METHYLTHIOADENOSINE, A POTENT INHIBITOR OF SPERMINE SYNTHASE FROM BOVINE BRAIN

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1. Introduction

Much effort has recently been made to develop specific inhibitors of polyamine synthesis which may help in elucidating the role of polyamines in cellular metabolism and in cell proliferation in particular [1]. Furthermore, inhibitors of polyamine synthesis may find applications as antiproliferative agents. Four enzymes are involved in the synthesis of polyamines in eukaryotic cells, i.e., ornithine decarboxylase, *S*-adenosylmethionine decarboxylase, and two propylamine transferases, one catalysing the synthesis of spermidine, the other producing spermine [2]. Most of the inhibitors presently available are inhibitors of the two decarboxylases, whereas little is known about inhibitors acting at the propylamine transferase step [1].

We have recently described an efficient method for the purification of spermine synthase from mammalian tissues using spermine–Sepharose affinity chromatography [3]. This enzyme has now been purified to an apparent homogeneity. The details of the purification procedure and the properties of the pure enzyme will be described elsewhere. In this report we demonstrate that the purified spermine synthase from bovine brain is strongly inhibited by methylthioadenosine, one of the products of the propylamine transferase reaction. This observation may be important for understanding the regulation of polyamine synthesis as well as for the development of new inhibitors of polyamine synthesis in eukaryotic cells.

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2. Materials and methods

D,L-[2-^{14}C]methionine (spec. act. 4.08 mCi/mmol) was supplied by New England Nuclear Corp. Labelled *S*-adenosylmethionine was synthesized from D,L-[2-^{14}C]methionine essentially as in [4]. Radioactive decarboxylated *S*-adenosylmethionine (*S*-methyladenosylhomocysteamine) was prepared from labelled *S*-adenosylmethionine using *S*-adenosylmethionine decarboxylase from *E. coli* purified through step 3 by the method in [5], as the enzyme. The product was purified on a Dowex 50-H^+ column and finally by preparative paper electrophoresis as in [6].

Spermidine trihydrochloride and dithiothreitol were purchased from Calbiochem. Spermidine was recrystallized from hot 6 N HCl–ethanol solution. *S*-adenosyl-L-homocysteine and methylthioadenosine (5′-deoxy-5′-methylthioadenosine) were supplied by Sigma Chemical Co. Adenosine was obtained from E. Merck, Darmstadt, and Whatman phosphocellulose paper P81 from Reeve Angel, London. CH-Sepharose 4B was a product of Pharmacia Fine Chemicals, Uppsala.

Spermine synthase was purified from bovine brain principally as in [3]. A second affinity chromatography on spermine–Sepharose was included as a final step. This procedure yielded an apparently homogeneous enzyme protein, as shown by polyacrylamide and SDS–gel electrophoresis (R.-L. P., A. R., T. Eloranta, in preparation). The final preparation had spec. act. ~400 nmol spermine produced/min . mg protein^{-1}.

The assay of spermine synthase activity was performed using radioactive decarboxylated

S-adenosylmethionine as substrate as detailed in [3,7]. Under standard assay conditions the incubation mixture contained, in total vol. 0.1 ml; 0.1 M potassium phosphate buffer (pH 7.4), 1 mM spermidine; 0.04 mM decarboxylated *S*-adenosylmethionine; 5 mM dithiothreitol; 0.05–0.1 μg enzyme protein. Protein was measured as in [3].

3. Results and discussion

The effect of the concentration of decarboxylated *S*-adenosylmethionine on the spermine synthase reaction is shown in fig.1. The affinity of the purified enzyme for decarboxylated *S*-adenosylmethionine was very high, and an app. K_m of ~1 μM for this substrate can be calculated from fig.1. The integrated Michaelis-Menten equation (see [8]) gave a K_m of 0.6 μM. Figure 1 also demonstrates that purified spermine synthase was powerfully inhibited by one of the reaction products, i.e., methylthioadenosine,

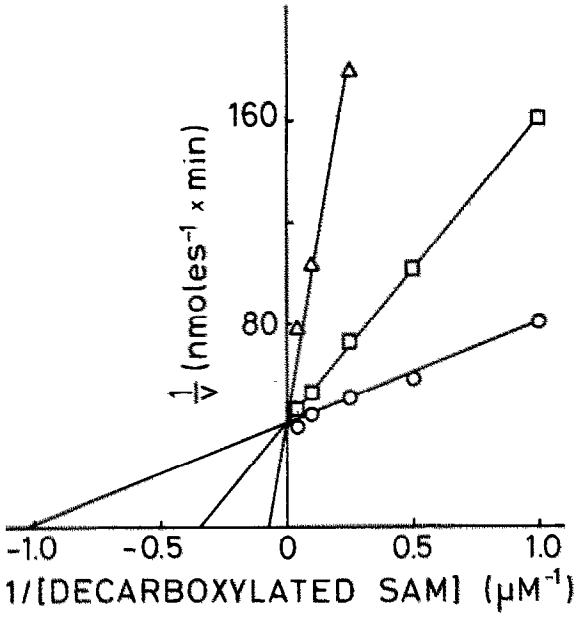

Fig.1. Inhibition of purified spermine synthase by methylthioadenosine. Spermine synthase activity (nmol spermine formed/min) was assayed in the absence (○) or presence of 1 μM (□) or 5 μM (△) methylthioadenosine, 2 mM spermidine, 1–25 μM decarboxylated *S*-adenosylmethionine (SAM) and 0.05 μg of enzyme protein. Incubation time was 5 min.

Table 1
Reversal of methylthioadenosine-induced inhibition of spermine synthase by adenosine

Addition	mM	Spermine synthase act. (pmol/min)
None (control)	–	48.6 (100)
Adenosine	0.2	52.3 (108)
Adenosine	1.0	48.6 (100)
Methylthio-adenosine (MTA)	0.01	18.9 (39)
MTA	0.01	27.8 (57)
Adenosine	0.2	
MTA	0.01	33.7 (69)
Adenosine	1.0	

Spermine synthase activity was measured in the presence of 40 μM decarboxylated *S*-adenosylmethionine, 1.0 mM spermidine and the compounds shown. Incubation time was 5 min. The % of the control activity is shown in parantheses

which acted as a competitive inhibitor with respect to decarboxylated *S*-adenosylmethionine. An app. K_i value of ~0.3 μM was calculated for methiothioadenosine. As shown in table 1, adenosine itself at ⩽ 1 mM was without effect or was slightly stimulatory but it partially reversed the inhibition produced by methylthioadenosine. The observation that *S*-adenosylhomocysteine (0.01–0.5 mM) or *S*-adenosylmethionine (0.01–0.1 mM) neither affected the spermine synthase reaction nor reversed the methylthioadenosine-induced inhibition (results not shown) also speaks for the specificity of the inhibition by the methylthio compound.

The physiological significance of the inhibition of spermine synthase by methylthioadenosine is difficult to assess at the present time, because neither decarboxylated *S*-adenosylmethionine nor methylthioadenosine accumulate in significant amounts in animal tissues. The only tissue which so far has been shown to contain measurable amounts of decarboxylated *S*-adenosylmethionine (0.2 μmol/g wet wt) is the eye of the sea catfish [9]. It appears that in most animal tissues it is ⩽ 1 μM. Little is also known about the content of methylthioadenosine of tissues. In rat ventral prostate < 0.2 μmol methylthioadenosine/g tissue was found [10]. In rabbit liver after feeding with large quantities of methionine it was 0.3 μmol/g liver (see [10]). It is known that many tissues contain a highly active nucleoside phosphorylase, different

from purine nucleoside phosphorylase, which catalyses the phosphorolytic cleavage of methylthioadenosine [10–12]. This gives a plausible explanation for the low concentration of methylthioadenosine in tissues. This also probably explains the observation [13] that crude prostatic spermine synthase was only partially inhibited by methylthioadenosine at 1 mM, i.e., 100-times higher than that used in the experiment described in table 1 of this paper. However, taking into account the very low concentration of decarboxylated *S*-adenosylmethionine of tissues and assuming that methylthioadenosine is present in tissues at 10^{-6}–10^{-5} M, it appears possible that methylthioadenosine at least partly regulates the rate of spermine synthesis in vivo. More careful analytical work on tissue methylthioadenosine and decarboxylated *S*-adenosylmethionine is definitely needed to solve this problem.

In conclusion, our results indicate that methylthioadenosine may play a role in the regulation of spermine synthesis in animal tissues. They also suggest that methylthioadenosine and, more importantly, its nucleosidase-resistant derivatives should be tested in vivo as inhibitors of polyamine synthesis and as possible antiproliferative agents.

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