THE PURIFICATION OF S-ADENOSYL-L-METHIONINE DECARBOXYLASE FROM RAT LIVER: INABILITY TO SEPARATE DECARBOXYLATION FROM SPERMIDINE SYNTHESIS

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Received June 14, 1971

SUMMARY

S-adenosyl-L-methionine decarboxylase has been purified more than 350-fold from rat liver by a new procedure. Some characteristics of the decarboxylase are described. Higher stages of purification did not abolish the stoichiometric relationship between CO_2 formation and spermidine production.

The polyamines are small nitrogenous bases whose synthesis and concentrations are enhanced in rapid growth systems, i.e., neoplastic tissue, regenerating rat liver, and in embryonic development (1-8). Those amines that occur in mammalian tissue and have been most widely studied are putrescine, spermidine, and spermine. Characterization of the enzymes responsible for the synthesis of these compounds may lead to a better understanding of their biologic role.

The enzyme pathway involved in spermidine synthesis has been studied most extensively in bacteria, where it is synthesized by two separate enzymes, (1) an S-adenosyl-L-methionine decarboxylase which decarboxylates S-adenosyl-L-methionine, and (2) a propylamine transferase which transfers a propylamine moiety from decarboxylated S-adenosyl-L-methionine to putrescine to form spermidine (9). Recently Pegg and Williams-Ashman (10) described the presence of a cell-free system responsible for the decarboxylation of S-adenosyl-L-methionine in rat ventral prostate and liver. They showed further that the ventral prostate system was more active than the liver system and that the activity of the preparations was specifically stimulated by putrescine, with

the stoichiometric formation of CO_2 and spermidine. Later these same authors reported the partial purification and characterization of the prostate enzyme in which they were unable to separate the decarboxylase activity from the propylamine transferase activity (11). Recently it has been reported that the putrescine-activated S-adenosyl-L-methionine decarboxylase in late stages of purification is no longer coupled to spermidine formation (12). This later finding suggested that the prostate enzyme responsible for the formation of spermidine from putrescine might indeed be separable into two distinct enzyme systems.

In contrast to the prostate, the liver S-adenosyl-L-methionine decarboxylase has not been studied as extensively. Russell and Lombardini (13) and Raina et al. (14) have shown in 100,000 x g supernatant solutions that S-adenosyl-L-methionine decarboxylase activity and spermidine formation increase several fold following partial hepatectomy. In addition, these same authors have shown close stoichiometry between decarboxylation and spermidine formation. Because of lack of purification of the liver enzyme(s) to date, description of the enzyme cofactor requirements, kinetics, and similarities to the prostate preparation have not been possible.

This paper describes a procedure for purification of the putrescine-activated S-adenosyl-L-methionine decarboxylase of normal rat liver and describes some of the characteristics of this enzyme system.

EXPERIMENTAL PROCEDURES

Putrescine 1,4,^{14}C dihydrochloride (17.7 μc/μmole) was obtained from New England Nuclear Corporation. S-adenosyl-L-methionine ^{14}C (47 μc/μmole) was obtained from Amersham-Searle. Enzyme activity was determined by measuring the liberation of $^{14}CO_2$ from ^{14}C carboxyl-labeled S-adenosyl-L-methionine, as previously described (11). The standard assay contained 10 μmoles of sodium phosphate buffer, pH 7.2, 0.5 μmole putrescine, 0.085 μmole S-adenosyl-L-

methionine 1-^{14}C;* 3.9 μmoles pyridoxal phosphate, 0.1 mg albumin and 0.05-0.1 ml of enzyme preparation in a total volume of 0.25 ml in 13 ml conical centrifuge tubes.

When the formation of spermidine from unlabeled S-adenosyl-L-methionine was estimated, 0.33 μmole of putrescine 1,4,^{14}C and 4.0 μmoles of cold putrescine were added to an assay containing the same concentration of reactants in 2.0 ml. The reaction was stopped by the injection of 2.0 ml of 5% w/v trichloroacetic acid containing 0.4 μmole of spermidine and spermine. After removal of the protein precipitate by centrifugation the supernatant was washed three times with five volumes of ether. The ether was discarded and the solution put directly on 5 cm x 0.25 cm^2 Dowex 50-H+ column. The labeled putrescine was removed with 200 ml of 0.8 N HCl. The labeled spermidine was removed with 20 ml of 6 N HCl and evaporated to dryness under reduced pressure and then dissolved in 0.5 ml of 0.01 N HCl. Of this solution 0.1 ml was spotted on 3MM Whatman chromatography paper and subjected to electrophoresis in 0.1M citric acid, pH 4.3. The chromatograms were developed with ninhydrin and the appropriate spots were cut out, eluted, and their radioactivity determined, as previously described (15).

Purification of liver S-adenosyl-L-methionine decarboxylase

The livers from Sprague-Dawley rats weighing 100-125 g were homogenized with four volumes of 0.05M phosphate buffer, pH 7.2, containing 1.0mM EDTA, 0.1mM Cleland's Reagent, 3μmM pyridoxal phosphate, and 0.5M sucrose. All operations were carried out at 0-2° C. Cellular debris was removed from the crude homogenate by centrifugation at 40,000 x g for 20 minutes. The resulting supernatant solution was clarified by further centrifugation at 100,000 x g for 1 hr and then by passage through cheesecloth to remove the lipid layer. An 8 ml aliquot from this solution was applied to a Sephadex (G-100 or G-150) column (45 cm x 4.9 cm^2 which had been equilibrated previously with the homogenizing

* The concentration of S-adenosyl-L-methionine was nonsaturating. However, in those experiments in which saturating levels were used, the values were consistently two times those reported here.

buffer). The enzyme was eluted with equilibrating buffer and the active fractions were pooled and dialyzed against 100 volumes of 0.01M phosphate buffer, pH 7.2, containing 1mM EDTA, 0.1mM Cleland's Reagent, 3μM pyridoxal phosphate, and 0.5M sucrose. Thirty ml of the dialyzed preparation were applied to a DEAE-cellulose column (25 cm x 4.9 cm^2, previously equilibrated with the same buffer as used in the dialysis). After washing the column with 90 ml of the equilibrating buffer, the active enzyme was eluted with a linear gradient of elution from 0-1M KCl in the equilibrating buffer. The total volume of the gradient was 300 ml and fractions 4 ml in volume were collected. Six or seven of the most active fractions were pooled and dialyzed against 100 volumes of 0.01M phosphate buffer, pH 7.2, containing 1mM EDTA, 0.1mM Cleland's Reagent, and 3μM pyridoxal phosphate. Twenty ml of this solution was applied to a column of calcium hydroxylapatite (Bio-Gel HT, Bio-Rad Laboratories, 3 cm x 4.9 cm^2, previously equilibrated with the buffer used to dialyze the DEAE-cellulose preparation). The column was washed with an additional 25 ml of the equilibrating buffer and then washed with 25 ml of 0.1M, 0.2M, 0.3M, and 0.4M phosphate buffer, pH 7.2, containing 1mM EDTA, 0.1mM Cleland's Reagent and 3μM pyridoxal phosphate. The active enzyme was eluted with 0.2M buffer with all the activity occurring in two 4 ml fractions which were pooled.

RESULTS AND DISCUSSION

The results of the procedure employed in the purification of liver S-adenosyl-L-methionine decarboxylase are shown in Table 1. The final fraction represented a purification of greater than 350-fold (60% recovery). Sephadex gel filtration was chosen as the initial step following ultracentrifugation because of the deleterious effects of ammonium sulfate on enzyme activity. It was observed that at 40% saturation ammonium sulfate caused the loss of between 60 to 90% of enzyme activity. Cleland's Reagent, EDTA, pyridoxal phosphate, sucrose, and phosphate buffer, pH 7.2, were found to maintain the stability of the enzyme during purification. The DEAE-cellulose eluate was stable at -20° C

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

Step	Volume	Protein*	Specific Activity	Purification	Recovery
	ml	mg	Units+ per mg protein		%
I. 100,000 x g supernatant	9	270	0.05	--	--
II. Sephadex gel filtrate	32	96	0.21	4.2	130
III. DEAE-cellulose column chromatography	22	2.5	6.19	123.0	100
IV. Calcium hydroxyl-apatite column chromatography	8	0.22	18.6	372.0	60

* Proteins were determined according to the method of Lowry et al. (17).

+ 1 enzyme unit = 1 mμmole of $^{14}CO_2$ liberated in 30 min. in the standard assay.

Table 2. Comparison of average molecular weight of S-adenosyl-L-methionine decarboxylase determined by two methods.

Enzyme Source	Fraction	Method	Molecular Weight	Average
Liver	100,000 x g supernatant	Sephadex gel filtration[a] G-150	50,000	
		Sucrose density centrifugation[b]		
		Alcohol dehydrogenase[c]	57,000	52,500
		Catalase[c]	48,000	
	DEAE-cellulose eluate	Sephadex gel filtration[a] G-100	44,000	43,500
		G-150	43,000	

[a] Method of Andrews (19)

[b] Method of Martin and Ames (18)

[c] Standard proteins used as markers in centrifugation.

for six months and the calcium hydroxylapatite at -20° C for almost a month. All fractions were fairly stable to lyophilization with loss of only 20-40% of enzyme activity. Polyacrylamide gel electrophoresis of a lyophilized preparation of the calcium hydroxylapatite eluate revealed four faint bands.

Certain physical characteristics of the enzyme were determined. The pH optimum for decarboxylation was 7.2 in 0.1M phosphate buffer and 7.35 in 0.1M Tris-HCl buffer. The experimentally determined Km for S-adenosyl-L-methionine was 3.6×10^{-5}M and the Km for putrescine when the S-adenosyl-L-methionine concentration was held at 0.28μM was 3.3×10^{-4}M. The molecular weight of the enzyme was determined to be approximately 50,000 by Sephadex gel filtration and by sucrose density centrifugation (Table 2). In the presence of 50μM Cleland's Reagent and 2mM pyridoxal phosphate, inhibition of enzyme activity was accomplished with both sulfhydryl group inhibitors and inhibitors of pyridoxal phosphate systems such as NSD-1055 (4-bromo-3 hydroxybenzyloxyamine dihydrogen phosphate) (16). The enzyme preparation after DEAE-cellulose chromatography required pyridoxal phosphate for maximal activity. The activity of the DEAE-cellulose preparation and the calcium hydroxylapatite preparation were stimulated sixfold by the presence of 2mM putrescine. Spermidine could be substituted for putrescine at all stages of the purification, but there was always a 2:1 preference for putrescine over spermidine.

From the above data it can be seen that the kinetics and pH optima of the liver enzyme system are very similar to those previously reported for the prostate (10,11). In contrast to the prostatic enzyme, however, higher stages of purification did not abolish the stoichiometric relationship between CO_2 formation and spermidine production (Table 3). Several possibilities might explain our inability to separate the decarboxylase activity from the propylamine transferase activity. Firstly, it may be that higher degrees of purification than we have achieved are needed to separate these two activities. Secondly, the two activities might be more tightly coupled in the liver than in the prostate. Thirdly, although unlikely, it is possible that one enzyme

complex exists in the liver, whereas two enzymes occur in the prostate. However, the low molecular weight of the liver enzyme system would make it appear unlikely that there are two separate proteins.

Table 3. Stoichiometry of the decarboxylation of S-adenosyl-L-methionine and the formation of spermidine.

Enzyme Source	Enzyme Fraction	Activity in mμmoles/30 min per mg protein	
		$^{14}CO_2$ production*	^{14}C spermidine synthesis*
Liver	100,000 x g supernatant	0.056	0.064
	DEAE-cellulose eluate	7.2	8.7

* See EXPERIMENTAL PROCEDURES for detailed methodology of these determinations.

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