SEPARATION AND PARTIAL PURIFICATION OF S-ADENOSYL-METHIONINE DECARBOXYLASE AND SPERMIDINE AND SPERMINE SYNTHASES FROM RAT LIVER

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Summary. Putrescine-activated S-adenosylmethionine decarboxylase and spermidine and spermine synthases, the enzymes catalyzing the synthesis of spermidine and spermine from S-adenosylmethionine and the appropriate amine, have been separated and partially purified from the soluble fraction of rat liver. During the purification of S-adenosylmethionine decarboxylase the stoichiometry between the decarboxylation of S-adenosylmethionine and the formation of spermidine in the presence of putrescine was lost.

The synthesis of spermidine from S-adenosyl-L-methionine (adenosylmethionine) and putrescine in E. coli is catalyzed by two different enzymes: (a) a magnesium-activated adenosylmethionine decarboxylase (1, 2) and (b) a propylamino transferase that transfers the propylamino group from 5'-deoxy-5'-S-(3-methylthiopropylamine) sulphonium adenosine (decarboxylated adenosylmethionine) to putrescine to yield spermidine (spermidine synthase) (3). The synthesis of spermidine in animal tissues seems to occur in an analogous manner although the individual enzymes involved differ from those described in E. coli. Adenosylmethionine decarboxylase from animal or some other eukaryotic sources does not require any metal cofactor but is intensively stimulated by putrescine and, to a lesser degree, by spermidine (4, 5, 6, 7, 11). In addition to the propylamino transferase activity responsible for the synthesis of spermidine there appears to be another protein catalyzing the transfer of propylamino group from decarboxylated adenosylmethionine to spermidine to yield spermine (spermine synthase) (8, 9). To date the separation of adenosylmethionine decarboxylase activity from the propylamino transferase activities has been demonstrated by partially purified preparations from rat prostate (7, 10), rat brain (9) and from yeast (11). Our preliminary data obtained with rat liver preparations (8) were also in agreement with the above results.

Very recently, however, Feldman et al. (12) reported that even a considerable purification of adenosylmethionine decarboxylase activity from rat liver did not abolish the stoichiometric coupling between CO_2 formation and

spermidine production from adenosylmethionine and putrescine by the purified decarboxylase preparation. Based on molecular weight determinations the authors concluded that it is unlikely that there were two separate proteins responsible for the decarboxylation of adenosylmethionine and the concomitant synthesis of spermidine. Because these results were contradictory to those obtained earlier with prostatic, liver, brain and yeast preparations (7,8,9,10,11), a careful re-examination of the liver system seemed desirable.

In the present communication we will show that also in rat liver at least three separable enzyme activities appear to be involved in the synthesis of spermidine and spermine from adenosylmethionine and the appropriate amine.

MATERIAL AND METHODS

Unlabelled adenosylmethionine and adenosylmethionine labelled at the carboxyl group (adenosylmethionine-1-^{14}C) were prepared enzymatically as described earlier (5,6). Decarboxylated adenosylmethionine was synthesized with the aid of partially purified *E. coli* adenosylmethionine decarboxylase (1) and purified on a Dowex 50-H^+ column followed by paper electrophoretic separation of decarboxylated adenosylmethionine from adenosylmethionine (9,11).

The assay conditions for adenosylmethionine decarboxylase (7,8), spermidine synthase (9,10) and spermine synthase (9) have recently been described in detail. Adenosylmethionine decarboxylase activity was assayed in the presence of 0.2 mM adenosylmethionine-1-^{14}C and a saturating concentration of putrescine (2.5 mM). The reaction mixture for spermidine synthase contained 0.5 mM putrescine-^{14}C and 0.1 mM decarboxylated adenosylmethionine. Spermine synthase was assayed in the presence of 0.5 mM spermidine-^{14}C and 0.1 mM decarboxylated adenosylmethionine. The other ingredients in the incubation mixtures have been described elsewhere (7,8, 9,10). One unit of enzyme activity is defined as the amount of the enzyme catalyzing the release of 1 nmole of CO_2 from adenosylmethionine-1-^{14}C, or the formation of 1 nmole of spermidine from putrescine-1-^{14}C, or the production of 1 nmole of spermine from spermidine-^{14}C in 30 min at 37° under conditions described above.

Purification of adenosylmethionine decarboxylase and spermidine synthase from rat liver. The present procedure is a modification of the procedures described recently (7,9,10). The 100 000 x g supernatant fraction (Crude extract) of liver homogenate, prepared in 25 mM Tris-HCl buffer, pH 7.2, containing 0.3 mM EDTA and 10 mM 2-mercaptoethanol was fractionated with solid ammonium sulphate (Mann special enzyme grade) at 0°. The fractions obtained by 0.3 - 0.6 and 0.6 - 0.75 saturation of ammonium

sulphate were dissolved in a small volume of 25 mM sodium phosphate buffer, pH 7.2, containing 0.3 mM EDTA and 0.5 mM dithiothreitol, and passed through a Sephadex G-25 column (Pharmacia, Sweden, 3 x 15 cm) previously equilibrated against the same buffer. The desalted samples were designated as Fraction 2A and 2B, respectively.

The proteins precipitated by ammonium sulphate between 0.3 - 0.6 saturation (Fraction 2A) were applied on a DEAE cellulose column (Whatman DE 52, 3 x 34 cm) equilibrated with the above buffer. The column was washed with 120 ml of the equilibration buffer and eluted with a linear gradient of 0.1 to 0.4 M NaCl prepared in the equilibration buffer (total gradient volume was 1000 ml). Adenosylmethionine decarboxylase activity was eluted between 335-450 ml. The most active fractions (65 ml) were pooled (Fraction 3A) for further purification. Adenosylmethionine decarboxylase activity was clearly separated from spermidine synthase activity that was eluted between 225-325 ml (see Fig. 1A). The most active fractions (66 ml) containing spermidine synthase activity were pooled (Fraction 3B) and used for further purification.

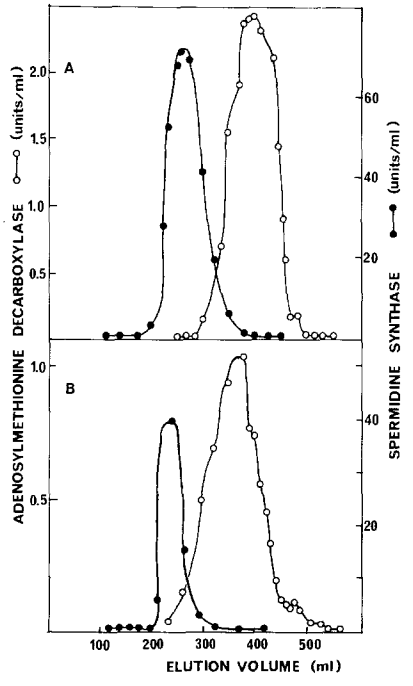

Fig. 1. Separation of liver adenosylmethionine decarboxylase and spermidine synthase activities on DEAE cellulose column. A. the ammonium sulphate fraction (Fraction 2A) was chromatographed on DEAE cellulose column as described in the text. B. a portion of the crude extract (Fraction 1, no previous ammonium sulphate fractionation) was chromatographed on the same column under identical elution conditions.

The Fraction 3A (adenosylmethionine decarboxylase) was chromatographed on a hydroxylapatite column (Bio-Gel HT, Bio-Rad Laboratories, 3 x 15 cm) previously equilibrated against 25 mM potassium phosphate buffer, pH 7.2, containing 0.5 mM dithiothreitol. The sample was washed into the column with a few ml of the equilibration buffer and a linear gradient of 0.025 M to 0.4 M potassium phosphate, pH 7.2, was connected with the column (total gradient volume was 500 ml). Adenosylmethionine decarboxylase activity was eluted from the column between 200-310 ml. The most active fractions (50 ml) were pooled (Fraction 4A), concentrated by ultrafiltration in a pressurized ultrafiltration cell (Amicon Corp.) to 5 ml and applied to a Sephadex G-200 column (2.5 x 30 cm) previously equilibrated against 25 mM Tris-HCl, pH 7.2, containing 50 mM KCl, 0.3 mM EDTA and 0.5 mM dithiothreitol. The adenosylmethionine decarboxylase activity was eluted at 1.8 to 2.0 times the void volume. The most active fractions were pooled (20 ml) and designated as Fraction 5A.

After the chromatography on DEAE cellulose the fractions containing spermidine synthase activity (Fraction 3B) were concentrated by ultrafiltration to 18 ml and chromatographed on a hydroxylapatite column as described above. Spermidine synthase activity emerged from the column close to the void volume but was nevertheless adsorbed. The most active fractions were pooled (34 ml. Fraction 4B), concentrated by ultrafiltration to 5 ml and applied to Sephadex G-200 column (see above). The elution volume of spermidine synthase activity from G-200 column was very close to that of adenosylmethionine decarboxylase. The most active fractions were pooled (16.5 ml) and designated as Fraction 5B.

RESULTS AND DISCUSSION

Table 1 summarizes the results of fractionation of the 100 000 x g supernatant fraction (Crude extract) of rat liver homogenate. The present procedure resulted in an about 900-fold purification of adenosylmethionine decarboxylase, resolving it from the spermidine synthase activity. The yield of adenosylmethionine decarboxylase activity in the final preparation (Fraction 5A) was 25 %. The purification of spermidine synthase activity was about 80-fold and the yield 15 % in the final preparation (Fraction 5B). This preparation did not contain any detectable adenosylmethionine decarboxylase activity. A partial separation of spermine synthase activity from the two above activities was already achieved by the ammonium sulphate fractionation. In agreement with our earlier results (8, 9), most of the spermine synthase activity was precipitated at higher concentrations of ammonium sulphate (0.60 - 0.75 saturation). Due to the low basal activity of spermine synthase

Table 1

Separation and partial purification of adenosylmethionine decarboxylase, spermidine synthase and spermine synthase from rat liver

Fraction	Enzyme activity, units/mg protein		
	Adenosyl-methionine decarboxylase	Spermidine synthase	Spermine synthase
1. Crude extract	0.04	2.02	0.02
2A. Ammonium sulphate fraction (0.3-0.6 saturation)	0.07	2.48	0.01
2B. Ammonium sulphate fraction (0.6-0.75 saturation)	0.01	0.09	0.09
3A. DEAE cellulose (pool I)	1.38	0.94	a.
3B. DEAE cellulose (pool II)	0.02	31.1	a.
4A. Hydroxylapatite of 3A	9.09	a.	a.
4B. Hydroxylapatite of 3B	a.	61.7	a.
5A. Sephadex G-200 of 4A	30.5	a.	a.
5B. Sephadex G-200 of 4B	a.	168	a.

Crude extract was obtained from 138 g of rat liver and processed as described in the text. a. = no detectable activity.

further attempts to purify this enzyme from the liver were abandoned. No spermine synthase activity was detected in the more refined preparations of adenosylmethionine decarboxylase and spermidine synthase. These results are consistent with the concept (8, 9) that separate proteins catalyze the synthesis of spermidine and spermine.

Chromatography of ammonium sulphate fraction (Fraction 2A) on DEAE cellulose column resulted in a distinct separation of adenosylmethionine decarboxylase activity from spermidine synthase activity with only little overlapping as shown in Fig. 1A. The stoichiometry between the decarboxylation of adenosylmethionine-1-^{14}C and the synthesis of spermidine from adenosylmethionine-2-^{14}C in the presence of putrescine (cf. refs. 9,10) was largely lost at this stage of purification: with the Fraction 3A the production of spermidine from adenosylmethionine-2-^{14}C was only 40 % as compared with the release of CO_2 from adenosylmethionine-1-^{14}C. The more refined preparations (4A, 5A, as well as 4B, 5B) did not promote any synthesis of spermidine from adenosylmethionine-2-^{14}C in the presence of

putrescine. Combination of Fractions 5A and 5B (cf. 10) readily catalyzed the synthesis of spermidine from adenosylmethionine and putrescine (results not shown).

The present results differ from those reported by Feldman et al. (12). The possibility that an exposure to high ionic strength, i. e. ammonium sulphate fractionation, might be the cause of the difference by dissociation of the hypothetical enzyme complex to components or subunits seems unlikely because adenosylmethionine decarboxylase activity was separated from spermidine synthase activity on DEAE cellulose chromatography even if ammonium sulphate fractionation was omitted (Fig. 1B). The inability to separate adenosylmethionine decarboxylase activity from spermidine synthase activity of a previous study (12) may be due to the large excess of the latter enzyme (some 50-100-fold) as compared with adenosylmethionine decarboxylase in crude extracts of rat liver (Table 1 and ref. 8).

Fig. 2 illustrates the results of gel filtration experiments with adenosylmethionine decarboxylase at different stages of the purification procedure. Adenosylmethionine decarboxylase activity from the crude extracts emerged from Sephadex G-200 column approximately at the same volume as did adenosylmethionine decarboxylase purified some 300-fold and containing no spermidine synthase activity. It therefore appears that the removal of spermidine synthase activity from adenosylmethionine decarboxylase did not appreciably change the molecular size of adenosylmethionine decarboxylase of rat liver.

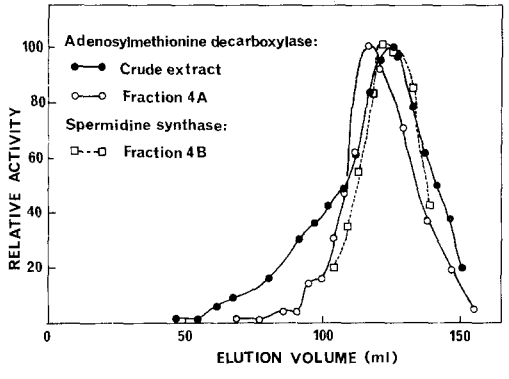

Fig. 2. Gel filtration of adenosylmethionine decarboxylase and spermidine synthase on Sephadex G-200 column. The gel filtrations were performed as described in the text using crude (Fraction 1) or partially purified (Fraction 4A) adenosylmethionine decarboxylase and partially purified (Fraction 4B) spermidine synthase preparations.

Even after the removal of spermidine synthase activity from the liver adenosylmethionine decarboxylase preparation, the decarboxylation was still strongly stimulated by putrescine. With the Fraction 5A, 2.5 mM putrescine resulted in about 7-fold stimulation of the decarboxylase activity. The experimentally determined K_m value for putrescine as an effector in the adenosylmethionine decarboxylase reaction was 0.005 mM. The apparent K_m value for putrescine as the substrate in spermidine synthesis (Fraction 5B) was about 0.1 mM both in the presence of 0.1 and 0.2 mM decarboxylated adenosylmethionine.

Several lines of evidence seem to indicate that the synthesis of spermidine and spermine in rat liver is accomplished by at least three different enzymes: a putrescine-activated adenosylmethionine decarboxylase and two propylamino transferases, one catalyzing the transfer of the propylamino group from decarboxylated adenosylmethionine to putrescine (spermidine synthase), the other to spermidine (spermine synthase). This is supported by the following observations: (i) Adenosylmethionine decarboxylase and spermidine synthase activities can be separated by ion exchange and adsorption chromatography even under "mild" conditions, i.e. without exposing the enzymes to high ionic strength. (ii) On gel filtration the size (Stokes radius) of adenosylmethionine decarboxylase in crude extracts appeared to be the same as after the spermidine synthase had been removed. Because the size of spermidine synthase, free of adenosylmethionine decarboxylase activity, was very close to that of purified adenosylmethionine decarboxylase (Fig. 2), one should expect a clear change in the molecular size of adenosylmethionine decarboxylase during the purification, if there was an enzyme complex that had been artificially dissociated. These results, however, must be regarded as preliminary ones and need further confirmation. (iii) The different affinities of these enzymes for putrescine (as an effector and a substrate) may also lend some support to this hypothesis. However, these data do not exclude the possible existence of a functional enzyme complex between adenosylmethionine decarboxylase and spermidine synthase during the synthesis of spermidine.

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