**Biosynthesis of Methionine in *Saccharomyces cerevisiae***

**KINETICS AND MECHANISM OF REACTION OF S-ADENOSYL METHIONINE : HOMOCYSTEINE METHYLTRANSFERASE***

**STANLEY K. SHAPIRO, ALDONA ALMENAS, AND JOHN F. THOMSON**

*From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439*

(Received for publication, November 23, 1964)

It is now firmly established that S-adenosylmethionine is the most important biological methyl donor in a variety of transmethylation reactions (1). However, in spite of the large number of methyltransferases that have been described, little information is available concerning the enzymatic mechanism of the methyl transfer from S-adenosylmethionine to a substrate resulting in the formation of a methylated product and S-adenosylhomocysteine. In previous work (2) we have described a methyltransferase obtained from *Saccharomyces cerevisiae* that catalyzes the following reaction

\[
\text{Homocysteine} + \text{S-adenosylmethionine} \rightarrow \text{methionine} + \text{S-adenosylhomocysteine} + \text{H}^+ \quad (1)
\]

Since this S-adenosylmethionine:homocysteine methyltransferase has been highly purified, an investigation of Reaction 1 as a model system for studies on the mechanism of methyl transfer in methionine biosynthesis was undertaken. The purified yeast enzyme seems well suited for such studies because the reaction requirements are relatively simple (2) compared with the complex requirements of the 5-methyltetrahydrofolate-homocysteine methyltransferases that have been isolated from *Escherichia coli* (3, 4).

**EXPERIMENTAL PROCEDURE**

**Source of Enzymes**—S-Adenosylmethionine:homocysteine methyltransferase was extracted from *S. cerevisiae* and was purified by alcohol fractionation and DEAE-cellulose chromatography (2). The enzyme preparation selected for use in most of the experiments had a specific activity 70 times that of the crude yeast extract. For some experiments, cell-free extracts of *Aerobacter aerogenes* (ATCC 8724) were prepared by sonic disintegration (5).

**Compounds**—S-Adenosylmethionine (L and D isomers) and S-adenosyl-L-thionine were isolated from yeast cells (6, 7). S-Adenosyl-L-homocysteine and S-riboyl-L-homocysteine were prepared according to the methods of Duerre (8). The preparation of dimethylthiodenosine and S-riboyl-L-methionine has been described by Parks and Schlenk (9). S-Methyl-L-methionine was synthesized chemically (10). L-Homocysteine was prepared from commercial L-homocysteine thiolactone (2). All other compounds were commercial products.

**Reaction Conditions and Assay for Methionine**—Standard reaction mixtures contained 0.1 ml of enzyme, 4 μmoles of L-homocysteine, 4 μmoles of S-adenosyl-L-methionine-14CH₃, 0.05 μmol of Zn++, and 0.1 M phosphate buffer, pH 7.0, in a total volume of 0.5 ml. Reaction mixtures minus homocysteine were prepared as controls for the complete reaction mixtures. All reaction components except the methyl donor were preincubated for 15 min at 37°C, and after addition of the methyl donor, the complete reaction mixtures were incubated for 1 hour. Methionine formation was measured by the tracer assay of Shapiro and Yphantis (11) with some modifications. The reaction mixtures were rapidly cooled to 0°C, an aliquot of 0.1 ml was placed on a Dowex 50 column (0.5 × 2 cm) in the Li⁺ form, and the methionine produced was quantitatively recovered after five washes of 0.2 ml of water. Paper chromatograms of the eluates were examined for radioactivity with an automatic scanning and recording device (12), and methionine was the only radioactive product detected. The 1.1-ml eluates were mixed with 90 ml of 50% toluene and 50% absolute ethanol containing 0.4% 2,5-diphenyloxazole. Radioactivity was measured in a Packard Tri-Carb spectrometer, and the amount of methionine produced was calculated on the basis of the specific radioactivity of the methyl donor, S-adenosyl-L-methionine-14CH₃. In the absence of 14C-labeled methyl donor, a modification (5) of the nitroprusside test of McCarthy and Sullivan (13) was used to measure methionine.

**Paper Chromatography**—Ascending chromatograms on Whatman No. 1 paper were developed with either 1-butanol-acetic acid-water (60:15:25, v/v) or pyridine-acetic acid-water (60:15:25, v/v). A ninhydrin spray was used to detect amino acids (2), and a platinic iodide spray was used for sulfur-containing compounds (14).

**Electrophoresis**—S-Adenosylmethionine and S-adenosylhomocysteine were separated and identified by paper electrophoresis with a Savant high voltage electrophoresis apparatus. Complete separation of the two compounds is achieved in 1 hour at 2500 volts and 120 amperes with a buffer consisting of 3% formic acid and 12% acetic acid, pH 2.0.

**Sedimentation Coefficient**—The separation cell method of Yphantis and Waugh (15) was used to determine the sedimentation coefficient of the enzyme. For this purpose, 23-fold purified enzyme (2) was suspended in 0.1 M phosphate buffer, pH 6.8, and was centrifuged for an effective time of 55 min at 52,640 rpm in a Spinco analytical centrifuge, model E.

*1 Enzyme dilutions were prepared so that 0.1 ml of the dilution per 0.5 ml of reaction mixture resulted in the synthesis of 0.02 to 0.2 μmole of methionine.*
RESULTS

Substrate Specificity of Reaction.—The specificity of the reaction for the methyl donor is shown in Table I. S-Adenosyl-L-methionine is active, whereas its isomer, S-adenosyl-L-methionine, does not serve as a methyl donor. S-Methyl-L-methionine shows slightly less activity than S-adenosyl-L-methionine. Dimethylacetothetin, dimethylpropiothetin, and betaine are inactive as methyl donors although these compounds are active in the threonine methyltransferase system of liver (16). Two other sulfonium compounds, dimethylthioadenosine, and S-ribosyl-L-methionine are inactive as methyl donors. The use of S-adenosyl-L-ethionine in a reaction mixture resulted in the formation of 0.15 pmole of L-ethionine per 0.5 ml.

The specificity of the reaction for the methyl acceptor is shown in Table II. The reaction is specific for homocysteine; no other methyl acceptors have been found. Among the inactive compounds are S-ribosyl-L-homocysteine, 2-mercaptoethylanol, and L-cysteine. The yeast enzyme utilizes L- or D-homocysteine as well as L-homocysteine thiolactone. In contrast, extracts of A. aerogenes utilize only L-homocysteine, and the D isomer and L-homocysteine thiolactone do not serve as methyl acceptors. This indicates that D-homocysteine and L-homocysteine thiolactone are not converted to L-homocysteine by the conditions of the incubation.

Optimum Conditions for Reaction.—The incorporation of various divalent metal ions into reaction mixtures containing L-homocysteine, S-adenosyl-L-methionine, and phosphate buffer resulted in stimulation of Reaction 1. As shown in Table III, 0.0001 m Zn++ resulted in a 25% greater yield of methionine and was therefore included in the standard reaction mixture as described under "Experimental Procedure."

In order to obtain a linear rate of reaction with respect to time, it was necessary to preincubate all reaction components except S-adenosyl-L-methionine for 15 min before the addition of the methyl donor. As shown in Fig. 1, preincubation of homocysteine or enzyme alone does not result in a linear rate of transmethylation upon the addition of the methyl donor. However, when both homocysteine and enzyme are preincubated, the rate of reaction is linear. Under these conditions, it was possible to obtain a linear rate of methionine formation as a function of S-adenosylmethionine concentration. On the other hand, it was not possible to obtain a linear rate of reaction as a function of homocysteine concentration, especially at low levels of homocysteine. Thus, it seemed that homocysteine might serve two functions, that of a reducing agent as well as that of a methyl acceptor.

Experiments were carried out in which various reducing agents were added to standard reaction mixtures. It may be seen (Table IV) that at a level of 0.1 µmole of homocysteine per 0.5 ml of reaction mixture, the addition of various reducing agents results in an increase in the absolute amount of methionine pro-
Effect of preincubation on the rate of methionine biosynthesis. Compounds to the left of the semicolon were preincubated at 37° for 15 min. Compounds to the right of the semicolon were added after preincubation, and the complete reaction mixtures were incubated for the times indicated. Reaction conditions and assay are described in "Experimental Procedure." H, l-homocysteine; E, enzyme; AM, S-adenosyl-l-methionine-14CH3, 86,000 cpm per µmole.

**TABLE IV**

<table>
<thead>
<tr>
<th>Homocysteine µmole/0.5 ml</th>
<th>Reducing agent</th>
<th>Methionine formed µmole/0.5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Thiodiglycol</td>
<td>0.02</td>
</tr>
<tr>
<td>0.1</td>
<td>2-Mercaptoethanol*</td>
<td>0.03</td>
</tr>
<tr>
<td>0.1</td>
<td>2-Mercaptoethanol</td>
<td>0.05</td>
</tr>
<tr>
<td>0.1</td>
<td>2-Mercaptoethanol†</td>
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</tr>
<tr>
<td>0.1</td>
<td>L-Cysteine</td>
<td>0.04</td>
</tr>
<tr>
<td>0.1</td>
<td>2,3-Dimercaptoalan</td>
<td>0.04</td>
</tr>
<tr>
<td>2.0</td>
<td>Thiodiglycol</td>
<td>0.09</td>
</tr>
<tr>
<td>2.0</td>
<td>2-Mercaptoethanol</td>
<td>0.09</td>
</tr>
<tr>
<td>2.0</td>
<td>L-Cysteine</td>
<td>0.10</td>
</tr>
<tr>
<td>2.0</td>
<td>2,3-Dimercaptoalan</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Concentration, 0.012 M.
† Concentration, 0.004 M.

Effect of reducing agents on methionine biosynthesis

The compounds listed below were added to standard (except for the indicated changes in homocysteine concentration) reaction mixtures. The methyl donor, S-adenosyl-l-methionine-14CH3, had a specific radioactivity of 150,000 cpm per µmole. Reaction conditions and assay are described in "Experimental Procedure."

Effect of 2-mercaptoethanol (0.006 M) on the biosynthesis of methionine. Compounds to the left of the semicolon were preincubated at 37° for 15 min. Compounds to the right of the semicolon were added after preincubation, and the complete reaction mixtures were incubated for 45 min. Reaction conditions and assay are described in "Experimental Procedure." H, l-homocysteine; MER, 2-mercaptoethanol; E, enzyme; AM, S-adenosyl-l-methionine-14CH3, 115,000 cpm per µmole.

**Fig. 3.** Biosynthesis of methionine as a function of L-homocysteine concentration. Standard reaction mixtures + 0.006 M 2-mercaptoethanol were used. Reaction conditions and assay are described in "Experimental Procedure." AM, S-adenosyl-l-methionine-14CH3, 200,000 cpm per µmole; S, L-homocysteine; v, reaction velocity, micromoles of methionine per 0.5 ml in 30 min.
duction. The effect of the reducing agents at higher levels of homocysteine is not as great as at the lower levels of methyl acceptor. The most effective reducing agent is 2-mercaptoethanol, and optimum activity is reached at 3 μmoles per 0.5 ml of reaction mixture (Table IV). Consequently, all subsequent reaction mixtures included 0.006 M 2-mercaptoethanol. It should be pointed out that under these conditions, 2-mercaptoethanol does not serve as a methyl acceptor. Reaction mixtures without homocysteine did not contain any radioactivity in the column effluents, and analyses of column effluents of complete reaction mixtures did not reveal any radioactive compound other than methionine after paper chromatography or paper electrophoresis. At concentrations up to 100 times that needed for homocysteine to act as a methyl acceptor, 2-mercaptoethanol is inactive.

In order to clarify the effect of 2-mercaptoethanol, standard assays were performed in which various combinations of 2-mercaptoethanol, enzyme, and homocysteine were preincubated for 15 min after which the remaining requisite components and methyl donor were added to make each reaction mixture complete for subsequent incubation. The results of these experiments, shown in Fig. 2, indicate that whether added before or after a preincubation period, the addition of 2-mercaptoethanol to standard reaction mixtures results in a marked increase in the formation of methionine. Preincubation of 2-mercaptoethanol with enzyme is more effective than preincubation of 2-mercaptoethanol with homocysteine, which seems to indicate that the primary effect of 2-mercaptoethanol is to serve as a reducing agent for the enzyme. Furthermore, the preincubation mixture of homocysteine + enzyme, with 2-mercaptoethanol added before final incubation, is also more effective than that of 2-mercaptoethanol + homocysteine. However, the greatest increase in methionine formation occurs when 2-mercaptoethanol is preincubated with both enzyme and homocysteine. Thus, it is proba-

![Figure 4](https://via.placeholder.com/150)

**Fig. 4.** Biosynthesis of methionine as a function of S-adenosyl-L-homocysteine concentration. Standard reaction mixtures + 0.006 M 2-mercaptoethanol were used. Reaction conditions and assay are described in "Experimental Procedure." Abbreviations: H, L-homocysteine; S, S-adenosyl-L-methionine-14CH3, 200,000 cpm per μmole per 0.5 ml in 30 min.

![Figure 5](https://via.placeholder.com/150)

**Fig. 5.** Biosynthesis of methionine as a function of the concentration of the product, L-methionine and S-adenosyl-L-homocysteine. Standard reaction mixtures + 0.006 M 2-mercaptoethanol were used. The methyl donor, S-adenosyl-L-methionine-14CH3, had a specific radioactivity of 200,000 cpm per μmole. Reaction conditions and assay are described in "Experimental Procedure."

![Figure 6](https://via.placeholder.com/150)

**Fig. 6.** Double reciprocal plots of initial reaction velocities against concentrations of S-adenosylmethionine. Standard reaction mixtures + 0.006 M 2-mercaptoethanol were incubated for 30 min. S-Adenosyl-L-methionine-14CH3 had a specific radioactivity of 200,000 cpm per μmole. Reaction conditions and assay are described in "Experimental Procedure." AM, S-adenosylmethionine, micromoles per ml; v, reaction velocity, micromoles of methionine per 0.5 ml in 30 min. Solid symbols (Curves 1 and 2), 0.5 μmole of methionine per ml; open symbols (Curves 3 and 4), no methionine. Triangles (Curves 1 and 3), 1.0 μmole of homocysteine per ml; circles (Curves 2 and 4), 4.0 μmoles of homocysteine per ml. Pairs of curves with three other concentrations of homocysteine were also obtained but were not plotted for the sake of clarity. The lines that did not deviate significantly from linearity were fitted by the method of least squares.
Fig. 7. Intercepts of 1/v versus 1/AM plots as a function of 1/AM. Standard reaction mixtures + 0.006 M 2-mercaptoethanol were incubated for 30 min. S-Adenosyl-L-methionine-14CH3 had a specific radioactivity of 200,000 cpm per pmole. Reaction conditions and assay are described in “Experimental Procedure.” AM, S-adenosylmethionine, micromoles per ml; H, homocysteine, micromoles per ml; v, reaction velocity, micromoles of methionine per 0.5 ml in 30 min. Curve 1, 0.47 pmole of S-adenosylhomocysteine per ml; Curve 2, no S-adenosylhomocysteine. Curve 3 (data from another experiment): circles, no methionine; triangles, 0.5 pmole of methionine per ml. The lines were fitted by the method of least squares.

The reaction velocity was measured in a series of reaction mixtures containing varying amounts of S-adenosylmethionine (AM) and homocysteine (H), both without product and with either methionine or S-adenosylhomocysteine (AH) added. In the presence of methionine, a plot of 1/v against 1/AM showed a marked departure from linearity (Fig. 6, Curves 1 and 2), whereas a plot of 1/v against 1/H showed a change in slope but no change in intercept in the presence of methionine, so that when these intercepts were then plotted against 1/AM (Fig. 7, Curve 3), the line obtained was virtually identical with that obtained in the absence of methionine. In the presence of the other product, AH, the intercepts of both 1/v versus 1/H and 1/v versus 1/AM plots were altered; the secondary plots of these intercepts against the other substrate showed that there is a

Table V

Inhibition of methionine biosynthesis

The compounds listed below were added to standard reaction mixtures. The methyl donor, S-adenosyl-L-methionine-14CH3, had a specific radioactivity of 105,000 cpm per pmole. Reaction conditions and assay are described in “Experimental Procedure.”

<table>
<thead>
<tr>
<th>Compound (4 µmoles/0.5 ml)</th>
<th>Inhibition</th>
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<tbody>
<tr>
<td>L-Methionine ..................</td>
<td>83</td>
</tr>
<tr>
<td>d-Methionine ..................</td>
<td>1</td>
</tr>
<tr>
<td>DL-Methionine sulfoxide .....</td>
<td>2</td>
</tr>
<tr>
<td>DL-Methionine sulfone .......</td>
<td>2</td>
</tr>
<tr>
<td>DL-Methionine sulfoximine ...</td>
<td>1</td>
</tr>
<tr>
<td>DL-Methioninol ................</td>
<td>1</td>
</tr>
<tr>
<td>DL-Methionine methyl ester ...</td>
<td>70</td>
</tr>
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</tr>
<tr>
<td>N-Acetyl-DL-methionine .......</td>
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</tr>
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<td>N-Benzoyl-DL-methionine ......</td>
<td>1</td>
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<td>N-Carbobenzoxy-DL-methionine</td>
<td>2</td>
</tr>
<tr>
<td>N-Hydroxymethyl-DL-methionine</td>
<td>78</td>
</tr>
<tr>
<td>L-Ethionine ..................</td>
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* DL compounds were used at a concentration of 8 µmoles/0.5 ml.

conduct studies on the kinetics of methionine biosynthesis with the S-adenosylmethionine:homocysteine methyltransferase of S. cerevisiae. The rate of methionine formation was studied as a function of homocysteine concentration at three levels of S-adenosylmethionine and as a function of S-adenosylmethionine concentration at three levels of homocysteine. Figs. 3 and 4 show that the resultant curves are virtually identical in each case. Since the intercepts are fairly similar, it appears that the ratio of substrates bound to the enzyme is of the same order. There is evidence of substrate inhibition of the reaction at the highest level of S-adenosylmethionine and at the lowest level of homocysteine (Fig. 4).

Product inhibition studies were undertaken to obtain information concerning the order of addition of the two substrates. Fig. 5 shows the rate of methionine formation as a function of the concentration of the product, methionine. There is 50% inhibition of Reaction 1 at levels as low as 0.4 µmole of added methionine per standard reaction mixture, and a similar rate of inhibition of the reaction (Fig. 5) occurs as a function of the other product, S-adenosylhomocysteine. A series of experiments was undertaken at the 25% level of inhibition caused by methionine in which the levels of both substrates were varied in the presence and absence of this product. The same series of experiments was performed at the 25% level of inhibition caused by the other product, S-adenosylhomocysteine, and the results were analyzed according to methods developed for kinetic studies of two substrate systems (17, 18).

The reaction velocity was measured in a series of reaction mixtures containing varying amounts of S-adenosylmethionine (AM) and homocysteine (H), both without product and with either methionine or S-adenosylhomocysteine (AH) added. In the presence of methionine, a plot of 1/v against 1/AM showed a marked departure from linearity (Fig. 6, Curves 1 and 2), whereas a plot of 1/v against 1/H showed a change in slope but no change in intercept in the presence of methionine, so that when these intercepts were then plotted against 1/AM (Fig. 7, Curve 3), the line obtained was virtually identical with that obtained in the absence of methionine. In the presence of the other product, AH, the intercepts of both 1/v versus 1/H and 1/v versus 1/AM plots were altered; the secondary plots of these intercepts against the other substrate showed that there is a

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* DL compounds were used at a concentration of 8 µmoles/0.5 ml.
straight line relationship for the intercept plot against 1/AM (Fig. 7, Curve 1) but not against 1/H (Fig. 8, Curve 1).

These data are consistent with several mechanisms involving an ordered binding, with homocysteine the first substrate bound and S-adenosylhomocysteine the first product released. There is also evidence of product inhibition or both product and substrate inhibition, but the data are not precise enough to distinguish among the various possibilities.

It may be noted in Fig. 6 that Curves 3 and 4 are nearly parallel. This observation implies that the coefficient of the reciprocal of the product of the substrates, $\phi_{12}$, in the Daykel equation (17)

$$\frac{E_0}{v} = \phi_1 + \frac{\phi_2}{[H]} + \frac{\phi_3}{[AM]} + \frac{\phi_{12}}{[H][AM]}$$

is essentially zero. The fact that the lines in Figs. 3 and 4 have common intercepts is consistent with these data. The rate equation would then be similar to that postulated for transaminases (19).

On the basis of Curve 2 in Fig. 7 and Curve 2 in Fig. 8, the Michaelis constants can be estimated as $3.2 \times 10^{-4}$ m for homocysteine and $8.6 \times 10^{-4}$ m for S-adenosylmethionine. When the Michaelis constants are calculated from the slopes of the lines in Figs. 3 and 4, the $K_m$ values are estimated to be $4.8 \times 10^{-4}$ m and $6.2 \times 10^{-4}$ m for homocysteine and S-adenosylmethionine, respectively. However, the former values are considered more reliable since they are derived from more data.

Inhibition of Reaction—The marked product inhibition of Reaction 1 (Fig. 5) led to an investigation into the molecular basis of this inhibition. As may be seen in Table V, d-methionine does not inhibit the reaction. Similarly, the three sulfur derivatives of methionine are inactive. If the carboxyl group of methionine is replaced by a hydroxyl group, there is no inhibition of the reaction. However, the methyl and ethyl esters of methionine are quite active as inhibitors. The only substitution on the amino group that causes inhibition is the $N$-hydroxymethyl derivative of methionine. All other $N$-substituted derivatives are inactive. Both the higher homologue, L-ethionine, and the lower homologue, methyl-L-cysteine, are inactive as inhibitors. The methyl donors of the thion-homocysteine methylpherase system (16), dimethylacetothetin, dimethylpropiothetin, and betaine, are not inhibitors of Reaction 1.

Sedimentation Coefficient—The sedimentation coefficient ($s_{20, w}$) of S-adenosylmethionine-homocysteine methyltransferase is $3.3 \pm 0.6 \times 10^{-13}$ sec. From this value it was estimated that the molecular weight of the enzyme is of the order of 30,000. This agrees with a similar estimate based on gel filtration of the enzyme on a Sephadex G-75 column (2). In the course of these experiments, 98 ± 3% of the total enzyme activity added to the separation cell was recovered.

DISCUSSION

The participation of S-adenosylmethionine in the biosynthesis of methionine is now generally recognized (3, 4, 20). However, its function as a direct methyl donor has been established only for the S-adenosylmethionine:homocysteine methyltransferases of microbial cells, seeds of higher plants, and rat liver (20). The current investigation has permitted insight into the mechanism of this reaction in yeast cells (S. cerevisiae). Although S-methyl-L-methionine can replace S-adenosyl-L-methionine as a methyl donor in Reaction 1, it is proposed that the latter compound is the “natural” substrate because only S-adenosylmethionine has been identified in yeast cells.

The homocysteine methyltransferase of S. cerevisiae utilizes only S-adenosyl-L-methionine and not the D-isomer as a methyl donor. The activity of either S-adenosyl-L-methionine or S-adenosyl-L-methionine suggests that only the methionine moiety is involved at the active site of the enzyme. The product inhibition studies support this hypothesis in that inhibition is dependent on the maintenance of the molecular integrity of methionine.

In contrast to the stereospecificity for the methyl donor, either D- or L-homocysteine can serve as the methyl acceptor in Reaction 1. This suggests either that the enzyme shows no stereospecificity with respect to the methyl acceptor or that the purified yeast extract contains a homocysteine racemase. Similarly, the extracts apparently contain a lactonase which permits conversion of homocysteine thiolactone to free homocysteine.

With only one or two exceptions, metal ions are not involved in the numerous methyltransferases that have been described (21). The participation of a metal in the S-adenosylmethionine: homocysteine methyltransferase of S. cerevisiae is indicated by the data in this report as well as in previous reports (2, 22), although the function and identification of the metal involved remain to be elucidated.

Another unusual feature of Reaction 1 is the fact that homocysteine serves both as a methyl acceptor and as a reducing agent for the enzyme. When its reducing function is replaced by 2-mercaptoethanol, homocysteine shows a rate of binding to the enzyme which is virtually identical with that of S-adenosylmethionine.

The mechanism of reaction of various homocysteine methyltransferases has been discussed in other reports (3, 4, 23-25). The present investigation represents the first study of the mechanism of methionine biosynthesis where S-adenosylmethionine is the direct methyl donor. The results reported in this paper have led to the hypothesis of a reaction involving an ordered binding in which homocysteine is the first substrate to react with the enzyme and methionine is the last product released.

Although net synthesis of methionine does not result from Reaction 1, net synthesis would occur if S-adenosylmethionine were regenerated from S-adenosylhomocysteine. This type of reaction has not been demonstrated in a system in vitro, but experiments with whole cells of S. cerevisiae suggest that such a reaction occurs. This has been discussed more fully elsewhere (2, 20).

SUMMARY

The optimum conditions, kinetics, and mechanism of reaction for the biosynthesis of methionine via the S-adenosylmethionine: homocysteine methyltransferase of Saccharomyces cerevisiae have been described. Divalent metal ions increase the rate of methionine biosynthesis. The methyl acceptor, homocysteine, also acts as a reducing agent for the enzyme, but the latter function is replaceable by 2-mercaptoethanol. Only L- or D-homocysteine can serve as the methyl acceptor whereas both S-adenosyl-L-methionine and S-methyl-L-methionine act as methyl donors. S-Adenosyl-L-ethionine serves as an ethyl donor for the biosynthesis of ethionine. The Michaelis constants for S-adenosyl methionine and homocysteine are $8.6 \times 10^{-4}$ m and $3.2 \times 10^{-4}$ m, respectively. Product inhibition of the reaction is highly specific with regard to the methionine molecule. The hypothesis

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of an ordered binding in the reaction with homocysteine the first substrate bound and methionine the last product released is supported. The sedimentation coefficient of the enzyme is $3.3 \pm 0.6 \times 10^{-13}$ sec.

Acknowledgment—The authors wish to express their thanks to Dr. David A. Yphantis of the Rockefeller Institute for his assistance with the determination of the sedimentation coefficient.

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Biosynthesis of Methionine in *Saccharomyces cerevisiae*: KINETICS AND MECHANISM OF REACTION OF S-ADENOSYLMETHIONINE:HOMOCYSTEINE METHYLTRANSFERASE

Stanley K. Shapiro, Aldona Almenas and John F. Thomson


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