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Enzymic Decarboxylation of *S*-Adenosyl-L-methionine in Rat Liver: Possible Interaction of Putrescine with the Prosthetic Group

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S-Adenosyl-L-methionine decarboxylase (EC 4.1.1.50) has been purified more than 1000-fold from rat liver. The molecular weight of the decarboxylase was calculated to be 68 000. No evidence was obtained indicating that pyridoxal phosphate acts as the prosthetic group of the enzyme. On the other hand, the decarboxylase apparently contains some carbonyl group(s) participating in the catalysis as supported by the inhibition of *S*-adenosyl-L-methionine decarboxylation in the presence of $NaBH_4$, phenylhydrazine or NaCN. Putrescine, the specific activator of mammalian *S*-adenosyl-L-methionine decarboxylase, might interact, directly or indirectly, with the carbonyl group(s) of the enzyme as suggested by the protection of the decarboxylase activity against borohydride reduction by the diamine.

Two soluble proteins are needed for the synthesis of spermidine from putrescine and *S*-adenosyl-L-methionine (Ado-Met) both in *prokaryotic* and *eukaryotic* cells. The first (*S*-adenosyl-L-methionine decarboxylase, Ado-Met decarboxylase, EC 4.1.1.50) catalyses the decarboxylation of Ado-Met and the second (spermidine synthase, EC 2.5.1.16) transfers the propylamino moiety from decarboxylated Ado-Met to putrescine.[1–4] The decarboxylation of Ado-Met apparently is the rate limiting reaction in the synthesis of spermidine in *eukaryotic* organisms.[3,4] Ado-Met decarboxylase from *Escherichia coli*, which has been purified to homogeneity, is specifically activated by magnesium,[1] and the enzyme contains, instead of pyridoxal phosphate, one or two mol of covalently bound pyruvate per one mol of enzyme, probably functioning as the prosthetic group.[5] Mammalian Ado-Met decarboxylase, unlike the *prokaryotic* enzyme, does not require magnesium ions, but is strongly and specifically activated by putrescine.[6,7] Ado-Met decarboxylase from rat prostate [6,7] and from rat liver [8] is inactivated by 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate treatment, which inhibits some enzymes requiring pyridoxal phosphate.[9] Moreover, a prior treatment of the prostatic Ado-Met decarboxylase with pyridoxal phosphate, but not with free pyridoxal, was shown to reverse the inhibitory action of the drug.[6,7] It has also been reported [8] that mammalian Ado-Met decarboxylase loses a part of its activity during dialysis against dilute buffers, but the full activity can be restored by the addition of pyridoxal phosphate in the incubation mixture.

The present communication reports an attempt to explore the nature of the prosthetic group of mammalian Ado-Met decarboxylase and to elucidate the mechanism of the activation of the decarboxylase by putrescine. No evidence was obtained to indicate that pyridoxal phosphate would function as the prosthetic group of Ado-Met decarboxylase from rat liver. On the other hand, the results show that the specific activation of mammalian Ado-Met decarboxylase by putrescine may involve an interaction of the amine with some carbonyl group(s) of the decarboxylase, may be with its prosthetic group.

MATERIAL AND METHODS

D,L-Methionine-1-^{14}C was purchased from the New England Nuclear Corporation (sp.act. 5.13 – 5.65 mC/mmol) or from the Radiochemical Centre, Amersham, Bucks., UK (sp.act. 55

mC/mmol), L-canaline, *p*-hydroxymercuribenzoate (HgBzOH), and *N*-ethylmaleimide (MalNEt) were supplied by Sigma. Methyl glyoxal bis(guanylhydrazone) (MGBG) was a product of Aldrich Chemical Company, and pyridoxal phosphate (PLP) was obtained from E. Merck AG, Darmstadt, Germany. NSD 1055 (4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate) was a gift to Dr. A. Raina from Smith and Nephew Research, Ltd. Dithiothreitol, putrescine, spermidine, and spermine were purchased from Calbiochem. Other chemicals were of analytical grade.

Radioactive and unlabelled Ado-Met was synthesized as described earlier.[10] The incubation conditions for the assay of Ado-Met decarboxylase activity have been described elsewhere.[4]

Partial purification of Ado-Met decarboxylase from rat liver. Female rats of the Wistar strain, weighing 250 – 350 g, received 10 mg MGBG per rat intraperitoneally at 24 and 12 h before sacrifice. MGBG has been shown to be a specific inhibitor of putrescine activated Ado-Met decaboxylase *in vitro*.[11] Using the principles of the method described earlier,[4] which included ammonium sulfate fractionation (0.35 – 0.60), chromatography on DEAE cellulose and on hydroxylapatite followed by four gel filtrations on Sephadex G-150, Sephahex G-100, Sephadex G-100, and Sephadex G-75, it was possible to purify Ado-Met decarboxylase over 1000-fold. The final enzyme preparation catalyzed a release of more than 1100 nmol of CO_2 from Ado-Met per mg protein per 30 min under standard assay conditions. The enzyme, however, contained some impurities as revealed by polyacrylamide gel electrophoresis.

Determination of the molecular weight. The sedimentation coefficient for Ado-Met decarboxylase was calculated from sucrose density gradient centrifugation experiments[12] using bovine liver catalase (Sigma) and yeast alcohol dehydrogenase (Boehringer, Mannheim) as markers. The molecular Stokes radius and the diffusion coefficient for the decarboxylase were calculated as described by Ackers and Steere[13] using Blue Dextran 2000, potassium dichromate, yeast alcohol dehydrogenase and bovine albumin (Armour Pharmaceutical Company) for the calibration of Sephadex G-150 column. The partial specific volume of the decarboxylase was assumed to be 0.74 ml/g.

Isoelectric focusing was performed as described earlier.[14] Protein was measured by the method of Lowry *et al.*[15]

RESULTS AND DISCUSSION

Table 1 summarizes some properties of highly purified Ado-Met decarboxylase from rat liver. These results are in good agreement with results obtained earlier with crude enzyme preparations from rat ventral prostate[16] and from rat liver.[4]

Consistent with earlier reports.[7,17] the addition of 5 mM dithiothreitol to the incubation mixture reverses the complete inhibition of Ado-Met decarboxylase by 1 mM HgBzOH or 0.3 mM $HgCl_2$. Furthermore, a pretreatment of the decarboxylase with 5 mM MalNEt for 1 h in ice resulted in 94 % inhibition which was not reversed by exposure to 5 mM dithiothreitol.

The activity of purified rat liver Ado-Met decarboxylase was stimulated 6-fold by 2.5 mM putrescine and nearly 2-fold by 5 mM 1,3-diaminopropane under standard assay conditions. Spermidine (10 mM) did not change the enzyme activity whereas Mg^{2+} (10 mM) was somewhat inhibitory. From various metals tested Zn^{2+} (1 mM) and Co^{2+} (1 mM) almost totally inhibited the decarboxylase activity in tris-HCl buffer, pH 7.4, but most of the activity could be restored by 5 mM dithiothreitol or 1 mM EDTA in the incubation mixture.

Common inhibitors of enzymes requiring pyridoxal phosphate had only slight inhibitory effect on the activity of purified rat liver Ado-Met decarboxylase when assayed in the presence

Table 1. Some properties of partially purified Ado-Met decarboxylase from rat liver.

K_m for Ado-Met (in the presence of 2.5 mM putrescine)	0.020 mM
K_m for Ado-Met (in the absence of putrescine)	0.350 mM
K_m for putrescine as the activator (in the presence of 0.2 mM Ado-Met)	0.010 mM
pH optimum	7.0 – 7.5
Isoelectric point	5.3
Stokes radius	3.03 nm
Sedimentation coefficient	5.1 S
Diffusion coefficient	7.03 F
M.W.	68 000

Table 2. Inhibition of rat liver Ado-Met decarboxylase by inhibitors of enzymes requiring pyridoxal phosphate. The incubations were performed by adding neutralized solutions of inhibitors to the standard incubation mixture.

Inhibitor	Concentration (mM)	Relative activity − putrescine	+putrescine
None	–	100	100
Isonicotinic acid hydrazine	10	82	84
Semicarbazide	10	52	96
Canaline	5	97	103
NSD 1055	5	0	0

Table 3. Partially purified rat liver Ado-Met decarboxylase preparations (Sephadex G-100 liberating 603 nmol of CO_2 from Ado-Met in standard incubation conditions: protein concentration varied between 0.03 and 0.1 mg per ml) were exposed to 10 mM $NaBH_4$ for 10 min at 37 °C. After the reduction the enzyme preparations were diluted with 25 mM potassium phosphate, pH 7.4, for subsequent assays.

Prior incubation in the presence of	Activity without $NaBH_4$ reduction nmol CO_2 liberated in 30 min (%)	Activity after $NaBH_4$ reduction
Experiment 1		
No additions	5.07 (100)	0.55 (11)
Putrescine (5 mM)	6.81 (100)	4.56 (67)
Spermidine (10 mM)	5.60 (100)	0.44 (8)
Spermine (10 mM)	6.10 (100)	0.12 (2)
Magnesium (20 mM)	6.22 (100)	2.48 (40)
1,3-Diaminopropane (5 mM)	6.73 (100)	2.89 (43)
Experiment 2		
No additions	3.16 (100)	0.19 (6)
Putrescine (5 mM)	2.85 (100)	2.13 (75)
Ado-Met (0.4 mM)	0.91 (100)	0.10 (11)
Putrescine (5 mM)+ Ado-Met (0.4 mM)	1.56 (100)	0.21 (14)
Ado-Met (0.4 mM)+ putrescine (5 mM)	0.92 (100)	0.11 (12)
Experiment 3		
No additions	3.31 (100)	0.13 (4)
Putrescine (3 mM)	4.95 (100)	4.05 (82)
Ado-Met (0.3 mM)	2.45 (100)	0.09 (4)
Ado-Met (0.3 mM)+ putrescine (3 mM)	4.35 (100)	0.99 (23)

of putrescine (Table 2). Semicarbazide (10 mM), however, clearly inhibited the decarboxylation of Ado-Met when assayed in the absence of putrescine. Furthermore, a prior treatment of the enzyme with 10 mM semicarbazide for 10 min at 37 °C totally inactivated the enzyme (not tabulated). Five mM NSD 1055 in the incubation resulted in a complete inhibition of Ado-Met decarboxylase activity. In contrast to those experiments with Ado-Met decarboxylase from rat ventral prostate,[6,7] the activity could not be restored by a prior incubation of the enzyme with 1 mM PLP. A treatment of the decarboxylase with imidazolium and cysteine in citrate buffer, pH 7.2, which is known to cleave the covalent bond between PLP and the apoenzyme in rabbit muscle phosphorylase b,[18] reduced the activity of Ado-Met decarboxylase

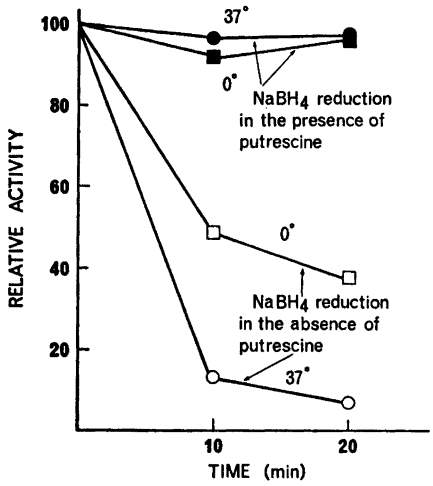

Fig. 1. A prior incubation of Ado-Met decarboxylase (Sephadex G-150 fraction, 0.075 mg protein per ml, decarboxylating 110 nmol of Ado-Met in standard assay conditions) with 5 mM putrescine before reduction with $NaBH_4$ protects the enzyme activity from the reductant. After incubating for 10 min at 37 °C with $NaBH_4$, the enzyme preparations were diluted with 25 mM potassium phosphate for the assay of enzyme activity.

from rat liver by less than 10 %. This inhibition could not be reversed by an inclusion of 1 mM PLP in the incubation mixture.

Carbonyl group reagents, sodium borohydride (10 mM), sodium cyanide (5 mM) or phenylhydrazine (1 mM) readily abolished the activity of rat liver Ado-Met decarboxylase. However, when the decarboxylase was incubated in the presence of 5 mM putrescine, prior to the exposure to $NaBH_4$, the inactivation of the enzyme was prevented as demonstrated in Fig. 1. As shown in Table 3, a prior incubation of the decarboxylase with magnesium or 1,3-diaminopropane also protected the enzyme activity from $NaBH_4$ reduction. On the other hand, when the enzyme was preincubated with the substrate, Ado-Met, the activity of the decarboxylase was completely inhibited by $NaBH_4$ reduction. Furthermore, the addition of the substrate together with putrescine to the enzyme mixture, prior to the treatment with $NaBH_4$, abolished the protective effect of putrescine against the reduction (Table 3).

Covalently bound pyruvate almost certainly functions as the prosthetic group of Ado-Met decarboxylase from *E. coli*.[5] Based mainly on experiments with NSD 1055, which inhibits the decarboxylation of Ado-Met by crude enzyme preparations from the cytosol fraction from rat ventral prostate [6,7] and from rat liver,[8] it has been suggested that PLP might act as the prosthetic group of mammalian Ado-Met decarboxylase. This suggestion has not been supported by experiments using Ado-Met decarboxylase from rat brain.[19]

In the absence of direct evidence it would be premature to conclude that mammalian Ado-Met decarboxylase contains carbonyl group(s) that functions as the prosthetic group. Nevertheless it is possible that this group(s) could be a part of the active center of the decarboxylase as shown by Recsei and Snell [20] for histidine decarboxylase from *Lactobacillus* 30a.

The mechanism of the specific activation of *eukaryotic* Ado-Met decarboxylase by putrescine still remains to be elaborated, but the present data suggest that putrescine might interact with the prosthetic group (carbonyl group) of the enzyme. This interaction may or may not involve a conformational change of the enzyme.

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