thiolactone by treatment with 2N KOH (9). Silica gel used for grinding cells was 28-200 mesh.

S-Adenosylhomocysteine and SAH-adenosine-8-^{14}C were prepared enzymatically from yeast. In a volume of 50 ml of 0.05M potassium phosphate buffer, pH 6.8, were included adenosine, or adenosine-8-^{14}C (500 μmoles), DL-homocysteine (1000 μmoles), and 200 units of SAH hydrolase prepared by acetone fractionation (see Results section). The reaction mixture was incubated for two hours at 37C. One-tenth ml of thiodiglycol was added and protein was removed by boiling for 3 minutes and centrifugation. The volume of the supernatant was reduced by a few ml by lyophilization. S-Adenosylhomocysteine could be separated from adenosine,

homocysteine, and other residual material by column chromatography on Sephadex G-10 using 50 cm x 1.9 cm columns. The column was equilibrated and eluted with distilled water containing 0.05 ml thiodiglycol per liter, with a flow rate of 30 ml per hr. at 20C. S-Adenosylhomocysteine elutes first, followed by adenosine and then homocysteine. The SAH peak was pooled and lyophilized, and redissolved in distilled water. S-Adenosylhomocysteine could be crystallized by freezing at -20C for several days at a concentration of 40 to 60 μmoles per ml. The SAH and SAH-adenosine-8-^{14}C was examined by paper chromatography (see Identification of Reaction Products) and no ultraviolet absorbing or ninhydrin positive material could be found except SAH, and all radioactivity was confined to SAH-adenosine-8-^{14}C. About 98 per cent of the SAH formed in the reaction mixture could be recovered by this method.

S-Adenosylmethionine was prepared from Baker's yeast (32) and purified by the Dowex 50-Na^+ column procedure of Shapiro and Ehninger (35). Methylthioadenosine was prepared by heating SAM in a boiling water bath under acid conditions for 1 hr. and purifying by chromatography (33).

All other chemicals were reagent grade chemicals purchased from standard manufacturers

Adenosine Deaminase

Adenosine deaminase from intestinal mucosa was purchased from Sigma Chemical Corporation, St. Louis, Missouri. The preparation contained approximately 200 units per mg protein. One unit is defined as the amount of enzyme which causes the deamination of one μmole of adenosine to inosine per minute at pH 7.5 at 25C. The reaction can be followed by observing the optical density decrease at 265 nm which occurs when adenosine is converted to inosine. At pH 7.5 the ΔE^{M}_{265} between adenosine and inosine is 8,100.

Growth Conditions

Yeast cells used for the purification of SAH hydrolase were grown in the medium of Snell (38), as modified by Duerre (8), in a 10 liter fermenter with maximal aeration at 30C. Cells were harvested at the end of the logarithmic growth phase using a Sharples continuous flow centrifuge. The cells were rinsed with several volumes of cold distilled water and were frozen, lyophilized, and stored in vacuo over $CaCl_2$ at -15C until used.

Cells used for yeast cell ghost experiments were grown in the synthetic medium detailed by Roman (30). Experimental conditions and preparation of inocula have been described elsewhere (24). Yeast cells were harvested during the midlog phase by millipore filtration and converted

to ghosts by the method of Schlenk and Zydek-Cwick (34) using protamine sulfate.

Determination of Enzymatic Activity

The standard reaction mixture contained 10 μmoles adenosine, 10 μmoles of freshly prepared DL-homocysteine, 50 μmoles of potassium phosphate buffer, pH 6.8, and enzyme in a volume of 1 ml. The reaction was started by the addition of enzyme, and was incubated for one half hour at 37C. The reaction was stopped by placing on ice for two minutes, and then adding 1 ml of freshly prepared 6% meta-phosphoric acid. Precipitated protein was removed by centrifugation at 28,000 x g at 2C. An aliquot of the supernatant was removed and used to measure the disappearance of -SH groups by the nitroprusside test of Grunert and Phillips (18). In a 3 ml cuvette were placed 0.1 ml of deproteinized reaction mixture and 0.9 ml of potassium phosphate buffer (0.05M, pH 6.8). One ml of nitroprusside solution was added followed immediately by 1 ml of cyanide solution. The optical density at 520 nm was measured in a Beckman DU spectrophotometer 30 seconds after the addition of nitroprusside solution. In this procedure 0.5 μmoles of DL-homocysteine has an OD_{520} of 1.300. A zero time control was included to calculate the disappearance of -SH on exposure to oxygen.

Protein was measured by the method of Lowry et al. (26), or when the OD 280/260 was greater than 1, it was measured by the method of Warburg and Christian (43).

One unit of enzyme was defined as that amount necessary to cause the disappearance of 1 μmole of -SH per one half hour at 37C.

Identification of Reaction Products

S-Adenosylhomocysteine, DL -homocysteine, and adenosine could be separated by ascending paper chromatography in several solvent systems. With butanol:acetic acid:water (60:15:25) the R_f's were SAH (.21), SAH sulfoxide (.13), homocysteine (.48), adenosine (.54), and inosine (.44). With acetone:water (4:1) the R_f's were SAH (.08), SAH sulfoxide (.03), homocysteine (.55), inosine (.52), and adenosine (.60). Ultraviolet absorbing compounds were located with a UV lamp, amino acids were located by spraying with 0.25 per cent ninhydrin in acetone, and sulfur compounds were located by the platinic iodide spray (42). In these chromatographic solvent systems SAH had an R_f equivalent to published values (16), and identical with SAH prepared from rat liver enzyme (6) and purified by phosphotungstic acid precipitation (35). S-Adenosylhomocysteine was ninhydrin positive, absorbed ultraviolet light, and produced a yellow spot with platinic iodide reagent. Its absorption spectrum and E_{max} were identical to published values (6).

RESULTS

Preparation of S-Adenosylhomocysteine Hydrolase

Step 1. Extraction of the Enzyme from Lyophilized Yeast

Twenty grams of lyophilized yeast were mixed with 20 g of silica gel and ground in a mortar to a fine powder (2-3 minutes). It was observed that grinding without silica could also extract the enzyme, but yields were slightly lower. The ground cells were mixed with 160 ml of cold 0.05M potassium phosphate buffer, pH 6.8; silica gel and cell debris were removed by centrifugation at 28,000 x g at 0C for 15 minutes. The supernatant liquid was decanted and saved. The specific activity of the enzyme in dialyzed or undialyzed crude extracts remained quite variable, ranging between 0.4 and 0.7.

Step 2. Acetone Fractionation

Acetone fractionation was performed in a salted ice bath with continuous stirring while maintained at -3 to -10C. Acetone was added slowly to the crude extract to a final concentration of 10 per cent (v/v) prior to bringing the temperature below 0C to prevent freezing. Acetone was added dropwise (about 3 ml per minute) to a concentration of 40 per cent (v/v). After stirring for 15 minutes, the

solution was centrifuged at 28,000 x g at -5C for 15 minutes. The supernatant was treated as before with acetone added to 60 per cent concentration. After centrifugation, the supernatant was discarded and the precipitate was re-dissolved in potassium phosphate buffer. After allowing the solution to stand overnight, a precipitate developed which was removed by centrifugation.

Step 3. Ammonium Sulfate Fractionation

Fractionation with ammonium sulfate originally resulted in loss of enzymatic activity. Ammonium sulfate reduces the pH to about 5 and the enzyme is labile at this pH. However it was found that ammonium sulfate could be used if the solution was maintained at a higher pH.

To the 40-60 per cent acetone fraction was added an equal volume of a freshly prepared solution of cold aqueous ammonium sulfate (0.30 g/ml) which had been titrated to a pH of 8.0 with concentrated ammonium hydroxide. The solution was allowed to mix for 10 minutes, and then solid ammonium sulfate was added (0.1 g/ml) with continuous stirring to a concentration of 0.25 g per ml. After stirring for 15 minutes, the solution was centrifuged at 28,000 x g. The supernatant was treated as above with ammonium sulfate to a concentration of 0.35 g per ml and centrifuged. The precipitate was resuspended in a few ml of 0.5M potassium phosphate buffer, pH 7.5.

Step 4. Chromatography on Sephadex G-200

The 0.25-0.35 ammonium sulfate fraction was added to a column of Sephadex G-200 (Figure 2). The enzyme was eluted after the void volume and in the first portion of the only major peak. If the enzyme was chromatographed on Sephadex G-100 the enzyme eluted in the void volume. The most active fractions (specific activity > 4) were pooled and chromatographed on hydroxylapatite.

Step 5. Chromatography on Hydroxylapatite

The pooled fractions from the Sephadex G-200 column were added to a column of hydroxylapatite (Figure 3). The enzyme is eluted with 0.1M phosphate buffer and elutes after the major protein peak in this fraction. If a shorter column is used the enzyme elutes with the major protein peak. The most active fractions (specific activity > 13) were pooled, frozen, and concentrated by lyophilization. The enzyme was not affected by lyophilization or when maintained frozen for several months.

Table 1 summarizes the results of the fractionation procedure. The enzyme was purified about 25 fold with a 15 per cent recovery and a specific activity of 15. The purified enzyme was free of adenosine deaminase, methionine activating enzyme (28), SAM:homocysteine methyltransferase (37), and adenosinekinase (3, 25). It showed no activity

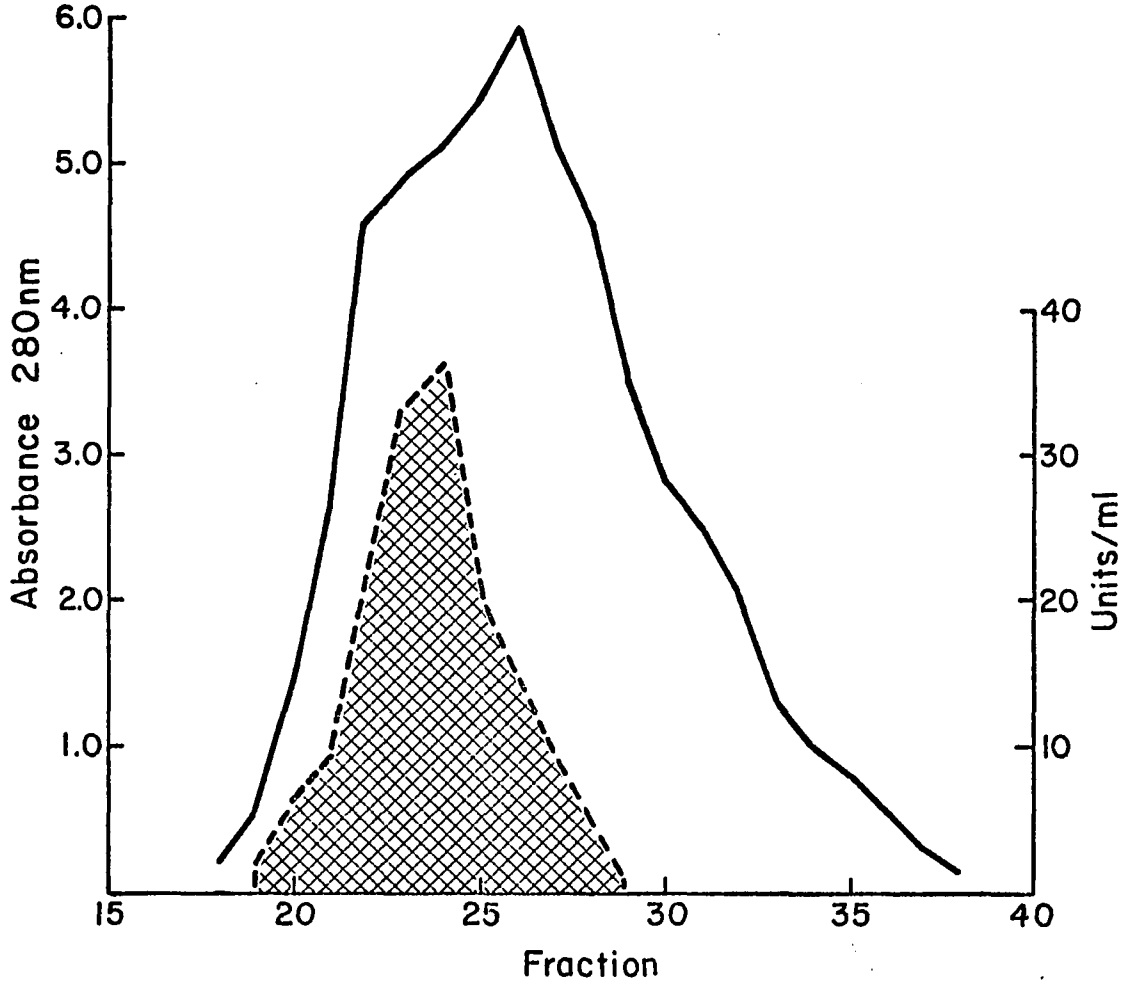

Figure 2. Fractionation of SAH hydrolase on Sephadex G-200 -- Solid line, optical density at 280 nm; shaded area, enzymatic activity as units per ml of effluent. A total of 391 mg of protein in a volume of 4.3 ml were added to the column (2.9 x 51 cm). The column was eluted with 0.05M potassium phosphate buffer, pH 6.8, and fractions of 6.5 ml were collected at a flow rate of 10 ml per hour.

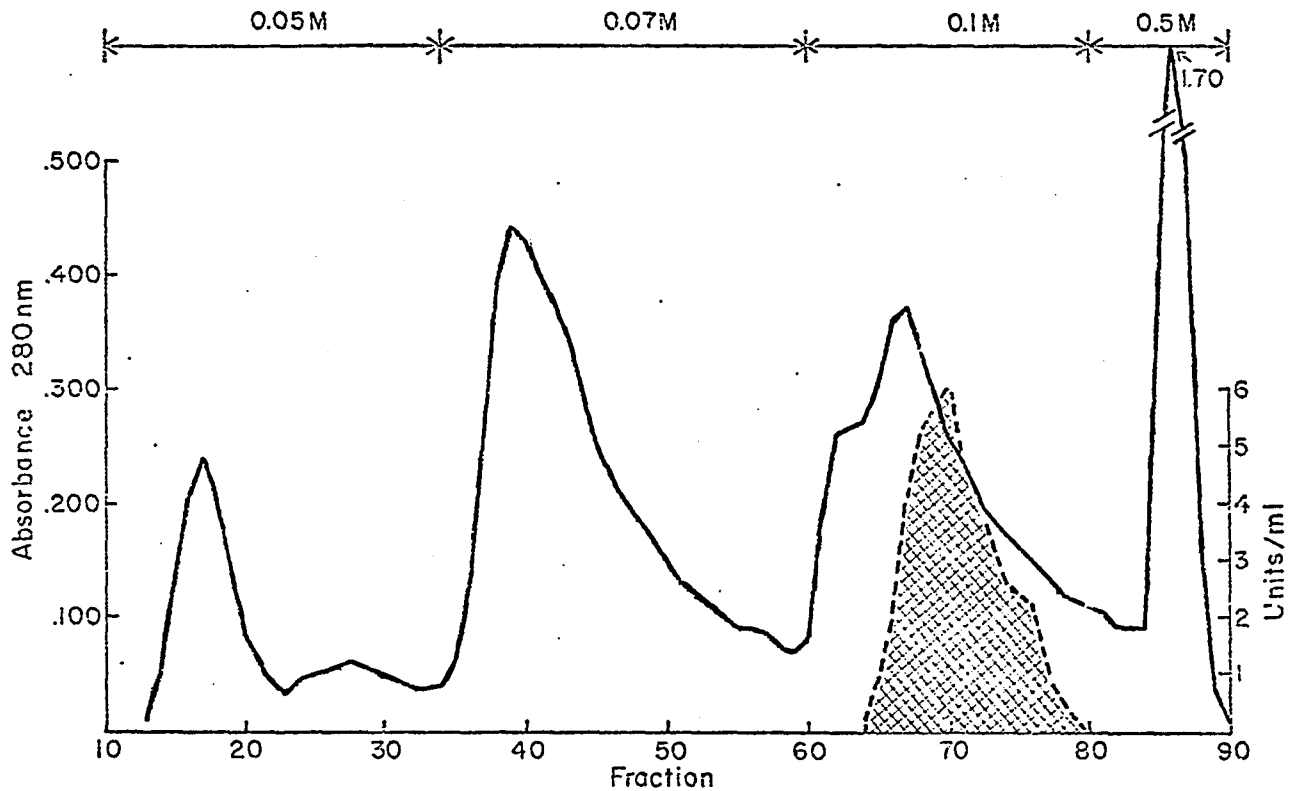

Figure 3. Fractionation of SAH hydrolase on hydroxylapatite -- Solid line, optical density at 280 nm; shaded area, enzymatic activity as units per ml. A total of 120 mg of protein are added to the column (2.9 x 19 cm), and fractions of 6.5 ml were collected at a flow rate of approximately 30 ml per hour. The column was eluted with stepwise molarity gradients of potassium phosphate buffer, pH 6.8, as indicated.

Table 1. Summary of purification of S-adenosylhomocysteine hydrolase from Saccharomyces cerevisiae.

Step	Vol	Total protein	Total activity	Specific activity	Purification	Yield
	ml	mg				%
1. Crude extract	118	2,360	1,416	.60	1.0	100
2. Acetone	18.5	999	1,480	1.48	2.4	100
3. Ammonium sulfate	4.3	391	1,118	2.85	4.9	79
4. Sephadex G-200	25.0	138	700	5.1	8.5	49
5. Hydroxylapatite	58.5	3.6	220	15.1	25.1	15.5

towards SAM, MTA, or cystathionine when included in the standard reaction mixture.

Specificity of SAH Hydrolase

Table 2 shows compounds substituted for adenosine and homocysteine in the reaction mixtures. Activity towards homocysteine and adenosine substituents was measured by disappearance of -SH groups and paper chromatography. Activity towards adenosine using homocysteine substituents was measured only by paper chromatography. For analysis by paper chromatography, enzymatic activity was stopped by placing the reaction mixtures on ice, and spotting 30 µl in 5 µl amounts directly on the paper. Each reaction mixture was compared to controls containing the enzyme plus one of the two substrates and a control without enzyme but containing both substrates. Conditions, solvents used, and location and identification of spots are discussed in the materials and methods section. None of the compounds in Column A could substitute for adenosine and none of the compounds in Column B could substitute for homocysteine except DL-homocysteine thiolactone, which showed a faint ultraviolet absorbing, ninhydrin positive spot at an R_f corresponding to that of SAH. Presumably a small amount of the thiolactone is hydrolyzed to the free amino acid in aqueous solutions.

Table 2. Compounds substituted for adenosine or homocysteine in specificity studies involving SAH hydrolase.[a]

A. Replacements for Adenosine	B. Replacements for Homocysteine
deoxyadenosine	D-homocysteine
2' adenosine monophosphate	L-cysteine
3' adenosine monophosphate	mercaptoethanol
guanosine[b]	thioglycollic acid
deoxyguanosine	DL-homocysteine thiolactone
cytidine	
uridine	
D-ribose	
l-serine	

[a]Reaction mixtures contained 50 μmoles potassium phosphate buffer (pH 6.8), 2 units of purified SAH hydrolase (Sp. act. 15), and either compounds of column A (5 μmoles) plus DL-homocysteine or compounds of column B (5 μmoles) plus adenosine (10 μmoles). Reaction mixtures were incubated at 37C for 2 hours. For measurement of product formation see text.

[b]Of the 5 μmoles in the reaction mixture, most was as the insoluble precipitate.

Enzymatic Decomposition of SAH

The purified enzyme could form SAH from adenosine and homocysteine but was virtually inactive against SAH. However, if SAH is incubated with SAH hydrolase and adenosine deaminase, SAH can be degraded to adenosine (inosine) and homocysteine. This is demonstrated in curve 1, Figure 4. The change in optical density at 265 nm indicates the deamination of adenosine to inosine. L-Homocysteine (curve 2) when included in the reaction mixture causes product inhibition. Curve 3 shows the activity of adenosine deaminase towards adenosine, which is at the same concentration as SAH in curve 1. L-Homocysteine did not interfere with the action of adenosine deaminase towards adenosine. When SAH was incubated with adenosine deaminase no change in optical density occurred. When SAH was incubated with SAH hydrolase for 1 hour, heated in a boiling water bath for one minute, cooled to room temperature, and adenosine added, no change in optical density occurred.

No alteration of the reaction rate of curve 1 occurred if methionine, spermidine, putrescine, serine, cysteine, glutathione, or cystationine were included in the reaction mixture at concentrations equivalent to SAH, nor could MTA replace SAH in the reaction mixture.

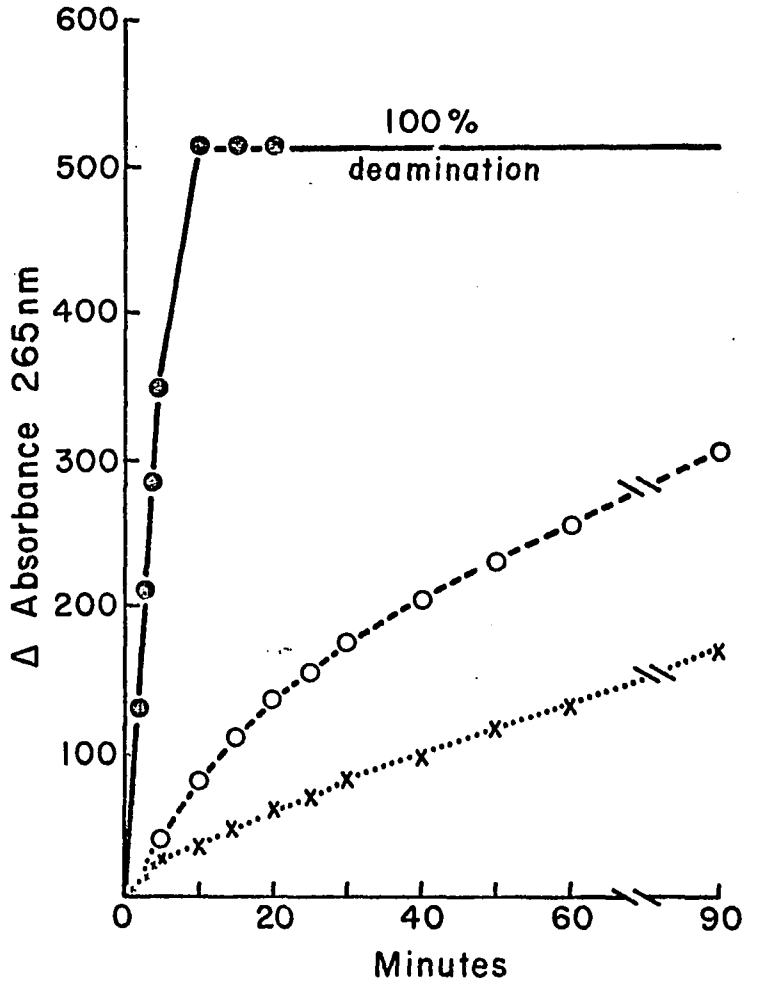

Figure 4. Enzymatic decomposition of SAH by SAH hydrolase and adenosine deaminase -- In a volume of 3 ml of 0.05M potassium phosphate buffer, pH 6.8, at 25C, were included 0.22 μmole SAH, 6 units SAH hydrolase (specific activity 17), and 0.02 units adenosine deaminase (curve 1). Curve 2: in addition, 0.22 μmole L-homocysteine. Curve 3: reaction mixture contains only 0.22 μmole adenosine and 0.02 units of adenosine deaminase. Conversion of adenosine to inosine is measured by the change in optical density at 265 nm.

S-Adenosylhomocysteine Hydrolase in Yeast Cell Ghosts

There was a possibility that SAH hydrolase could react differently if studied within the milieu of the cell. Such an opportunity can be explored by using yeast cell ghosts (34). Figure 5 shows that in yeast cell ghosts SAH hydrolase activity is similar to the activity of the purified enzyme. The yeast cell ghosts can synthesize SAH from adenosine and homocysteine, but can cause only a slight hydrolysis of SAH to adenosine and homocysteine.

Examination of adenosine-8-^{14}C controls by paper chromatography showed all radioactivity was confined to the ultraviolet absorbing adenosine spot, and no radioactivity could be found in the spot corresponding to inosine.

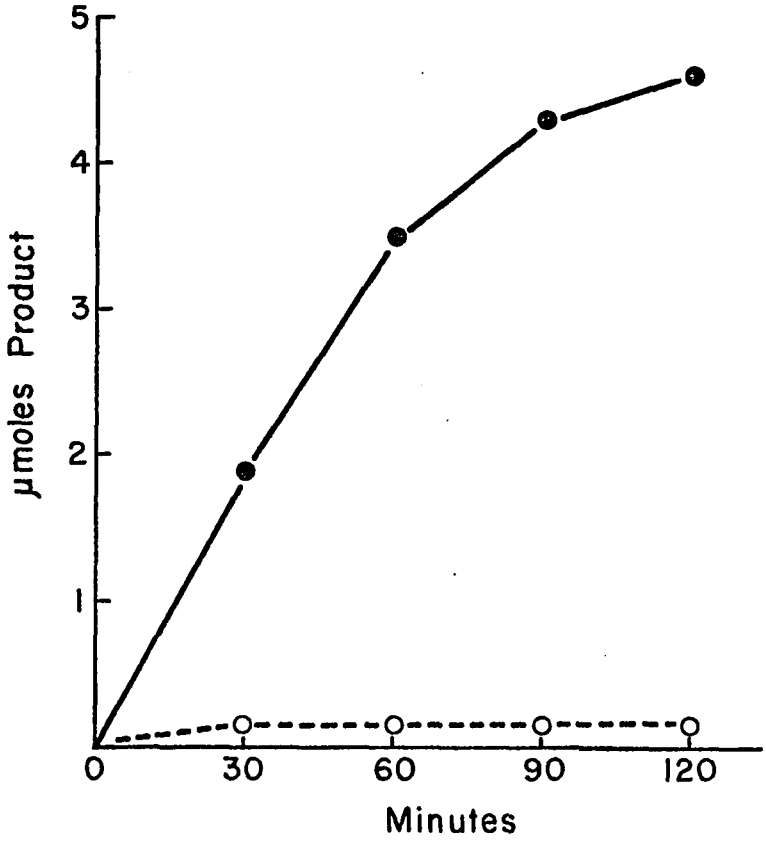

Figure 5. Activity of SAH hydrolase in yeast cell ghosts -- The reaction mixtures contained 50 µmoles of potassium phosphate buffer, pH 6.8, 20 mg dry weight of yeast cell ghosts, and either 10 µmoles of DL-homocysteine plus 10 µmoles of adenosine (●) or 5 µmoles of SAH (○) in 1 ml, incubated at 37C. Enzymatic activity was determined by the nitroprusside test.

DISCUSSION

In vivo studies of SAH utilization in yeast by Knudsen et al. (24) and Duerre (8) using radioactively labeled SAM, SAH, and adenine indicated that SAH was degraded to adenosine and homocysteine, with most of the adenosine subsequently entering nucleic acids, and homocysteine being methylated to form methionine.

In crude extracts of yeast an enzyme was demonstrated which mediates the reaction (8,24):

$$\text{adenosine} + \text{homocysteine} \rightleftharpoons \text{S-adenosylhomocysteine} + H_2O$$

The equilibrium of the enzyme favored the synthesis of SAH. This enzyme in yeast is similar in activity to an enzyme purified from rat liver (5), and different from an enzyme from bacteria (7). This latter enzyme cleaved SAH to s-ribosylhomocysteine and adenine.

In the present work, the enzyme from yeast was purified 25 fold and some of its catalytic properties examined. As in the crude extracts of yeast, the purified enzyme could synthesize SAH from adenosine and homocysteine. However, when SAH was the substrate, only slight amounts could be hydrolyzed to adenosine and homocysteine. Differences in pH or temperature did not significantly alter SAH

hydrolysis. The enzyme was specific for adenosine and homocysteine since no other nucleosides, ribose, or -SH compounds tested could replace either adenosine or homocysteine in the reaction mixture (Table 2). The purified enzyme was also free of SAM synthetase and could not degrade SAM or MTA. Sephadex G-200 and G-100 chromatography during purification indicated the molecular weight of the enzyme was between 10^5 and 2×10^5. The enzyme differs from the rat liver enzyme by its lability to ammonium sulfate fractionation.

Yeast cell ghosts provided an opportunity to study the enzyme under conditions which might simulate enzymatic activity in vivo. Yeast cell ghosts are prepared by the action of small basic proteins such as cytochrome c or protamine sulfate on the yeast cell membrane, which tears holes in the membrane large enough to allow low molecular weight compounds to leak out, but small enough to keep high molecular weight compounds (MW $\geq$ 13,000) such as enzymes and nucleic acids within the cells (34). The extent of disruption in macromolecular organization, intracellular arrangement, or compartmentalization is not known. Several enzymes, however, have been shown to function in ghosts (34). It was thought possible that SAH hydrolase might show greater hydrolytic activity towards SAH if it were examined in what might be a more natural state within the yeast cell ghost. Figure 3 shows that the enzymatic activity in yeast

cell ghosts closely parallels activity of the enzyme in vitro. Only a small amount of SAH is hydrolyzed, whereas SAH is readily synthesized from adenosine and homocysteine.

The difference between the theorized degradation of SAH to adenosine and homocysteine in vivo (8, 24) and the observed synthesis of SAH from adenosine and homocysteine in vitro and in yeast cell ghosts, can be resolved by postulating that the degradation of SAH is driven in vivo by enzymes that remove adenosine and homocysteine as they are formed. This possibility is demonstrated in Figure 3, where an increased amount of SAH is hydrolyzed by SAH hydrolase when adenosine is removed by converting it to inosine in the presence of commercial adenosine deaminase. When homocysteine is included in the reaction mixture, a slower rate of SAH hydrolysis is observed, indicating product inhibition by homocysteine. If an enzymatic system was included in the reaction mixture which could remove homocysteine (by methylating it to methionine, for example) there should be an even higher rate of SAH hydrolysis.

Two enzymes have been demonstrated in yeast extracts that are capable of methylating homocysteine to methionine (8, 37). and it is quite possible that these enzymes can account for the removal of homocysteine in vivo. This author is aware of no reports of the demonstration of adenosine deaminase in yeast. Research results in process

in this laboratory indicate that using undialyzed crude extracts of yeast incubated with adenosine-8-^{14}C, small amounts of inosine-8-^{14}C can be formed. However the deaminase activity appeared to be too low to drive SAH hydrolysis to any degree. Furthermore, adenosine deaminase activity could not be demonstrated in yeast cell ghosts, although it is possible that the method of preparing the ghosts is injurious to the enzyme.

A more likely candidate for an enzyme to drive SAH hydrolysis is adenosinekinase. This enzyme has been demonstrated in yeast by Caputto (3) and has been purified severalfold by Kornberg and Pricer (25). Adenosinekinase catalyzes the reaction:

adenosine + adenosine triphosphate $\longrightarrow$
adenosine monophosphate + adenosine diphosphate

This enzyme is currently under study in our laboratory and results indicate it is present in crude extracts in sufficient quantity to drive SAH hydrolysis by converting the adenosine moiety of SAH to adenosine monophosphate.

S-Adenosylhomocysteine is known to be a product inhibitor of several tRNA methylases (23), and maybe other methylases as well. Kjellin-Straby (22) has postulated from studies on various methionine mutants of Saccharomyces cerevisiae, that SAM:SAH ratios may have a controlling effect on RNA synthesis, although true intracellular

measurements of these compounds is difficult due to compartmentalization and lability. In low SAM concentrations and high SAH concentrations methylation of unmethylated or submethylated tRNA would be inhibited by SAH. Presumably, submethylated tRNA would not be functional, and in turn could have feedback effects on other areas of nucleic acid or protein synthesis.

The equilbrium of SAH hydrolysis in yeast could be an interesting control mechanism in itself. Should enzymatic removal of adenosine and homocysteine be decreased, a buildup of these compounds within the cell would lead to synthesis of SAH, which, in turn, could lead to product inhibition of tRNA methylases (and maybe other methylases as well) with consequent inhibition of other cellular activities.

The pathway of SAM and SAH biosynthesis and utilization indicates that it can be partially cyclical with purines being drawn off for nucleic acid synthesis and amino acids being drawn off for spermine and homoserine biosynthesis. Input for the cycle comes from purine biosynthesis and methionine biosynthesis. Purine entering SAM and SAH can theoretically be recycled via adenosine and nucleoside phosphates back into SAM and SAH or terminate by entering nucleic acids. Homocysteine, originating from SAH can be remethylated to form methionine, the methionine condensing with ATP to form SAM, etc. The cyclical nature of the

pathway prompts one interesting question. Does the adenosine monophosphate in nucleic acids originate from a pool of adenosylphosphate compounds formed directly as a result of purine synthesis or from a pool of adenosyl compounds which first had to pass through the ATP-SAM-SAH-adenosine pathway prior to incorporation into nucleic acids? Knudsen et al. (24) have shown, using an adenine requiring mutant of S. cerevisiae, that exogenously supplied SAM and SAH can satisfy the purine requirements of this microorganism to the same extent as exogenously supplied adenine. If the latter pathway were true, it could provide a control mechanism for coordinating nucleic acid synthesis (including methylation of nucleic acids) with purine synthesis. The amount of nucleic acid synthesis may depend on the amount of SAM, MTA, and SAH being formed.

The close similarities between SAH hydrolysis in yeast and in rat liver would suggest that control functions and utilization of SAH, adenosine, and homocysteine are similar in eucaryotic cells. Since SAH is readily degraded to S-ribosylhomocysteine and adenine in bacteria (7), different mechanisms would have to function. The pathway of SAH utilization in different organisms may reflect another difference between eucaryotes and procaryotes.

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