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FORMATION OF CO_2 FROM THE CARBOXYL GROUP OF S-ADENOSYLMETHIONINE BY LIVER MEMBRANE-ASSOCIATED ENZYMES INVOLVES THE DEMETHYLATION-TRANSSULPHURATION PATHWAY

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SUMMARY

The activity released from membrane fragments into the supernatant fraction of rat liver homogenate by Triton X-100 and forming $^{14}CO_2$ from carboxyl-labeled S-adenosylmethionine (1) is not a true S-adenosylmethionine decarboxylase. It did not produce decarboxylated S-adenosylmethionine but was also able to use S-adenosylhomocysteine as a substrate. The formation of CO_2 from these two substrates was absolutely dependent on the presence of cytosol proteins and low-molecular weight compounds and it accounted for 5 to 10% of the total S-adenosylmethionine degrading activity of the supernatant fraction. The reaction showed an initial lag period and was inhibited by every intermediate of the transsulphuration pathway. It is concluded that the formation of CO_2 from S-adenosylmethionine involves the demethylation-transsulphuration route from S-adenosylmethionine to α-ketobutyric acid which is finally decarboxylated.

INTRODUCTION

It was reported by Sturman in 1976 that the activity of S-adenosylmethionine decarboxylase in rat liver homogenates was mainly localized in the crude nuclear fraction, but that this activity was not involved in the biosynthesis of polyamines (1). Marked changes were also observed in the subcellular distribution of the S-adenosylmethionine decarboxylating activity in rat liver during regeneration and development (2). Since these observations were in complete disagreement with the reported properties of S-adenosylmethionine decarboxylase (3,4), a detailed study of the decarboxylating activity liberated from membrane fragments by Triton X-100 seemed desirable.

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EXPERIMENTAL

S-Adenosyl-[1-^{14}C]homocysteine was synthesized as follows. The reaction mixture, containing in a total volume of 1 ml, 50 mM potassium phosphate (pH 7.4), 2 mM adenosine, 10 μM adenosine deaminase inhibitor, 2 mM glycine, 0.4 mM S-adenosyl-L-[1-^{14}C]-methionine (7 mCi/mmol) and 10-15 mg of crude supernatant protein from rat liver, was incubated at 37°C for 15 min. Under these conditions 50-70% of the substrate was demethylated to S-adenosyl-L-[1-^{14}C]homocysteine. The reaction was terminated by adding 200 μl of 50% (w/v) trichloroacetic acid. After centrifugation, excess acid was removed by three successive ether extractions. The supernatant was then applied to a Cellex-P (H^+ form) column and eluted with a stepwise HCl-gradient (5). The fractions containing S-adenosyl-[1-^{14}C]homocysteine were combined, evaporated to dryness at 25°C, dissolved immediately in 1 ml of water and stored at -20°C. The radiopurity of the product was more than 95% as determined by paper electrophoresis and by ascending paper chromatography as described earlier in detail (5).

L-Canaline, L-(+)-cystathionine, DL-homoserine and α-ketobutyric acid were obtained from Sigma Chemical Co. 4-Bromo-3-hydroxybenzyloxyamine (NSD 1055) was from Lederle Laboratories and Triton X-100 from BDH Chemicals Ltd.

Livers of albino Wistar rats were homogenized in 2 vol. of ice-cold 0.25 M sucrose containing 1 mM mercaptoethanol and 1 mM dithiothreitol with or without 1% Triton X-100. Crude subcellular fractions were obtained essentially as described by Sturman (1). Nuclei were prepared by the method of Chauveau et al. (6) and mitochondria as described by Chappell and Hansford (7). Each pellet was washed twice with ice-cold 0.25 M sucrose, homogenized in 0.25 M sucrose containing 1 mM mercaptoethanol, 1 mM dithiothreitol and 1% Triton X-100 and centrifuged for 45 min at 105 000 g (5). The supernatants were used for the assay of decarboxylating activity (8). Ultrafiltrates of the supernatant fractions were prepared using cell model 12 equipped with a Diaflo^R UM 10 membrane supplied by Amicon Corp.

The sources, purification and preparation procedures of other chemicals and materials as well as assay methods and other experimental details are described in the earlier reports (5,8-10).

RESULTS AND DISCUSSION

The soluble cytosol fraction of rat liver homogenate possessed a very high S-adenosylmethionine degrading activity. This activity was entirely dependent on dialysable low-molecular weight compounds, and could be largely restored by the addition of 1 mM glycine to dialysed enzyme preparations. On the basis of chromatographic and electrophoretic analysis (5) identical radioactive products were produced whether S-adenosyl-L-[1-^{14}C]methionine or S-adenosyl-L-[2-^{14}C]methionine was used as a substrate. The initial degradation product appeared to be S-adenosylhomocysteine, and its further

catabolism could be prevented by inclusion in the incubation mixture of 2 mM adenosine and 10 μM adenosine deaminase inhibitor, which profoundly inhibits the cleavage of S-adenosylhomocysteine by a specific hydrolase (EC 3.3.1.1; for references see 11). Similar results were also obtained when the supernatant fraction of liver homogenate prepared in 1% Triton X-100 was used as enzyme source. All these observations support the view that the bulk of S-adenosylmethionine is catabolized through the demethylation-transsulphuration route in rat liver (12) and emphasize the important role of so called competing methyltransferase systems (13).

To compare the relationships between the activity of S-adenosylmethionine decarboxylase (EC 4.1.1.50) present in the soluble supernatant fraction of liver homogenate and the decarboxylating activity released from membranes by Triton X-100 (1), production of CO_2 and decarboxylated S-adenosylmethionine, and polyamine synthesis were studied simultaneously. In the mixtures containing soluble supernatant fraction, as in those containing Triton-supernatant of liver homogenates, only about 0.5% of the initial radioactivity was incorporated into spermidine, which was equivalent to the amount of $^{14}CO_2$ production in the mixtures containing soluble supernatant enzymes (cf. Table 1). Since the decarboxylating activity released by Triton X-100 was not accompanied with spermidine production nor with an accumulation of decarboxylated S-adenosylmethionine, and was completely independent of the presence of putrescine, it apparently did not catalyse decarboxylation of S-adenosylmethionine, but rather some of its degradation products.

To clarify whether the decarboxylating activity released by Triton X-100 was connected with the highly active demethylation-transsulphuration route, the properties of this activity were studied in more detail. It was observed that the activity was

Table 1. Relationship of S-adenosylmethionine decarboxylating activity with spermidine production in the supernatant fraction of liver homogenate prepared with or without 1% Triton X-100

Reaction product	(pmoles formed/30 min at 37°C per g wet wt. of liver)	
	- Triton X-100	+ Triton X-100
CO_2	4950	44820
Decarboxylated S-adenosylmethionine	n.d.	n.d.
Spermidine	3580	4270
Spermine	n.d.	n.d.

Each reaction mixture contained, in a total volume of 0.5 ml, 0.1 M potassium phosphate (pH 7.4), 2 mM putrescine, 71 mg (-Triton) or 175 mg (+Triton) of enzyme protein, corresponding to 75 mg of wet liver, and 0.08 mM substrate (4.0 mCi/mmol) being either S-adenosyl-L-[1-^{14}C]methionine or S-adenosyl-L-[2-^{14}C]methionine. Assay methods for the detection of produced CO_2 (8), decarboxylated S-adenosylmethionine (10) and polyamines (9,10) have been described earlier. n.d., not detectable (<5% of the values for spermidine formation).

strongly inhibited by dilution and potassium ions. Of the buffers tested, 100 mM glycylglycine gave highest activities with a pH-optimum at 7.4. The activity was apparently not dependent on peroxide formation, since it was not inhibited by catalase. As shown in Table 2, no single subcellular fraction was active, but combination of the crude nuclear and supernatant fractions restored activity. Furthermore, both the ultrafiltrate and the soluble proteins of the supernatant fraction appeared to be involved. When the soluble proteins were fractionated by $(NH_4)_2SO_4$ precipitation, the essential soluble proteins were obtained by the combination of only two fractions, corresponding to 30-45% and 55-75% $(NH_4)_2SO_4$ saturation. Interestingly, the former fraction evidently included at least S-adenosylhomocysteine hydrolase, EC 3.3.1.1 (14), and cystathionine β-synthase,

Table 2. Subcellular requirements for the S-adenosylmethionine decarboxylating activity released by Triton X-100

Fraction	%	Fraction	%
Whole homogenate	100	Crude nuclear (CN)	<1
Supernatant (S)	3	CN + S	145
Dialysed supernatant (DS)	3	CN + S + ML	122
Ultrafiltrate from supernatant (US)	0	CN + S + ML + MS	116
Microsomal (MS)	0	CN + DS	19
MS + S	5	CN + US	<1
Mitochondrial-lysosomal (ML)	0	CN + ML + MS	2
ML + S	7	CN + US + ML + MS	2
ML + MS + US	0	Purified nuclei (N)	0
Purified mitochondria (M)	0	N + S	5
M + S	8	N + M + S	7

Each reaction mixture contained, in a total volume of 0.25 ml, 0.1 M glycylglycine (pH 7.4), 2 mM dithiothreitol, 1% Triton X-100, 0.2 mM S-adenosyl-L-[1-^{14}C]methionine (2.0 mCi/mmol) and enzyme preparation from the subcellular fractions indicated, each corresponding to 35 mg of wet liver.

EC 4.2.1.22 (15), whereas cystathionine γ-lyase, EC 4.4.1.1, was probably exclusively in the latter fraction (15). Indeed, as shown in Table 3, the activity was markedly inhibited by sulphydryl (PCMB) and pyridoxal phosphate (L-canaline, NSD 1055) reagents and by every intermediate of the demethylation-transsulphuration route. It is well known that the enzymes responsible for the formation and degradation of cystathionine are dependent on pyridoxal phosphate and require thiol groups for activity (15).

The CO_2-producing activity liberated $^{14}CO_2$ from S-adenosyl-L-[1-^{14}C]homocysteine, apparently by a mechanism similar to that forming $^{14}CO_2$ from S-adenosyl-L-[1-^{14}C]methionine, as can be inferred from the inhibition pattern (Table 3), subcellular location and other requirements. However, the $^{14}CO_2$ production from 0.2 mM

Table 3. Effect of various compounds on the decarboxylating activity released from membranes by Triton X-100

Additions	Inhibition (-) or stimulation (+), % substrate	
	S-Adenosyl-L-[1-^{14}C]methionine	S-Adenosyl-L-[1-^{14}C]homocysteine
0.2 mM S-Adenosyl-L-methionine		+218
2 mM S-Adenosyl-L-methionine		- 82
1 mM S-Adenosyl-L-homocysteine	-87	
1 mM Adenosine and 10 μM adenosine deaminase inhibitor	-97	- 96
1 mM L-Homocysteine	-58	
1 mM L-Cystathionine	-59	- 73
1 mM L-Homoserine	-72	- 68
1 mM α-Ketobutyric acid	-76	- 60
1 mM L-Canaline	-94	- 94
1 mM NSD 1055	-75	- 85
1 mM p-Chloromercuribenzoate	-80	- 93

Supernatant fraction of liver homogenate prepared in 1% Triton X-100 was used as enzyme source. For other details see the legend for Table 2.

S-adenosyl-L-[1-^{14}C]homocysteine was only one third of that from 0.2 mM S-adenosyl-L-[1-^{14}C]methionine. Although the production of $^{14}CO_2$ from labeled S-adenosylhomocysteine was greatly reduced in the presence of 2 mM S-adenosylmethionine which was probably due to the dilution of the specific radioactivity by the formation of unlabeled S-adenosylhomocysteine, 0.2 mM S-adenosylmethionine was unexpectedly stimulatory to a great extent. This can probably be explained by the activation of the transsulphuration pathway by S-adenosylmethionine (16).

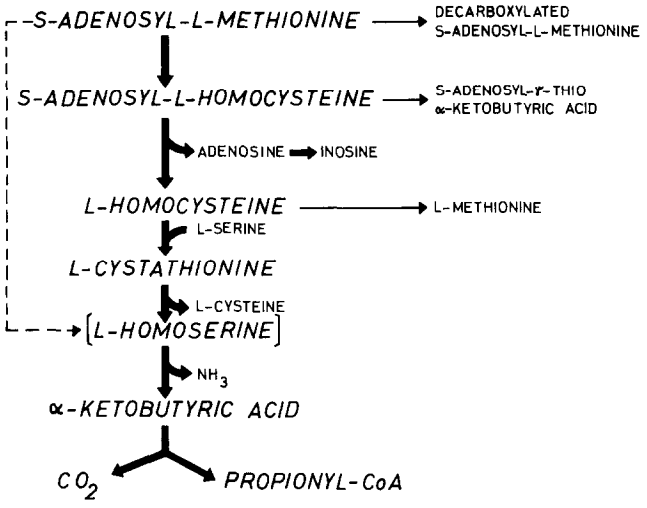

Fig. 1. Catabolism of S-adenosyl-L-methionine in rat liver.

From our results it can be concluded that the membrane-associated activity liberating CO_2 from S-adenosylmethionine reported by Sturman (1) involves several enzymes of the demethylation-transsulphuration pathway followed by decarboxylation of α-ketobutyric acid as illustrated in Fig. 1 (17). The rapid increase of the activity after birth (2) can be explained by the maturation of cystathionine β-synthase (18). However, there is some evidence indicating that S-adenosylmethionine may also be converted to α-ketobutyric acid via pathways distinct from the transsulphuration route (19,20).

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