Comparison of inhibitors of *S*-adenosylmethionine decarboxylase from different species

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S-Adenosyl-L-methionine decarboxylases were purified from rat ventral prostate, yeast (*Saccharomyces cerevisiae*), slime mould (*Physarum polycephalum*) and bacteria (*Escherichia coli*) and tested for inhibition by a variety of nucleosides related to *S*-adenosylmethionine and by methyl- and ethyl-glyoxal bis(guanyldiazide). Although the enzymes from these different sources are markedly different with respect to activation by cations, the inhibition by nucleosides was quite similar. Very little inhibition was seen when analogues of *S*-adenosylmethionine with a different base were tested or when the ribose ring was opened or the positive charge on the sulphur atom was not present. Some derivatives in which the amino acid portion of the molecule was altered were more potent inhibitors, but again there was little difference between the enzymes from different sources. 5'-(Dimethylsulphonio)-5'-deoxyadenosine and *S*-adenosyl-3-methylthiopropylamine were the most inhibitory substances and had similar K_i values, suggesting that the aminopropyl group does not contribute significantly to the binding. All of the *S*-adenosylmethionine decarboxylases were strongly competitively inhibited by methylglyoxal bis(guanyldiazide) and even more powerfully by its ethyl analogue, although the putrescine-activated enzymes from prostate and yeast were more sensitive than the bacterial and slime-mould enzymes. All of the *S*-adenosylmethionine decarboxylases tested bound to a column of methylglyoxal bis(guanyldiazide) linked to Sepharose and were not eluted by 0.5 M-NaCl, but could be released by 1 mM concentrations of the drug, providing a rapid and efficient method for their purification.

AdoMet decarboxylase is an essential enzyme for the biosynthesis of polyamines in mammalian cells, lower eukaryotes and many micro-organisms (Tabor & Tabor, 1976; Williams-Ashman & Pegg, 1981; Pegg & McCann, 1982). This enzyme provides decarboxylated AdoMet, which is used as an aminopropyl donor by the enzymes that form spermidine and spermine. All of the preparations of AdoMet decarboxylase so far examined, namely those from *Escherichia coli* (Wickner *et al.*, 1970; Markham *et al.*, 1982), yeast (Cohn *et al.*, 1977) and rat liver (Pegg, 1977; Demetriou *et al.*, 1978), contain covalently bound pyruvate that is essential for catalytic activity and forms a Schiff base intermediate with the substrate (Pankaskie & Abdel-Monem, 1980; Allen & Klinman, 1981; Markham

et al., 1982). Despite this similarity there are significant species differences in AdoMet decarboxylases. The enzyme from *E. coli* has six identical subunits of mol.wt. 17000, each containing one pyruvate molecule (Markham *et al.*, 1982), whereas the yeast AdoMet decarboxylase has two subunits of mol.wt. 41000 (Cohn *et al.*, 1977). Although there is some difference between values reported for the mammalian enzyme by various laboratories, the most recent consensus is that the mammalian enzyme contains two subunits of mol.wt. 32000 (Pegg, 1977, 1979; Sakai *et al.*, 1979; Seyfried *et al.*, 1982). More importantly, AdoMet decarboxylases from different species fall into three classes according to their activation by cations. AdoMet decarboxylases from *E. coli* (Wickner *et al.*, 1970), *Lathyrus sativus* seedlings (Suresh & Adiga, 1977), *Azobacter vinelandii* (Coppoc *et al.*, 1971), *Klebsiella aerogenes* (Stevens *et al.*, 1978), *Pseudomonas aeruginosa* (Pösö *et al.*, 1976) and *Phaseolus aureus* sprouts (Coppoc *et al.*, 1971)

Abbreviations used: AdoMet, *S*-adenosyl-L-methionine; *S*-adenosyl-L-methionine dialdehyde derivative, 2'-O-[(*R*)-formyl(adenin-9-yl)methyl]3'-*S*-(*R*)-methioninyl-3'-deoxy-(*S*)-glyceraldehyde.

require Mg^{2+} for activity. AdoMet decarboxylases from all mammalian tissues (Pegg & Williams-Ashman, 1969; Williams-Ashman & Pegg, 1981), from *Saccharomyces cerevisiae* (Jänne *et al.*, 1971; Pösö *et al.*, 1975a) and from a wide variety of filamentous fungi (Hart *et al.*, 1978) require putrescine for maximal activity. AdoMet decarboxylases from *Tetrahymena pyriformis* (Pösö *et al.*, 1975b), *Physarum polycephalum* (Mitchell & Rusch, 1973) and *Dictyostelium discoideum* (Stevens *et al.*, 1978) are not activated by cations. The activation of AdoMet decarboxylase by putrescine is an important regulatory mechanism that links the supply of decarboxylated AdoMet to that of putrescine and favours the conversion of putrescine into spermidine. Therefore, those species that have putrescine-activated AdoMet decarboxylases contain the polyamines in considerable excess over the precursor, putrescine (Pegg & Hibasami, 1979).

A number of studies have been carried out on inhibitors of AdoMet decarboxylases. Such inhibitors may have considerable pharmacological interest and could be used to study the function of polyamines (Pegg & McCann, 1982). Virtually all of these studies have been carried out only with the enzyme from mammalian sources and the effects of a number of nucleosides on it have been described (Pegg, 1969; Zappia *et al.*, 1977; Yamanoha & Samejima, 1980; Pankaskie & Abdel-Monem, 1980; Kolb *et al.*, 1982). Methylglyoxal bis(guanylhydrazone) was found to be a potent inhibitor of putrescine-activated AdoMet decarboxylases by Williams-Ashman & Schenone (1972). They also noted that it was inhibitory but much less active against the Mg^{2+} -activated enzyme from *E. coli*. Subsequent work supported these findings (Pegg, 1973; Hölttä *et al.*, 1973; Corti *et al.*, 1974; Pösö *et al.*, 1975a,b; Williams-Ashman *et al.*, 1977). It was reported that the AdoMet decarboxylase from *P. polycephalum* was strongly inhibited (Mitchell & Rusch, 1973), but that from *T. pyriformis* was resistant (Pösö *et al.*, 1975b). These results suggest that different inhibitors may show selective inhibition of one or other form of AdoMet decarboxylase, but some of the comparisons already made were only quantitative and were carried out with crude enzyme preparations. Therefore, it appeared that a more detailed comparison of the effects of potential inhibitors on AdoMet decarboxylases from each of the classes was worthwhile. In order to carry out this study under comparable conditions it was necessary to purify the enzymes to remove other proteins that could interact with or degrade the potential inhibitors. This is of particular importance since artifactual resistance of the mouse liver AdoMet decarboxylase to inhibition by methylglyoxal bis(guanylhydrazone) was reported by Heby & Russell (1974). Powerful inhibition was observed by other

groups who partially purified the enzyme (Corti *et al.*, 1974; Williams-Ashman *et al.*, 1977).

The present paper, therefore, describes convenient purification methods for AdoMet decarboxylases from rat ventral prostate, *E. coli*, *S. cerevisiae* and *P. polycephalum* and the effects on their activity of a number of potential inhibitors. It was found that despite the differences in activators for these enzymes, the inhibitions observed for each substance and, therefore, presumably the active site were remarkably similar.

Materials and methods

Chemicals

S-Adenosyl-L-[carboxy- ^{14}C]methionine (sp. radioactivity 55 mCi/mol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. AdoMet, putrescine and general biochemical reagents were products of the Sigma Chemical Co., St. Louis, MO, U.S.A. AdoMet was repurified before use by chromatography on Dowex-50 (H^+ form) as previously described (Pegg & Williams-Ashman, 1969). Methylglyoxal bis(guanylhydrazone) was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Methylglyoxal bis(guanylhydrazone)-Sephacrose was prepared by the coupling of CH-Sephacrose 4B (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) to the drug using a water-soluble carbodiimide as previously described (Pegg, 1974). The final product contained about $2 \mu\text{mol}$ of bound drug per ml of gel. Ethylglyoxal bis(guanylhydrazone) was a gift from Dr. E. Mihich, Roswell Park Memorial Institute, Buffalo, NY, U.S.A. Other preparations of this drug were synthesized by a published procedure (Podrebarac *et al.*, 1963). Most of the structural analogues of AdoMet used in this study were generously provided by Dr. R. T. Borchardt, Department of Biochemistry, University of Kansas, Lawrence, KS, U.S.A. Their synthesis is described by Borchardt *et al.* (1976). Decarboxylated S-adenosylmethionine (S-adenosyl-3-methylthio-propylamine) was prepared as previously described (Hibasami *et al.*, 1980). 5'-(Dimethylsulphonio)-5'-deoxyadenosine was synthesized by the reaction of 5'-methylthioadenosine with methyl iodide (Zappia *et al.*, 1978).

Enzyme purification

AdoMet decarboxylase was isolated from rat ventral prostates from 30 male Sprague-Dawley-strain rats weighing 400–500 g. The same procedure was used as that published for the isolation of the enzyme from rat liver (Pösö & Pegg, 1982). After initial purification by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography, the enzyme solution was dialysed overnight against 2.5 mM-putrescine / 2.5 mM-dithiothreitol / 25 mM-Tris / HCl,

pH 7.5, and applied to a column (1.6 cm $\times$ 18 cm) of methylglyoxal bis(guanyldiazide) attached to Sepharose that had previously been equilibrated with the same buffer. The column was washed with 200 ml of the equilibration buffer followed by 100 ml of 0.3 M-NaCl and 200 ml of 0.5 M-NaCl in this buffer. The enzyme was then eluted by addition of 1 mM-methylglyoxal bis(guanyldiazide) to the 0.5 M-NaCl/25 mM-Tris/HCl (pH 7.5)/2.5 mM-putrescine/2.5 mM-dithiothreitol. The active fractions were concentrated to a volume of 1 ml and freed from the inhibitor by repeated use of an Amicon ultrafiltration cell. The final enzyme was recovered in 45% yield and was purified 7000-fold. It had a specific activity of 0.5 μ mol of CO₂ released/min per mg under the standard assay conditions. AdoMet decarboxylase was isolated from *Saccharomyces cerevisiae* (500 g) by the method of Cohn *et al.* (1977) through the DEAE-cellulose step. The enzyme was then dialysed and chromatographed on the affinity column exactly as described above for the prostatic enzyme. The final preparation represented a 5600-fold purification in 30% yield and had a specific activity of 0.6 μ mol of CO₂ released/mg of protein per min. AdoMet decarboxylase was isolated from *E. coli* (250 g of frozen *E. coli* B obtained from Miles Laboratories, Elkhart, IN, U.S.A.) by the procedure of Wickner *et al.* (1970) up to the end of the DEAE-cellulose chromatography and omitting the heat step. The preparation was then dialysed against 50 mM-Tris/HCl/5 mM-MgCl₂/1 mM-dithiothreitol and applied to a column (1.6 cm $\times$ 18 cm) of methylglyoxal bis(guanyldiazide) attached to Sepharose. After washing the column with 200 ml of 50 mM-Tris/HCl/5 mM-MgCl₂/1 mM-dithiothreitol, it was eluted successively with 200 ml of 0.1 M-NaCl in this buffer, 200 ml of 0.5 M-NaCl in this buffer and 50 ml of 1 mM-methylglyoxal bis(guanyldiazide)/50 mM-Tris/HCl/1 mM-dithiothreitol. The final solution eluted the enzyme in the first 20 ml, which was concentrated and freed from the inhibitor as described above. The final preparation represented a 1200-fold purification in 45% yield and had a final specific activity of 6 μ mol of CO₂ released/mg per min under the standard assay conditions. AdoMet decarboxylase was isolated from dried pellets of *Physarum polycephalum* microplasmodia (Mitchell & Rusch, 1973), which were kindly provided by Dr. J. L. A. Mitchell, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, U.S.A. After extraction in 50 mM-sodium phosphate (pH 7.5)/2.5 mM-dithiothreitol the extract was centrifuged and applied to a column of DEAE-cellulose as previously described for the rat liver enzyme (Pösö & Pegg, 1982). The column was eluted with a linear gradient of 0.1–0.4 M-NaCl in this buffer and the active fractions were concentrated and dialysed against 25 mM-Tris/HCl (pH 7.5)/2.5 mM-dithio-

threitol and applied to a column of methylglyoxal bis(guanyldiazide) linked to Sepharose. All details of this chromatography were as for the prostate enzyme, except that the buffer did not contain putrescine. The final enzyme preparation was obtained in 30% yield and represented a 2800-fold purification. The enzyme had a specific activity of 0.4 μ mol of CO₂ released/mg per min. All of the purified AdoMet decarboxylases were stored at –20°C at a concentration of 0.5–2 mg of protein/ml. The putrescine-activated AdoMet decarboxylases were stored in a solution of 2.5 mM-putrescine/2.5 mM-dithiothreitol/25 mM-Tris/HCl (pH 7.5); putrescine was omitted from the solutions for storage of the enzymes from *E. coli* and *P. polycephalum*. The AdoMet decarboxylase preparations from prostate, yeast and *E. coli* gave single bands corresponding to molecular-weight values of 33 000, 40 000 and 18 000 respectively when analysed by polyacrylamide-gel electrophoresis under denaturing conditions as described by Pösö & Pegg (1982) but several bands were visible in the preparation from *P. polycephalum*.

Assay of AdoMet decarboxylase

The reaction was followed by measuring the production of ¹⁴CO₂ from *S*-adenosyl-L-[carboxy-¹⁴C]methionine (Pegg & Williams-Ashman, 1969). All assays were carried out at 37°C for 15–30 min under conditions in which the activity was proportional to the protein added and to the time of incubation. The assay mixture contained 200 μ M-*S*-adenosyl-L-[carboxy-¹⁴C]methionine (0.2 μ Ci), 50 mM-sodium phosphate, pH 7.5, 2.5 mM-dithiothreitol and the enzyme in a total volume of 0.25 ml. For assays of the prostate and yeast enzyme, 2.5 mM-putrescine was included. For assays of the *E. coli* enzyme this was replaced by 10 mM-MgCl₂. *K_m* and *K_i* values were determined graphically as described by Todhunter (1979), using linear regression analysis to determine the slopes.

Results

AdoMet decarboxylases were purified from *E. coli*, *S. cerevisiae*, *P. polycephalum* and rat ventral prostate and the effects of a number of nucleosides related to AdoMet on their activities were determined (Table 1). The enzymes were assayed in the presence of 0.2 mM-AdoMet, saturating concentrations of activating cations (putrescine for the enzymes from yeast or prostate and Mg²⁺ for enzyme from *E. coli*). Only a few of the compounds tested were potent inhibitors of the reaction. Most of the analogues in which the base of AdoMet was changed were totally inactive towards all of the AdoMet decarboxylases. These included changes to

Table 1. Inhibition of *S*-adenosylmethionine decarboxylase from different sources by analogues of *S*-adenosylmethionine. Compounds with the structures indicated were added to the assays at the concentrations shown and the activity found expressed as a percentage of that in the absence of any inhibitor. The inhibitor was tested first at 1 mM. If less than 10% activity occurred at this concentration, the assays were repeated at 0.1 mM. Where less than 10% activity was seen with 0.1 mM, the assay was repeated with 0.01 mM inhibitor.

Concentration added	Concentration (mM)	Activity of <i>S</i> -adenosylmethionine decarboxylase (%)			
		Prostate	<i>S. cerevisiae</i>	<i>P. polycephalum</i>	<i>E. coli</i>
None	—	100	100	100	100
<i>S</i> -Adenosyl-D-methionine	1	53	74	48	90
<i>S</i> -Adenosyl- <i>S</i> -methyl-L-cysteine	1	8	16	19	33
<i>S</i> -Adenosyl-4-methylthiobutyrate	1	10	17	17	29
<i>S</i> -Adenosyl-3-methylthiopropylamine	0.1	17	26	31	27
5'-(Dimethylsulphonio)-5'-deoxyadenosine	0.1	21	23	26	35
<i>S</i> -Adenosyl-L-ethionine	1	79	88	66	91
<i>S</i> -Adenosyl-L-methionine dialdehyde derivative	1	80	76	87	84
<i>S</i> -Inosyl-L-methionine	1	72	81	65	75
<i>S</i> -Guanosyl-L-methionine	1	100	99	102	104
<i>S</i> -Uridyl-L-methionine	1	100	95	85	91
<i>S</i> -Cytidyl-L-methionine	1	95	100	95	101
<i>N</i> ⁶ -Methyl- <i>S</i> -adenosyl-L-methionine	1	100	98	101	100
<i>N</i> ⁶ -Dimethyl- <i>S</i> -adenosyl-L-methionine	1	97	100	97	91
3-Deaza- <i>S</i> -adenosyl-L-methionine	1	90	91	76	77
7-Deaza- <i>S</i> -adenosyl-L-methionine (<i>S</i> -tubercidinyl-L-methionine)	1	77	89	72	70
8-Aza- <i>S</i> -adenosyl-L-methionine	1	61	74	62	84
Methylglyoxal bis(guanyldiazide)	0.01	12	13	76	79
Ethylglyoxal bis(guanyldiazide)	0.01	2	1	41	53
<i>S</i> -Adenosyl-L-homocysteine	1	101	103	102	104
<i>S</i> -Adenosyl-L-homocysteine sulphoxide	1	89	96	95	98

pyrimidines, guanine or additions of methyl groups to the *N*⁶-position of adenine. Analogues containing hypoxanthine, 3-deaza-adenine, tubercidin (7-deaza-adenine) or 8-aza-adenine were slightly inhibitory, but reduced activity by less than 40% in all cases. These results indicate that the active sites of the enzymes are highly specific for adenosine and that even simple changes such as the presence of an *N*⁶-methyl group abolish the binding. The ribose ring and the positively charged sulphonium centre are also critical for binding since the 2',3'-dialdehyde derivative in which the ring is opened was only weakly inhibitory and *S*-adenosyl-L-homocysteine and *S*-adenosyl-L-homocysteine sulphoxide were inactive.

Alterations to the amino acid group did produce compounds with inhibitory activity. Changing the amino acid to L-ethionine or to D-methionine produced weak inhibitors (Table 1). The *S*-methyl-L-cysteine derivative, which has one methylene group less than methionine, was a stronger inhibitor, as was the deaminated derivative, *S*-adenosyl-4-methylthiobutyrate. However, the strongest inhibitors were decarboxylated *S*-adenosylmethionine (*S*-adenosyl-3-methylthiopropylamine) and 5'-(dimethylsulphonio)-5'-deoxyadenosine, which pro-

duced more than 60% inhibition at 0.1 mM concentrations (Table 1). Although there were slight differences between the enzymes from various sources in response to these nucleotides, these differences were not particularly marked. The most potent inhibitors of AdoMet decarboxylase tested were methyl- and ethyl-glyoxal bis(guanyldiazide). As indicated in Table 1, these inhibitors were somewhat selective, producing greater inhibition of the prostate and yeast enzymes, but both agents inhibited even the bacterial enzyme by more than 40% at 0.1 mM and ethylglyoxal bis(guanyldiazide) inhibited the *P. polycephalum* and *E. coli* enzymes by about 50% at 0.01 mM. Ethylglyoxal bis(guanyldiazide) was easily the strongest inhibitor of AdoMet decarboxylases from all sources tested in the present study.

Further experiments were carried out with the more potent inhibitors, which were tested as inhibitors over a range of concentrations and concentrations of AdoMet. The structures of these compounds are indicated in Fig. 1. All of the inhibitors were competitive with respect to AdoMet and the observed values of K_m for AdoMet and K_i for the inhibitors are listed in Table 2. There was very little difference in the K_i values for decarboxylated

Fig. 1. Structures of inhibitors described in Table 2

(a) Derivatives of *S*-adenosylmethionine: 5'-(dimethylsulphonio)-5'-deoxyadenosine, $R = -CH_3$; *S*-adenosyl-3-methylthiopropylamine, $R = -CH_2CH_2CH_2NH_2$; *S*-adenosyl-4-methylthiobutyrate, $R = -CH_2CH_2CH_2CO_2H$; *S*-adenosyl-*S*-methyl-L-cysteine, $R = -CH_2CH(NH_2)CO_2H$ (L-). (b) Derivatives of glyoxal bis(guanylhyazone): methylglyoxal bis(guanylhyazone), $R = -CH_3$; ethylglyoxal bis(guanylhyazone), $R = -CH_2CH_3$.

AdoMet and 5'-(dimethylsulphonio)-5'-deoxyadenosine, which for all four enzymes were in the range 5–13 μM . The bis(guanylhyazone) derivatives had K_i values of less than 1 μM for AdoMet decarboxylase of prostate and yeast. The K_i values were 15–40 times greater for the enzyme from *P. polycephalum* or *E. coli*. However, even the less active methylglyoxal bis(guanylhyazone) gave a K_i for the latter enzymes of about 10–13 μM and ethylglyoxal bis(guanylhyazone) gave a K_i of 1.6–2.6 μM . Therefore, these derivatives are actually quite potent inhibitors of these microbial enzymes.

The high affinity of all of the AdoMet decarboxylases for these bis(guanylhyazones) was used to purify the enzymes, as described in the Materials and methods section. Enzyme from all four sources was strongly retained on columns of methylglyoxal bis(guanylhyazone) linked to Sepharose provided that it was applied in the presence of the appropriate activating cation (5 mM-Mg²⁺ for enzyme from *E. coli*, 2.5 mM-putrescine for yeast and prostate). The enzymes were not released from this adsorbant by washing with 0.5 M-NaCl in the loading buffer, which released more than 95% of the attached protein, but appeared in the first 10 ml after the addition of 1 mM-methylglyoxal bis(guanylhyazone) to this washing solution. Indeed in all cases the AdoMet decarboxylases were released in the presence of 1 mM-methylglyoxal bis(guanylhyazone)/0.1 M-NaCl/50 mM-Tris/HCl (pH 7.5) and the activating cation (see above). This indicates that the attachment to the column is not due simply to ionic interaction between the positively charged ligand and the enzymes, but to more specific attachment, probably involving the active site.

Discussion

The present results indicate that despite the marked differences in activators, subunit composition and molecular weight between AdoMet decarboxylases from various species, the active sites are very similar. In order to bind at the active site, nucleoside derivatives must contain adenine, an intact ribose ring and a positive charge at the sulphonium centre (Table 1). It has been known for some time that the sulphur atom can be replaced by selenium without preventing the decarboxylation by mammalian AdoMet decarboxylases (Pegg, 1969). Pankaskie & Abdel-Monem (1980) and Kolb *et al.* (1982) have shown that replacement of this sulphur atom by a nitrogen atom still allows strong interaction with the active site of rat liver AdoMet decarboxylase. This implies that the positive charge is the critical feature. The amino acid moiety attached to the sulphonium centre is not needed for binding, as indicated by the strong inhibition by 5'-(dimethylsulphonio)-5'-deoxyadenosine, which has only a methyl group attached to this site. The present paper is the first to describe the potent inhibition of AdoMet decarboxylases from microorganisms by this agent, but while these experiments were in progress Kolb *et al.* (1982) noted that it inhibited crude rat liver AdoMet decarboxylase with a K_i of 2 μM , which is in reasonable agreement with our results for the prostatic enzyme.

The strong inhibition of AdoMet decarboxylase by decarboxylated AdoMet has been observed previously by several groups, including Pegg & Williams-Ashman (1969), Jänne *et al.* (1971), Pösö *et al.* (1975a,b) and Yamanoha & Samejima (1980).

Table 2. *Inhibition constants for S-adenosylmethionine decarboxylases from different sources*

The assays were carried out as described in the Materials and methods section and the apparent K_m for AdoMet and K_i for the substances shown were determined.

Compound	Inhibition constant (K_i) for enzyme shown (μM)			
	Prostate	<i>S. cerevisiae</i>	<i>P. polycephalum</i>	<i>E. coli</i>
S-Adenosylmethionine	65*	115*	95*	30*
Methylglyoxal bis(guanyldiazide)	0.3	0.6	10	13
Ethylglyoxal bis(guanyldiazide)	0.07	0.1	2.6	1.6
5'-(Dimethylsulphonio)-5'-deoxyadenosine	6	9	10	7
S-Adenosyl-3-methylthiopropylamine	5	12	13	8
S-Adenosyl-4-methylthiobutylamine	24	132	112	50
S-Adenosyl-S-methyl-L-cysteine	39	158	131	74

* K_m for this substrate.

Our results agree with those of Pösö *et al.* (1975a) in that there is little difference in the sensitivity of the enzyme from various sources to this inhibitory product, in contrast with previous reports that the enzyme from *E. coli* was resistant to such inhibition (Jänne *et al.*, 1971). The K_i values for decarboxylated AdoMet are very similar to those for 5'-(dimethylsulphonio)-5'-deoxyadenosine, indicating that the aminopropyl group is no more effective in binding to the active site than the methyl group. Therefore, despite previous suggestions that the terminal amino group plays an important role in binding to the enzyme (Zappia *et al.*, 1977), it appears that this is not a major factor. The other compounds containing an adenosyl group and a sulphonium centre were considerably less inhibitory, particularly with the yeast and *P. polycephalum* enzymes. This indicates that the groups involved, namely D-methionine, L-ethionine, deaminated methionine and S-methyl-L-cysteine do not fit so well into the site as the decarboxylated methionine or dimethyl sulphide moieties. This is probably due to steric limitations at the active site, which allows effective binding only of the AdoMet substrate, the decarboxylated AdoMet product and the relatively small 5'-(dimethylsulphonio)-5'-deoxyadenosine.

The important finding of the sensitivity of mammalian AdoMet decarboxylases to inhibition by methylglyoxal bis(guanyldiazide) was first made by Williams-Ashman & Schenone (1972). Since then, many papers have confirmed this finding and the value for K_i given in Table 2 for the prostate and yeast enzymes is consistent with the potent inhibition observed in these studies (Höltta *et al.*, 1973; Pegg, 1973; Corti *et al.*, 1974; Pösö *et al.*, 1975a,b; Porta *et al.*, 1977; Pösö & Pegg, 1982). The present results also confirm that ethylglyoxal bis(guanyldiazide) is a stronger inhibitor than the methyl derivative not only for mammalian AdoMet decarboxylases as previously reported (Corti *et al.*,

1974; Pösö & Pegg, 1982), but also for AdoMet decarboxylases from micro-organisms. Ethylglyoxal bis(guanyldiazide) may penetrate cells less well than methylglyoxal bis(guanyldiazide) but intracellular contents sufficient to interfere with polyamine synthesis should be easy to achieve. Even the enzyme from *P. polycephalum*, which was least sensitive, had a K_i of less than 3 μM . Although it has widely been implied that such bis(guanyldiazides) are not active against bacterial AdoMet decarboxylases this is based only on considerable differences in sensitivity of *E. coli* and mammalian AdoMet decarboxylases to methylglyoxal bis(guanyldiazide) (Table 1; Williams-Ashman & Schenone, 1972). In fact, as shown in Table 2, even methylglyoxal bis(guanyldiazide) has a K_i of 13 μM for the bacterial enzyme and ethylglyoxal bis(guanyldiazide) is easily the most active inhibitor of this enzyme presently available having a K_i of 1.6 μM .

It might be argued that the micro-organisms contain more than one form of AdoMet decarboxylase, which differ in their sensitivity to methylglyoxal bis(guanyldiazide), and that by purifying the enzyme on the basis of affinity chromatography with this reagent we have selected for the most sensitive form. This is unlikely to be so, since the yield of enzyme at this stage was in all cases very good (better than 80% of that applied to the affinity column) and even crude extracts before this chromatography were strongly inhibited by the drug (results not shown).

The results reported in Table 2 for the AdoMet decarboxylase from *E. coli* are in broad general agreement with a recent report from Markham *et al.* (1982). They found a K_m of 60 μM for AdoMet and K_i values of 20 μM for methylglyoxal bis(guanyldiazide) and 20 μM for decarboxylated AdoMet. The slight discrepancies between these values and those reported in Table 2 may be due to the different

assay conditions and temperature of assay or to some differences between the enzyme preparations. Early preparations of *E. coli* AdoMet decarboxylase were found to have less than stoichiometric amounts of pyruvate per subunit and had higher K_m values and a lower V_{max} than more recent preparations isolated under less harsh conditions (Wickner *et al.*, 1970; Markham *et al.*, 1982). This appears to be related to a loss of pyruvate from some subunits during purification.

Purification of AdoMet decarboxylase from rat liver by affinity chromatography on methylglyoxal bis(guanylhydrazone) was first introduced by Pegg (1974) and this method has now been used in many laboratories to isolate the enzyme from various rodent (Pegg, 1977, 1979; Demetriou *et al.*, 1978; Sakai *et al.*, 1979; Yamanoha & Samejima, 1980; Pankaskie & Abdel-Monem, 1980; Pösö & Pegg, 1982), bovine (Seyfried *et al.*, 1982) and yeast (Pösö *et al.*, 1975a; Cohn *et al.*, 1977) cells. It is shown here that this method is equally applicable to those AdoMet decarboxylases that are not putrescine-activated even though they have somewhat lower affinities for methylglyoxal bis(guanylhydrazone). While our experiments were in progress, Markham *et al.* (1982) also observed that this affinity chromatography was suitable for the purification of AdoMet decarboxylase from *E. coli*. It also should be noted that the use of ethylglyoxal bis(guanylhydrazone) for affinity chromatography may be advantageous for any AdoMet decarboxylases in which the binding to the methyl derivative is insufficient.

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