ACTIVATION OF CYSTATHIONINE SYNTHASE BY ADENOSYLMETHIONINE AND ADENOSYLETHIONINE

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SUMMARY. The administration of ethionine results in a rapid and marked increase in cystathionine synthase in rat liver. The specific activity doubles within 20 minutes following a dose of 400 mg/kg. Pretreatment with either cycloheximide or actinomycin D fails to prevent this response. Preincubation of crude liver extracts with ethionine, methionine or ATP does not affect the specific activity. However, preincubation with ATP together with either methionine or ethionine leads to a marked increase in cystathionine synthase. This finding is duplicated by the preincubation of partially-purified cystathionine synthase with S-adenosylmethionine.

Recently, Koracevic reported that the specific activity of L-serine sulfhydrase (EC 4.2.1.22) in rat liver increases following the administration of ethionine (1). Pretreatment with actinomycin D, cycloheximide, DL-methionine sulfone, L-methionine-DL-sulfoximine and ATP did not alter this response. However, DL-methionine did inhibit the ethionine-induced change. Koracevic was unable to demonstrate any effect of ethionine *in vitro* and concluded that the ethionine effect was indirect and was mediated by either a metabolite of ethionine or an ethionine-induced activator.

L-Serine sulfhydrase activity is considered a property of cystathionine-β-synthase (EC 4.2.1.22) (2). For this reason, we decided to employ a direct assay of cystathionine synthase as a means to reexamine and to extend Koracevic's interesting observation.

METHODS. We employed male Sprague-Dawley rats weighing 150-200g in the routine studies. The animals received Wayne Lab-Blox with the exception of the studies in which we maintained them on purified diets containing 3.5% casein or 55% casein (General Biochemicals).

We have described our methods for the partial purification of cystathionine synthase (3) and for the assay of this enzyme (4). Protein was determined by the Lowry method (5).

All reagents were of reagent grade. Prior to use, we purified S-adenosylmethionine by ion-exchange chromatography (6).

We used the t-test for unpaired samples for the statistical comparisons.

RESULTS. Table 1 summarizes the studies of the effect of ethionine administered in vivo. We present the data as the specific activity of the enzyme but we obtained equally significant differences when we used units/g liver; units/liver or units/liver/g body weight. The activity of cystathionine synthase was twice the control value 20 minutes after a single injection of L-ethionine (400 mg/

TABLE 1

EFFECT OF ETHIONINE ON CYSTATHIONINE SYNTHASE IN RAT LIVER

Pretreatment	Ethionine		Cystathionine Synthase
	Dosage (mg/kg)	Duration (min)	(% Control)
None	400mg	20	202*
"	"	40	396**
"	"	60	384**
"	"	120	461**
"	"	180	418**
Cyclohex.	"	"	389**
Act. D	"	"	386**
LPD Diet	"	"	309*
HPD Diet	"	"	323*
None	100mg	"	318**
"	200mg	"	472**
"	400mg	"	418**
"	600mg	"	498**

Each group contained at least 5 rats. Results for the specific activity of cystathionine synthase are given as the percentage of the value obtained in appropriate control animals. P values for the comparison of treated and control animals = * $P < .02$; ** $P < .001$.

Pretreatments: cycloheximide (20 mg/kg) or actinomycin D (2 mg/kg) by intraperitoneal injection 30 minutes before the ethionine. LP Diet (3.5% Casein) or HP Diet (55%) were fed for 7 days prior to the experiment.

Ethionine was injected intraperitoneally as a solution of 20 mg/ml in isotonic saline. The interval between the ethionine injection and the sacrifice of the animal is indicated as "duration".

kg). At 40 minutes the enzyme activity was four-times the control value and remained in this range for three hours. Pretreatment with cycloheximide or actinomycin D did not impair the ethionine-induced increase. Furthermore, we noted similar effects of ethionine in rats prefed diets which varied in protein content. We did not analyze completely the relationship of dose to response. As indicated in Table 1, we noted a significant increase in cystathionine synthase three hours after we administered ethionine at a lower dosage (100 mg/kg). In additional studies, we found that ethionine reactivity was greater in female rats. There was no change in cystathionine synthase 60 minutes after the injection of 100 mg/kg ethionine in male rats. This same treatment resulted in a 50% increase in hepatic enzyme in female rats ($p < .05$).

During the studies indicated in Table 1, we found that ethionine had little effect on the activities of methionine adenosyltransferase (EC 2.5.1.6) (4); betaine-homocysteine methyltransferase (EC 2.1.1.5) (7); 5-methyltetrahydrofolate homocysteine methyltransferase (EC 2.1.1.13) (8); and cystathionase (EC 4.4.1.1) (4).

Both the rapidity of the response to ethionine as well as the failure of cycloheximide and actinomycin to inhibit this response suggested that the increase in cystathionine synthase did not result from changes in the rate of synthesis or degradation of the enzyme. We considered the following alternative mechanisms: (1) crude liver extracts from ethionine-treated rats catalyzed the synthesis of a compound other than cystathionine; (2) ethionine-treatment resulted in a metabolite which was a positive effector of the cystathionine synthase reaction and (3) ethionine (or an ethionine derivative) caused the formation of active enzyme from an inactive precursor or activated further the normally active tetramer (9).

We excluded the first possibility. The product migrated with cystathionine during paper chromatography in both 2-propanol-88% formic acid-water (7:1:2) and methanol-pyridine-1N HCl (37:4:8). In addition, peroxide treatment yielded a compound inseparable from cystathionine sulfoxide and performate

oxidation resulted in a substance which co-chromatographed with cystathionine sulfone (10).

We could not demonstrate the presence of a positive effector in extracts prepared from the livers of ethionine-treated rats. Neither dialysis nor gel filtration reduced the specific activity of cystathionine synthase.

However, the cystathionine synthase content in a crude liver extract from untreated rats increased by 70% when preincubated with the TCA-soluble extract from livers of ethionine treated animals. The TCA-soluble "activator" was removed by AG-50 (Na^+) and was absent from TCA extracts of control livers.

We were able to demonstrate the formation of the "activator" in vitro. The post-microsomal fraction derived from the livers of untreated rats was preincubated with ethionine and/or ATP in a medium optimal for the synthesis of adenosylethionine. We removed the reactants before we measured the cystathionine synthase activity. As illustrated by Table 2, preincubation with ethionine or ATP alone had no effect on enzyme activity. Preincubation with ethionine + ATP caused a marked increase in cystathionine synthase. This was not inhibited by methionine. Indeed, methionine + ATP was as effective as ethionine + ATP.

Preincubation with purified S-adenosylmethionine activates partially-purified preparations of cystathionine synthase. The results in Table 3 suggest that the activation is a linear function of the concentration of adenosylmethionine.

DISCUSSION. Our results indicate that both S-adenosylmethionine and S-adenosylethionine activate cystathionine synthase. We require more detailed studies with maximally purified enzyme preparations before we can define the mechanism. The fact that equivalent activation occurs with partially-purified enzyme and with crude extract suggests enhanced catalysis due to some modification of the enzyme structure. However, we cannot exclude the conversion of an inactive zymogen to the active enzyme. Similarly, the equal effectiveness of adenosylmethionine and adenosylethionine makes it unlikely that transalkylation is

TABLE 2

IN VITRO EFFECT OF METHIONINE AND ETHIONINE ON CYSTATHIONINE SYNTHASE IN LIVER EXTRACTS

Preincubation Media			Specific Activity
Met	Eth	ATP	(nmoles/135 min/mg prot)
0	0	0	312
+	0	0	276
0	+	0	314
0	0	+	254
+	0	+	720
0	+	+	772
+	+	+	792

The unsupplemented preincubation medium contained 0.12M Tris, pH 7.6; 1M KCl; 40 mM $MgCl_2$; 5.5 mM reduced glutathione and liver extract (post-microsomal supernatant containing 50mg protein) in a total volume of 2.35ml. Where indicated we added L-methionine (10 µmoles); L-ethionine (10 µmoles) or ATP (22.5 µmoles). Following the 60 minute preincubation, we exchanged the buffer system to 100 mM potassium phosphate, pH 7.5 containing 1 mM reduced glutathione by means of gel filtration through Sephadex G-25 columns. Cystathionine synthase activity was measured in samples of this final preparation which contained approximately 0.75 mg protein.

involved in the activation process.

An apparent inconsistency exists between the in vivo and in vitro studies. Methionine has no effect in the intact rat (1) whereas methionine plus ATP or S-adenosylmethionine alone are activators in vitro. We suggest that this reflects the rapid utilization of S-adenosylmethionine in vivo. Farber et al. have shown that the injection of methionine at a dose of 1 g/kg leads to hepatic concentrations of adenosylmethionine in the range of 0.2 µmoles/g. An equivalent amount of ethionine results in concentrations of adenosylethionine which approximate 3.0 µmoles/g (11). It is possible that the chronic

TABLE 3

ACTIVATION OF CYSTATHIONINE SYNTHASE BY S-ADENOSYLMETHIONINE

Preincubation Ado Met (mM)	Specific Activity (nmoles/mg prot/135 min)
0	1726
0.125	1924
0.25	2200
0.625	2614
1.25	4591

We preincubated 4.5 mg of partially-purified cystathionine synthase in 0.5 ml of media containing 5 mM potassium phosphate, pH 7.5 and the indicated concentration of S-adenosylmethionine. Following the preincubation, and prior to the enzyme assay, we exchanged media as noted in Table 2.

administration of methionine would result in a greater increase in the concentration of S-adenosylmethionine. In these situations the activation of cystathionine synthase may be a homeostatic response. The utilization of homocysteine in the irreversible transsulfuration sequence would limit the resynthesis of methionine by means of the methylation of homocysteine (7,12). In this regard, it is interesting to note that adenosylmethionine inhibits the synthesis of methyltetrahydrofolate (13).

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