STIMULATION OF THE DECARBOXYLATION OF S-ADENOSYLMETHIONINE BY PUTRESCINE IN MAMMALIAN TISSUES

A. E. Pegg and H. G. Williams-Ashman

Brady Urological Institute
and
Department of Pharmacology and Experimental Therapeutics
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

Received December 6, 1967

Putrescine and L-methionine are known to serve as precursors for the biosynthesis of spermidine by both bacterial and mammalian cells in vivo (Raina, 1963; Tabor and Tabor, 1964; Siimes and Jänne, 1967). Two separate enzymes involved in this process have been partially purified from E. coli. The first of these catalyzes the decarboxylation of S-adenosyl-L-methionine (SAM) to 5'-deoxy-5'-S-(3-methylthiopropylamine)sulfoniumadenosine ("decarboxylated-SAM"). The second enzyme promotes transfer of the propylamine moiety of decarboxylated-SAM to putrescine, with the formation of spermidine and thiomethyladenosine (Tabor, 1962a,b). With the exception of a preliminary report on the incorporation of labeled putrescine into the polyamine fraction of minced rat prostate (Tabor, Rosenthal, and Tabor, 1956), no work on the biosynthesis of polyamines by cell-free preparations of mammalian tissues appears to have been carried out. This paper describes the presence in rat ventral prostate and liver of an enzyme system catalyzing the release of CO_2 from the carboxyl group of SAM. The ventral prostate, which is very rich in spermine and spermidine (Rhodes and Williams-Ashman, 1964) is much more active than liver in this respect. The decarboxylase activity of partially purified preparations from ventral prostate is markedly and specifically stimulated by putrescine, with the stoichiometric formation of CO_2 and spermidine. The nature of the enzyme(s) responsible for these

transformations is discussed.

METHODS AND MATERIALS

DL-Methionine-1-C^{14} (3.42 mC/mmole) and putrescine-1,4-C^{14} (5.22 mC/mmole) were purchased from New England Nuclear. Carboxyl-labeled SAM was synthesized using SAM synthetase purified from rat liver (Cantoni and Durell, 1957). The product was purified by chromatography on Dowex-50, as was unlabeled SAM obtained from Calbiochem. Bacterial SAM decarboxylase was prepared from frozen E. coli cells according to Tabor (1962a). Decarboxylated-SAM was prepared with the aid of this enzyme.

SAM decarboxylase activity was measured by assaying release of $C^{14}O_2$ from SAM-$C^{14}OOH$. The reactions were carried out in small Erlenmeyer flasks fitted with rubber caps which carried a polypropylene well. Each well contained 0.2 ml of a mixture of ethanolamine and methyl cellosolve (2:1, v/v). The reactions were halted by injection of 0.5 ml of 0.5 N H_2SO_4 through the rubber cap. The flasks were incubated for a further 15 min to ensure that all bound CO_2 was released from the medium. The well and its contents were then removed, and placed in counting vials containing 15 ml of scintillation fluid, and radioactivity determined according to Calvin, Kosto, and Williams-Ashman (1967). Incorporation of labeled putrescine into spermidine was determined by the method of Tabor (1962b). All enzymic reactions were carried out at 37° with agitation in air.

Liver and ventral prostate tissue were excised from adult, male, Sprague-Dawley rats weighing more than 250 g. The tissue was homogenized in 5-10 vol of 0.25 M sucrose, 1 mM sodium EDTA of pH 7.2. The homogenate was centrifuged at 10,000 x g for 10 min and the supernatant fluid centrifuged at 100,000 x g for 90 min. The final supernatant fluid was further fractionated by addition of suitable aliquots of saturated $(NH_4)_2SO_4$, neutralized with NH_4OH, at 0°. The precipitates of protein were dissolved in a medium containing 20 mM Tris-HCl of pH 7.6 (at 4°), 1 mM 2-mercaptoethanol, 0.1 mM disodium EDTA, and dialyzed against 30 vol of the same medium at 4° for at

least 12 hr. Protein was estimated by the method of Lowry et al. (1951), with bovine serum albumin as standard.

RESULTS AND DISCUSSION

When rat liver or ventral prostate homogenates were fractionated by differential centrifugation, most of their ability to catalyze the decarboxylation of SAM-$C^{14}OOH$ was found to reside in the material that was not sedimentable after ultracentrifugation for 90 min at 100,000 x g. In the test system described in Table 1, one mg of protein from fresh, undialyzed soluble liver extracts catalyzed a linear rate of formation of 5-10 μμmoles of CO_2 in 30 min; ventral prostate extracts were much more active, giving rise to 150-300 μμmoles of CO_2. The latter rates of reaction were found with suboptimal levels of the labeled SAM substrate (0.02 mM). When the initial SAM levels were saturating (> 0.2 mM), the rates of decarboxylation with liver or prostate were more than twice the aforementioned values. Decarboxylation of SAM by undialyzed soluble extracts of liver or ventral prostate was increased not more than two-fold by addition of excess amounts (2.5 mM) of putrescine. Dialysis of extracts from either tissue, however, resulted in a marked diminution of CO_2 release, which was entirely restored by addition of 2.5 mM putrescine to the reaction mixture. The protein precipitated by ammonium sulfate between 45% and 65% saturation from crude soluble ventral prostate extracts contained most of the SAM decarboxylating activity, and exhibited about 2-3 times the specific activity of initial extracts when supplemented with putrescine. Table 1 shows that CO_2 liberation from SAM by such a dialyzed ammonium sulfate fraction was stimulated 11-fold by addition of putrescine.

The addition of spermidine resulted in a much smaller but significant stimulation, whereas L-ornithine had no effect, and comparable levels of spermine or cadaverine were inhibitory. The direct addition of unlabeled decarboxylated-SAM inhibited CO_2 release from SAM-C^{14}-OOH alone, but was without influence on the greatly enhanced rate of decarboxylation observed

Table 1

Effect of various substances on decarboxylation of SAM by ventral prostate

Additions	μμmoles $C^{14}O_2$ released/ 30 min/mg protein
Nil	57
Putrescine (2.5 mM)	690
Spermidine (5 mM)	92
L-Ornithine (2 mM)	58
Spermine (2.5 mM)	38
Cadaverine (2.5 mM)	28
Unlabeled decarboxylated-SAM (0.025 mM)	34
Unlabeled decarboxylated-SAM (0.025 mM) plus putrescine (2.5 mM)	683

Each vessel contains 150 μmoles of Tris-HCl of pH 7.3; 0.5 μmoles of 2-mercaptoethanol; 0.05 μmoles of sodium EDTA; 0.02 μmoles SAM (3.8 cpm/μμmole) and 3 mg of protein of ventral prostate 45-65% ammonium sulfate fraction in a total volume of 1 ml. Incubated 30 min at 37°.

in the presence of putrescine.

We confirmed the findings of Tabor (1962a) that the SAM decarboxylase of E. coli exhibits an almost complete requirement for Mg^{++} ions, and that the reaction with this bacterial enzyme readily proceeds nearly to completion in the presence of Mg^{++}. The E. coli SAM decarboxylase was unaffected by putrescine (2.5 mM) when measured in the absence or presence of $MgCl_2$ (20 mM). On the contrary, the ventral prostate SAM decarboxylase activity determined in the absence of added putrescine was inhibited about 50% by 20 mM $MgCl_2$, which did not affect CO_2 formation in the presence of 2.5 mM putrescine.

The decarboxylation of SAM in the absence or presence of putrescine catalyzed by the dialyzed ventral prostate ammonium sulfate fraction was not enhanced by exogenous pyridoxal phosphate. The reactions were, however, inhibited by fairly high concentrations of either isonicotinic acid hydrazide (INH) or 4-bromo-3-hydroxybenzyloxyamine (NSD 1055). The latter substances are known to inhibit a number of pyridoxal phosphate requiring enzymes (Levine, Sato, and Sjoerdsma, 1965). Table 2 shows that inhibition of SAM decarboxylation by INH or NSD 1055 was reversed by pyridoxal phosphate.

These experiments suggest, but by no means prove, that pyridoxal phosphate is involved in the action of the prostatic SAM decarboxylating system.

Table 2

Inhibition of SAM decarboxylation by isonicotinic acid hydrazide and NSD 1055 and reversal by pyridoxal phosphate

Additions	μμmoles $C^{14}O_2$/30 min/mg protein in presence of:	
	No added putrescine	Putrescine (2.5 mM)
Nil	84	605
Pyridoxal-P (1 mM)	71	558
NSD 1055 (0.3 mM)	49	263
NSD 1055 (0.3 mM) plus pyridoxal-P (1 mM)	64	580
INH (5 mM)	63	443
INH (5 mM) plus pyridoxal-P (1 mM)	82	598

The experimental conditions are shown in Table 1.

The mechanism of stimulation of SAM decarboxylation by putrescine in mammalian tissues remains uncertain. It is possible that the liver and prostatic enzyme preparations contain a SAM decarboxylase which is directly activated, perhaps in an allosteric fashion, by putrescine. It can also be imagined that the SAM decarboxylase is powerfully inhibited by the product of the reaction (decarboxylated-SAM). If it is assumed that the same preparations also contain a propylamine transferase (spermidine synthetase; Tabor, 1962b) which, in the presence of putrescine, utilizes decarboxylated-SAM to form spermidine and thiomethyladenosine, then the latter reactions might effectively remove the decarboxylated-SAM and prevent the latter substance from inhibiting the SAM decarboxylase. In accordance with this notion, we have observed that the same dialyzed ventral prostate ammonium sulfate fractions catalyze the synthesis of spermidine from putrescine and decarboxylated-SAM added as such, at rates which are comparable to the synthesis of spermidine found when an excess of SAM and putrescine are used as substrates (cf. Table 3). It can be seen from Table 1, however, that inhibition of SAM decarboxylation by unlabeled decarboxylated-SAM, as catalyzed by the prostate

preparations in the absence of added putrescine, is only about 40% under conditions where the levels of added decarboxylated-SAM are more than 100 times those that would be expected to be formed from the labeled SAM substrate.

Table 3

Stoichiometry of CO_2 release and spermidine formation during decarboxylation of SAM in the presence of putrescine

S-Adenosyl-methionine concentration (mM)	Specific Radioactivity (cpm/μμmole) of SAM	Putrescine (mM)	μμmoles product formed per 30 min per mg protein	
			Spermidine	CO_2
0.02	3.8	0	--	58
0.02	3.8	0.25	330	341
0.08	0.95	0	--	93
0.08	0.95	0.25	610	598
0.08	0.95	2.50	803	768
0.15	0.52	0	--	126
0.15	0.52	2.50	912	960
0.24	0.31	0	--	121
0.24	0.31	0.25	633	681
0.24	0.31	2.50	965	1,110

The specific radioactivity of the SAM-C^{14} in various tubes is shown; this labeled SAM was used for estimation of CO_2 release. When spermidine formation was determined, parallel tubes were set up containing equivalent concentrations of unlabeled SAM, and labeled putrescine at 0.25 mM (5.5 cpm/μμmole) or 2.5 mM (0.55 cpm/μμmole). The polyamine fraction from chromatography on Dowex-50 was separated and its radioactivity determined. Paper electrophoretic experiments (Raina, 1953) showed that spermidine was the only radioactivity substance formed that entered this fraction. Other conditions and ventral prostate enzyme preparation as in Table 1. The tubes were incubated for 30 min at 37°.

Table 3 shows the result of an experiment in which formation of spermidine from labeled putrescine and unlabeled SAM was measured under exactly the same conditions where the release of $C^{14}O_2$ from SAM-$C^{14}OOH$ was determined, in the absence and presence of unlabeled putrescine. It is evident that there was a close correspondence between the formation of CO_2 and spermidine at all concentrations of SAM and putrescine examined. These findings raise the possibility of an alternative explanation for the stimulation of SAM decarboxylation by putrescine in mammalian tissues, viz., that the decarboxylation of SAM and the synthesis of spermidine are catalyzed by a single enzyme (or closely linked enzyme complex) via a reaction which does

not involve free decarboxylated-SAM as an intermediate. Experiments designed to test this hypothesis are in progress.

ACKNOWLEDGMENT

This work was supported in part by a research grant (HD-01453) from the U.S. Public Health Service.

REFERENCES

Calvin, H.I., Kosto, B., and Williams-Ashman, H.G., Arch. Biochem. Biophys., 118, 670 (1967).
Cantoni, G.L., and Durrell, J., J. Biol. Chem., 225, 1033 (1957).
Levine, R.J., Sato, T.L., and Sjoerdsma, A., Biochem. Pharmacol., 14, 139 (1965).
Lowry, O.H., Rosebrough, N.J., Farr, A., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).
Raina, A., Acta Physiol. Scand., 60, Suppl. 218 (1963).
Rhodes, J.B., and Williams-Ashman, H.G., Med. Exp., 10, 281 (1964).
Siimes, M., and Jänne, J., Acta Chem. Scand., 21, 815 (1967).
Tabor, H., and Tabor, C.W., Pharmacol. Rev., 16, 245 (1964).
Tabor, H., Rosenthal, S.M., and Tabor, C.W., Federation Proc., 15, 367 (1956).
Tabor, C.W. in "Methods in Enzymology" Vol. 5, Colowick, S.P., and Kaplan, N.O. (eds), Academic Press, New York, p.756 (1962a).
Tabor, C.W., in "Methods in Enzymology" Vol. 5, Colowick, S.P., and Kaplan, N.O. (eds), Academic Press, New York, p.761 (1962b).