Biochem. J. (1975) **151**, 67–73
Printed in Great Britain

S-Adenosylmethionine Decarboxylase from Baker's Yeast

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(*Received* 14 *April* 1975)

1. *S*-Adenosyl-L-methionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) was purified more than 1100-fold from extracts of *Saccharomyces cerevisiae* by affinity chromatography on columns of Sepharose containing covalently bound methylglyoxal bis(guanylhydrazone) {1,1'[(methylethanediylidene)dinitrilo]diguanidine} [Pegg, (1974) *Biochem. J.* **141**, 581–583]. The final preparation appeared to be homogeneous on polyacrylamide-gel electrophoresis at pH8.4. 2. *S*-Adenosylmethionine decarboxylase activity was completely separated from spermidine synthase activity [5'-deoxyadenosyl-(5'),3-aminopropyl-(1),methylsulphonium-salt–putrescine 3-aminopropyltransferase, EC 2.5.1.16] during the purification procedure. 3. Adenosylmethionine decarboxylase activity from crude extracts of baker's yeast was stimulated by putrescine, 1,3-diaminopropane, cadaverine (1,5-diaminopentane) and spermidine; however, the purified enzyme, although still stimulated by the diamines, was completely insensitive to spermidine. 4. Adenosylmethionine decarboxylase had an apparent K_m value of 0.09mM for adenosylmethionine in the presence of saturating concentrations of putrescine. The omission of putrescine resulted in a five-fold increase in the apparent K_m value for adenosylmethionine. 5. The apparent K_a value for putrescine, as the activator of the reaction, was 0.012mM. 6. Methylglyoxal bis(guanylhydrazone) and *S*-methyladenosylhomocysteamine (decarboxylated adenosylmethionine) were powerful inhibitors of the enzyme. 7. Adenosylmethionine decarboxylase from baker's yeast was inhibited by a number of conventional carbonyl reagents, but in no case could the inhibition be reversed with exogenous pyridoxal 5'-phosphate.

The main, if not necessarily the only, function of *S*-adenosyl-L-methionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) in most organisms is apparently to furnish the propylamine moiety for the biosynthesis of spermidine and spermine. The older view originally based on the pioneering work of Pegg & Williams-Ashman (1969), supposing that only one enzyme or inseparable enzyme complex in mammalian tissues would be responsible for the decarboxylation of adenosylmethionine and the subsequent propylamine transfers, has been revised after more extensive purification and separation of the enzymes involved (Jänne & Williams-Ashman, 1971; Raina & Hannonen, 1971; Hannonen *et al.*, 1972*a*,*b*; Pegg, 1974).

S-Adenosylmethionine decarboxylase from baker's yeast resembles the corresponding enzyme from animal tissues by being intensively and also specifically stimulated by minute amounts of putrescine (Jänne *et al.*, 1971*b*; Coppoc *et al.*, 1971). Adenosylmethionine decarboxylase from most eukaryotic organisms differs in this respect from the enzyme of prokaryotic organisms such as *Escherichia coli* (Wickner *et al.*, 1970) and *Azotobacter vinelandii* (Coppoc *et al.*, 1971). Adenosylmethionine decarboxylase from the latter sources is insensitive to putrescine (and to other related amines) but absolutely requires Mg^{2+} ions for the catalytic activity. The classification of adenosylmethionine decarboxylases into putrescine-activated eukaryotic enzymes and Mg^{2+}-dependent prokaryotic enzymes, however, has a number of important exceptions. Thus adenosylmethionine decarboxylase from bean sprouts (Coppoc *et al.*, 1971), from the slime mould *Physarum polycephalum* (Mitchell & Rusch, 1973) and from the protozoan *Tetrahymena pyriformis* (Pösö *et al.*, 1975) is not stimulated by putrescine. In contrast with the plant enzyme, which requires Mg^{2+} (Coppoc *et al.*, 1971), the plasmodium enzyme (Mitchell & Rusch, 1973) and the protozoan enzyme (Pösö *et al.*, 1975) do not need any metal ion.

Adenosylmethionine decarboxylase activity in rat tissues is relatively easy to separate from the two propylamine transferases (spermidine synthase and spermine synthase) (Jänne & Williams-Ashman, 1971; Raina & Hannonen, 1971; Hannonen *et al.*, 1972*a*), whereas there was only partial separation of the adenosylmethionine decarboxylase activity from the spermidine synthase activity in extracts of baker's yeast with conventional chromatographic methods (Jänne *et al.*, 1971*b*).

In the present paper adenosylmethionine decarboxylase from *Saccharomyces cerevisiae* has been

purified by affinity chromatography on columns of Sepharose containing bound methylglyoxal bis-(guanylhydrazone) {1,1'[(methylethanediylidene)-dinitrilo]diguanidine}, the inhibitor of the enzyme, as introduced by Pegg (1974). The enzyme was stimulated by putrescine even in highly refined forms not contaminated by the spermidine synthase activity. Spermidine, which stimulated the enzyme activity in crude extracts, had no effect on purified preparations. All spermidine synthase activity was resolved from the adenosylmethionine decarboxylase activity by a single passage of the enzyme through the affinity-chromatography column. The yeast enzyme, like the mammalian counterpart, was powerfully inhibited by methylglyoxal bis(guanylhydrazone) and by decarboxylated adenosylmethionine, the product of the reaction. Purified adenosylmethionine decarboxylase was also inhibited by the conventional carbonyl inhibitors; however, these inhibitions could not be reversed to any appreciable extent by the addition of exogenous pyridoxal 5'-phosphate.

Experimental

Materials

Unlabelled and labelled *S*-adenosyl-L-methionine were prepared enzymically essentially as described by Pegg & Williams-Ashman (1969).Decarboxylated adenosylmethionine was prepared with the aid of adenosylmethionine decarboxylase from *E. coli* partially purified by $(NH_4)_2SO_4$ fractionation (Wickner *et al.*, 1970). Decarboxylated adenosylmethionine (together with any remaining adenosylmethionine) were isolated from the reaction mixture by passage through a Dowex 50 (H^+ form) column (1cm×5cm) and the final separation of decarboxylated adenosylmethionine from adenosylmethionine was done by preparative paper electrophoresis by the method of Jänne *et al.* (1971*b*).

[1,4-^{14}C]Putrescine (sp. radioactivity 17.5mCi/mmol) was purchased from New England Nuclear Corp. (Dreieichenhain, Germany). Putrescine, spermidine and spermine (as their hydrochloride salts) were purchased from Calbiochem, San Diego, Calif., U.S.A., and cadaverine hydrochloride was from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. 4-Bromo-3-hydroxybenzyloxyamine (NSD-1055) was the product of Smith and Nephew Ltd., Harlow, Essex, U.K. Methylglyoxal bis(guanylhydrazone) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A.

CH-Sepharose 4B (agarose with a C-6 'spacer' with a carboxyl group for coupling) was purchased from Pharmacia (Uppsala, Sweden) and *N*-ethyl-*N*-(3-dimethylaminopropyl)carbodi-imide hydrochloride was obtained from Fluka AG, Buchs SG, Switzerland. The coupling of methylglyoxal bis-(guanylhydrazone) to CH-Sepharose with the aid of carbodi-imide was performed as recommended by the manufacturer of the CH-Sepharose (Pharmacia) and as also described in detail by Pegg (1974).

Preparation of the yeast extract

Baker's yeast (*Saccharomyces cerevisiae*) was obtained from Oy Alko, Helsinki, Finland. The packed cells were suspended in an equal volume (w/v) of 25mM-Tris–HCl buffer, pH7.4, containing 0.1mM-EDTA and 1mM-dithiothreitol (hereafter termed 'standard buffer'). The cell suspension was extruded twice through a chilled (−25°C) pressure cell (X-press, Biox Ab Nacka, Sweden) at 147MPa. The resulting homogenate was centrifuged at 15000$g_{max.}$ for 15min and the supernatant fraction (crude extract) was used for further purification.

Analytical methods

The activity of adenosylmethionine decarboxylase was assayed in the presence of 0.2mM-adenosyl-[*carboxyl*-^{14}C]methionine and 2.5mM-putrescine as described by Jänne & Williams-Ashman (1971). The activity of spermidine synthase was assayed in the presence of 0.5mM-[1,4-^{14}C]putrescine and 0.1mM-decarboxylated adenosylmethionine. The ingredients of the incubation mixture and the isolation of the radioactive spermidine after the incubation are given elsewhere (Jänne *et al.*, 1971*a*). The incubation time was 30min at 37°C for both reactions. The decarboxylation of adenosylmethionine and the synthesis of spermidine were linear with time and the amount of the enzyme under experimental conditions used. The enzyme activities were measured in duplicate except when monitoring column chromatographies.

Polyacrylamide-gel electrophoresis was carried out at 4°C by the method of Davis (1964).

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

The double-reciprocal lines were computed by the least-squares method.

Purification of adenosylmethionine decarboxylase from baker's yeast

To a crude extract (fraction 1) prepared from about 200g of yeast cells, 0.2vol. of 10% (v/v) streptomycin sulphate (a gift from Orion Oy, Helsinki, Finland) was slowly added. The suspension was left, with occasional stirring, at 0°C for 30min and then centrifuged at 15000$g_{max.}$ for 15min. The supernatant fraction (fraction 2) was further fractionated with solid $(NH_4)_2SO_4$ (special enzyme grade; Mann/Schwarz, Orangeburg, N.Y., U.S.A.) at 0°C. The proteins precipitated between 40 and 60%

saturation of $(NH_4)_2SO_4$ were pelleted at 15000 $g_{max.}$, dissolved in a small volume of the standard buffer and dialysed against 50vol. of the buffer for 16h. The dialysed $(NH_4)_2SO_4$ fraction (fraction 3) was applied to a DEAE-cellulose column (4.5cm×37cm; DE-52, Whatman Biochemicals Ltd., Maidstone, Kent, U.K.) previously equilibrated with the standard buffer. The column was first washed with 200ml of the buffer and connected to a linear gradient of 0.1–0.4M-NaCl in the standard buffer (gradient volume was 1000ml). Adenosylmethionine decarboxylase activity emerged from the column at about 0.3–0.35M-NaCl. The most active fractions (160ml) were pooled and dialysed overnight against the standard buffer containing 0.1mM-putrescine. The dialysed DEAE-cellulose fraction (fraction 4) was applied to a Sepharose 4B column (2.4cm×13cm) containing bound methylglyoxal bis(guanylhydrazone) (see under 'Materials') previously equilibrated with the standard buffer. The column was washed with 200ml of the standard buffer followed by 200ml of 0.5M-NaCl in the buffer, and the adenosylmethionine decarboxylase activity was eluted from the column with the standard buffer containing 0.3M-NaCl and 1mM-methylglyoxal bis(guanylhydrazone) (Pegg, 1974). The enzyme activity emerged as a sharp peak soon after the front of the elution buffer. The active fractions were pooled, dialysed overnight against the standard buffer containing 0.1mM-putrescine and designated as fraction 5.

Results

Purification of the enzyme

The purification steps of adenosylmethionine decarboxylase from yeast extracts are presented in Table 1. The procedure resulted in a purification of more than 1100-fold compared with the crude yeast extract. Fraction 5 appeared to be homogeneous on polyacrylamide-gel electrophoresis at pH8.4. The activity of spermidine synthase was co-purified until the DEAE-cellulose step, where a partial separation of the activities was achieved. However, a complete separation of spermidine synthase and adenosylmethionine decarboxylase activities was achieved by a single passage of fraction-4 preparation through the affinity-chromatography column (Table 1).

After the affinity chromatography fraction-5 enzyme was rapidly inactivated in the absence of putrescine. Dialysis of fraction-5 preparation for 16h against buffers not containing putrescine resulted in 80–90% loss of the enzyme activity. When stored at 0°C in the presence of 0.1mM-putrescine 50% of the enzyme activity was lost in 10 weeks. Freezing and thawing of adenosylmethionine decarboxylase preparations from the DEAE-cellulose stage invariably resulted in a large loss of activity.

Table 1. *Purification of adenosylmethionine decarboxylase from baker's yeast*

A crude extract prepared from about 200g of yeast cells was processed as described in the text. The activities are expressed as nmol of product formed in 30min. Abbreviation: MGBG, methylglyoxal bis(guanylhydrazone).

Fraction	Total protein (mg)	Adenosylmethionine decarboxylase activity (nmol/mg of protein) (*a*)	Purification (fold)	Spermidine synthase activity (nmol/mg of protein) (*b*)	Purification *a*/*b*	Ratio
1. Crude extract	10000	3.5	1	4.5	1	0.8
2. Streptomycin sulphate precipitation	10600	2.9	0.8	3.9	0.9	0.7
3. $(NH_4)_2SO_4$ fraction	2080	5.3	1.5	6.2	1.4	0.9
4. DEAE-cellulose	400	32.5	9.3	13.4	3.0	2.4
5. MGBG–Sepharose	1.1	4060	1160	—	—	—

Table 2. *Effect of various amines on yeast adenosylmethionine decarboxylase activity*

Adenosylmethionine decarboxylase activity was assayed at 37°C in 100mM-potassium phosphate buffer, pH7.4, as described in the text, with either crude extract (0.14mg of protein) or fraction-5 preparation (5μg of protein) being used as the source of the enzyme. The activities are expressed as nmol of CO_2 liberated/30min.

Addition	Concentration (mM)	Adenosylmethionine decarboxylase activity (nmol of CO_2/30min) Crude extract	Purified enzyme
None	—	0.050	0.270
Putrescine	2.5	1.120	1.190
1,3-Diaminopropane	2.5	0.450	0.640
Cadaverine	2.5	0.410	0.690
Spermidine	2.5	0.170	0.270
Spermine	2.5	0.070	0.170

Stimulation of the decarboxylation of adenosylmethionine by various amines

Adenosylmethionine decarboxylase from various rat tissues is known to be stimulated not only by putrescine but also by spermidine (Pegg & Williams-Ashman, 1969) and by 1,3-diaminopropane and cadaverine (Williams-Ashman & Schenone, 1972; Hannonen, 1975).

Table 2 shows adenosylmethionine decarboxylase activity from dialysed yeast extracts was stimulated by putrescine (24-fold) and the activity was also markedly enhanced by the addition of 1,3-diaminopropane (tenfold), cadaverine (1,5-diaminopentane) (ninefold) or spermidine (more than threefold). However, when purified preparations (fraction 5) were used as the source of the enzyme, spermidine was no longer able to stimulate the decarboxylation of adenosylmethionine. The aliphatic diamines stimulated the enzyme activity at all stages of purification, although the extent of the stimulation clearly decreased during the purification (Table 2).

Affinity for adenosylmethionine and putrescine

As Fig. 1 shows, the apparent K_m value of the purified enzyme for adenosylmethionine (fraction 5) was about 0.09mM, which is in good agreement with the earlier results with crude yeast extracts (Coppoc *et al.*, 1971). In the absence of putrescine the apparent K_m value for adenosylmethionine increased to 0.5mM, i.e. more than fivefold. The maximum velocities of the reaction in both the absence and the presence of putrescine appeared to be approximately equal.

The apparent activator constant K for putrescine was about 10μM when measured in the presence of 0.2mM-adenosylmethionine (Fig. 2). The stimulation of the decarboxylation by diaminopropane and cadaverine in the presence of 0.2mM-adenosylmethionine was about one-half of that with

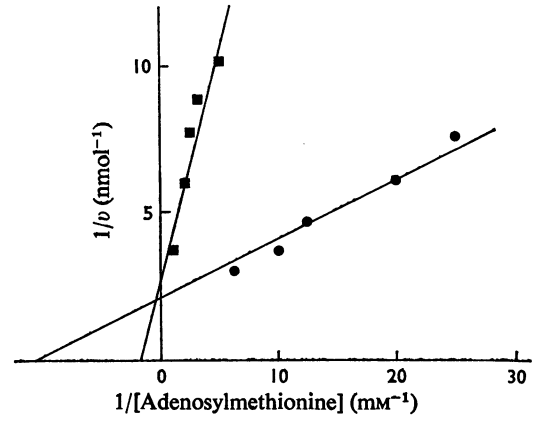

Fig. 1. *Effect of putrescine on the affinity of adenosylmethionine decarboxylase for adenosylmethionine*

Adenosylmethionine decarboxylase activity was measured in fraction-5 preparation (μg of protein) in the absence or the presence of putrescine. ■, No putrescine; ●, 2.5mM-putrescine.

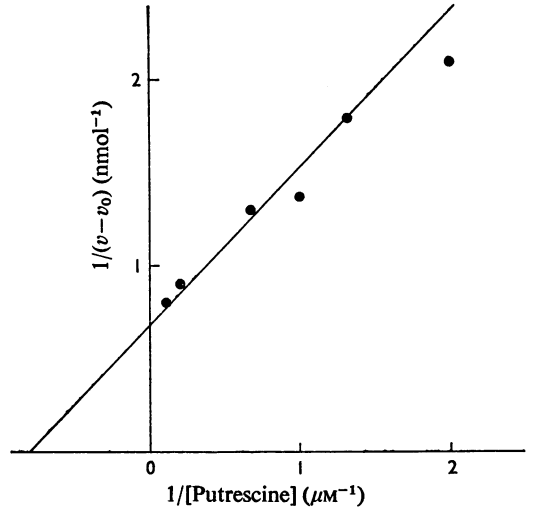

Fig. 2. *Activation constant for putrescine*

Adenosylmethionine decarboxylase activity was measured in fraction-5 preparation (5μg of protein) in the presence of 0.2mM-adenosylmethionine and various concentrations of putrescine. v_0, Reaction velocity in the absence of putrescine; v, reaction velocity in the presence of putrescine.

Table 3. *Effect of methylglyoxal bis(guanylhydrazone) on adenosylmethionine decarboxylase and spermidine synthase activities in crude extracts of baker's yeast*

Adenosylmethionine decarboxylase and spermidine synthase activities were assayed at 37°C in 100mM-potassium phosphate buffer, pH7.4, as described in the text by using crude extracts of baker's yeast as the source of the enzyme.

Methylglyoxal bis(guanylhydrazone) (μM)	Adenosylmethionine decarboxylase (% of control)	Spermidine synthase (% of control)
0 (control)	100	100
0.5	7	143
1.0	3	152
2.0	2	167
5.0	0.5	163

putrescine. The apparent affinity of adenosylmethionine decarboxylase for 1,3-diaminopropane was close to that for putrescine, thus indicating that the effect was probably due to 1,3-diaminopropane itself and not to any contaminating putrescine.

Inhibition of adenosylmethionine decarboxylase activity by decarboxylated adenosylmethionine and methylglyoxal bis(guanylhydrazone)

As reported (Jänne *et al.*, 1971*b*) the product of the reaction decarboxylated adenosylmethionine is a powerful inhibitor of the eukaryotic adenosylmethionine decarboxylase. Adenosylmethionine decarboxylase from baker's yeast was inhibited by decarboxylated adenosylmethionine both in crude extracts and in the most purified form. The apparent K_i value for decarboxylated adenosylmethionine was close to 1 μM. Inhibition was competitive at relatively low concentrations of the product, but became more complex at higher concentrations of decarboxylated adenosylmethionine (results not shown).

Methylglyoxal bis(guanylhydrazone) is a powerful and specific inhibitor of mammalian putrescine-activated adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972). The inhibition of partially purified rat liver adenosylmethionine decarboxylase by the compound appears to be competitive with respect to the substrate adenosylmethionine and uncompetitive with respect to the activator putrescine (Hölttä *et al.*, 1973). The inhibition of purified adenosylmethionine decarboxylase from baker's yeast (fraction-5 preparation) by methylglyoxal bis(guanylhydrazone) apparently influenced both the slope and intercepts in the double-reciprocal plot. The most marked changes occur in the apparent K_m value of the enzyme for its substrate adenosylmethionine. Calculated on the basis of a competitive-inhibition pattern, the inhibition constant was less than 1 μM.

Table 4. *Effect of various carbonyl reagents on yeast adenosylmethionine decarboxylase activity*

Purified adenosylmethionine decarboxylase (fraction-5 preparation) was incubated in the presence of various carbonyl inhibitors at the concentrations indicated. After 30min at 37°C in 100mM-potassium phosphate buffer, pH7.4, a sample was taken and the adenosylmethionine decarboxylase activity was assayed under standard incubation conditions (this resulted in a 15-fold dilution of the inhibitor in the final incubation mixture), in the absence or the presence of 0.1mM-pyridoxal 5'-phosphate. The enzyme activity is expressed as nmol of CO_2 liberated in 30 min. NSD-1055, 4-Bromo-3-hydroxybenzyloxyamine.

Prior incubation with	Concentration (mM)	Adenosylmethionine decarboxylase activity: −Pyridoxal 5'-phosphate	Adenosylmethionine decarboxylase activity: +Pyridoxal 5'-phosphate
Expt. 1			
No additions	—	1.48	1.45
Isonicotinic acid hydrazide	5	1.10	1.23
Semicarbazide	5	0.003	0.005
NSD-1055	1	0.003	0.002
Expt. 2			
No additions	—	1.09	1.05
KCN	50	0	0.01
Isonicotinic acid hydrazide	10	0.51	0.56
Semicarbazide	1	0.86	0.82
NSD-1055	0.2	0.45	0.55
Canaline	1	0.57	0.59

A noteworthy feature was found in the behaviour of spermidine synthase activity when adenosylmethionine decarboxylase was inhibited by methylglyoxal bis(guanylhydrazone) in crude yeast extracts. As Table 3 shows, the addition of inhibitor at concentrations that almost totally abolished the decarboxylation of adenosylmethionine resulted in a concomitant stimulation of spermidine synthase activity. Table 3 also shows that the activity of the latter enzyme increased more than 50% at micromolar concentrations of methylglyoxal bis(guanylhydrazone). The stimulation of spermidine synthase activity by the inhibitor of adenosylmethionine decarboxylase also might imply that both enzymes are actually competing for free putrescine and the inhibition of adenosylmethionine decarboxylase would stimulate spermidine synthase (with putrescine as the substrate) by making more putrescine available to the latter enzyme. Methylglyoxal bis(guanylhydrazone) did not stimulate spermidine synthase from rat liver. This, however, might be due to the fact that in rat liver the total activity of spermidine synthase exceeds that of adenosylmethionine decarboxylase by a factor of 50 or even more (Hannonen *et al.*, 1972*b*), whereas in baker's yeast both activities are roughly comparable (Table 1).

Table 5. *Protection of adenosylmethionine decarboxylase by diamines against borohydride reduction*

Purified adenosylmethionine decarboxylase (dialysed fraction-5 preparation) was incubated at 37°C for 30min in the absence or presence of 30mM-$NaBH_4$ with the additions indicated. After the incubation a sample was taken and adenosylmethionine decarboxylase was assayed under standard incubation conditions. The enzyme activity is expressed as nmol of CO_2 liberated in 30min.

Prior incubation with	Concentration (mM)	Adenosylmethionine decarboxylase activity (nmol of CO_2/30min)	(%)
No additions	—		
$-NaBH_4$		1.02	100
$+NaBH_4$		0.04	4
Adenosylmethionine	0.2		
$-NaBH_4$		1.21	100
$+NaBH_4$		0.06	5
Putrescine	2.5		
$-NaBH_4$		1.50	100
$+NaBH_4$		1.10	73
Cadaverine	2.5		
$-NaBH_4$		1.30	100
$+NaBH_4$		0.87	67
1,3-Diaminopropane	2.5		
$-NaBH_4$		1.27	100
$+NaBH_4$		0.93	73

Inhibition of adenosylmethionine decarboxylase activity by carbonyl reagents

As Table 4 shows, the purified adenosylmethionine decarboxylase from baker's yeast was inhibited by prior incubation in conventional carbonyl inhibitors. 4-Bromo-3-hydroxybenzyloxyamine (NSD-1055), which is known to inhibit some pyridoxal enzymes (Leinweber, 1968), also inhibited yeast adenosylmethionine decarboxylase. It is remarkable that relatively high concentrations of isonicotinic acid hydrazide and canaline 2-amino-4-oxyaminobutyric acid (Rahiala *et al.*, 1971) were needed to produce any inhibition. As also shown in Table 4, in no case could the enzyme activity be restored with exogenous pyridoxal 5'-phosphate.

When the dialysed fraction-5 enzyme was incubated in the presence of 30mM-$NaBH_4$ for 30min at 37°C a virtually total loss of enzyme activity occurred (Table 5). Inclusion of the substrate adenosylmethionine apparently did not protect the enzyme against the borohydride reduction (Table 5). When the reduction was done in the presence of putrescine, 1,3-diaminopropane or cadaverine, all capable of stimulating the enzyme activity, about 70% of the enzyme activity was retained. Practically the same result was obtained from a series of similar experiments. This might conceivably indicate, among other things, that the activation by aliphatic diamines of the yeast adenosylmethionine decarboxylase could involve interactions between the prosthetic group of the enzyme and activators.

Discussion

Adenosylmethionine decarboxylase from extracts of baker's yeast closely resembles the corresponding enzyme from different animal tissues. Yeast adenosylmethionine decarboxylase, activated by putrescine (and some other aliphatic diamines), is powerfully inhibited by methylglyoxal bis(guanylhydrazone) as well as by the product of the reaction (decarboxylated adenosylmethionine). Even the kinetic parameters, such as the apparent K_m value for adenosylmethionine, the K_a value for the activator putrescine and the K_i values for the inhibitors mentioned above, for adenosylmethionine decarboxylase from rat tissues and baker's yeast are remarkably similar (Hölttä *et al.*, 1973; Hannonen, 1975).

It also appears that adenosylmethionine decarboxylase and spermidine synthase are separate proteins in both animal tissues and yeast, although a complete separation of the two activities in extracts of *Saccharomyces cerevisiae* appears more difficult to achieve by conventional chromatographic methods than in rat liver extracts (Jänne *et al.*, 1971*b*; Hannonen *et al.*, 1972*b*).

The specific activity of our fraction-5 preparation of yeast adenosylmethionine decarboxylase was close to that reported by Pegg (1974) for rat liver enzyme, i.e. 135 and 166nmol of CO_2 liberated/min per mg of protein respectively. These values are more than 100-fold higher than those reported by Feldman *et al.* (1972) for the adenosylmethionine decarboxylase from rat liver. In a subsequent paper Manen & Russell (1974) purified adenosylmethionine decarboxylase from rat liver to a specific activity approximately twice as high as the previous preparation (Feldman *et al.*, 1972). However, on the basis of the data given by Pegg (1974), the specific activity of their preparation does not represent more than a few per cent of the apparently homogeneous adenosylmethionine decarboxylase from rat liver. Accordingly claims that adenosylmethionine decarboxylase is inseparable from spermidine synthase activity (Feldman *et al.*, 1972; Manen & Russell, 1974), on the basis of this kind of purification of the enzyme, appear to be slightly unrealistic.

Like adenosylmethionine decarboxylase from various tissues of the rat, the enzyme is also stimulated by other diamines, such as 1,3-diaminopropane and cadaverine. The stimulation by spermidine, which enhanced the enzyme activity more than threefold in crude extracts, however, completely disappeared during the purification of the enzyme. The reason for that, as well as for the decrease in the magnitude of the stimulation by putrescine, might

conceivably be the fact that spermidine (or putrescine) is actively removing decarboxylated adenosylmethionine for the synthesis of spermine (or spermidine) in crude extracts containing the synthase activities, thus abolishing the possible product inhibition by the decarboxylated adenosylmethionine. At the present time there is no experimental evidence whatsoever for the existence of several adenosylmethionine decarboxylases in either rat liver or baker's yeast.

The nature of the prosthetic group of the putrescine-activated adenosylmethionine decarboxylase from baker's yeast still remains unsolved. It is obvious that the prosthetic group is a carbonyl group, but there is no direct experimental evidence that either pyridoxal phosphate or any other type of carbonyl group functions as the prosthetic group. Neither is the mechanism of putrescine stimulation known at the moment. The experiments showing that the aliphatic diamines, in addition to being capable of stimulating the decarboxylation, can also protect the enzyme against the reduction by borohydride, either the purified liver enzyme (Hannonen, 1975) or yeast enzyme (Table 5), may indicate that the activation of adenosylmethionine decarboxylase by diamines involves some specific interactions between the carbonyl group(s) of the enzyme and the amino group(s) of the activators. However, larger quantities of pure enzyme are needed to show that directly.

References

Coppoc, G. L., Kallio, P. & Williams-Ashman, H. G. (1971) *Int. J. Biochem.* **2**, 673–681

Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427

Feldman, M. J., Levy, C. C. & Russell, D. H. (1972) *Biochemistry* **11**, 671–677

Hannonen, P. (1975) *Acta Chem. Scand. B* **29**, 295–299

Hannonen, P., Jänne, J. & Raina, A. (1972*a*) *Biochim. Biophys. Acta* **289**, 225–231

Hannonen, P., Jänne, J. & Raina, A. (1972*b*) *Biochem. Biophys. Res. Commun.* **46**, 341–348

Hölttä, E., Hannonen, P., Pispa, J. & Jänne, J. (1973) *Biochem. J.* **136**, 669–676

Jänne, J. & Williams-Ashman, H. G. (1971) *Biochem. Biophys. Res. Commun.* **42**, 222–229

Jänne, J., Schenone, A. & Williams-Ashman, H. G. (1971*a*) *Biochem. Biophys. Res. Commun.* **42**, 758–764

Jänne, J., Williams-Ashman, H. G. & Schenone, A. (1971*b*) *Biochem. Biophys. Res. Commun.* **43**, 1362–1370

Leinweber, F.-J. (1968) *Mol. Pharmacol.* **4**, 337–348

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Manen, C.-A. & Russell, D. H. (1974) *Biochemistry* **13**, 4729–4735

Mitchell, J. L. A. & Rusch, H. P. (1973) *Biochim. Biophys. Acta* **297**, 503–516

Pegg, A. E. (1974) *Biochem. J.* **141**, 581–583

Pegg, A. E. & Williams-Ashman, H. G. (1969) *J. Biol. Chem.* **244**, 682–693

Pösö, H., Sinervirta, R., Himberg, J.-J. & Jänne, J. (1975) *Acta Chem. Scand. B* in the press

Rahiala, E.-L., Kekomäki, M., Jänne, J., Raina, A. & Räihä, N. C. R. (1971) *Biochim. Biophys. Acta* **227**, 337–343

Raina, A. & Hannonen, P. (1971) *FEBS Lett.* **16**, 1–4

Wickner, R. B., Tabor, C. W. & Tabor, H. (1970) *J. Biol. Chem.* **245**, 2132–2139

Williams-Ashman, H. G. & Schenone, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 288–295