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# Effect of putrescine on the synthesis of *S*-adenosylmethionine decarboxylase

Takaaki KAMEJI and Anthony E. PEGG*
Department of Physiology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033, U.S.A.

The synthesis of *S*-adenosylmethionine (AdoMet) decarboxylase was studied by translating the rat prostate mRNA for this enzyme in a reticulocyte lysate. The protein was formed as a precursor of $M_r$ 37000, which was converted into the enzyme subunit of $M_r$ 32000 in the lysates. The presence of putrescine had no effect on the synthesis of the precursor of AdoMet decarboxylase, but accelerated its conversion into the enzyme subunit. Spermidine, spermine, decarboxylated AdoMet, AdoMet and methylglyoxal bis(guanylhydrazone) were not able to substitute for putrescine in this effect. These results indicate that, in addition to its direct activation of mammalian AdoMet decarboxylase, putrescine could increase the amount of the enzyme by increasing its production.

## INTRODUCTION

AdoMet decarboxylase is an essential enzyme in the biosynthesis of the polyamines spermidine and spermine in eukaryotes and many prokaryotes [1–3]. Mammalian AdoMet decarboxylase is activated by putrescine, providing a physiological regulatory mechanism by which an increase in cellular putrescine can increase the supply of decarboxylated AdoMet to act as an aminopropyl donor for spermidine synthase [4–6]. We have shown that in the rat AdoMet decarboxylase is synthesized as a precursor molecule of $M_r$ 37000, which is then converted into the enzyme subunit of $M_r$ 32000 [7]. It is probable that this conversion generates the enzyme-bound pyruvate which acts as a prosthetic group for this enzyme [4–6].

It is firmly established that the content of AdoMet decarboxylase protein is inversely related to the cellular concentration of the polyamines [5–11]. A number of factors contribute to this regulation, including polyamine-mediated changes in the mRNA content [7] and the rate of degradation of the protein [8–10]. Additionally, it appears that both spermidine and spermine inhibit the translation of mRNA for AdoMet decarboxylase. This inhibition can be demonstrated by the direct addition of the polyamines to reticulocyte lysates supplemented with AdoMet decarboxylase mRNA [12]. As part of our continuing studies on this inhibition, we examined the effect of putrescine on the translation. Putrescine did not affect the synthesis of AdoMet decarboxylase, but had a striking effect on the processing of the precursor.

## EXPERIMENTAL

α-Difluoromethylornithine was generously given by Merrell Dow Research Institute, Cincinnati, OH, U.S.A. Methylglyoxal bis(guanylhydrazone) was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. L-[$^{35}$S]Methionine (approx. 1500 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, IL, U.S.A. Protein A bacterial adsorbent was purchased from ICN Immunobiochemicals, Lisle, IL, U.S.A. Oligo(dT)–cellulose (type III) was obtained from Collaborative Research, Lexington, MA, U.S.A. Other biochemical reagents were products of Sigma Chemical Co., St. Louis, MO, U.S.A. Reticulocyte lysates were purchased from BRL, Bethesda, MD, U.S.A. In some experiments the lysates were freed from putrescine and polyamines by gel filtration [12].

The content of AdoMet decarboxylase mRNA in rat prostate was increased by treating the rats with α-difluoromethylornithine for 3 days as previously described [10]. Total RNA was extracted from rat prostate by the guanidinium/hot-phenol method [13]. Poly(A)-containing RNA was isolated from the total RNA by two passages over oligo(dT)–cellulose. The poly(A)-containing RNA (0.25 μg) was heated to 65 °C for 10 min and then translated in a total volume of 30 μl containing 10 μl of the reticulocyte lysate, 1.4 mM-$MgCl_2$, 0.5 mM-ATP, 0.15 mM-GTP, 0.2 mM-glucose 6-phosphate, 0.2 mM-dithiothreitol and the putrescine concentration indicated. After incubation at 30 °C for 60 or 90 min as indicated, the reaction was stopped by the addition of a chilled solution containing 10 mM-Tris/HCl, pH 7.4, 0.1% SDS, 0.1% Triton X-100, 2 mM-EDTA, 5 mM-methionine and 0.1% bovine serum albumin. Newly synthesized AdoMet decarboxylase was immunoprecipitated by the addition of 3 μl of a specific antiserum against AdoMet decarboxylase [11,12]. After incubation at room temperature for 30 min, 30 μl of 10% Protein A bacterial adsorbent was added, the mixture shaken for 30 min at room temperature and the precipitate removed by centrifugation at 15000 *g* for 1 min. The precipitate was washed with 4 × 1 ml of ice-cold 10 mM-Tris/HCl (pH 7.4)/0.1% SDS/2 mM-EDTA/0.1% Triton X-100/5 mM-methionine. The washed precipitate was dissolved by boiling in 40 μl of 62.5 mM-Tris/HCl (pH 6.8)/2.3% SDS/5% 2-mercaptoethanol/10% glycerol and subjected to polyacrylamide-

Abbreviation used: AdoMet, *S*-adenosylmethionine.

* To whom reprint requests should be addressed.

gel electrophoresis (10% -acrylamide gels) in the presence of SDS [14]. The gels were treated with Amplyfy (Amersham-Searle), dried and exposed to Kodak XAR film at −70 °C for fluorography. The amounts of AdoMet decarboxylase and its precursor that were synthesized were quantified by densitometric scanning of the films [12].

## RESULTS

As previously described [10], the translation of prostate mRNA in a reticulocyte lysate system followed by immunoprecipitation of the products corresponding to AdoMet decarboxylase indicated that proteins of $M_r$ 37000 and 32000 were formed (Fig. 1). The 32000-$M_r$ protein corresponded exactly to the subunit of AdoMet decarboxylase. The total production of AdoMet decarboxylase was somewhat greater in lysates which had been freed from polyamines by gel filtration, but the percentage that was present as the 32000-$M_r$ band was slightly decreased (Fig. 1 and Table 1). The gel-filtration

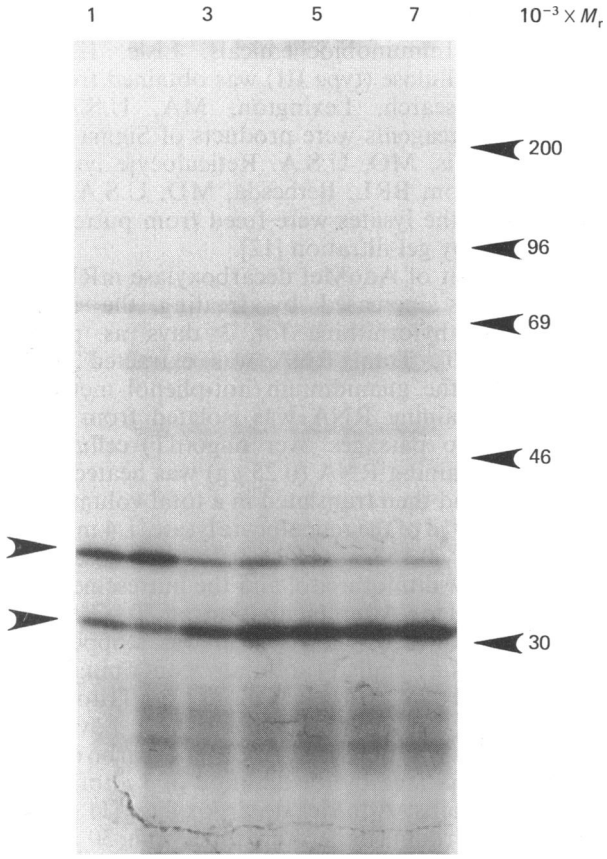


**Fig. 1. Effect of putrescine on synthesis of AdoMet decarboxylase by reticulocyte lysates**

The mRNA from rat prostate was translated in a reticulocyte lysate incubated for 90 min at 30 °C. Lane 1 shows results with a non-gel-filtered lysate; lanes 2–7 show results with a gel-filtered lysate supplemented with the following concentrations of putrescine: 0, lane 2; 0.05 mM, lane 3; 0.1 mM, lane 4; 0.2 mM, lane 5; 0.4 mM, lane 6; 0.8 mM, lane 7. The arrows indicate the position of the 37000-$M_r$ precursor and the 32000-$M_r$ subunit of AdoMet decarboxylase. The positions and sizes ($\times 10^{-3}$) of $M_r$ markers are also shown.

**Table 1. Effect of putrescine on the translation of prostate AdoMet decarboxylase mRNA**

Results obtained by densitometric scanning of the two bands corresponding to AdoMet decarboxylase in gels such as those in Fig. 1 were expressed in arbitrary units. The second column gives the percentage of the AdoMet decarboxylase that was present as the 32000-$M_r$ enzyme subunit. The first row shows results with a control non-filtered lysate. The other rows show results with a lysate freed from endogenous putrescine and polyamines by gel filtration and supplemented with the putrescine concentration indicated. The results shown are means of two or three estimations which agreed within ±10% of the mean.

| | AdoMet decarboxylase synthesis | |
|---|---|---|
| Putrescine concn. (μM) | Total (units) | % as 32000-$M_r$ band |
| Approx. 2 (non-gel-filtered lysate) | 0.77 | 43 |
| 0 | 1.00 | 31 |
| 50 | 0.84 | 74 |
| 100 | 1.17 | 78 |
| 200 | 1.14 | 78 |
| 400 | 1.02 | 86 |
| 800 | 1.12 | 87 |

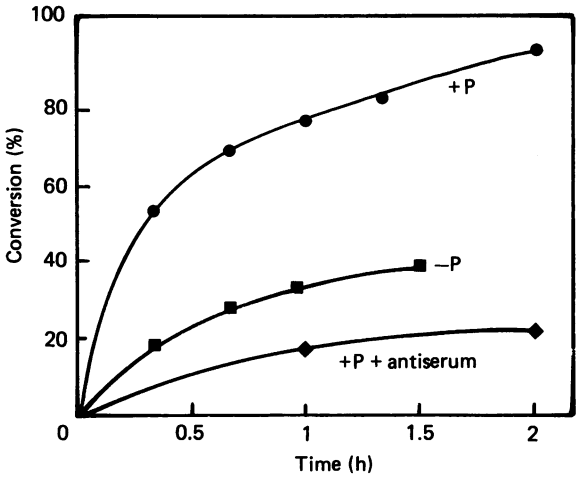


**Fig. 2. Time course of the effect of putrescine on conversion of AdoMet decarboxylase precursor**

After 60 min incubation of the reticulocyte lysate system in the absence of putrescine to allow labelling of the AdoMet decarboxylase precursor and subunit, 0.1 mM-cycloheximide was added to stop protein synthesis, and the incubation was continued for the time shown in the presence of 0.2 mM-putrescine (P) (●), in the absence of putrescine (■) and in the presence of 0.2 mM-putrescine plus antiserum to AdoMet decarboxylase (◆). The samples were then analysed for the content of labelled AdoMet decarboxylase and its precursor by polyacrylamide-gel electrophoresis. The Figure shows the quantitative results for the conversion of the remaining 37000-$M_r$ precursor into the 32000-$M_r$ protein during the incubation in the presence of cycloheximide, which were calculated from densitometric scanning of the gels.

procedure decreased the spermidine content of these lysates by more than 90% (from about 0.6 to 0.06 mM) and decreased their putrescine content from about 5 $\mu$M to below the limit of detection. When putrescine was added to the gel-filtered reticulocyte lysates there was little change in the total amount of synthesis of AdoMet decarboxylase, as indicated by the sum of the two bands, but the addition of putrescine led to a concentration-dependent increase in the percentage of the product that was present as the 32000-$M_r$ protein (Fig. 1 and Table 1).

These results suggest that putrescine was affecting the conversion of the 37000-$M_r$ precursor into the 32000-$M_r$ protein. More detailed studies of this conversion were therefore carried out by allowing the synthesis to occur for 1 h in the reticulocyte lysate and then adding cycloheximide to block further protein synthesis. At the same time as the cycloheximide was added, 0.2 mM-putrescine was added to some samples and the time course of production of the 32000-$M_r$ protein was determined (Fig. 2). The results show clearly that putrescine accelerated the conversion. If the antiserum to AdoMet decarboxylase was added to the lysates during this incubation, it inhibited the processing irrespective of the concentration of putrescine that was present (Fig. 2 and Table 2).

The same procedure was used to investigate the concentration-dependence and specificity of the action of putrescine. A maximal effect required more than 0.2 mM, but even 0.05 mM-putrescine gave a substantial increase in the rate of processing (Table 2). The stimulation of the conversion of the precursor of AdoMet decarboxylase was a specific effect of putrescine. As shown in Table 3, the addition of 1,3-diaminopropane, spermidine, spermine, AdoMet, decarboxylated AdoMet or methylglyoxal bis(guanylhydrazone) did not mimic the action of putrescine when added at equivalent concentrations (2 mM). These substances also had no effect at 0.2 mM (results not shown), but methylglyoxal bis(guanylhydrazone) had a slight effect at 10 mM (Table 3).

**Table 2. Effect of putrescine concentration on conversion of AdoMet decarboxylase precursor**

After 60 min incubation in the absence of putrescine to allow labelling of the AdoMet decarboxylase precursor and subunit, 0.1 mM-cycloheximide was added to stop protein synthesis, and the incubation was continued for 1 h in the presence of the concentration of putrescine shown. The results shown are means of three estimating which differed by less than 10% of the mean.

| Addition | Percentage of AdoMet decarboxylase as: 32000-$M_r$ band | 37000-$M_r$ band |
|---|---|---|
| Initial value* | 34 | 66 |
| None | 68 | 32 |
| 0.05 mM-putrescine | 83 | 17 |
| 0.1 mM-putrescine | 86 | 14 |
| 0.2 mM-putrescine | 90 | 10 |
| 0.2 mM-putrescine + control antiserum | 97 | 3 |
| 0.2 mM-putrescine + anti-(AdoMet decarboxylase) serum | 70 | 30 |
| 0.4 mM-putrescine | 93 | 7 |
| 0.8 mM-putrescine | 96 | 4 |
| 1.2 mM-putrescine | 94 | 6 |
| 1.2 mM-putrescine + control antiserum | 96 | 4 |
| 1.2 mM-putrescine + anti-(AdoMet decarboxylase) serum | 79 | 21 |

* Before addition of cycloheximide.

**Table 3. Effect of different amines on conversion of AdoMet decarboxylase precursor**

The experiment was carried out as in Table 2, but the amines shown were added during the incubation in the presence of cycloheximide.

| Compound added | Percentage of AdoMet decarboxylase as: 32000-$M_r$ band | 37000-$M_r$ band |
|---|---|---|
| Initial* | 34 | 66 |
| No addition | 72 | 28 |
| 0.2 mM-putrescine | 89 | 11 |
| 2 mM-putrescine | 96 | 4 |
| 2 mM-spermidine | 73 | 27 |
| 2 mM-spermine | 78 | 22 |
| 2 mM-1,3-diaminopropane | 78 | 22 |
| 2 mM-AdoMet | 68 | 32 |
| 2 mM-decarboxylated AdoMet | 69 | 31 |
| 2 mM-methylglyoxal bis-(guanylhydrazone) | 77 | 23 |
| 10 mM-methylglyoxal bis-(guanylhydrazone) | 85 | 15 |

* Before addition of cycloheximide.

## DISCUSSION

The present work and our previous publications [7,12] on the translation of prostatic mRNA for AdoMet decarboxylase indicate that this enzyme is synthesized as a precursor and that the synthesis of this precursor and its conversion into the enzyme subunit can be studied in the reticulocyte lysate system. However, at present little is known about the conversion process. In particular, it is not yet clear whether the formation of the 32000-$M_r$ protein requires enzymes present in the lysate or occurs autocatalytically, as is the case with the precursor to histidine decarboxylase from *Lactobacillus* [15,16], which resembles AdoMet decarboxylase in having pyruvate at its active site. Snell and colleagues have shown that the conversion of the precursor of histidine decarboxylase into the enzyme subunit generates the pyruvate prosthetic group at the *N*-terminus [15]. It is likely, although not proven rigorously, that the processing of AdoMet decarboxylase does generate the pyruvate group in the subunit, since this product, but not the precursor, binds to an affinity column of methylglyoxal bis(guanylhydrazone) linked to Sepharose [7]. Putrescine is known to bind to the mammalian AdoMet decarboxylase and to bring about a configurational change, since it activates the enzyme [4–6]. It is possible that such a change in

structure in response to putrescine binding may also aid in the conversion of the precursor.

The physiological significance of the stimulation of processing of the AdoMet decarboxylase precursor by putrescine is difficult to evaluate at present. There have been no reports indicating that the precursor is present in cell extracts, but it is a common procedure in the preparation of such extracts to homogenize in the presence of putrescine. Detailed studies with immunoblotting techniques using extracts prepared as rapidly as possible in the absence of putrescine will be needed to establish the extent to which the precursor may accumulate under various conditions. Although processing did occur in the absence of putrescine, it is still possible that its acceleration by putrescine is important in the production of the enzyme. AdoMet decarboxylase has a very rapid turnover, with a half-life of less than 1 h in many tissues [4–6]. If the precursor molecule is also subject to this rapid degradation, a significant fraction of it could become degraded without ever becoming active. If this is the case, the increased rate of conversion of the precursor into the subunit in the presence of putrescine could increase the amount of active enzyme.

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