The effects of 1-aminooxy-3-aminopropane and *S*-(5′-deoxy-5′-adenosyl)methylthioethylhydroxylamine on ornithine decarboxylase and *S*-adenosyl-L-methionine decarboxylase from *Escherichia coli*

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1-Aminooxy-3-aminopropane (APA) was shown to be a potent competitive inhibitor ($K_i = 1.0$ nM) of partially purified *Escherichia coli* ornithine decarboxylase. APA did not inhibit *S*-adenosyl-L-methionine decarboxylase and spermidine synthase from *E. coli*. *S*-(5′-Deoxy-5′-adenosyl)methylthioethylhydroxylamine (AMA), which is a structural analogue of decarboxylated *S*-adenosyl-L-methionine, was for the first time shown to be an irreversible inhibitor of bacterial *S*-adenosyl-L-methionine decarboxylase and a competitive inhibitor ($K_i = 47$ μM) of bacterial ornithine decarboxylase. AMA had no effect on spermidine synthase.

Ornithine decarboxylase *S-Adenosyl-L-methionine decarboxylase* *Enzyme inhibition* *(Escherichia coli)*

1. INTRODUCTION

The polyamines normally found in bacteria are putrescine and spermidine [1]. Spermine is not synthesized by most bacteria; one exception is *Acetobacter* [2]. Inhibition of polyamine biosynthesis prevents or retards bacterial growth [3–6]. The most widely used inhibitor is 2-difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC) [7]. Analogous inhibitors acting by the same general mechanism against ODC [8–10] or other decarboxylases [6,11] have been synthesized.

No good inhibitor of bacterial *S*-adenosyl-L-methionine decarboxylase (ADC) has been discovered yet. The inhibition of ADC caused by methylglyoxalbis(guanylhydrazone) (MGBG) has a K_i which is higher than that observed with eukaryotic enzymes [12]. Other potential inhibitors of ADC are Berenil and Pentamidine [13].

Inhibitors of bacterial spermidine synthase (putrescine aminopropyltransferase) such as dicyclohexylamine [14] and *S*-adenosyl-1,8-diamino-3-thiooctane (AdoDATO) [15] have been synthesized. These compounds, both alone and in combination with other inhibitors of polyamine biosynthesis, have been shown to retard bacterial growth [3,4,15].

This paper describes two potent inhibitors of polyamine biosynthesis in *E. coli*; 1-aminooxy-3-aminopropane (APA) and *S*-(5′-deoxy-5′-adenosyl)methylthioethylhydroxylamine (AMA).

2. MATERIALS AND METHODS

2.1. *Chemicals*

L-[1-^{14}C]Ornithine (48 mCi/mmol) and *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine (52 mCi/mmol) were purchased from CEA Research International (Gif-sur-Yvette, France). L-[*propylamine*-^{14}C]Decarboxyadenosylmethionine (42 nCi/nmol) was prepared as described in [16]. APA

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and AMA were a gift from Dr Khomutov. All other biochemicals were obtained from Sigma (St. Louis, MO).

2.2. *Enzymes*

The enzymes were extracted from lyophilized cells of *E. coli* strain B (ATCC 11303) obtained from Sigma. The lyophilized cells were first treated with acetone and partial purification of ODC was then performed as described by Hölttä et al. [17]. ADC-containing fractions from chromatography on DEAE-cellulose were dialysed and used for ADC measurements. A dialysed $(NH_4)_2SO_4$ precipitate of the bacterial extracts was used as the source of spermidine synthase.

2.3. *Enzyme assays*

ODC activity was measured as in [18]. The incubation mixture contained 100 mM Tris-HCl buffer (pH 8.5), 4 mM EDTA, 4 mM dithiothreitol, 0.4 mM pyridoxal 5-phosphate, 0.15 μCi L-[1-^{14}C]ornithine, 20–400 μM L-ornithine and the enzyme sample. The incubations were carried out at 37°C for 20 min, under conditions where the reaction is linear with respect to time. The time-dependent inhibition of ADC was carried out as described [10]. At time zero 50 μl enzyme stock solution was mixed with 50 μl assay medium (without substrate) and 50 μl inhibitor in water and incubated at 37°C. At various times 20 μl aliquots were transferred into 580 μl assay medium (giving a 30-fold dilution) containing 166 mM Tris-HCl buffer (pH 7.4), 3.3 μM EDTA, 6.7 mM $MgCl_2$, 0.057 μCi *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine and 15 μM *S*-adenosyl-L-methionine. The incubation was carried out for 30 min at 37°C and stopped by the injection of 0.5 ml of 2.5 M H_2SO_4. Spermidine synthase was measured exactly as described earlier, using method I [19]. Protein was measured by the method of Bradford [20].

3. RESULTS

The effect of APA on partially purified *E. coli* ODC is shown in fig.1. APA, which is a structural analogue of putrescine, was a powerful inhibitor of ODC. The inhibition was competitive with respect to ornithine and the K_i for the reaction was calculated to be 1.0 nM (fig.1). APA has previously been shown to be an irreversible inhibitor of liver ADC and a competitive inhibitor of bovine brain spermidine synthase [21]. Therefore, APA was tested on ADC and spermidine synthase from *E. coli*. No inhibition occurred with concentrations up to 1 mM (not shown).

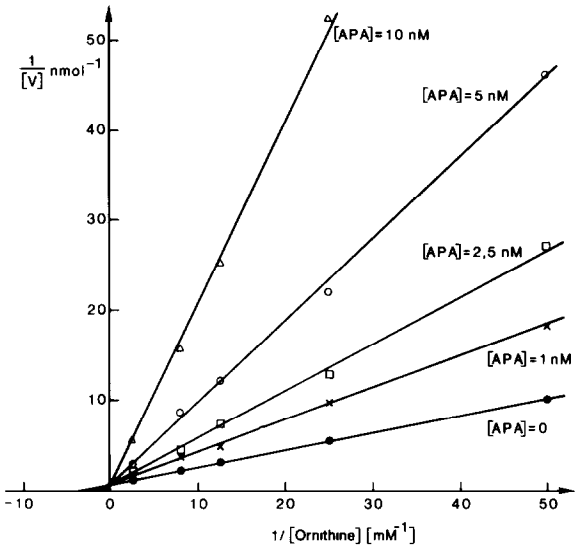

Fig.1. Inhibition of partially purified *E. coli* ODC by APA. ODC activity was measured as described in the text. Concentrations of ornithine were varied from 20 to 400 μM. The inhibitor concentrations are shown.

Incubation of *E. coli* ADC with AMA, which is shown for the first time to be an inhibitor of polyamine biosynthesis, resulted in a time-dependent loss of enzyme activity which followed pseudo first-order kinetics (fig.2). The loss of activity was related to the concentration of inhibitor. By plotting the time for half-inactivation ($t_{1/2}$) against the reciprocal of the inhibitor concentration ($1/I$) a straight line was obtained (fig.2, inset), showing that the rate of inactivation varied linearly with the AMA concentration in this concentration range.

To verify the irreversibility of the inactivation ADC was incubated with AMA (0.5 mM) for 30 min, which caused 99% inhibition. Prolonged dialyses of the inactivated ADC against standard buffer did not restore the activity, thus demonstrating the irreversibility of the inhibition (not shown).

Surprisingly, AMA also inhibited *E. coli* ODC.

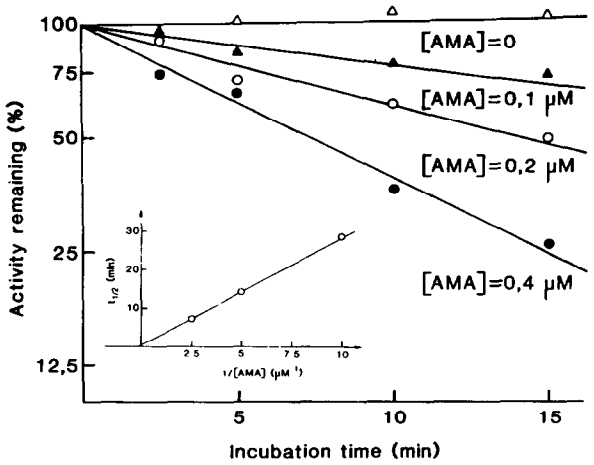

Fig.2. Time- and concentration-dependent inhibition of *E. coli* ADC by AMA. ADC was assayed as described in the text and the concentrations of AMA are indicated. (Inset) Times of half-inactivation ($t_{1/2}$) plotted vs the reciprocal of the inhibitor concentration.

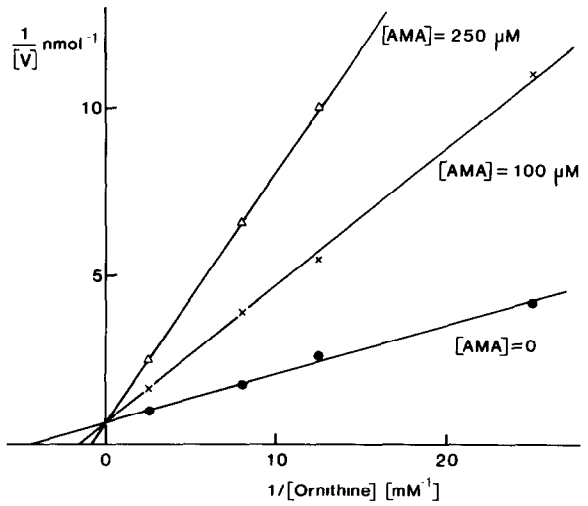

Fig.3. Inhibition of partially purified *E. coli* ODC by AMA. Assay conditions were the same as in fig.1. The concentrations of AMA are indicated.

It competed with ornithine in a competitive manner, having a K_i of 47 μM (fig.3). When AMA was tested with spermidine synthase no inhibition occurred with concentrations up to 1 mM (not shown).

4. DISCUSSION

APA is an analogue of canaline which is a potent inhibitor of several pyridoxal phosphate-dependent enzymes, including ODC [22]. However, we have shown earlier that the inhibition of mammalian ODC caused by APA is specific since it does not involve pyridoxal phosphate, but occurs via competition with ornithine [21], just as in fig.1. The inhibition of *E. coli* ODC is a little stronger (K_i = 1.0 nM) when compared with mammalian ODC (K_i = 3.2 nM) [21], and APA seems to be the strongest inhibitor of ODC reported so far.

Interestingly, APA did not inhibit *E. coli* ADC nor spermidine synthase. In contrast, the mammalian enzymes are inhibited [21]. In the case of ADC this might be explained by the fact that eukaryotic ADC is stimulated by putrescine whereas the bacterial enzyme is not [23].

Because AMA is a structural analogue of decarboxylated *S*-adenosyl-L-methionine, it was shown for the first time that AMA is a new irreversible inhibitor of *E. coli* ADC. AMA is a very strong and rapid inhibitor of ADC (fig.2). Compared to other inhibitors of ADC [24], including Berenil and Pentamidine [13], AMA seems to be the most effective reported so far.

Surprisingly, AMA inhibited *E. coli* ODC (fig.3) but not spermidine synthase. AMA competed with ornithine in a competitive manner. Possibly the thioethylhydroxylamine group of AMA can bind to an ornithine site on the enzyme.

Since inhibition of bacterial polyamine biosynthesis seems to inhibit growth of bacteria [3–6,25,26] these two potent inhibitors or their analogues may be useful drugs for specific inhibition of bacterial growth.

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