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INACTIVATION OF BETAINE-HOMOCYSTEINE METHYLTRANSFERASE BY ADENOSYLMETHIONINE AND ADENOSYLETHIONINE

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SUMMARY: Preincubation of betaine-homocysteine methyltransferase, prepared from rat liver, with either S-adenosylmethionine or S-adenosylethionine results in a marked loss of enzyme activity. Gel filtration did not restore activity. However both S-adenosylhomocysteine and L-homocysteine, when added to the preincubation medium, inhibited the inactivation of betaine-homocysteine methyltransferase.

The locus formed by the enzymes which utilize homocysteine as a substrate is central to the regulation of methionine metabolism in mammalian liver (1). At this metabolic site homocysteine either may be converted irreversibly to cystathionine or may be conserved by remethylation to methionine. The original hypothesis was that 5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase, E.C. 2.1.1.13) is more significant than betaine-homocysteine methyltransferase (E.C. 2.1.1.5) for methionine conservation (2). Recent studies which demonstrated an increase in the hepatic content of betaine-homocysteine methyltransferase in the livers of rats fed diets devoid of methionine (3), as well as experiments with an *in vitro* model of the regulatory locus (4), led us to question the initial concept. In this context, we reexamined the factors which modify the activity of rat liver betaine-homocysteine methyltransferase. We confirmed the inhibition of the enzyme by both reaction products, methionine and N,N-dimethylglycine (5). Inactivation of betaine-homocysteine methyltransferase by S-adenosylmethionine and S-adenosylethionine, which has not been reported previously, is the basis for this report.

MATERIALS AND METHODS

Enzyme Preparation. Our routine preparation provides enrichment of 15-20 fold with a yield of approximately 25%. We modified our previous

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method (5). Following heat-treatment and an initial gel filtration (Sephadex G-25), the extract was concentrated by membrane filtration (Diaflo XM 300). We placed the concentrate on a column of CM Bio Gel A equilibrated with 1mM potassium phosphate, pH 7.5. The column was washed with the same buffer before we eluted the enzyme in 10mM potassium phosphate, pH 7.5.

Assays. We have described our assay for betaine-homocysteine methyltransferase (3). The system contains 2mM betaine and 7mM L-homocysteine. For protein determinations, we used the Bio Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA).

Reagents. All chemicals were of reagent grade. We purified S-adenosylmethionine and S-adenosylethionine by adsorption on Bio Rex 70 and subsequent elution with 4M acetic acid. The eluate was lyophilized and the residue was redissolved in water. Extraction with diethylether removed residual acetic acid.

RESULTS

As indicated by the data in Table I, preincubation of the partially-purified preparation of betaine-homocysteine methyltransferase with S-adenosylmethionine or S-adenosylethionine resulted in a loss of enzymatic activity. The magnitude of this decrease was a function of the content of adenosyl-derivative in the preincubation medium. Neither S-adenosylhomocysteine nor 5'-methylthioadenosine affected the activity of betaine-homocysteine methyltransferase.

The mechanism for this decrease was not reversible inhibition of the enzyme since we could not restore activity by gel filtration of the inactivated

Table I
INACTIVATION OF BETAINE-HOMOCYSTEINE METHYLTRANSFERASE

Compound	Content (nmoles)	Product Formation (% control)
Adenosylmethionine	12	103
"	28	68
"	60	41
"	100	27
Adenosylethionine	11	54
"	28	46
"	55	27
"	110	10
Adenosylhomocysteine	55	85
"	110	116
Methylthioadenosine	55	95
"	110	95

We preincubated 0.125 ml of a mixture of partially-purified enzyme preparation and the compound indicated for 30 minutes. Subsequently we added the reagents for the routine assay of betaine-homocysteine methyltransferase.

Table II
CONDITIONS FOR INACTIVATION OF BETAINE-HOMOCYSTEINE METHYLTRANSFERASE BY S-ADENOSYLETHIONINE

Preincubation		Specific Activity (nmol/60 min/mg Prot)	
Ado Et	Temp	Direct	Gel Filtration
0	37°	754	858
+	37°	85	85
+	0°	568	677
0/+[a]	37°	538	793

2 ml of enzyme preparation was preincubated for 30 minutes at the temperature indicated and in a final volume of 2.25 ml. Where noted the mixture contained 1.4 µmoles of S-adenosylethionine. Following preincubation, we assayed betaine-homocysteine methyltransferase in both untreated samples ("Direct") and samples treated by passage through columns of Sephadex G-25.

[a] 0/+ - The S-adenosylethionine was added after the preincubation.

preparation (Table II). Furthermore both compounds, S-adenosylmethionine and S-adenosylethionine, were less effective when added immediately before the enzyme assay. Finally the temperature of the preincubation was significant. Treatment with S-adenosylethionine at 0° resulted in a loss of 17% of the control activity as compared to an 88% decline when we preincubated at 37°.

Our standard incubation duration for the assay of betaine-homocysteine methyltransferase was 30 minutes. Since the addition of either S-adenosylmethionine or S-adenosylethionine directly to the assay system resulted in less inactivation than that which we observed following preincubation of the enzyme with these compounds, we considered the possibility that a constituent of the assay system was protective. Betaine was ineffective, however, the addition of homocysteine to the preincubation medium did inhibit the inactivation. We studied this effect in greater detail (Table III). High concentrations of L-homocysteine were necessary. Equivalent amounts of DL-homocysteine, L-cysteine, glutathione (reduced) and dithiothreitol failed to limit the inactivation. Consequently the effect of L-homocysteine was not that of a non-specific thiol. The addition of S-adenosylhomocysteine, at a concentration more than 10-times that of S-adenosylethionine, partially inhibited

Table III
INHIBITION OF ADENOSYLETHIONINE - INDUCED INACTIVATION OF BETAINE - HOMOCYSTEINE METHYLTRANSFERASE

Compound, μmoles		Enzyme Activity (% Control)
None		8
S-Adenosylhomocysteine,	0.5	9
"	1.5	18
"	4.8	24
L-Homocysteine,	1.0	7
"	2.0	6
"	5.0	9
"	10.0	50
DL-Homocysteine,	10.0	2
L-Cysteine,	10.0	0
Glutathione,	10.0	4
Dithiothreitol,	10.0	2

We preincubated a mixture containing partially purified enzyme, S-adenosylethionine (110 nmoles), and the compound indicated in a final volume of 0.425 ml. After 30 minutes at 37°, we initiated the assay for betaine-homocysteine methyltransferase by adding the appropriate reagent mixture.

the inactivation. We observed a further reduction in the inactivation when we increased the S-adenosyl-homocysteine to 4.8 μmoles. Neither L-homocysteine nor S-adenosylhomocysteine reactivated enzyme treated previously with S-adenosylethionine.

DISCUSSION

The mechanism by which S-adenosylmethionine and S-adenosylethionine inactivate betaine-homocysteine methyltransferase is not defined in the present study. Both the failure of gel filtration to restore activity and the fact that preincubation resulted in a greater effect than did direct inclusion of the compounds in the enzyme assay system indicate that we are not observing reversible enzyme inhibition. We cannot discriminate between the possibilities of the irreversible binding of the total S-adenosyl compounds (or some portion of the molecule) and the catalysis of some change in the primary structure of the enzyme protein. The equivalence of S-adenosylmethionine and S-adenosylethionine makes it unlikely that enzymatic transalkylation is the cause. On

the other hand recent studies suggest that the activation of cystathionine-β-synthase by adenosylethionine may result from the conversion to a more active form (6). Perhaps a similar mechanism pertains to the inactivation of betaine-homocysteine methyltransferase.

The inactivation occurs at concentrations of metabolites which may be found in vivo. Preincubation of enzyme with 224μM S-adenosylmethionine resulted in a loss of 30% of the betaine-homocysteine methyltransferase activity. The level of this compound in the livers of rats fed a high protein diet approximates 160 nmol/g and higher levels occur following the administration of methionine (7). However we were unable to demonstrate any change in hepatic betaine-homocysteine methyltransferase within two to four hours of the intraperitoneal injection of ethionine in a dose of 800mg/kg body weight (data not shown). Nevertheless we must consider the possibility that the tissue content of S-adenosylmethionine may affect the activity of betaine-homocysteine methyltransferase in vivo.

Inactivation of betaine-homocysteine methyltransferase adds another potential component to the complex mechanism for the regulation of methionine metabolism in mammalian tissues. In the past we have focussed attention on both factors which regulate the tissue content of the relevant enzymes as well as the regulatory potential implicit in the kinetic characteristics of these enzymes (1). However the metabolites in the pathway may affect the activities of enzymes for which they are neither substrates nor products. S-adenosylmethionine appears to be of particular importance. This compound activates cystathionine-β-synthase (8) and, by inhibiting 5,10-methylene-tetrahydrofolate reductase (EC 1.1.99.15) (9), limits the synthesis of 5-methyltetrahydrofolate. These effects, together with the inactivation of betaine-homocysteine methyltransferase, suggest that S-adenosylmethionine may function as a "switch". At low tissue concentrations, the two homocysteine methyltransferases would be unimpaired and the resynthesis of methionine would be facilitated. Conversely, high concentrations of S-adenosylmethionine would

activiate cystathionine synthase while limiting homocysteine remethylation. Under these circumstances, irreversible transsulfuration would be enhanced.

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