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Purification of Rat Liver *S*-Adenosyl-L-methionine Decarboxylase

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The amount of *S*-adenosyl-L-methionine decarboxylase present in rat liver was enhanced 17-fold by administration of methylglyoxal bis(guanylhydrazone),* a specific inhibitor of the enzyme. The enzyme was purified 1400-fold in 50% yield from such liver extracts by chromatography on columns of the inhibitor bound to Sepharose. The purified enzyme had no spermidine synthetase activity.

S-Adenosyl-L-methionine decarboxylase (EC 4.1.1.50) is an important enzyme in the biosynthetic pathway leading to polyamines since the product of the decarboxylation acts a donor of the propylamine groups of spermidine and spermine (Pegg, 1970; Tabor & Tabor, 1972). Studies of mammalian *S*-adenosyl-L-methionine decarboxylases have been hampered by the low activity in most tissues and the poor yield of partially purified enzyme obtained by using published procedures (Pegg & Williams-Ashman, 1969; Jänne & Williams-Ashman, 1971*a*; Hannonen *et al.*, 1972*a*). There is disagreement as to whether the liver enzyme which is activated by putrescine (Pegg & Williams-Ashman, 1969) can be separated from spermidine synthetase (Feldman *et al.*, 1972; Hannonen *et al.*, 1972*a*). The present paper describes a method for the isolation of highly purified *S*-adenosyl-L-methionine decarboxylase in good yield from rat liver.

Experimental

S-Adenosyl-L-methionine decarboxylase and spermidine synthetase activities were measured as described by Pegg & Williams-Ashman (1969). The assay medium contained 0.1M-potassium phosphate buffer, pH7.0, 2.5mM-dithiothreitol, 2.5mM-putrescine and 0.2mM-*S*-adenosyl-L-[*carboxy*-^{14}C]-methionine (1.3mCi/mmol) for the former activity or 0.2mM-*S*-adenosyl-L-[2-^{3}H]methionine (72.5mCi/mmol) for the latter. The radioactive compounds were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and were diluted to the appropriate specific radioactivity with material purchased from the Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.

Methylglyoxal bis(guanylhydrazone) was linked to Sepharose as follows; 15g dry wt. of CH-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was swollen overnight in 1 litre of 0.5M-NaCl, washed with 4 litres of 0.5M-NaCl and then with 4 litres of water. The washed gel (volume about 55ml) was suspended in a total volume of 100ml of water containing 1.2g of methylglyoxal bis(guanylhydrazone) dihydrochloride (R. N. Emanuel Ltd., Wembley, Middx., U.K.) and the pH was adjusted to 5.5 with 1M-NaOH. The mixture was stirred slowly and 3g of 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide dissolved in 5ml of water was added dropwise, and then the solution was shaken gently for 24h at room temperature. The gel was then washed with 8 litres of 0.5M-NaCl and stored in 10mM-Tris–HCl of (pH7.5) – 0.5M-NaCl at 4°C until required. The amount of the inhibitor bound to the Sepharose by this procedure has not been determined accurately, but approximate estimates based on the absorbance of the gel and the molar extinction coefficient of the free inhibitor (38400 at 283nm at pH1 and 33500 at 325nm at pH11) suggest that 0.4–5μmol of inhibitor was bound/ml of gel.

Protein was determined by the biuret method or by measuring the extinction at 280nm (Layne, 1957) with bovine serum albumin [Sigma (London) Chemical Co. Ltd.] as standard.

Purification of S-adenosyl-L-methionine decarboxylase. Twenty-four male rats (130g body wt.) of the Wistar strain bred in this Institute were given 80mg of methylglyoxal bis(guanylhydrozone) dihydrochloride (dissolved in 0.14M-NaCl at a concn. of 40mg/ml)/kg body wt. by intraperitoneal injection. The rats were killed 24h later and the livers removed and homogenized at 4°C with 3vol. of 2.5mM-putrescine – 1mM-dithiothreitol – 0.1mM-EDTA–10mM-Tris–HCl, pH7.5 (soln. A). All subsequent operations were performed at 0–4°C. The homogenate was centrifuged at 105000*g* for 3h and the supernatant fraction removed and treated with $(NH_4)_2SO_4$ as described by Jänne & Williams-Ashman (1971*a*). The proteins precipitated between

* The systematic name for this compound is 1,1′-methylethanediylidene)dinitro]diguanidine.

40 and 65% satn. were dissolved in 50ml of soln. A and dialysed for 15h against 100vol. of the same solution.

A column (8.5cm×2.5cm) was packed with the Sepharose linked to methylglyoxal bis(guanylhydrazone) and washed with 200ml of soln. A. The dialysed sample was then applied to the column at a flow rate of 40ml/h, and the column was washed with 100ml of soln. A followed by 100ml of soln. A containing 0.3M-NaCl. *S*-Adenosyl-L-methionine decarboxylase activity was then eluted from the column by washing with soln. A containing 0.3M-NaCl and 1mM-methylglyoxal bis(guanylhydrazone). Fractions of volume 10ml were collected and assayed for enzyme activity by adding samples of volume 2μl to the standard assay mixture. Although the inhibitor present in these samples produced considerable inhibition of activity even at this dilution, activity could readily be detected and was usually present in fractions 8–10. These fractions were brought to 70% satn. by addition of $(NH_4)_2SO_4$ and after being left at 0°C for 1h the precipitate formed was collected by centrifugation at 20000*g* for 20min. This precipitate was dissolved in 10ml of soln. A and dialysed against 200vol. of soln. A overnight. The dialysed solution was applied to the Sepharose column and the enzyme eluted as described above. The fractions containing activity were combined, diluted with water to give a concentration of 0.1M–NaCl and applied to a column (5cm×1cm) of DEAE-cellulose (Whatman DE 52) previously equilibrated with soln. A without EDTA. The column, run at a flow rate of 20ml/h, was then washed with 20ml of 0.1M-NaCl in soln. A and eluted with a linear gradient of 0.1–0.3M-NaCl (40ml of each) in soln. A. Fractions of volume 2ml were collected and almost all of the *S*-adenosyl-L-methionine decarboxylase activity was present in three fractions having a salt concentration of about 0.17M-NaCl. These fractions were dialysed for 12h against 100vol. of 0.1M-KCl–1mM-dithiothreitol–0.025M-potassium phosphate, pH7.0, and this procedure was repeated twice. The enzyme was then stored frozen at −70°C.

Results and discussion

Methylglyoxal bis(guanylhydrazone), which is a specific and potent inhibitor of mammalian *S*-adenosyl-L-methionine decarboxylases (Williams-Ashman & Schenone, 1972), was used in two ways in the purification of this enzyme from rat liver (Table 1). Administration of the inhibitor to rats 24h before death produced a 17-fold increase in the specific activity of *S*-adenosyl-L-methionine decarboxylase present in liver extracts (Table 1; similar 105000*g* supernatant extracts from control rats had a specific activity of 0.007unit/mg of protein). The enzyme from liver enriched in this way was retained by a column of Sepharose linked to the inhibitor by a 6-carbon spacer arm (Cuatrecasas, 1972) and was eluted by addition of the inhibitor to the eluting solution. A second passage through the column was found to be a more convenient procedure for routine preparations than more extensive washing of the column at the first passage. The eluted enzyme was freed from inhibitor, and some additional purification was achieved by DEAE-cellulose chromatography which gave a final preparation representing a 1400-fold purification in 50% yield. The purified enzyme had a specific activity about 6 times that of the enzyme obtained from rat prostate (Jänne & Williams-Ashman, 1971*a*) and gave a single band on electrophoresis in polyacrylamide gels at pH8.3 by the method of Jänne & Williams-Ashman (1971*b*).

The procedure was also used for the preparation of *S*-adenosyl-L-methionine decarboxylase from the livers of normal rats not treated with the inhibitor and gave a final preparation with a specific enzyme activity of 152units/mg of protein in 45% yield. The similarity between these values and those obtained from rats treated with methylglyoxal bis(guanylhydrazone) indicates that administration

Table 1. *Purification of S-adenosyl-L-methionine decarboxylase from rat liver*

Details of the purification steps are given in the text. The results shown are for livers from 24 male rats given 80mg of methylglyoxal bis(guanylhydrazone)/kg body wt. 24h before death. The initial liver extracts contained some inhibitor, but were assayed at sufficient dilution for this not to affect the activity. Eluates from the Sepharose–methylglyoxal bis(guanylhydrazone) columns were freed from inhibitor by extensive dialysis against soln. A before assay. One unit of activity represents the formation of 1nmol of CO_2/min incubation time at 37°C under the standard assay conditions.

Fraction	Protein (mg)	Units (nmol/min)	Specific activity (nmol/min per mg)	Yield (%)
105000*g* supernatant	14780	1773	0.12	100
$(NH_4)_2SO_4$ precipitate (40–65% satd.)	5886	1708	0.29	96
First Sepharose–methylglyoxal bis(guanylhydrazone) eluate	28	1344	48	76
Second Sepharose–methylglyoxal bis(guanylhydrazone) eluate	10	1123	112	63
DEAE-cellulose eluate	6	996	166	56

of the inhibitor causes a real increase in the amount of the enzyme present in the liver rather than to any change in the concentration of some activator or inhibitor of the enzyme.

The properties of the highly purified *S*-adenosyl-L-methionine decarboxylase were similar to those previously reported with less active preparations from mammalian tissues (Pegg & Williams-Ashman, 1969; Jänne & Williams-Ashman, 1971*a*; Hannonen *et al.*, 1972*a*). Decarboxylation was stimulated about eightfold by the presence of 2.5mM-putrescine when the enzyme was assayed at pH 7.0, but the preparation was completely inactive in the formation of spermidine from putrescine and *S*-adenosyl-L-methionine (less than 1 pmol of spermidine formed/min per mg of protein). Initial tissue extracts from normal liver showed the previously reported stoicheiometry between CO_2 release and spermidine formation (Pegg & Williams-Ashman, 1969; Feldman *et al.*, 1972), but all spermidine synthetase activity was lost after the first passage through the Sepharose column. It is unlikely therefore that there is a strong affinity between the proteins responsible for decarboxylation of *S*-adenosyl-L-methionine and synthesis of spermidine. Feldman *et al.* (1972) reported that they were unable to separate these two activities during a procedure leading to a 350-fold purification of rat liver *S*-adenosyl-L-methionine decarboxylase which they thought to be near homogeneity as judged by polyacrylamide-gel electrophoresis. However, the specific activity of their preparation was less than 1% of that obtained in the present study. Stoicheiometry between spermidine formation and CO_2 production when crude enzyme preparations are employed is probably due to the fact that spermidine synthetase is present in much greater amounts than *S*-adenosyl-L-methionine decarboxylase and has a very low K_m for decarboxylated *S*-adenosyl-L-methionine (Pegg, 1970; Hannonen *et al.*, 1972*a*).

Pyridoxal phosphate did not stimulate the decarboxylation of *S*-adenosyl-L-methionine by the purified liver enzyme even after extensive dialysis. It is known that the bacterial *S*-adenosyl-L-methionine decarboxylase, which is not activated by putrescine, has a bound pyruvate group which is essential for enzyme activity (Wickner *et al.*, 1970) and it is possible that the mammalian enzyme has a similar prosthetic group.

The activity of *S*-adenosyl-L-methionine decarboxylase in mammalian cells is lost rapidly after application of inhibitors of protein synthesis (Russell & Taylor, 1971; Hannonen *et al.*, 1972*b*; Fillingame & Morris, 1973; Kay & Lindsay, 1973; Pegg *et al.*, 1973). Methylglyoxal bis(guanylhydrazone) greatly decreases this loss and probably acts in this way to produce the increased activities of enzyme seen in animals or cells treated with the inhibitor (Fillingame & Morris, 1973; Pegg *et al.*, 1973). Studies of the turnover of *S*-adenosyl-L-methionine decarboxylase in the presence and absence of the inhibitor may therefore provide a useful system for investigation of the control of enzyme synthesis and degradation, and the method of isolation of the enzyme described in this paper may be a valuable tool in such studies.

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