INHIBITION OF AMINOPROPYLTRANSFERASES BY *S*-ADENOSYLMETHIONINE

Effect of simultaneous administration of *S*-adenosylmethionine and methylglyoxal bis(guanylhydrazone) on polyamine concentrations in regenerating rat liver

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1. Introduction

There has been intensive research activity into the biosynthetic pathway and the function of polyamines in the past decade [1–4]. Considerable progress in elucidating the function of the polyamines has been made by utilizing inhibitors of their biosynthesis [2,4–8]. All of the inhibitors now in use act on either ornithine or *S*-adenosylmethionine (SAM) decarboxylases [6–9]. None has been shown to produce substantial depletion of intracellular spermine.

Here we demonstrate that partially purified aminopropyltransferases from rat liver were strongly inhibited by SAM and that administration of SAM in combination with methylglyoxal bis(guanylhydrazone) (MGBG) caused a decrease in the concentration not only of spermidine, but also of spermine in the liver and kidney of partially hepatectomized rats. This observation may be useful for understanding the regulation of polyamine synthesis in eukaryotic cells.

2. Materials and methods

2.1. *Chemicals*

S-Adenosyl-L-[*methyl*-^{14}C]methionine (spec. act. 50–60 mCi/mmol) was purchased from New England Nuclear Corp. SAM was supplied by Sigma Chemical Co. Decarboxylated SAM, both unlabeled and labeled in the methyl group was prepared by the action of SAM decarboxylase from *Escherichia coli* and purified by chromatography on Dowex-50-H$^+$ and high-voltage paper electrophoresis [10]. MGBG was obtained from Aldrich Chemical Co. All other chemicals were products of Nakarai Chemicals Ltd.

2.2. *Animals*

Male Sprague-Dawley rats (160 g) were used for all experiments. Partial hepatectomy was performed under light ether anaesthesia as in [11]. SAM and MGBG were dissolved in 0.14 M NaCl before administration and injected intraperitoneally. Animals were sacrified by decapitation at 72 h after the operation.

2.3. *Preparation of spermidine and spermine synthases*

Spermidine and spermine synthases (aminopropyltransferases) were purified from rat liver as in [12]. Extracts containing both enzyme activities were fractionated into spermidine synthase and spermine synthase preparations by the treatment with ammonium sulfate followed by DEAE-cellulose, Hydroxyapatite and Sephadex G-200 column chromatography. The enzyme preparations used here were about 200-fold (Sephadex G-200 fraction, spermidine synthase) and 130-fold (hydroxyapatite fraction, spermine synthase) of purification over the specific activity present in the crude ultracentrifugal extracts.

2.4. *Assay of aminopropyltransferase activities*

The aminopropyltransferase activity was determined by measuring the production of [*methyl*-^{14}C]-methylthioadenosine from decarboxylated *S*-adenosyl-[*methyl*-^{14}C]methionine in the presence of putrescine (spermidine synthase) or of spermidine (spermine synthase) as in [13]. The assay medium contained 100 mM sodium phosphate buffer (pH 7.5), 42 μM

decarboxylated *S*-adenosyl-[*methyl*-^{14}C]methionine (2 μCi/μmol), 5 mM dithiothreitol, 0.5 mM putrescine or 0.5 mM spermidine and the enzyme preparation in 0.2 ml total vol. Assays were incubated at 37°C for 30 min. The production of methylthioadenosine was entirely dependent on putrescine (spermidine synthase) and spermidine (spermine synthase).

2.5. *Determination of polyamines*

The amounts of putrescine, spermidine and spermine present in the liver and kidney were determined as in [14]; homogenization of the tissues in 0.1 N HCl, extraction of amines into *n*-butanol at alkaline pH and separation by paper electrophoresis followed by staining with ninhydrin.

3. Results and discussion

As shown in table 1, SAM was strongly inhibitory giving 77% inhibition of spermidine synthase and 92% inhibition of spermine synthase at 1 mM. Spermine synthase was more sensitive to the inhibitor than spermidine synthase and 74% of the activity was inhibited even at 0.1 mM. This inhibition was not due to the conversion of SAM to the decarboxylated derivatives (which if formed could dilute the specific activity of the labeled substrate) because the enzyme preparations used were practically free of SAM decarboxylase activity (data not shown). This was confirmed by addition to the assay mixture of 10 μM MGBG which did not affect the inhibition by SAM (table 1). MGBG is a potent inhibitor of SAM decarboxylase [15]. Further, a similar inhibition of spermidine and spermine synthases by SAM was confirmed using labeled putrescine or spermidine (data not shown) [16]. Detailed kinetics and the mode of action of SAM to the spermidine and spermine synthases will be published elsewhere.

Table 1
Inhibition of rat liver aminopropyltransferases by *S*-adenosylmethionine (SAM)

Addition to assay	SAM (mM)	% Inhibition	
		Spermidine synthase	Spermine synthase
SAM	1.0	77	92
	0.1	33	74
SAM + MGBG	1.0	79	94
(10 μM)	0.1	34	76

Spermidine synthase and spermine synthase activities were measured with the addition of the compounds shown. Details of the incubation conditions were shown in the text. The results were expressed as % inhibition due to the addition of compounds

Although several potent inhibitors of polyamine production are available, including α-difluoromethylornithine which is an irreversible inhibitor of ornithine decarboxylase and rapidly reduces cellular putrescine and spermidine levels [7,8], none of the agents currently in use has significant effects on the

Table 2
Effect of repeated administration of *S*-adenosylmethionine (SAM) and methylglyoxal bis(guanylhydrazone) (MGBG) on the polyamine concentrations in 72 h-regenerating liver

Admin.	Polyamine concentration (nmol/g wet liver)		
	Putrescine	Spermidine	Spermine
None	62.5 ± 6.5	1875.1 ± 67.8	504.2 ± 26.6
SAM	68.8 ± 12.2	1883.5 ± 70.5	515.5 ± 30.2
MGBG	616.7 ± 25.4[b]	1716.8 ± 71.9	509.2 ± 20.9
SAM + MGBG	862.7 ± 52.3[a]	1395.9 ± 62.8[a]	333.4 ± 18.8[a]

[a] Differs from MGBG-administrated value, SAM-administrated value and non-administrated value ($p < 0.01$)
[b] Differs from both SAM-administrated value and non-administrated value ($p < 0.001$)

Groups of 3 rats were partially hepatectomized 72 h before death. SAM (100 mg/kg) and MGBG (50 mg/kg) were injected intraperitoneally at 24, 48 and 68 h after operation

Table 3
Effect of repeated administration of *S*-adenosylmethionine (SAM) and methylglyoxal bis(guanylhydrazone) (MGBG) on the polyamine concentrations in the kidney of rats bearing 72 h-regenerating liver

Admin.	Polyamine concentration (nmol/g wet kidney)		
	Putrescine	Spermidine	Spermine
None	55.8 ± 7.8	454.2 ± 21.8	585.0 ± 24.9
SAM	50.2 ± 6.9	422.4 ± 18.9	560.8 ± 26.5
MGBG	354.2 ± 23.4[b]	384.2 ± 19.8	502.5 ± 27.7
SAM + MGBG	429.2 ± 26.3[a]	335.4 ± 18.4[a]	404.2 ± 20.9[a]

[a] Differs from MGBG-administrated value, SAM-administrated value and non-administrated value ($p < 0.01$)
[b] Differs from both SAM-administrated value and non-administrated value ($p < 0.001$)

See the legend to table 2 for experimental details

concentration of spermine. In this connection, SAM was expected to lower the cellular spermine level, particularly if given simultaneously with a SAM decarboxylase inhibitor such as MGBG.

Table 2 shows the effect of the administration of SAM and MGBG on polyamine content in the remnant livers of partially hepatectomized rats. The administration of SAM in combination with MGBG caused more decreases in the concentrations of spermidine and spermine than the administration of MGBG alone. A significant increase in putrescine content may be mainly due to inhibition of diamine oxidase by MGBG [17]. The administration of only SAM did not significantly affect the cellular concentration of spermidine and spermine. This might be interpreted that the increased level of SAM effective for the enzyme inhibition after the administration is gradually decreased by the activity of the decarboxylase in tissues and also that the increased level of a decarboxylated product, a substrate for the spermidine and spermine synthases could compensate the SAM inhibition against the enzymes.

In the kidneys of rats bearing regenerating livers, almost the same effect of the drugs on polyamine levels as that in the liver was observed (table 3). Spermine concentration was depressed more by the administration of SAM simultaneously with MGBG than by the administration of the latter alone. An increase of putrescine concentration by the administration of MGBG was also observed in the kidney of partially hepatectomized rats.

The concentration of SAM in adult rats has been reported to be ~70 and 50 nmol/g wet wt for liver and kidney, respectively [18,19]. It is conceivable that increases in the SAM content induced by the simultaneous administration of SAM and MGBG might exert inhibitory effect on spermidine and spermine synthesis.

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