SEPARATION OF ENZYME ACTIVITIES CATALYSING SPERMIDINE AND SPERMINE SYNTHESIS IN RAT BRAIN

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1. Introduction

Previous work from Williams-Ashman's laboratory [1, 2] has demonstrated that the enzymic synthesis of spermidine and spermine in rat ventral prostate involves putrescine-activated decarboxylation of S-adenosyl-L-methionine (SAM) and the transfer of the propylamine group from decarboxylated SAM to putrescine or spermidine, yielding spermidine or spermine, respectively. Some evidence has also been provided which suggests that the same protein may catalyse both the conversion of putrescine to spermidine and the conversion of spermidine to spermine with SAM of decarboxylated SAM as the propylamine donor [2]. In disagreement with the latter notion, we observed that ammonium sulphate fractionation of soluble extracts from regenerating rat liver resulted in a partial separation of spermidine and spermine synthesizing enzyme activities [3]. The present paper describes separation and partial purification of enzymes from rat brain, which catalyse the synthesis of spermidine (spermidine synthase) or spermine (spermine synthase) from decarboxylated SAM and the appropriate amine. Both these enzyme preparations seem to be uncontaminated by the other and free from a significant amount of SAM decarboxylase activity. While this study was in progress, Jänne et al. [4, 5] reported that a prostatic enzyme catalysing the synthesis of spermidine, but not spermine, from decarboxylated SAM can be separated from putrescine-activated SAM decarboxylase.

2. Materials and methods

Labelled SAM was separated from either DL-methionine-2-^{14}C or DL-methionine-1-^{14}C largely as described by Pegg and Williams-Ashman [1]. Decarboxylated SAM was prepared from unlabelled SAM with the aid of crude SAM decarboxylase isolated from *E. coli* [3] and purified on a Dowex 50-H$^+$ column followed by preparative paper electrophoresis using 0.05 M citric acid buffer. The final preparation did not contain any SAM as a contaminant.

Putrescine-activated decarboxylation of SAM-^{14}COOH, as well as the synthesis of spermidine and spermine from SAM-2-^{14}C were determined as described earlier [3, 6]. The standard assay mixture for spermidine synthase contained, in a total volume of 0.2 ml, 20 μmoles of sodium phosphate pH 7.5 (final), 1 μmole of dithiotreitol [3], 0.1 μmole (0.2 μCi) of ^{14}C-putrescine, 0.03 μmole of decarboxylated SAM and the enzyme solution. The assay medium for spermine synthase was the same except that ^{14}C-putrescine was replaced by 0.1 μmole (0.15 μCi) of ^{14}C-spermidine. After 30 min incubation at 37 °C the reaction was stopped with one ml of 10% trichloroacetic acid. The radioactive products were purified by treatment of the samples on Dowex 50-H$^+$ columns [1, 2], followed by separation by paper electrophoresis [3]. Under the conditions used the rates of spermidine and spermine synthesis were linear for at least 30 min and proportional to the amount of added enzyme protein. The systems were not fully saturated with respect to putrescine, spermidine and decarboxylated SAM.

Separation of polyamine synthesizing enzyme activities from rat brain was carried out as follows.

The 100,000 *g* supernatant fraction of brain homogenate was obtained as described earlier [3]. All subsequent operations were performed at temperatures below 4 °C. The supernatant was made upto 1 mM with dithiothreitol (DTT) and fractionated with solid ammonium sulphate (Mann special enzyme grade) as indicated in table 2. The precipitates were dissolved in a small volume of 25 mM tris – 1 mM DTT – 0.3 mM EDTA, pH 7.2 (at 25 °C), and dialysed against the same solution for 12 hr. Dialysed samples were then applied on a DEAE cellulose column (Whatman DE52; 2.5 × 25 cm), previously equilibrated with the dialysing buffer. The column was washed with 100 ml of 25 mM tris – 2 mM DTT – 0.3 mM EDTA, pH 7.2, and then eluted with a linear gradient of 0 to 500 mM KCl made in the same buffer. Fractions of 10 ml were collected. The most active fractions (4–7 tubes) were pooled and fractionated with solid ammonium sulphate. Precipitates were dissolved in a small volume of 20 mM sodium phosphate – 1 mM DTT – 0.3 mM EDTA, pH 7.2, and dialysed against 100 volumes of the same buffer. Finally the dialysed samples were applied on a hydroxylapatite column (Hypatite C, Clarkson Chemicals Co., Inc. USA; 1.5 × 11 cm) previously equilibrated with the dialysing buffer. The column was first washed with 10–12 ml of the dialysing buffer, which eluted almost all the spermidine synthase activity (cf. [5]), followed by 20 ml of 40 mM sodium phosphate. The latter eluted most of the spermine synthase. The column was then connected to a linear gradient of 40 to 400 mM sodium phosphate containing 2 mM DTT and 0.3 mM EDTA (total volume 150 ml). SAM decarboxylase was eluted at about 0.12–0.18 M KCl. Fractions of 4 to 5 ml were collected. The most active fractions were pooled and concentrated with solid ammonium sulphate. The dialysed enzyme preparations were made up to 5 mM with DTT. They could be stored for at least a week at 0 °C without significant loss of enzyme activity.

Protein was determined by the method of Lowry et al. [6] or by ultraviolet absorption [7].

3. Results and discussion

The data in table 1 indicate considerable differences between various rat tissues in the activities of the enzymes synthesizing spermidine and spermine from SAM. The ratio of spermidine synthesis to spermine synthesis varied from 2.0 (brain) to 4.6 (testis). The brain was found to be fairly active in spermine synthesis. Because larger amounts of this tissue were more easily available as compared to rat prostate, explored earlier in Williams-Ashman's laboratory [1, 2], the brain was chosen for fractionation studies.

Table 1
Spermidine and spermine synthesizing enzyme activities of various rat tissues.

Tissue	Polyamine synthesis from SAM-2-^{14}C (pmoles/mg protein per 30 min)	
	Spermidine	Spermine
Ventral prostate	3460	832
Brain	563	279
Testis	505	110
Thymus	322	148
Pancreas	243	113
Spleen	104	53
Kidney	85	38
Liver	78	25

Two-month-old male Sprague-Dawley rats were used for analysis. Polyamine synthesis was assayed in the standard incubation medium as described earlier [3], using dialysed supernatant fractions of tissue homogenates as enzyme source. The values are the mean of two pooled samples, each obtained from three animals.

Table 2 summarizes the results of fractionation of the 100,000 *g* supernatant of rat brain. In agreement with our earlier results with regenerating liver [3], ammonium sulphate fractionation resulted in a partial separation of spermidine and spermine synthase activities. A further separation was achieved by gradient elution on a DEAE cellulose column and the second ammonium sulphate fractionation (3A and 3B). Hydroxylapatite treatment resulted in an enzyme fraction (4A-1) with very high spermidine synthase activity, but without any detectable spermine synthase or SAM decarboxylase activity. This result is consistent with the work of Jänne et al. [5] with prostatic preparations. Most of the spermine synthase was eluted with 0.04 M potassium phosphate. This fraction (4B) was practically free of spermidine synthase and SAM decarboxylase activity. Neither of these fractions (4A-1 and 4B) catalysed the synthesis of significant amounts of spermidine or spermine from SAM-2-^{14}C (cf. table

Table 2
Fractionation of polyamine synthesizing enzyme activities of rat brain.

Fraction		Polyamine synthesis from decarboxylated SAM nmoles/mg protein per 30 min		SAM decarboxylase nmoles $^{14}CO_2$/mg protein per 30 min
		Spermidine	Spermine	
1	Dialysed supernatant	2.65	1.50	0.39
2A	Ammonium sulphate fract. (30–50%) of 1	8.65	1.58	0.84
3A	DEAE cellulose and ammonium sulphate fract. (0–50%) of 2A	17.7	0.49	1.93
4A-1	Hydroxylapatite fract. (0.02 M) of 3A	402.0	n.d.	n.d.
4A-2	As above (0.12–0.17 M)	2.7	0.07	10.1
2B	Ammonium sulphate fract. (50–75%) of 1	1.32	2.30	0.29
3B	DEAE cellulose and ammonium sulphate fractionation (60–75%) of 2B	0.10	7.96	0.22
4B	Hydroxylapatite fract. (0.04 M) of 3B	0.09	25.8	0.02

n.d. = not detectable.

3). Fraction 4A-2 showed highest SAM decarboxylase activity and still contained some spermidine synthase, but only traces of spermine synthase. As with prostatic preparations [4] SAM decarboxylase (4A-2) was stimulated many fold by putrescine and to a lesser degree by spermidine, with practically no concomitant synthesis of spermidine or spermine (cf. table 3). Starting from the dialysed supernatant the overall purification of SAM decarboxylase and spermidine and spermine synthases was about 25, 150 and 17 fold respectively, and the recovery 6–8%.

Table 3
Spermine synthesis of S-adenosylmethionine by combined enzyme fractiond from rat brain.

Enzyme fraction	Spermine synthesis from SAM-2-^{14}C pmoles/30 min	Production of CO_2 from SAM-^{14}COOH pmoles/30 min
4B	19	14
4A-2	9	372
4B plus 4A-2	311	335

Spermine synthesis from SAM-2-^{14}C and decarboxylation of SAM-^{14}COOH were assayed in the presence of 2 mM spermidine [3], but without added putrescine. Enzyme fractions, 0.17 mg protein of 4A-2 and 0.15 mg of 4B per assay, were the same as shown in table 2.

The mixture of two enzyme fractions, one containing SAM decarboxylase, the other spermine synthase, catalysed the synthesis of spermine from SAM-2-^{14}C (table 3). This result, analogous to that reported by Jänne et al. [5] for SAM decarboxylase and spermidine synthase and confirmed by us, strongly suggests that at least three proteins, either separately or as enzyme complex(es), are needed for synthesis of spermidine and spermine in animal tissues. These include a SAM decarboxylase and two propylamine transferases, one specific for putrescine and the other for spermidine. It would be of interest to determine whether these enzyme activities change in parallel fashion e.g., in regenerating liver [6] and after various hormone treatments.

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