Vol. II, Goodwin, T. W., Ed., London, Academic Press, p 721.
Goodwin, T. W., and Mercer, E. I. (1963), *Biochem. Soc. Symp. 24,* 37.
Green, T. R., and Baisted, D. J. (1972), *Biochem. J. 130,* 983.
Grob, E. C., Kirschner, K., and Lynen, F. (1961), *Chimia 15,* 308.
Holloway, P. W., and Popjak, G. (1967), *Biochem J. 104,* 57.
Kandutsch, A. A., Paulus, H., Levin E., and Bloch, K. (1964), *J. Biol. Chem. 239,* 2507.
Kurokawa, T., Ogura, K., and Seto, S. (1971), *Biochem. Biophys. Res. Commun. 45,* 251.
Loomis, W. D., and Battaile, J. (1966), *Phytochemistry 5,* 423.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.
Lynen, F., Agranoff, B. W., Eggerer, H., Henning, W., and Moeslein, E. M. (1959), *Angew. Chem. 71,* 657.
Momose, K., and Rudney, H. (1972), *J. Biol. Chem. 247,* 3930.
Mysels, K. J., and Scholten, P. C. (1962), *Science 136,* 693.
Nandi, D. L., and Porter, J. W. (1964), *Arch. Biochem. Biophys. 105,* 7.
Ogura, K., Nishino, T., and Seto, S. (1968), *J. Biochem. 64,* 197.
Ogura, K., Shinka, T., and Seto, S. (1972), *J. Biochem. 72,* 1101.
Oster, M. O., and West, C. A. (1968), *Arch. Biochem. Biophys. 127,* 112.
Popjak, G. (1969), *Methods Enzymology 9,* 363.
Robinson, D. R., and West, C. A. (1970a), *Biochemistry 9,* 70.
Robinson, D. R., and West, C. A. (1970b), *Biochemistry 9,* 80.
Stoddart, J. L. (1969), *Phytochemistry 8,* 831.
Vesterberg, O. (1971), *Biochim. Biophys. Acta 243,* 345.
Wells, L. W., Schelble, W. J., and Porter, J. W. (1964), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 23,* 426.
West, C. A., Oster M., Robinson, D., Francis, L., and Murphy, P. (1968), in Biochemistry and Physiology of Plant Growth Substances, Wightman, F., and Setterfield, G., Ed., Ottawa, Runge Press, p 313.

# Comparative Properties of Rat Liver and Sea Urchin Eggs *S*-Adenosyl-L-methionine Decarboxylase†

Carol-Ann Manen and Diane H. Russell*

ABSTRACT: *S*-Adenosyl-L-methionine decarboxylase has been extensively purified from both rat liver and sea urchin eggs utilizing a new procedure which includes affinity chromatography. This has resulted in a preparation from sea urchin eggs with a very high specific activity, *i.e.,* over 500 units/mg of protein. Acrylamide gel electrophoresis indicated that a high degree of purity had been obtained; *i.e.,* over 60% of the remaining protein was *S*-adenosyl-L-methionine decarboxylase. The properties of the enzyme isolated from sea urchin eggs are very similar to those previously reported for rat liver, with the exception that metal ions have no effect on the enzyme from sea urchin eggs. The molecular weight of the enzyme isolated from both sources is very similar (approximately 50,000). Both preparations, after extensive purification, still exhibit coupling of the decarboxylation of *S*-adenosyl-L-methionine and the formation of either spermidine or spermine depending upon which amine is added as the receptor for the propylamine moiety.

It has been established that the mammalian enzyme, *S*-adenosyl-L-methionine decarboxylase, has vastly different requirements than the enzyme performing the same task in *Escherichia coli* (Tabor and Tabor, 1964; Pegg and Williams-Ashman, 1969). In the mammalian system, evidence indicated that the decarboxylation of *S*-adenosyl-L-methionine and the transfer of the propylamine moiety from decarboxylated *S*-adenosine-L-methionine to putrescine to form spermidine, or to spermidine to form spermine, might be catalyzed by one enzyme or an enzyme complex. It was not possible to demonstrate any decarboxylated *S*-adenosyl-L-methionine as a free intermediate in crude homogenates and this reaction required either putrescine or spermidine, or a similar substrate, as a receptor molecule for the propylamine moiety (Pegg and Williams-Ashman, 1969; Williams-Ashman and Schenone, 1972). While the enzyme isolated from *E. coli* required magnesium, the mammalian enzyme did not require any metal ions (Tabor and Tabor, 1964; Feldman *et al.,* 1971). The mammalian enzyme is strongly inhibited by known inhibitors of pyridoxal phosphate requiring enzymes (Pegg and Williams-Ashman, 1969; Feldman *et al.,* 1972). Also, J. Sturman and L. Kremzner (personal communication) have demonstrated recently a definite requirement for pyridoxal phosphate by *S*-adenosyl-L-methionine decarboxylase assayed in liver extracts of vitamin $B_6$ (pyridoxal phosphate) deficient rats. The enzyme isolated from *E. coli* requires covalently bound pyruvate as a prosthetic group (Wickner *et al.,* 1970).

Recently, certain investigators have reported that it is possible to separate the decarboxylase function from the propylamine transfer function (Jänne and Williams-Ash-

† From the Department of Pharmacology, University of Arizona College of Medicine, Tucson, Arizona 85724. *Received September 19, 1973.* Supported by U. S. Public Health Service Grant 1-RO1-CA-14783 from the National Cancer Institute.

TABLE I: Purification of *S*-Adenosyl-L-methionine Decarboxylase from Sea Urchin Eggs.

| Fraction | Vol (ml) | Protein (mg) | Sp Act. (Units/mg of Protein) Putrescine Stimulated | Spermidine Stimulated | Purification | % Recovery |
|---|---|---|---|---|---|---|
| Ultracentrifuged supernatant | 15 | 75.2 | 90 | 40 | | |
| Sephadex gel | 45 | 6.9 | 360 | 170 | 4.2 | 38 |
| DEAE-cellulose | 10 | 1.4 | 1,570 | 760 | 17.6 | 32 |
| Affinity[a] | 5 | 0.2 | 17,850 | 8730 | 206.0 | 23 |

[a] Sodium *p*-chloromercuribenzoate affinity chromatography.

man, 1971; Hannonen *et al.*, 1972). They have indicated also that even a third function, a spermine synthase, can be separated (Raina and Hannonen, 1971). However, several laboratories have reported that in crude supernatants one enzyme or one enzyme complex catalyzed both functions, and that the measurement of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxyl*-$^{14}C$]methionine was concomitant with either spermidine or spermine synthesis depending on whether putrescine or spermidine was added as the receptor molecule for the propylamine moiety (Russell and Lombardini, 1971; Russell and Potyraj, 1972; Mitchell and Rusch, 1973).

Evidence indicates that the enzyme(s) responsible for spermidine and spermine syntheses are indeed one system. Spermidine synthesis (putrescine-stimulated *S*-adenosyl-L-methionine decarboxylase activity) was stimulated in the castrated rat uterus after intraperitoneal injection of 17$\beta$-estradiol, and both putrescine-stimulated and spermidine-stimulated *S*-adenosyl-L-methionine decarboxylase respond to inhibitors of protein and RNA synthesis in precisely the same manner (Russell and Potyraj, 1972). The half-life of the spermidine synthesizing enzyme, measured after cycloheximide administration is similar to that for the spermine synthesizing system, *i.e.*, 60 min (Russell and Taylor, 1971; Russell and Potyraj, 1972). Therefore, there is evidence in both rat liver and rat uterus to support the concept of one enzyme which functions as both a decarboxylase and a propylamine transferase for the synthesis of both spermidine and spermine. The rate limiting factor in either case is the activity of *S*-adenosyl-L-methionine decarboxylase.

In an attempt to clarify this situation, we have enhanced the purification of the rat liver enzyme over that previously reported, and have purified the enzyme from another source, sea urchin eggs. Sea urchin eggs were used because of the high specific activity of *S*-adenosyl-L-methionine decarboxylase detectable in crude ultracentrifuged supernatant preparations. Also, sea urchins contain high concentrations of spermine, unlike mammalian systems (Manen and Russell, 1973), making it possible to effectively study the conversion of spermidine to spermine. In a recent review of polyamine biosynthesis, it was postulated that the inability to separate the enzymes in rat liver might be due to the low specific activity of the final preparation, even though it was purified over 400-fold (Tabor and Tabor, 1972). Therefore, we have utilized the technique of affinity chromatography as a further step in purification of both the rat liver enzyme and the enzyme isolated from sea urchin eggs. This technique has resulted in preparations of extremely high specific activity. In both cases, only one enzyme, *S*-adenosyl-L-methionine decarboxylase, appears to be responsible for the total synthesis of spermidine and spermine.

## Materials and Methods

Dithiothreitol, pyridoxal phosphate, putrescine dihydrochloride, spermidine trihydrochloride, and EDTA were purchased from Calbiochem. *S*-Adenosyl-L-[*carboxyl*-$^{14}C$]methionine (7.7 mCi/mmol), [$^{14}$C]putrescine (20.3 mCi/mmol), and [$^{14}$C]spermidine (20.0 mCi/mmol) were purchased from New England Nuclear Corporation. The [$^{14}$C]putrescine was purified by high-voltage electrophoresis before use. DEAE-cellulose (Whatman DE52) was prepared by the method of Peterson and Sober (1962). CNBr-activated Sepharose was purchased from Pharmacia and substituted according to Cuatrecasas and Anfinsen (1971) with minor modifications. Two grams of CNBr-activated Sepharose was allowed to swell in 0.001 M HCl for 20 min with gentle agitation, then washed with 250 ml of 0.001 M HCl and 250 ml of distilled $H_2O$. The washed Sepharose was resuspended in an equal volume of cold distilled $H_2O$ containing 2 mmol/ml of ethylenediamine-HCl. This solution was adjusted to pH 10.0 with 10 N NaOH before it was added to the Sepharose. After shaking the Sepharose–ethylenediamine solution at least 16 hr at 4°, the gel was washed with large volumes of distilled $H_2O$.

The packed aminoethyl-Sepharose was suspended in 12 ml of 40% dimethylformamide, 0.4 mmol of sodium *p*-chloromercuribenzoate was added, the pH was adjusted to 4.8, and then 5 mmol of 1,3-(dimethylaminopropyl)carbodiimide was added. The pH was maintained at 4.8 by continuous titration with 0.1 N NaOH for 1 hr. After reacting at room temperature for another 18 hr, the substituted agarose was washed over an 8-hr period with 4 l. of 0.1 M $NaHCO_3$ (pH 8.8).

Ripe *Lytechinus pictus* were purchased from Pacific Biomarine, Venice, Calif. Spawning was induced by injection of 0.5 M KCl. Eggs were collected and washed in artificial sea water. Sprague-Dawley male rats (100–125 g) were obtained from Zivic-Miller, Allison Park, Pa.

*Enzyme Preparation.* Twenty grams of freshly spawned eggs was disrupted in a glass–Teflon homogenizer with 4 vol of 0.05 M sodium–potassium phosphate buffer (pH 7.2), containing 1.0 mM EDTA, 0.1 mM dithiothreitol, 3 $\mu$M pyridoxal phosphate, and 0.5 M sucrose. After removal of most of the cellular debris by centrifugation at 40,000*g* for 20 min, the crude preparation was centrifuged at 100,000*g* for an additional hour. The supernatant solution was decanted and passed through cheesecloth to remove any suspended lipid material. Rat liver (5 g) was prepared in an identical manner.

*Enzyme Purification.* The operations described below were carried out at 0–5°. The purification at each step of a typical preparation is summarized in Table I. One enzyme

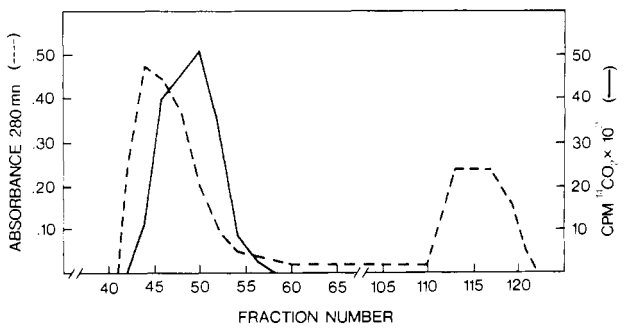


FIGURE 1: Sephadex gel filtration (G-75) of *S*-adenosyl-L-methionine decarboxylase isolated from sea urchin eggs. For details see the section on purification of the enzyme. Protein (- -) was measured by the absorbance at 280 nm. Enzyme activity (—) was assayed as described under Methods.

unit is equal to 1 $\mu$mol of $^{14}CO_2$ formed per minute. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

*Filtration on Sephadex G-75.* An aliquot (13 ml) of the enzyme preparation obtained in the previous step was applied to a reverse flow Sephadex G-75 column (100 cm × 5 $cm^2$) which had been equilibrated with 0.05 M sodium-potassium phosphate buffer (pH 7.2), containing 1.0 mM EDTA, 0.1 mM dithiothreitol, and 3 $\mu$M pyridoxal phosphate (buffer 1). The enzyme was eluted in 3.0-ml fractions with buffer 1 under a hydrostatic pressure of 20 cm at a flow rate of 35 ml/hr. The ten most active fractions (40–50) were pooled (Figure 1) and used in the next step.

*DEAE-Cellulose Chromatography.* The pooled enzyme fractions were dialyzed with a hollow fiber dialyzer (Bio-Rad Laboratories, Rockville Center, N.Y.) for 1 hr against 100 vol of 0.01 M sodium-potassium phosphate buffer (pH 7.2), containing 1 mM EDTA, 0.1 mM dithiothreitol, and 3 $\mu$M pyridoxal phosphate (buffer 2). Thirty milliliters of the dialyzed preparation was applied to a DEAE-cellulose column (50 cm × 1.1 $cm^2$) which had been equilibrated with buffer 2. After washing the column with 90 ml of buffer 2, the enzyme was eluted with 300 ml of a linear gradient of 0–1 M KCl, in buffer 2 (Figure 2). Fractions (3.0 ml) were collected and the five most active fractions (47–52) were pooled and again dialyzed as described above against 100 vol of 0.05 M sodium phosphate buffer (pH 7.2), containing 1.0 mM EDTA, 0.1 mM dithiothreitol, 3 $\mu$M pyridoxal phosphate, and 0.25 M sucrose (buffer 3).

An aliquot (30 ml) of the enzyme preparation was applied to a sodium *p*-chloromercuribenzoate-Sepharose column (10 cm × 0.6 $cm^2$) which had been previously equilibrated with buffer 3. After washing the column with approximately 20 ml of buffer 3, 1.0 ml of 50 mM dithiothreitol in the above buffer was applied to the column and the flow stopped for 2 hr. Then the enzyme was eluted with 50 mM dithiothreitol in buffer 3 and collected in 2.5-ml fractions (Figure 3). These fractions were dialyzed for 1 hr against two changes of 50 vol of buffer 3.

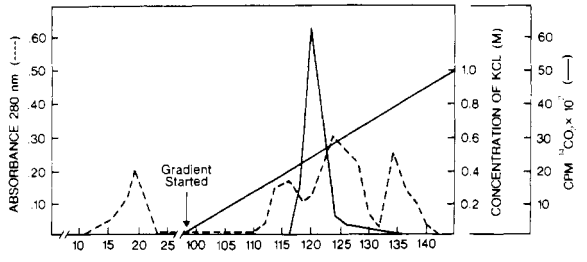


FIGURE 2: Chromatography of *S*-adenosyl-L-methionine decarboxylase isolated from sea urchin eggs on DEAE-cellulose. For details see the section on purification of the enzyme. Protein (- -) was measured by the absorbance at 280 nm. Enzyme activity (—) was assayed as described under Methods.

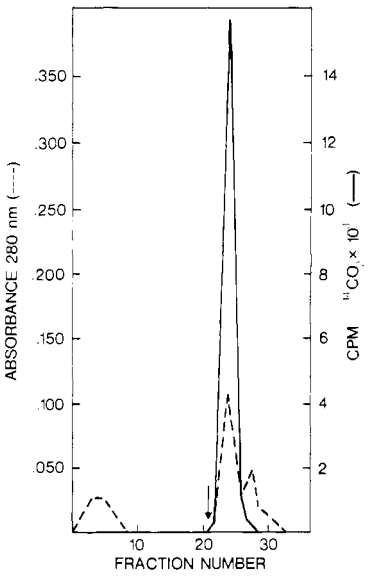


FIGURE 3: Affinity chromatography of *S*-adenosyl-L-methionine decarboxylase isolated from sea urchin eggs on a column containing sodium *p*-chloromercuribenzoate-ethylenediamine coupled to Sepharose 4B. For details of this preparation see the section on Materials. The vertical arrow indicates the introduction of 50 mM dithiothreitol which was used to elute the enzyme. Protein (- -) was measured by absorbance at 280 nm. Enzyme activity (—) was assayed as described under Methods.

*Disc Electrophoresis.* The purified enzyme was applied to a 7% acrylamide gel, 9 cm in length, topped with 250 $\mu$l of stacking gel, which had been pre-run for 1 hr in a Tris-glycine electrophoresis buffer (pH 9.0). After running at 4 mA/gel for 2 hr, the gels were either fixed and stained in Coomasie Blue (Figure 4) or sliced into 1-mm sections, incubated in the enzyme assay mixture described below for 4 hr, and assayed for enzyme activity.

*Assay for S-Adenosyl-L-methionine Decarboxylase Activity.* *S*-Adenosyl-L-methionine decarboxylase activity was determined by measuring the liberation of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxyl*-$^{14}$C]methionine as described by

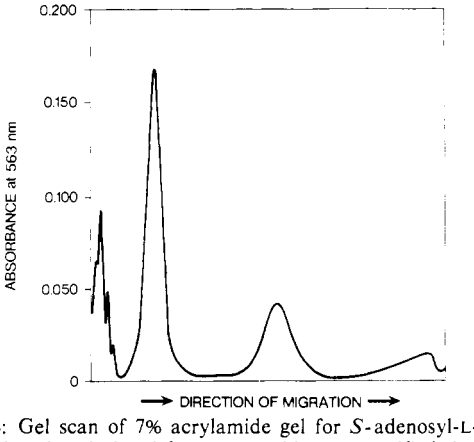


FIGURE 4: Gel scan of 7% acrylamide gel for *S*-adenosyl-L-methionine decarboxylase isolated from sea urchin eggs purified through affinity chromatography. The bands shown on this scan were the only bands present on the gel. *S*-Adenosyl-L-methionine decarboxylase activity was found only in the first major band. Assay of the gel slices for enzyme activity is described under Methods.

TABLE II: Purification of *S*-Adenosyl-L-methionine Decarboxylase from Rat Liver.

| Fraction | Vol (ml) | Protein (mg) | Sp Act. (Units/mg of Protein) | | Purification | % Recovery |
|---|---|---|---|---|---|---|
| | | | Putrescine Stimulated | Spermidine Stimulated | | |
| Ultracentrifuged supernatant | 15 | 315 | 1.3 | 0.6 | | |
| Sephadex gel | 30 | 95 | 4.6 | 2.3 | 3.3 | 100 |
| DEAE-cellulose | 10 | 5.1 | 157 | 77 | 115 | 90 |
| Affinity[a] | 6 | 0.4 | 1553 | 760 | 1138 | 73 |

[a] Sodium *p*-chloromercuribenzoate affinity chromatography.

Russell (1972). A saturating concentration of *S*-adenosyl-L-methionine (65 $\mu$M) was used in these assays. Either 2.5 mM putrescine or 5 mM spermidine was used in the assay as an acceptor for the propylamine moiety from *S*-adenosyl-L-methionine.

*Determination of [$^{14}C$]Spermidine Formation from [$^{14}C$]Putrescine.* Incubation conditions and concentrations were the same as those for the *S*-adenosyl-L-methionine decarboxylase assay with minor modifications. The assay was conducted in a total volume of 2.0 ml of 2.5 mM [$^{14}C$]putrescine was used as the propylamine acceptor. One flask was stopped immediately with acid and acted as the control. At the end of the assay, the center wells of the flasks were removed and counted for the liberation of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxyl*-$^{14}C$]methionine.

Dry sulfosalicylic acid was added to the flasks to obtain a final concentration of 4%. The flasks were cooled for 10 min on ice and centrifuged for 10 min at 10,000 rpm. The supernatant was applied manually to a Beckman 121C amino acid analyzer for separation of the polyamines; this methodology has been previously described (Marton *et al.*, 1973). The polyamines were eluted from the column with 2.35 M sodium citrate buffer (pH 4.68) and collected in 2.5-ml aliquots. Radioactivity in the putrescine and spermidine fractions was determined by counting 1.5-ml samples of the appropriate aliquots in a cocktail containing 15 ml of Aquasol and 2 ml of distilled $H_2O$.

*Determination of [$^{14}C$]Spermine from [$^{14}C$]Spermidine.* The assay was conducted as described for [$^{14}C$]spermidine formation from [$^{14}C$]putrescine with the exception that 1 mM [$^{14}C$]spermidine + 4 mM unlabeled spermidine was added as the receptor for the propylamine moiety from *S*-adenosyl-L-methionine.

*Molecular Weight.* The molecular weight of the enzyme was estimated by Sephadex gel (G-100) filtration (Andrews, 1964). The commercial source and molecular weight of the individual proteins used as standards are as follows: ribonuclease A (bovine pancreas), 13,700 (Hirs *et al.*, 1956); chymotrypsinogen A (bovine pancreas), 25,000

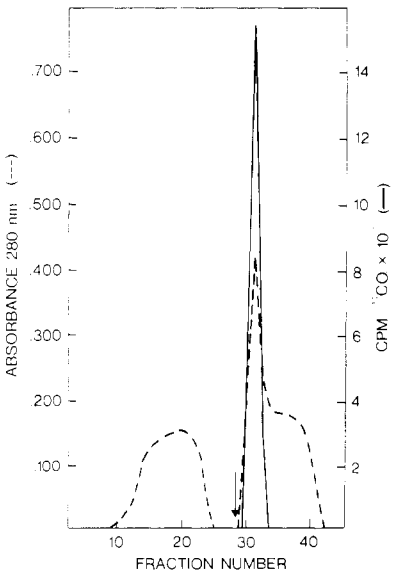


FIGURE 5: Affinity chromatography of *S*-adenosyl-L-methionine decarboxylase isolated from rat liver on a column containing sodium *p*-chloromercuribenzoate-ethylenediamine coupled to Sepharose 4B. For details of this preparation see the section on Materials. The vertical arrow indicates the introduction of 50 mM dithiothreitol which was used to elute the enzyme. Protein (- -) was measured by absorbance at 280 nm. Enzyme activity (—) was assayed as described under Methods.

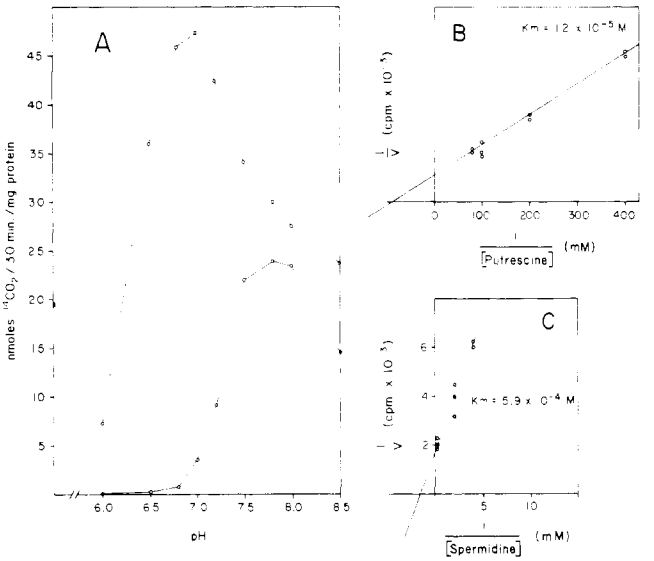


FIGURE 6: (A) Optimal pH for putrescine-stimulated (- -) and spermidine-stimulated (—) *S*-adenosyl-L-methionine decarboxylase isolated from sea urchin eggs and purified through DEAE-cellulose chromatography. Enzyme activity was assayed as described under Methods. (B) Double reciprocal plot of the velocity of the enzyme-catalyzed reaction in varying putrescine concentrations. The sea urchin enzyme after DEAE-cellulose chromatography was incubated in the standard assay mixture at pH 7.0 as described under Methods with the minor modifications that the putrescine concentration varied as indicated and the *S*-adenosyl-L-[*carboxyl*-$^{14}C$]methionine concentration was saturating (65 $\mu$M). (C) Double reciprocal plot of the velocity of the enzyme-catalyzed reaction in varying spermidine concentrations. The sea urchin enzyme after DEAE-cellulose chromatography was incubated in the standard assay mixture at pH 7.7 as described under Methods with the minor modifications that the spermidine concentration varied as indicated and the *S*-adenosyl-L-[*carboxyl*-$^{14}C$]methionine was saturating (65 $\mu$M).

TABLE III: Effect of Certain Inhibitors of Sulfhydryl Groups on the Activity of S-Adenosyl-L-methionine Decarboxylase Isolated from Sea Urchin Eggs.[a]

| Inhibitor | Sp Act. of Putrescine Stimulated (Units/mg of Protein) | % Inhibition | Sp Act. of Spermidine Stimulated (Units/mg of Protein) | % Inhibition |
|---|---|---|---|---|
| None | 1637 | | 787 | |
| Iodoacetamide | 546 | 67 | 300 | 62 |
| *p*-Chloromercuribenzoate | 23 | 99 | 23 | 97 |
| *N*-Ethylmaleimide | 37 | 98 | 50 | 94 |

[a] The concentration of inhibitor used in the assay was $2 \times 10^{-4}$ M. An enzyme fraction isolated from sea urchin eggs purified through DEAE-cellulose chromatography was used. The inhibitor was incubated with the enzyme for 10 min prior to addition of S-adenosyl-L-[*carboxyl*-$^{14}$C]methionine. Details of the assay are described under Methods.

(Desnuelle, 1960); ovalbumin, 45,000 (Warner, 1954); and aldolase (rabbit muscle), 160,000 (Kawahara and Tanford, 1966). All were obtained from Sigma.

## Results

*Purification of the Enzyme.* A typical purification procedure for S-adenosyl-L-methionine decarboxylase from unfertilized sea urchin eggs is shown in Table I. Approximately 60% of the protein remaining after affinity chromatography, as assessed by polyacrylamide gel electrophoresis, was proven to be S-adenosyl-L-methionine decarboxylase (Figure 4).

The purification procedure for the enzyme from rat liver is shown in Table II. The tenfold increment in purity after sodium *p*-chloromercuribenzoate affinity chromatography results in a purification of the original preparation that is over 1000-fold. At this point, however, the specific activity is still less than that of the enzyme prepared from sea urchin eggs by this same procedure. The elution curves for the isolation of the enzyme from rat liver are similar to those of Feldman *et al.* (1972). A typical elution curve for sodium *p*-chloromercuribenzoate affinity chromatography is shown in Figure 5.

*General Characteristics of the Enzyme Purified from Sea Urchins.* The pH optimum for the putrescine-stimulated activity of the enzyme isolated from sea urchin eggs after DEAE-cellulose chromatography is 7.0. This is within the range reported for the mammalian enzyme (Pegg and Williams-Ashman, 1969; Feldman *et al.*, 1972). The spermidine-stimulated activity, however, exhibited optimal activity between pH 7.5 and 8.0, a marked variation from the optimal pH of the putrescine-stimulated activity (Figure 6). All enzyme assays were conducted at the optimal pH for the particular assay.

Both putrescine and spermidine stimulated the activity of S-adenosyl-L-methionine decarboxylase with putrescine favored by a 2:1 ratio. This ratio remains constant throughout purification (Tables I and II). If putrescine or spermidine is not added to the assay mixture of the enzyme purified from sea urchin eggs, there is no detectable S-adenosyl-L-methionine decarboxylase activity. This differs from the mammalian enzyme which does exhibit some decarboxylase activity in the absence of exogenous polyamines (Pegg and Williams-Ashman, 1969).

Through the use of the double reciprocal plot, the apparent $K_m$ values for putrescine and spermidine of the enzyme fraction purified through DEAE-cellulose were calculated (Figure 6). In both cases, the Michaelis constants of the sea urchin system differed by an order of magnitude from those reported from the mammalian enzyme (Feldman *et al.*, 1972). This may account for both the lower levels of putrescine present in developing sea urchins and the higher levels of spermine that accumulate as compared to mammalian systems that are undergoing growth processes (Russell and Lombardini, 1971; Russell and McVicker, 1972).

In the sea urchin, both putrescine- and spermidine-stimulated S-adenosyl-L-methionine decarboxylase activities are inhibited by the presence of compounds that are known to inhibit the enzymatic activities of proteins containing sulfhydryl groups (Table III). Both activities are inhibited also by NSD-1055,[1] an inhibitor of pyridoxal phosphate requiring enzymes (Levine *et al.*, 1965) and by methylglyoxalbis(guanylhydrozone), a specific inhibitor of S-adenosyl-L-methionine decarboxylase (Williams-Ashman and Schenone, 1972). The addition of methylglyoxalbis(guanylhydrozone) to the assay, however, alters the ratio of putrescine- to spermidine-stimulated S-adenosyl-L-methionine decarboxylase activity. That is, the spermidine-stimulated activity is inhibited only 60–70% by methylglyoxalbis(guanylhydrozone) (5 $\mu$M) and this inhibition remains constant with increased concentrations of methylglyoxalbis(guanylhydrozone), whereas putrescine-stimulated activity can be inhibited almost completely (90%, 10 $\mu$M methylglyoxalbis(guanylhydrozone)).[2] This change in the ratio of putrescine- to spermidine-stimulated S-adenosyl-L-methionine decarboxylase activity is in agreement with the findings of Heby *et al.* (1973) and Heby and Russell (1974).

The effects of dithiothreitol and EDTA, both singly and combined, on putrescine-stimulated and spermidine-stimulated S-adenosyl-L-methionine decarboxylase also were studied. Dithiothreitol alone, even in concentrations as high as 5.0 mM, does not significantly affect the activity of S-adenosyl-L-methionine decarboxylase. Concentrations of EDTA greater than 1 mM inhibited both putrescine- and spermidine-stimulated S-adenosyl-L-methionine decarboxylase, but the spermidine-stimulated activity was more

[1] Abbreviation used is: NSD-1055, 4-bromo-3-hydroxybenzoyloxyamine dihydrogen phosphate.

[2] A more detailed study of the inhibition of putrescine- and spermidine-stimulated S-adenosyl-L-methionine decarboxylase activity by methylglyoxalbis(guanylhydrozone) and NSD-1055 has been submitted to the reviewers for examination and can be obtained by writing directly to the authors.

strongly inhibited than that of the putrescine-stimulated activity. That is, if the concentration of EDTA in the assay mixture is 10 mM, the spermidine-stimulated activity is completely inhibited, whereas the putrescine-stimulated activity was reduced by only 40%. If the concentration of either dithiothreitol or EDTA was held constant and the concentration of the other increased, there was a progressive inhibition of enzyme activity. This inhibition was more marked if the concentration of dithiothreitol was held constant and the concentration of EDTA was increased. At the highest combined concentrations (10 mM EDTA and 5.0 mM dithiothreitol), *S*-adenosyl-L-methionine decarboxylase was completely inhibited.[3]

In general, metals are inhibitory to enzyme activity; the enzyme isolated from rat liver is inhibited by the addition of $5 \times 10^{-4}$ M $MgCl_2$, $MnCl_2$, $ZnSO_4$, $NH_4Cl_2$, $CaCl_2$, $FeCl_2$, $CoCl_2$, and $CdCl_2$ (Feldman *et al.*, 1972). The enzyme isolated from sea urchins, however, is neither appreciably stimulated nor inhibited by the same concentrations of any of these metals. These results are not unexpected, given the relatively large concentrations of metals in sea water.

The molecular weight, as determined by Sephadex gel filtration after DEAE-cellulose chromatography, was estimated to be 56,000. Again, this correlates well with the estimated molecular weight of purified *S*-adenosyl-L-methionine decarboxylase from rat liver (Feldman *et al.*, 1972).

*Relationship of $^{14}CO_2$ Evolution from S-Adenosyl-L-[carboxyl-$^{14}$C]methionine to Spermidine or Spermine Formation.* The enzyme preparations from both rat liver and sea urchin eggs exhibit stoichiometry between the formation of [$^{14}$C]spermidine when [$^{14}$C]putrescine was added to the assay mixture and the evolution of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxyl*-$^{14}$C]methionine. This stoichiometry was present in the enzyme fraction at all stages. After sodium *p*-chloromercuribenzoate affinity chromatography, the enzyme isolated from sea urchins, when incubated with [$^{14}$C]putrescine and *S*-adenosyl-L-[*carboxyl*-$^{14}$C]methionine, formed 478 nmol of [$^{14}$C]spermidine and liberated 470 nmol of $^{14}CO_2$. The enzyme isolated from rat liver, purified through affinity chromatography, formed 46.0 nmol of [$^{14}$C]spermidine and liberated 45.7 nmol of $^{14}CO_2$. Also, in both preparations, this same relationship was found between the formation of [$^{14}$C]spermine when [$^{14}$C]spermidine was added to the assay and the evolution of $^{14}CO_2$ from added *S*-adenosyl-L-[*carboxyl*-$^{14}$C]methionine. The enzyme isolated from sea urchin eggs, after affinity chromatography, formed 20.4 nmol of [$^{14}$C]spermine and liberated 21.6 nmol of $^{14}CO_2$. The enzyme isolated from rat liver, also after affinity chromatography, formed 2.30 nmol of [$^{14}$C]spermine and liberated 2.35 nmol of $^{14}CO_2$.

## Discussion

*S*-Adenosyl-L-methionine decarboxylase is a key enzyme in the polyamine biosynthetic pathway. An understanding of the characteristics of this enzyme, therefore, may allow us to manipulate cellular polyamine concentrations and consequently, cellular metabolic functions.

*S*-Adenosyl-L-methionine decarboxylase isolated from unfertilized sea urchin eggs has been purified over 200-fold. Polyacrylamide gel electrophoresis indicated that approximately 60% of the protein remaining after purification was *S*-adenosyl-L-methionine decarboxylase. The enzyme isolated from sea urchin eggs contains sulfhydryl groups, has a molecular weight of approximately 56,000, and requires pyridoxal phosphate as a cofactor.

This enzyme has many properties in common with the enzyme isolated from mammalian sources. Both enzymes have similar molecular weights and require pyridoxal phosphate as a cofactor and either putrescine or spermidine as a stimulating factor. The differences between these two enzymes appear to be due to environmental adaptations. That is, the enzyme isolated from mammalian sources is inhibited by the presence of metal ions, while the enzyme isolated from sea urchin eggs is neither stimulated nor inhibited by the same concentrations, or higher concentrations, of metal ions. This is readily explainable on the basis of high concentrations of metal ions in sea water. The accumulation of putrescine, spermidine, and spermine also varies in sea urchins and can be directly correlated with the lower $k_m$ values for putrescine and spermidine exhibited by the enzyme isolated from sea urchins as compared to these constants calculated for the enzyme isolated from rat liver. This would mean that lower concentrations of putrescine and spermidine would be present and a higher concentration of spermine would accumulate. This is indeed the pattern of accumulation in both adult and developing sea urchins (Manen and Russell, 1973). In contrast, stimulated mammalian systems, such as regenerating rat liver (Russell and Lombardini, 1971) and lactating mammary gland (Russell and McVicker, 1972), have low concentrations of spermine during the stimulated period. The high concentrations of spermine present in developing sea urchins originally indicated that the sea urchin enzyme might be useful to study spermine synthesis.

Originally it was reported that mammalian systems, in contrast to prokaryotic systems, contained one protein that both decarboxylated *S*-adenosyl-L-methionine and transferred a propylamine moiety to putrescine or spermidine to form spermidine or spermine, respectively (Tabor and Tabor, 1964; Pegg and Williams-Ashman, 1969). Recently, it has been reported that this protein can be separated into three discrete enzymes: a decarboxylase, a spermidine synthase, and a spermine synthase (Jänne and Williams-Ashman, 1971; Raina and Hannonen, 1971; Hannonen *et al.*, 1972). However, the data presented in this paper concerning the characteristics of *S*-adenosyl-L-methionine decarboxylase isolated both from sea urchin eggs and rat liver support the hypothesis that all three reactions are catalyzed by the same enzyme.

The enzyme isolated from sea urchin eggs by the procedure described herein has the highest specific activity yet reported for the enzyme. Both this enzyme and the enzyme purified from rat liver by the same procedure, a 1000-fold purification, exhibited a stoichiometric relationship between the liberation of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxyl*-$^{14}$C]methionine and the formation of either [$^{14}$C]spermidine or [$^{14}$C]spermine depending on whether [$^{14}$C]putrescine or [$^{14}$C]spermidine had been added to the assay mixture. It has been postulated (Raina and Hannonen, 1971) that stoichiometry could be explained by the contamination of the decarboxylase with a small amount of synthase. The purity of the enzyme fractions from rat liver and

[3] A detailed study of the effects of varying concentrations of EDTA and dithiothreitol on putrescine- and spermidine-stimulated *S*-adenosyl-L-methionine decarboxylase activities has been submitted to the reviewers for examination and may be obtained by writing directly to the authors.

sea urchin makes this explanation unlikely. Previous methods for the determination of stoichiometry have depended upon high-voltage paper electrophoresis or column chromatography for the separation of the polyamines. Both of these methods result in a considerable overlap of the polyamine fractions. This means that tracer isotope separations are difficult. The use of the amino acid analyzer for separation resulted in clean, sharply defined fractions, separated by sufficient volume so that there was no overlap of the $^{14}C$-labeled isotopes in the different polyamine fractions (see Methods for complete details).

There are several unresolved areas in the literature concerning S-adenosyl-L-methionine decarboxylase. The first of these involved the use of decarboxylated S-adenosyl-L-methionine as a substrate for measuring *in vitro* reactions of mammalian systems. It has been reported (Raina and Hannonen, 1971) that spermidine synthase was present in concentrations 100-fold that of S-adenosyl-L-methionine decarboxylase when decarboxylated S-adenosyl-L-methionine was used as the substrate. However, of prime importance, decarboxylated S-adenosyl-L-methionine has not been found as a free intermediate in mammalian systems (Pegg and Williams-Ashman, 1969). Further, given the kinetics of the system, the amount of decarboxylated S-adenosyl-L-methionine that could be potentially available at any given time would not appear sufficient to catalyze the reaction (Cohen, 1971). That is, decarboxylated S-adenosyl-L-methionine has a $K_m$ so similar ($2.5 \times 10^{-5}$ M) to the $K_m$ for S-adenosyl-L-methionine ($5 \times 10^{-5}$ M), that it does not seem possible for decarboxylated S-adenosyl-L-methionine to serve as an efficient donor of a propylamine moiety to putrescine or spermidine.

A final argument centers on the molecular weight of S-adenosyl-L-methionine decarboxylase. Hannonen *et al.* (1972) reported the separation of a spermidine synthase, incapable of the decarboxylation of S-adenosyl-L-methionine, from purified S-adenosyl-L-methionine decarboxylase, incapable of spermidine synthesis, and then rechromatographed these two proteins on a Sephadex G-200 column with crude S-adenosyl-L-methionine decarboxylase that was capable of both decarboxylation and spermidine synthesis. Purified S-adenosyl-L-methionine decarboxylase, as well as S-adenosyl-L-methionine decarboxylase from crude supernatants with both decarboxylase and transferase activity, and presumed spermidine synthase, emerged in the same fractions, suggesting they all had identical molecular weights. If S-adenosyl-L-methionine decarboxylase were split into two or more parts of purification methods, the original protein (crude supernatant preparation) should have a molecular weight equal to the sum of these parts, *i.e.*, at least 100,000. These data appear inconsistent with the separation of S-adenosyl-L-methionine decarboxylase into component enzymes, but are consistent with the theory that there is only one enzyme involved in both the decarboxylation of S-adenosyl-L-methionine and the transfer of a propylamine moiety from decarboxylated S-adenosyl-L-methionine to putrescine or spermidine to form spermidine or spermine, respectively.

References


Andrews, P. (1964), *Biochem. J. 91,* 222.

Cohen, S. S. (1971), Introduction to the Polyamines, Englewood Cliffs, N. J., Prentice-Hall.

Cuatrecasas, P., and Anfinsen, C. B. (1971), *Methods Enzymol. 22,* 345.

Desnuelle, P. (1960), *Enzymes, 2nd Ed., 2,* 93.

Feldman, M. J., Levy, C. C., and Russell, D. H. (1971), *Biochem. Biophys. Res. Commun. 44,* 675.

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

Hannonen, P., Jänne, J., and Raina, A. (1972), *Biochem. Biophys. Res. Commun. 46,* 341.

Heby, O., and Russell, D. H. (1974), *Cancer Res. 34,* 886.

Heby, O., Sauter, S., and Russell, D. H. (1973), *Biochem. J. 136,* 1121.

Hirs, C. H. W., Moore, S., and Stein, W. J. (1956), *J. Biol. Chem. 219,* 623.

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

Kawahara, K., and Tanford, C. (1966), *Biochemistry 5,* 1578.

Levine, R. J., Satro, T. L., and Sjoerdsma, A. (1965), *Biochem. Pharmacol. 14,* 139.

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

Manen, C. A., and Russell, D. H. (1973), *J. Embryol. Exp. Morph. 29,* 331.

Marton, L. J., Vaughn, J. G., Hawk, I. A., Levy, C. C., and Russell, D. H. (1973), *in* Polyamines in Normal and Neoplastic Growth, Russell, D. H., Ed., New York, N. Y., Raven Press, pp 367–372.

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

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

Peterson, E. A., and Sober, H. A. (1962), *Methods Enzymol. 5,* 3.

Raina, A., and Hannonen, P. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 16,* 1.

Russell, D. H. (1972), *Cancer Res. 32,* 1459.

Russell, D. H., and Lombardini, J. B. (1971), *Biochim. Biophys. Acta 240,* 273.

Russell, D. H., and McVicker, T. A. (1972), *Biochem J. 130,* 71.

Russell, D. H., and Potyraj, J. J. (1972), *Biochem. J. 128,* 1109.

Russell, D. H., and Taylor, R. L. (1971), *Endocrinology 88,* 1397.

Tabor, H., and Tabor, C. W. (1964), *Pharmacol. Rev. 16,* 245.

Tabor, H., and Tabor, C. W. (1972), *Advan. Enzymol. 36,* 203.

Warner, R. C. (1954), *Proteins 11,* 435.

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

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