Catabolism and lability of *S*-adenosyl-L-methionine in rat liver extracts

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The fate of *S*-adenosyl-L-methionine was studied in rat liver extracts by analysing the distribution of radioactivity from labelled adenosylmethionine in decomposition products, which were separated from each other by chromatographic and electrophoretic means. Marked non-enzymic degradation to adenine, pentosyl-methionine, methylthioadenosine and homoserine was evident at pH 6.9–7.8. Enzymic cleavage to methylthioadenosine was stoichiometric with the accumulation of spermidine and could be totally prevented by inhibiting *S*-adenosyl-L-methionine decarboxylase. The results rule out the existence of adenosylmethionine cyclotransferase in rat liver and indicate that only two quantitatively significant enzymic processes are involved in hepatic adenosylmethionine degradation. Excluding non-enzymic decomposition, more than 99% of adenosylmethionine is demethylated and exclusively catabolized further by *S*-adenosyl-L-homocysteine hydrolase. Less than 1% of adenosylmethionine is decarboxylated and immediately utilized totally for polyamine biosynthesis.

S-Adenosyl-L-methionine is known to be cleaved, either enzymically or non-enzymically, at the six different sites indicated in Fig. 1. Sites I and IV (followed by cleavage at site II) are universally involved in two physiologically essential processes: biological methylation and polyamine biosynthesis (for references see Usdin *et al.*, 1982). At site II, cleavage is also by an enzyme transferring the 3-amino-3-carboxypropyl group of adenosyl-methionine to special uridyate residues. This has been observed in some forms of bacterial tRNA (Nishimura *et al.*, 1974) and probably occurs in other living cells as well (Williams-Ashman *et al.*, 1982). Enzymes utilizing adenosylmethionine in transamination (site V; Stoner & Eisenberg, 1975) or in reductive transadenosylation (site III; Knappe & Schmitt, 1976) have only been described in bacteria. Site VI can only be cleaved non-enzymically (Schlenk & Zydek-Cwick, 1969). In addition to site VI, sites I (Paik *et al.*, 1975), IV (Coppoc *et al.*, 1971), II and III (Schlenk & Zydek-Cwick, 1969) are also targets for non-enzymic cleavage.

Abbreviations used: MGBG, methylglyoxal bis(guan-ylhydrazone); Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; EHNA, *erythro*-9-(2-hydroxynon-3-yl)adenine.

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Fig. 1. Sites for enzymic and non-enzymic cleavage of *S*-adenosyl-L-methionine

Sites I, II, III and VI are subject to pH-dependent non-enzymic cleavage. Site I is also cleaved by a variety of methyltransferases and site II by 3-(3-amino-3-carboxypropyl)uridine synthase. Cleavage at site III occurs in connection with activation of some enzymes, e.g. pyruvate formate-lyase, lysine 2,3-aminomutase and tetrahydropteroylglutamate methyltransferase. Site IV is attacked by *S*-adenosyl-L-methionine decarboxylase and site V by a bacterial diaminopelargonic acid aminotransferase.

Since the discovery of methylthioadenosine as a potential inhibitor of spermine synthase (EC 2.5.1.22) (Pajula & Raina, 1979), enzymes attacking site II of adenosylmethionine cleavage have become especially interesting, owing to their possible regulatory role in mammalian growth processes. Several enzymes are known to cleave adenosylmethionine to methylthioadenosine in a variety of bacterial, yeast and plant cells, but very little evidence is available for the existence of such activities in animal cells (for references see Schlenk, 1983). In addition to the two polyamine-synthesizing propylamine transferases, only adenosylmethionine cyclotransferase (EC 2.5.1.4) activity has been reported to be quantitatively significant in the production of methylthioadenosine in mammals. Such activity, however, has only been demonstrated in the soluble fraction of pig (Swiatek *et al.*, 1973) and rat (Pietropaolo *et al.*, 1972) liver extracts. Some indirect evidence supporting the existence of adenosylmethionine cyclotransferase in the rat has also been reported (Edwards *et al.*, 1977; Wilson *et al.*, 1979).

Adenosylmethionine is very rapidly utilized in a variety of transmethylation reactions (Hoffman, 1980), but still retains a rather constant concentration under dramatically different conditions (Eloranta & Raina, 1977), despite serving to some extent as a sink for extra methionine (Eloranta, 1977). Adenosylmethionine metabolism, therefore, is obviously controlled by a very adaptable hepatic mechanism. The purpose of the present work was to search for catabolic routes of adenosylmethionine that could contribute to the remarkable adaptation to variations in methionine supply and the need for adenosylmethionine for transmethylation and polyamine biosynthesis. Special attention was focused on reactions producing methylthioadenosine, a metabolite that may influence cellular metabolism in several ways (for references see Schlenk, 1983).

Experimental

Chemicals

Labelled methionine and ATP were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and utilized for the synthesis of labelled *S*-adenosyl-L-methionine as described by Pegg & Williams-Ashman (1969). The *S*-adenosyl-L-methionine preparations were purified by Cellex-P chromatography (Eloranta *et al.*, 1976) and stored frozen in 1 mM-HCl. MGBG (dihydrochloride monohydrate), was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Adenine 9- β -D-arabinofuranoside (arabinosyladenine), Tris, L-canaline, L-homoserine, 5'-methylthioadenosine and Hepes were from Sigma Chemical Co., St.

Louis, MO, U.S.A., and EHNA was from Burroughs Wellcome Co., Research Triangle Park, NC, U.S.A. 5'-Methylthiotubercidin was synthesized as described by Raina *et al.* (1981). Details of other chemicals and materials are given elsewhere (Eloranta *et al.*, 1976).

Animals

Female albino rats of the Wistar strain weighing approx. 120 g were used. The rats were housed at 20°C and 50–60% humidity, maintained under regularly alternating 14 h periods of light and 10 h periods of dark and were fed (a standard diet made by Hankkija Oy, Turku, Finland) and watered *ad libitum*. Food was removed 12 h before they were killed.

Assays

The rats were decapitated under diethyl ether anaesthesia, and the livers were rapidly removed, washed in ice-cold 0.25 M-sucrose and homogenized with a Potter–Elvehjem homogenizer in 3 vol. of ice-cold 0.25 M-sucrose containing 1 mM-dithiothreitol, 1 mM-mercaptoethanol and 0.1 mM-EDTA with or without 1% (v/v) Triton X-100. Soluble fractions of the homogenates were prepared by centrifugation at 105 000 g_{av} for 1 h at 0°C. Dialysed preparations were made by passing the soluble fractions through a Sephadex G-25 column (Pharmacia Fine Chemicals, Uppsala, Sweden) with the appropriate homogenization buffer. When indicated, soluble proteins were separated from low- M_r compounds by ultrafiltration through a Diaflo UM 10 membrane (Amicon Corp., Lexington, MA, U.S.A.). The pellet was thoroughly washed with appropriate buffer solution and then dissolved in buffer to regain the initial volume. Acetone-desiccated preparations were made from the soluble fractions essentially as described by Wilson *et al.* (1979). Protein was determined in all preparations by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

All the incubations were carried out at 37°C, as were all buffer pH measurements. Reactions were stopped by adding trichloroacetic acid to give a final concentration of 8% (w/v). After centrifugation at 2000 g_{av} for 10 min at 4°C, the supernatant was separated and extracted three times with an equal volume of diethyl ether to remove most of the trichloroacetic acid. Radioactive reaction products were analysed in the ether-extracted supernatants either directly or after fractionation by Cellex chromatography (Eloranta *et al.*, 1976). The products were separated from each other jointly with authentic marker substances by paper-electrophoretic and t.l.c. methods and counted for radioactivity as pre-

viously described (Kajander *et al.*, 1976; Eloranta *et al.*, 1976, 1982a).

The activity of S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) was determined by the method of Pegg & Williams-Ashman (1969) and that of spermidine synthase (EC 2.5.1.16) as described by Raina *et al.* (1983). The assay method for methylthioadenosine phosphorylase (EC 2.4.2.28) has been reported by Cacciapuoti *et al.* (1978).

All the data given have been reproduced in at least two separate experiments, the results agreeing within $\pm 20\%$.

Results

Stability of S-adenosyl-L-methionine in experimental conditions

Since S-adenosyl-L-methionine is known to be labile at pH > 3 (Schlenk & Zydek-Cwick, 1969), the extent and type of its non-enzymic cleavage was carefully studied in the pH range 6.9–7.8. This covers most of the diverse pH values reported in studies of the degradation of this compound *in vitro*. As long as less than 20% of S-adenosyl-L-methionine was consumed, degradation rate increased with time and pH. Experiments with adenosylmethionines labelled with ^{14}C either in the methionine or in the adenine moiety indicated that all the degradation was due to cleavage at only sites II and VI (see Fig. 1), producing methylthioadenosine and homoserine or adenine and S-pentosylmethionine respectively. The rate of cleavage at both sites increased with increasing pH (Table 1). Site VI, however, was more sensitive than site II to non-enzymic hydrolysis at alkaline pH. Addition of bovine serum albumin (20 mg/ml) to the incubation mixture had no effect on the degradation. Non-enzymic formation of adenosylhomocysteine or adenosine from adenosylmethionine was not observed under any circumstances.

Catabolism of S-adenosyl-L-methionine via pathways unrelated to the transmethylation-trans-sulphuration route

Since all S-adenosyl-L-methionine catabolized by the trans-sulphuration route is first demethylated to S-adenosyl-L-homocysteine, the further degradation of the latter compound was thoroughly studied, with special interest in the existence of catabolic pathways independent of S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1).

Under the conditions described in Table 2, 36 μM -S-[8- ^{14}C]adenosyl-L-homocysteine (sp. radioactivity 5.5 Ci/mol) was equally well degraded in the presence of whole homogenate, supernatant fraction or soluble proteins of the liver.

Table 1. *Non-enzymic degradation of S-[8- ^{14}C]adenosyl-L-methionine at 37°C*

The incubation mixtures contained 20 mM-Hepes/NaOH, 75 mM-NaCl, 10 mM-EDTA, 5 mM-dithiothreitol, 2 mM-putrescine and 0.08 mM-S-[8- ^{14}C]adenosyl-L-methionine (sp. radioactivity 4.6 Ci/mol; radioactive purity 99.3%) in a final volume of 0.3 ml. At time points indicated, 0.1 ml portions were acidified with 20 μl of acetic acid and 20 μl samples were then analysed by t.l.c. (Eloranta *et al.*, 1982a) and paper electrophoresis at pH 3.5 (Eloranta *et al.*, 1976). Replicate incubations in 20 mM-Tris/HCl and 100 mM-potassium phosphate buffers gave similar results.

pH (37°C)	Incubation time (h)	Degradation products (% of total radioactivity)	
		Methylthioadenosine	Adenine
6.87	1	1.98	2.33
	2	4.74	3.42
7.06	1	2.17	3.12
	2	5.14	5.21
7.40	1	3.07	5.23
	2	6.67	7.90
7.79	1	4.10	8.51
	2	7.53	14.11

Each of the liver preparations, corresponding to 6.7 mg of wet tissue and thus to approx. 150 pmol of adenosylhomocysteine hydrolase subunits, was able to degrade completely within 20 min the 1800 pmol of [8- ^{14}C]adenosylhomocysteine present in 50 μl of the incubation mixture. Addition of 1 mM-adenosine or 1 mM-arabinosyladenine with 25 μM -EHNA, a combination known to give suicide inactivation of adenosylhomocysteine hydrolase (Eloranta *et al.*, 1982b), totally prevented the degradation of [8- ^{14}C]adenosylhomocysteine by the soluble proteins of the liver. Some slow degradation (less than 10%/h) was supported by whole homogenate as well as by the supernatant fraction in the presence of the above-stated inactivators. In both cases, however, all the degraded [8- ^{14}C]adenosylhomocysteine was recovered as [8- ^{14}C]adenosine, as judged by t.l.c., thereby indicating that a small fraction of adenosylhomocysteine hydrolase can be protected against inactivation not only *in vivo* (see Eloranta *et al.*, 1982b) but also in crude tissue preparations. It is thus evident that adenosylhomocysteine hydrolase is the sole adenosylhomocysteine-consuming activity in the liver under conditions and substrate concentrations closely resembling those *in vivo*.

As shown in Table 2, degradation of [1- ^{14}C]adenosylmethionine was as rapid in the supernatant fraction as in the whole homogenate of the liver, and seemed to be dependent on the

Table 2. *Enzymic degradation of S-adenosyl-L-[1-¹⁴C]methionine in liver extracts*

Incubations were carried on for 1 h at 37°C in 50 μ l of 0.25 M-sucrose containing 1 mM-dithiothreitol, 1 mM-2-mercaptoethanol, 0.1 mM-EDTA, 91 μ M-S-adenosyl-L-[1-¹⁴C]methionine (sp. radioactivity 2.1 Ci/mol), and the indicated liver extract each corresponding to 6.7 mg of wet liver. When indicated, S-adenosyl-L-homocysteine (AdoHcy), adenosine (Ado), L-homocysteine (Hcy), putrescine or MGBG (each at 1 mM) and EHNA (25 μ M) were included in the incubation mixture. ¹⁴CO₂ liberated during the incubation was trapped in 0.2 ml of 1 M-Hyamine hydroxide and counted for radioactivity as previously described (Eloranta *et al.*, 1976). Reactions were stopped by adding 10 μ l of 10% (w/v) HClO₄. Unchanged substrate was separated from the radioactive degradation products by adsorption to phosphocellulose discs that were then counted for radioactivity (Eloranta, 1977). All the values are corrected for non-enzymic degradation and expressed in percentages of the total radioactivity. For details of the methods used for analyses and for preparation of liver extracts, see the Experimental section.

Liver fraction	Additions	¹⁴ CO ₂ release (%)	Total degradation (%)
Whole homogenate	None	30.99	79.06
	AdoHcy, Ado, Hcy, EHNA	8.33	21.26
Supernatant	None	1.28	73.67
	AdoHcy, Ado, Hcy, EHNA	0.46	5.84
	MGBG	0.83	70.95
Soluble proteins	None	0.34	6.62
	Putrescine	0.90	7.76
	Putrescine, MGBG	0.34	6.61
	Ultrafiltrate	1.16	40.07

presence of the low-*M_r* compounds of the supernatant fraction. The degradation both in the whole homogenate and in the supernatant fraction was markedly and similarly inhibited by the combination of 1 mM-adenosylhomocysteine, 1 mM-adenosine, 1 mM-homocysteine and 25 μ M-EHNA no matter whether 1-¹⁴C-labelled (Table 2), *Me*-¹⁴C-labelled, 8-¹⁴C-labelled or *adenosine*-U-¹⁴C-labelled adenosylmethionine was used as substrate (results not shown). The significant production of ¹⁴CO₂ from both [1-¹⁴C]adenosylmethionine (Table 2) and [*Me*-¹⁴C]adenosylmethionine (results not shown) in the whole homogenate was inhibited to the same extent as was total degradation by this inhibitory combination. This indicated that both processes were largely dependent on the initial demethylation of adenosylmethionine. Obviously, the great majority of the ¹⁴CO₂ production from [1-¹⁴C]adenosylmethionine in the homogenate was due to further catabolism of the degradation products and involved membrane-bound enzymes (Table 2). One-third of the tiny fraction of ¹⁴CO₂-releasing activity still present in the supernatant was blocked by 1 mM-MGBG, and two-thirds by preventing further catabolism of adenosylhomocysteine (Table 2). It thus appears that, of the 79% degradation of [1-¹⁴C]adenosylmethionine in liver homogenate under the circumstances studied, less than 0.5% was due to adenosylmethionine decarboxylase and further polyamine biosynthesis, whereas the rest was solely connected to transmethylation reactions.

Since significant quantities of the radioactivity from [*Me*-¹⁴C]adenosylmethionine were found to be incorporated both into acid-precipitable macro-

molecules and into compounds not easily separable from adenosylmethionine by the methods used, detailed analysis of the degradation products was performed by using as substrate adenosylmethionine labelled at the adenosine moiety. Total degradation of 120 μ M-adenosylmethionine, including both *methionine*-2-³H label and either *adenosine*-U-¹⁴C or -8-¹⁴C label, was seen when it was incubated for 1 h at 37°C in 0.1 ml of liver homogenate (corresponding to 16.7 mg of wet tissue). The methionine moiety was cleaved from the adenosine moiety and catabolized to a variety of compounds separable from the catabolites of the adenosine moiety by t.l.c. Only traces of radioactivity were found as adenosylhomocysteine. When the incubation was carried out in the presence of 1.5 mM-adenosylhomocysteine, 1 mM-adenosine, 1 mM-L-homocysteine and 25 μ M-EHNA, the degradation rate of adenosylmethionine was significantly decreased, with adenosylhomocysteine becoming a major catabolite (Table 3). Since total inactivation of adenosylhomocysteine hydrolase was found to be impossible in the whole homogenate, a combination of adenosylhomocysteine, L-homocysteine and adenosine with the adenosine deaminase inhibitor EHNA was added to the reaction mixture. This blocked further catabolism of radioactive adenosylhomocysteine both by diluting it into a large adenosylhomocysteine pool and by switching adenosylhomocysteine hydrolase to favour the synthetic reaction. Owing to the very high capacity of this enzyme in the liver, however, some leakage is likely, and this explains the slight enzymic formation of radioactive adenosine under the conditions studied (Table 3). Some enzymic

Table 3. Degradation of *S*-[adenosine- U - ^{14}C]adenosyl-L-methionine and *S*-[8- ^{14}C]adenosyl-L-methionine in liver homogenate under conditions blocking the trans-sulphuration pathway

Incubation mixtures (Exp.) contained, in 0.1 ml, 1.5 mM-*S*-adenosyl-L-homocysteine, 1 mM-adenosine, 1 mM-L-homocysteine, 25 μ M-EHNA, ^{14}C -labelled 120 μ M-*S*-adenosyl-L-methionine substrate (sp. radioactivity 1.67 Ci/mol) and 50 μ l of liver homogenate corresponding to 16.7 mg of fresh tissue. For blank mixtures the homogenate was heated for 5 min at 100°C to destroy enzyme activities. Incubations were performed at 37°C for 1 h and stopped by adding 10 μ l of 50% (w/v) trichloroacetic acid. Radioactive compounds were then separated and analysed from the ether-extracted supernatants of the reaction mixtures by t.l.c. as described in the Experimental section. All the values are expressed as percentages of the total radioactivity present in the mixture.

Radioactive compound	Substrate ...	Radioactivity (% of total)			
		<i>S</i> -[adenosine- U - ^{14}C]AdoMet		<i>S</i> -[8- ^{14}C]AdoMet	
		Blank	Exp.	Blank	Exp.
<i>S</i> -Adenosyl-L-methionine		94.96	64.78	94.22	69.15
<i>S</i> -Adenosyl-L-homocysteine		0.55	26.86	1.24	21.60
Compounds co-migrating with ribose		0.50	2.45	0.62	0.63
Adenine		1.40	2.68	1.94	5.71
Adenosine		0.53	3.04	0.43	2.83
5'-Methylthioadenosine		2.06	0.19	1.55	0.08

degradation to adenine obviously occurred, in addition to the non-enzymic cleavage. Considering that the radioactivity in *S*-[adenosine- U - ^{14}C]adenosylmethionine is equally distributed between the adenine and the ribose portions, enzymic formation of adenine seems to be due to the action of methylthioadenosine phosphorylase, which also splits non-enzymically formed methylthioadenosine (Table 3). Radioactivity co-migrating in t.l.c. with ribose and some impurities of the substrate is suggested to be methylthio[U - ^{14}C]ribose 1-phosphate or its catabolite, since it was also formed from 5'-methylthio[U - ^{14}C]adenosine under identical conditions. It thus appears that under the conditions studied only two processes were responsible for the enzymic degradation of adenosylmethionine: (i) 29–23% was demethylated to adenosylhomocysteine and (ii) 1.4–2.3% was converted into methylthioadenosine. To what extent the latter process was connected to polyamine biosynthesis could not be accurately determined in whole homogenate.

Catabolism of *S*-adenosyl-L-methionine to 5'-methylthioadenosine

Preventing further catabolism of methylthioadenosine was found essential to be able to clarify the routes converting adenosylmethionine into methylthioadenosine and to study their quantitative role in adenosylmethionine consumption. Under the conditions described in Table 3, 5'-methylthio[U - ^{14}C]adenosine was cleaved by methylthioadenosine phosphorylase present in the liver homogenate at a rate exceeding 1 nmol/h per mg of wet tissue and was not degraded non-enzymically. This degradation rate is at least 100 times the capacity of liver to produce methylthio-

adenosine. Neither polyamines, EDTA and MGBG (all at 2 mM) nor 50 μ M-EHNA had any effect on the degradation of 90 μ M-[^{14}C]methylthioadenosine in liver homogenate. At a concentration of 2 mM, methylthiotubercidin was only weakly (20%) and adenine only moderately (60%) inhibitory. The only method capable of slowing further catabolism of ^{14}C -labelled methylthioadenosine in the homogenate was dilution with high concentrations of unlabelled methylthioadenosine. High concentrations of added methylthioadenosine, however, were considered undesirable, since they are likely to interfere with the processes to be studied.

Since all the documented methylthioadenosine-producing activities reside in the soluble supernatant fraction of tissues or cells and are not dependent on any low- M_r cofactors or regulators (for references see Schlenk, 1983), detailed studies of the activities converting adenosylmethionine into methylthioadenosine focused on the soluble proteins of the liver.

To minimize the activity of methylthioadenosine phosphorylase, soluble proteins were separated from phosphate and other low- M_r compounds by ultrafiltration. The protein pellet was thoroughly washed with 20 mM-Tris/HCl (pH 7.4 at 37°C) containing 1 mM-dithiothreitol, 1 mM-EDTA and 100 mM-KCl, and finally dissolved in the washing buffer to give the initial volume. This procedure had no significant effect on the activities of spermidine synthase and adenosylmethionine decarboxylase (measured in the presence of 1 mM-putrescine). The activity of methylthioadenosine phosphorylase was decreased, however, to 8% of that found in the whole homogenate and in the supernatant fraction. The remaining activity of

methylthioadenosine phosphorylase was abolished by 2mM-adenine and also by 2mM-methylthio-tubercidin. The activities of the former two enzymes were not affected by up to 2.5mM concentrations of adenine and adenosylhomocysteine or by the combination of 1mM-adenosine and 1mM-L-homocysteine with 25 μ M-EHNA. Although the activity of adenosylmethionine decarboxylase was completely abolished by 0.25mM-MGBG, even 2.5mM-MGBG had no effect on the activity of spermidine synthase. The latter enzyme was markedly inhibited, however, by 1–2mM concentrations of methylthioadenosine and methylthio-tubercidin. It seems likely, therefore, that the incubation conditions selected for further experiments (see the legend for Fig. 2) were adequate to prevent catabolism of adenosylhomocysteine and methylthioadenosine without significantly interfering with any soluble enzyme attacking adenosylmethionine at sites other than I (see Fig. 1). It must be emphasized that, in order to prove the existence of enzyme activities cleaving adenosylmethionine at sites II, III or VI, interfering formation of adenosine and adenine through the hydrolytic action of adenosylhomocysteine hydrolase and methylthioadenosine phosphorylase, respectively, should be prevented.

Approx. 30% of S-[8- 14 C]adenosyl-L-methionine was enzymically demethylated to adenosylhomocysteine and 5% was converted into methylthioadenosine (Fig. 2a) under the conditions studied. Formation of adenosylhomocysteine was not affected by 0.6mM-MGBG or 1mM-methylthioadenosine, whereas the enzymic formation of methylthioadenosine was completely prevented by MGBG and markedly decreased by methylthioadenosine, which also caused some accumulation of decarboxylated adenosylmethionine. In both cases, decreased enzymic formation of methylthioadenosine was accompanied by an equal increase in the sum of non-degraded and decarboxylated adenosylmethionine. Total radioactivity recovered as adenine (6%) and unidentified compounds (3%) is the same in all three experiments and equal to the non-enzymic adenine production (9%).

Considering that S-adenosyl-L-[2- 14 C]-methionine was to some extent contaminated by radioactive adenosylhomocysteine, methionine and homoserine, distribution of radioactivity from both of the labelled substrates is the same in all experiments with respect to adenosylmethionine consumption and adenosylhomocysteine accumulation (Fig. 2b). Pentosylmethionine, not separable from methionine by the methods used, is apparently formed only non-enzymically. Accumulation of radioactive homoserine (Fig. 2b) did not reflect any changes in enzymic methylthioadenosine production (Fig. 2a), but was equal in all three experi-

ments and somewhat lower than in the blank, which is probably due to further metabolism. Formation of homoserine thus appears to be the measure of non-enzymic methylthioadenosine synthesis and formation of spermidine the measure of enzymic synthesis. All decarboxylated adenosylmethionine is evidently converted into spermidine under the experimental conditions used, and substrate starts to accumulate if the spermidine synthase reaction is inhibited.

Formation of methylthioadenosine and homoserine was also studied in acetone-dried powdered Triton extracts of rat liver. Incubation conditions were analogous to those described in Fig. 2, except that 2mM-adenosylhomocysteine, 2mM-methylthioadenosine, 2mM-L-homoserine and 1mM-L-canaline, the last being an inhibitor blocking the conversion of homocysteine via cystathionine into homoserine, were included in the reaction mixtures. Accumulation of radioactive homoserine was found to be stoichiometric with non-enzymic synthesis and completely unrelated to enzymic formation of methylthioadenosine.

Discussion

The present results indicate that, of the very rapid utilization of adenosylmethionine in rat liver, well over 99% is used in transmethylation reactions, with the rest being directed to polyamine biosynthesis. Some conversion of adenosylmethionine into methylthioadenosine is likely to occur during the modification of certain uridylate residues of a few tRNA forms (Schlenk, 1983), but this is quantitatively negligible compared with the production of methylthioadenosine as a side product of polyamine biosynthesis. There was no indication of adenosylmethionine being subject to decomposition by oxidative transaminases, decarboxylases or other unspecific enzymes present in the whole homogenate or in the Triton X-100 extracts of the liver. The supernatant fraction of liver homogenate is easily contaminated, however, by oxidative activities releasing CO₂ from the degradation products formed from adenosylmethionine via the transmethylation-transsulphuration route. This contamination is not sensitive to MGBG and should be considered when measuring adenosylmethionine decarboxylase activity by the CO₂-trapping method (Pegg & Williams-Ashman, 1969).

The existence of hepatic adenosylmethionine cyclotransferase, also known as adenosylmethionine hydrolase or adenosylmethionine-splitting enzyme, is excluded by the present survey. Controversial results reported by Swiatek *et al.* (1973) and supported by the study by Wilson *et al.* (1979) are probably a consequence of non-enzymic

Fig. 2. Degradation of *S*-[8-¹⁴C]adenosyl-L-methionine and *S*-adenosyl-L-[2-¹⁴C]methionine by soluble liver proteins. Reaction mixtures contained in 0.5 ml: 20 mM-Tris/HCl, pH 7.4 at 37°C, 5 mM-dithiothreitol, 4 mM-EDTA, 2 mM-adenine, 2 mM-putrescine, 1 mM-adenosine, 1 mM-L-homocysteine, 25 μM-EHNA, 8 mg of soluble liver protein separated from small molecules of the supernatant fraction by ultrafiltration, and either 40 μM-*S*-[8-¹⁴C]adenosyl-L-methionine (sp. radioactivity 4.59 Ci/mol; *a*) or 40 μM-*S*-adenosyl-L-[2-¹⁴C]methionine (sp. radioactivity 4.08 Ci/mol; *b*). Protein was omitted from blanks (no. 1). Incubations were carried out in the absence (no. 2) or presence of either 0.6 mM-MGBG (no. 3) or 1 mM-methylthioadenosine (no. 4). After incubation at 37°C for 100 min, reactions were stopped by adding 0.1 ml of 50% (w/v) trichloroacetic acid. Precipitates were removed by centrifugation at 2000g for 10 min and 0.5 ml of the supernatant was ether-extracted and fractionated by Cellex (bed volume 3 cm³) chromatography with a stepwise HCl gradient formed from 20 ml each of 1 mM-HCl, 50 mM-HCl and 500 mM-HCl. Each 20 ml fraction was collected, evaporated to dryness and dissolved in 0.2 ml of either water (1 mM-HCl and 50 mM-HCl eluates) or 1 mM-HCl (500 mM-HCl eluates). Radioactive compounds present in the concentrated fractions were then analysed from 10 μl samples by paper-electrophoretic and t.l.c. methods as previously described (Kajander *et al.*, 1976; Eloranta *et al.*, 1976, 1982a). Accumulation of *S*-methyladenosylhomocysteamine was only seen in Expt. no. 4, and is shown as a shaded area (0.7% in both *a* and *b*) superimposed on adenosylmethionine bars. Spermidine is shown as a shaded area superimposed on homoserine bars. The shaded area in adenine bars indicates unidentified radioactive catabolites of adenine. *S*-Pentosylmethionine bars also include methionine.

adenosylmethionine cleavage to methylthioadenosine. The studies initially described by Pietropaolo *et al.* (1972) and later confirmed by other groups (see Usdin *et al.*, 1982) most likely dealt with conversion of adenosylmethionine into homoserine via the transmethylation-trans-sulphuration pathway. The data arising from the feeding experiments reported by Edwards *et al.* (1977) can now be fully explained also by the action of cysteine aminotransferase and effective methionine recycling (for references see Toohey, 1983). There is thus no solid support to the operation of adenosylmethionine cyclotransferase in mammalian tissues. On the contrary, cell-culture studies with some human leukaemic cell lines (Kamatani & Carson, 1980) as well as with some hamster and mouse cell lines (Boliska *et al.*, 1983) agree with the assumption that methylthioadenosine is only formed in mammalian metabolism from adenosylmethionine as a side product of polyamine biosynthesis.

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