

2-Mercaptoethylamine, a competitive inhibitor of spermidine synthase in mammalian cells

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Spermidine synthase from rat ventral prostate was inhibited by 2-mercaptoethylamine (MEA). Inhibition of spermidine synthase by MEA was competitive with respect to one of the substrates putrescine, but not competitive with respect to the other substrate decarboxylated *S*-adenosylmethionine. MEA markedly depressed spermidine and spermine contents in human erythroid leukemia K562 cells, suggesting that these changes resulted from the inhibitory effect of MEA on spermidine synthase *in situ*.

2-Mercaptoethylamine; Spermidine synthase inhibitor; Polyamine depletion; (Human leukemia cell)

1. INTRODUCTION

Inhibitors of polyamine biosynthesis have become the subjects of intensive research because of their potential usefulness in the elucidation of the biological function of polyamines in cellular metabolism and in cell proliferation. Many compounds have been reported as inhibitors for ornithine decarboxylase and *S*-adenosylmethionine decarboxylase [1]. Some of these compounds, however, have unwanted effects on cellular polyamine metabolism, including stabilization and/or activation of these decarboxylases, paradoxical increases of the cellular concentrations of putrescine or spermine, inhibition of diamine oxidase, and other effects probably unrelated to polyamine

metabolism, for instance, mitochondrial damages [1-4]. It may therefore be of worth to find inhibitors acting on the propylamine transferring steps and to develop agents of increased specificity. Pegg and Hibasami [5] have pointed out that the limiting factor in spermidine and spermine syntheses is the supply of one of their substrates decarboxylated AdoMet. The intracellular concentration of this substrate for spermidine and spermine synthases is very low and the turnover rates of these enzymes are relatively slow [6]. So these steps may well prove to be suitable sites at which polyamine biosynthesis can be effectively inhibited. Hibasami and Pegg [7], and Pajula and Raina [8] have reported that MTA, a by-product of both spermidine and spermine synthase reactions, potentially inhibited spermine synthase. However, very little attention has been paid to 3-mercapto-propylamine moiety of decarboxylated AdoMet.

Here we demonstrate that partially purified spermidine synthase from rat ventral prostate was inhibited by MEA, which has an analogous structure to the 3-mercapto-propylamine moiety of decarboxylated AdoMet, and that addition of MEA to the culture medium caused a decrease in

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Abbreviations: MEA, 2-mercaptoethylamine; MTA, 5'-methylthioadenosine; decarboxylated AdoMet, decarboxylated *S*-adenosylmethionine

the concentrations of spermidine and spermine in cultured human erythroid leukemia K562 cells.

2. MATERIALS AND METHODS

2.1. Chemicals

MEA and MTA were purchased from Sigma, St. Louis, MO, USA. *S*-Adenosyl-L-[methyl- 14 C]methionine (53.6 mCi/mmol) was obtained from New England Nuclear, Boston, MA, USA. Decarboxylated AdoMet, both unlabelled and labelled in the methyl group were prepared by the action of *S*-adenosyl-methionine decarboxylase from *Escherichia coli* (strain B) and purified by chromatography on Dowex-50-H⁺ and paper electrophoresis as described [9]. All other chemicals were products of Nakarai Chemicals Ltd, Kyoto, Japan.

2.2. Animals

Male Wistar strain rats (250–300 g) were purchased from Shizuoka agricultural co-operative association for experimental animals, Hamamatsu, Japan. The animals were given water and food ad libitum and ventral prostates were removed.

2.3. Cell culture

Human erythroid leukemia K562 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 50 IU/ml penicillin G potassium and 50 μ g/ml streptomycin sulfate in a humidified chamber containing 5% CO₂ in air at 37°C. Cell number was measured by using counting chamber and microscope. Viability was determined by trypan blue staining.

2.4. Purification of spermidine and spermine synthases

Spermidine and spermine synthases were purified from rat ventral prostate as described [10]. Extracts containing both enzyme activities were fractionated into spermidine synthase and spermine synthase preparations, respectively, by the treatment with ammonium sulfate followed by DEAE-cellulose and Ultragel ACA 43 column chromatography. The enzyme preparations used in this study were 265-fold (spermidine synthase) and 170-fold (spermine synthase) of purification over the specific activity present in the crude ultracentrifugal extracts. These preparations of synthases were free from each other and neither preparation contained significant amounts of *S*-adenosyl-methionine decarboxylase or of 5'-methylthioadenosine phosphorylase.

2.5. Assay for spermidine and spermine synthases

The enzyme activities were 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 described [11]. The assay mixture contained 100 mM sodium phosphate buffer, pH 7.5, 5 mM dithiothreitol, 41.2 μ M decarboxylated *S*-adenosyl-[methyl- 14 C]methionine, 0.5 mM putrescine or 0.5 mM spermidine and the enzyme preparation in 0.1 ml of total volume unless otherwise noted. Assays were performed in triplicate incubating at 37°C for 30 min. The production of methylthioadenosine was entirely dependent on putrescine and spermidine.

2.6. Determination of intracellular polyamine contents

Polyamines (putrescine, spermidine and spermine) were determined by HPLC as described [12]. K562 cells were harvested by low speed centrifugation (1000 \times g for 3 min), suspended in ice-cold phosphate buffered saline and centrifuged once more. The resulting cell pellets were suspended in 0.4 N perchloric acid and disintegrated by freeze-thawing 3 times. The samples were centrifuged at 10000 \times g for 30 min, and the supernatants were analyzed by HPLC (Shimazu LC-5A).

3. RESULTS AND DISCUSSION

Fig.1 shows the dose-response curve for MEA on the inhibition of activities of spermidine and spermine synthases. Spermidine synthase was much more sensitive to MEA than spermine synthase. In table 1, the effects of MEA were compared with those of MTA. Unlike MEA, MTA inhibited spermidine synthase less sensitively than spermine synthase. It may be noteworthy that small molecules of MEA, which is analogous to the 3-mercaptopropylamine moiety of decarboxylated AdoMet, retain an ability to inhibit spermidine synthase. On the other hand, 2-mercaptoethanol inhibited neither spermidine nor spermine synthases in the concentration range from 0.1 to 1 mM (not shown), indicating that the amino group of MEA was necessary to inhibit the enzyme activity.

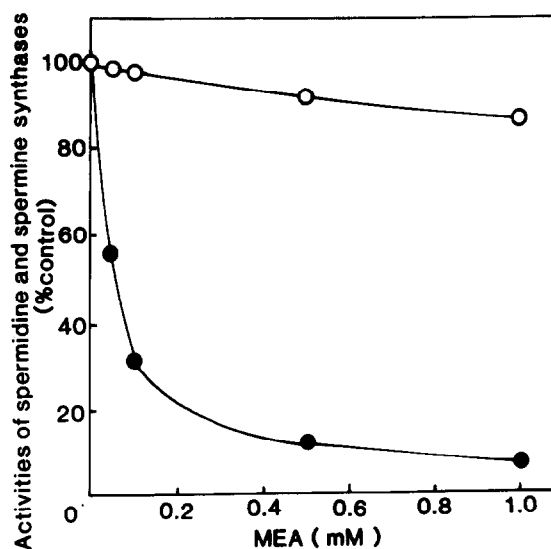


Fig.1. Inhibition of spermidine synthase (●) and spermine synthase (○) by MEA. The results are expressed as percentages of the control activity measured in the absence of MEA. Each point represents the mean of triplicate determinations.

Table 1

Comparison of inhibition of spermidine and spermine syntheses by MEA and MTA

Compound	Concentration (mM)	Inhibition (%)	
		Spermidine synthase	Spermine synthase
MEA	1	92.8	12.1
	0.1	66.5	1.4
	0.01	17.1	0.2
MTA	0.1	76.7	91.6
	0.01	16.8	57.2

MEA and MTA were tested by adding to the standard assay medium at the concentrations shown. Experimental details are given in section 2. The percentage inhibition due to the compounds are shown

The observation that MEA was not able to stimulate [*methyl*-¹⁴C]methylthioadenosine production by spermidine or spermine synthases (not shown) indicated that MEA was not a substrate for the two synthases.

Removal of MEA from the MEA-treated sper-

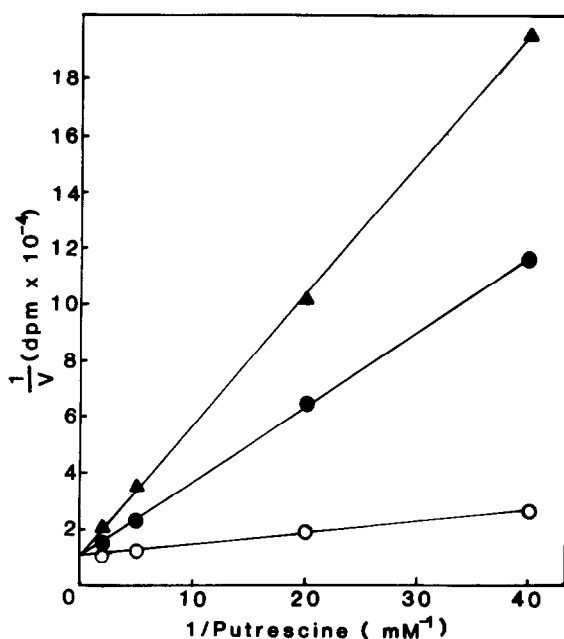


Fig.2. Competitive inhibition of spermidine synthase by MEA with putrescine as the variable substrate. Spermidine synthase activity was assayed in the absence (○) or presence of 25 μM (●) or 50 μM (▲) MEA, with 41.2 μM decarboxylated AdoMet, 0.025–0.5 mM putrescine and an enzyme preparation.

midine synthase preparation by dialysis restored the enzyme activity to that of the control enzyme preparation treated similarly except for exposure to the inhibitor. Thus, the inhibition of spermidine synthase by MEA was reversible.

Fig.2 shows the effect of the concentration of putrescine on the inhibition of spermidine synthase by MEA. The inhibition was competitive with respect to putrescine. The K_i value for MEA and K_m for putrescine were calculated to be 5 and 36 μM, respectively. MEA may construct a homologous conformation to putrescine, resulting in competitive inhibition of spermidine synthase.

Fig.3 shows the effect of the concentration of decarboxylated AdoMet on the inhibition of spermidine synthase by MEA. Although the extent of inhibition by MEA increased as the concentration of the inhibitor was increased, spermidine synthase activity in the presence of MEA was apparently constant throughout the concentration range from 8.2 to 82.4 μM of decarboxylated AdoMet.

As shown in table 2, MEA dose-dependently depressed the spermidine and spermine contents in

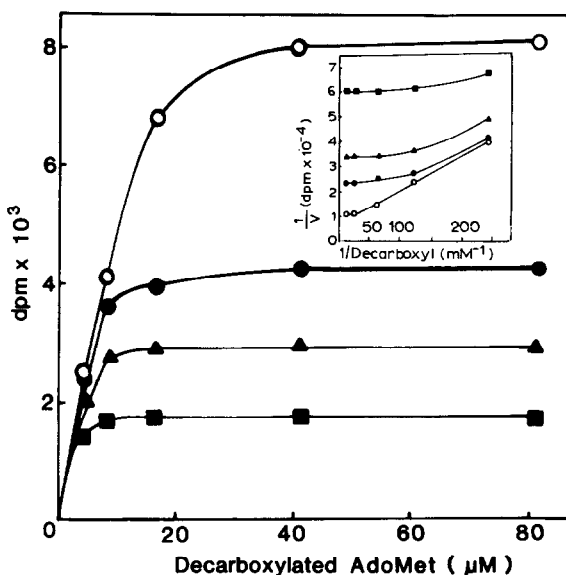


Fig.3. Inhibition of spermidine synthase by MEA with decarboxylated AdoMet as the variable substrate. Spermidine synthase activity was measured in the absence (○) or presence of 25 μM (●), 50 μM (▲) or 100 μM (■) MEA, with 0.5 mM putrescine, 4.1–82.4 μM decarboxylated AdoMet and the enzyme preparation. The insert shows the reciprocal plots of the experimental results.

Table 2

Effect of MEA on cellular contents of polyamines in human erythroid leukemia K562 cells

Treatment	Concn (mM)	Putrescine	Spermidine	Spermine
Control		0.19 (100)	7.11 (100)	8.98 (100)
MEA	0.8	0.25 (132)	4.48 (63)	5.78 (64)
	1.6	0.31 (163)	2.09 (29)	2.38 (27)

K562 cells were exposed to 0.8–1.6 mM MEA for 4 days and harvested to determine cellular polyamines and protein. The percent of the control (without treatment) is shown in parentheses. Each value is the mean of triplicate experiments and is expressed as nmol/mg cellular protein

human erythroid leukemia K562 cells, suggesting that these changes resulted from the inhibitory effects of MEA on spermidine synthase in situ. This inhibitor may be useful as an experimental tool for elucidating the physiological function of the polyamines.

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