A RAPID ASSAY METHOD FOR SPERMIDINE AND SPERMINE SYNTHASES. DISTRIBUTION OF POLYAMINE-SYNTHESIZING ENZYMES AND METHIONINE ADENOSYLTRANSFERASE IN RAT TISSUES

Aarne RAINA, Raija-Leena PAJULA and Terho ELORANTA
Department of Biochemistry, University of Kuopio, P.O. Box 138, 70101 Kuopio 10, Finland

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1. Introduction

Two enzymes involved in the biosynthesis of polyamines [1] which catalyse the transfer of the propylamine group of decarboxylated *S*-adenosylmethionine (*S*-methyladenosylhomocysteamine) to putrescine (spermidine synthase) or spermidine (spermine synthase) have not been extensively characterized from eucaryotic sources. The slow progress in this area is obviously due to the tedious and time-consuming methods used for the assay of these enzymes [2–5]. In this paper we describe a rapid and sensitive isotopic method for the assay of spermidine and spermine synthases. The method is based on the isolation of the radioactive polyamines formed from radioactive decarboxylated *S*-adenosylmethionine labelled in the propylamine moiety by using phosphocellulose ion exchange paper. Our results demonstrate marked differences between different tissues in the activities of spermidine and spermine synthases. No parallelism was found between the activities of these enzymes. Neither was there any correlation between the synthase activities and the activity of *S*-adenosylmethionine decarboxylase, which is in agreement with the view that these are three different enzymes.

2. Materials and methods

L-[*Me*-^{14}C]Methionine (specific activity 56 mCi/mmol), S-adenosyl-L-[*Me*-^{14}C]methionine (55 mCi/mmol) and DL-[1-^{14}C]ornithine monohydrochloride (58 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, UK. DL-[1-^{14}C]Methionine (8.69 mCi/mmol), DL-[2-^{14}C]methionine (4.08 mCi/mmol), [1,4-^{14}C]putrescine dihydrochloride (11.27 mCi/mmol), [1,4-^{14}C]spermidine trihydrochloride (10.22 mCi/mmol) and [1,4-^{14}C]spermine tetrahydrochloride (18.75 mCi/mmol) were purchased from the New England Nuclear Corporation, Boston, Mass., USA. Before use, the radioactive putrescine and spermidine were purified on a Dowex 50-H$^+$ column [4], and DL-[1-^{14}C]ornithine was treated with hydrochloric acid [6].

Labelled *S*-adenosylmethionine was synthesized from either DL-[1-^{14}C]methionine or DL-[2-^{14}C]methionine essentially as described by Pegg and Williams-Ashman [2]. Radioactive decarboxylated *S*-adenosylmethionine was prepared from *S*-adenosylmethionine labelled at the C-2 position of the methionine moiety with the aid of adenosylmethionine decarboxylase from *E. coli* purified through Step 3 by the method of Wickner et al. [7]. Decarboxylated *S*-adenosylmethionine was purified on a Dowex 50-H$^+$ column and finally freed of any contaminating *S*-adenosylmethionine by preparative paper electrophoresis [8].

Dithiothreitol, putrescine dihydrochloride and spermidine trihydrochloride were purchased from Calbiochem, Los Angeles, Calif., USA. Whatman phosphocellulose paper P81 was obtained from Reeve Angel and Co., London, UK.

The animals used were albino rats of the Wistar strain fed ad libitum with a standard diet. The rats were decapitated under ether anaesthesia and the tissues were rapidly removed, washed in ice-cold

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0.25 M sucrose and homogenized either with a Potter-Elvehjem or an Ultra Turrax (Janke et Kunkel) homogenizer in 3–10 vol of 0.25 M sucrose containing 1 mM mercaptoethanol, 0.1 mM EDTA and 1 mM dithiothreitol. The homogenates were centrifuged for 45 min at 105 000 g_{av}. The supernatant fraction was used for the enzyme assays as such or after dialysis for 16 h against 200 vol of 10 mM potassium phosphate buffer (pH 7.4) containing 50 mM KCl, 1 mM mercaptoethanol, 0.1 mM EDTA and 0.1 mM dithiothreitol.

The activities of L-ornithine decarboxylase (EC 4.1.1.17), *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) and L-methionine adenosyltransferase (EC 2.5.1.6) were determined in undialysed supernatant fractions as previously described [9]. The assay of spermidine and spermine synthases were carried out in conical glass-stoppered centrifuge tubes (vol 4 ml) using dialysed supernatant fractions as the enzyme source. The assay mixture for spermidine synthase (EC 2.5.1.16) contained, in a total volume of 0.1 ml, 0.1 M potassium phosphate buffer, pH 7.4, 0.5 mM putrescine, 0.03 mM ^{14}C-labelled decarboxylated *S*-adenosylmethionine, 5 mM dithiothreitol and 0.05–0.6 mg of the enzyme protein. The blank values were determined with an enzyme preparation heated at 80°C for 5 min. After incubation at 37°C for 30 min, the reaction was stopped by adding 10 μl of 1 M potassium hydroxide. The tubes were thoroughly mixed, closed tightly and incubated for 30 min at 100°C to decompose the unreacted decarboxylated *S*-adenosylmethionine [2,4]. After cooling, 20 μl of 1 M perchloric acid was added, and the acid supernatant was separated by centrifugation. For separation of radioactive spermidine, a phosphocellulose ion-exchange paper (Whatman P81) was used, cf. [10].

An aliquot of 20–40 μl of the perchloric acid supernatant was applied on a double layer of Whatman P81 discs (diameter 24 mm). The paper discs were then thoroughly wetted with distilled water, washed on a Büchner funnel with 300 ml of 25 mM hydrochloric acid, dried and counted for radioactivity in a toluene-based scintillant [10]. The washing procedure efficiently removed the degradation products derived from decarboxylated *S*-adenosylmethionine, while 94–95% of the input radioactive spermidine was retained by the paper. The recovery of spermidine was checked from time to time. For the assay of spermine synthase activity the above procedure was modified as follows. In the assay mixture putrescine was replaced by 1 mM spermidine. Washing the paper discs were carried out using 300 ml of 50 mM hydrochloric acid. More than 99% of the radioactive spermine present in the perchloric acid supernatant was retained by the paper. The identity with spermidine or spermine of the radioactive products retained by the phosphocellulose paper under the above conditions was confirmed by paper electrophoresis [11].

3. Results and discussion

The present method employing phosphocellulose ion exchange paper for the isolation of the radioactive polyamine formed in the spermidine or spermine synthase reaction is rapid and clearly superior to the earlier ones [2–5]. This is especially true for the assay of spermine synthase activity. Admittedly the radioactive substrate, i.e. decarboxylated *S*-adenosylmethionine, is fairly expensive and not easy to prepare On the other hand, only a small amount of this substrate is needed for the assay because the K_M values for decarboxylated *S*-adenosylmethionine are of the order of 10^{-6} M [1].

No systematic study of the distribution of spermidine and spermine synthases in various tissues have been reported so far. This kind of study would help in understanding factors involved in the regulation of polyamine synthesis. Table 1 shows the activities of ornithine decarboxylase, *S*-adenosylmethionine decarboxylase as well as spermidine and spermine synthases in tissues of male rats at the age of two months. The tissue distribution of methionine adenosyltransferase, an enzyme producing substrate for both polyamine synthesis and biological transmethylations, is shown for comparison. The highest specific activity of spermidine synthase was observed in the pancreas. This observation and the high activity of *S*-adenosylmethionine decarboxylase might explain the high content of spermidine found in this particular tissue [11]. It is noteworthy that spermidine synthase activity was high in the uterus, but very low in the skeletal muscle. The spermine synthase activity was in most tissues low as compared to the activity of spermidine synthase, but was remarkably high in the brain, cf. [12]. Mixing brain extracts with those

Table 1
Distribution of polyamine-synthesizing enzyme activities and methionine adenosyltransferase in rat tissues

Tissue	Ornithine decarboxylase	*S*-adenosyl-methionine decarboxylase	Spermidine synthase	Spermine synthase	Methionine adenosyl-transferase
	(pmol $^{14}CO_2$/mg protein per 30 min)		(pmol/mg protein per 30 min)		(pmol/mg protein per min)
Brain	14	296 ± 11	3090 ± 150	3220 ± 240	79 ± 10
Heart	37	50	1200	270	41
Kidneys	75	74	1450	509	307
Liver	7	68 ± 8	3220 ± 200	266 ± 33	5610 ± 260
Lungs	20	28	1980	394	38
Pancreas	7	289	37 800	563	558
Prostate	941	1170	18 100	2740	141
Skeletal muscle	8	39	250	277	14
Small intestine	201	151	5910	666	78
Spleen	12	86	4080	889	65
Testes	99	190 ± 21	2180 ± 230	207 ± 79	70 ± 6
Thymus	63	123	5710	1090	212
Uterus	38	100	9170	792	96

Except for uterus, tissues of male rats aged 10 weeks were used for analysis. For determination of ornithine decarboxylase activity, aliquots of 6 tissue extracts were pooled. The values for the other enzyme activities represent the means (± S.D.) of 2 or 3 pooled samples, each obtained by combining 3 and 2 organs, respectively.

obtained from liver, pancreas, prostate or skeletal muscle did not reveal the presence of inhibitors or activators of the synthases in these tissues (results not shown). In general, there was no parallelism in the activities of spermidine and spermine synthases in different tissues. Neither was there any correlation between the activity of *S*-adenosylmethionine decarboxylase and the activities of spermidine and spermine synthases. This result is in accordance with the view that separate enzymes catalyse the decarboxylation step and the subsequent transfer of the propylamine group [1].

The changes with age in the activities of polyamine-synthesizing enzymes in four rat tissues is shown in fig.1. Except for brain, the activities of spermidine and spermine synthases were highest in tissues of newborn animals. The very low activity of spermidine synthase found in the skeletal muscle may explain the low spermidine content of this tissue in adult rats [13]. In agreement with an earlier report [14], the activity of *S*-adenosylmethionine decarboxylase in the brain was found to be low at the time of birth.

The assay method for spermidine and spermine synthases described in this paper makes it more

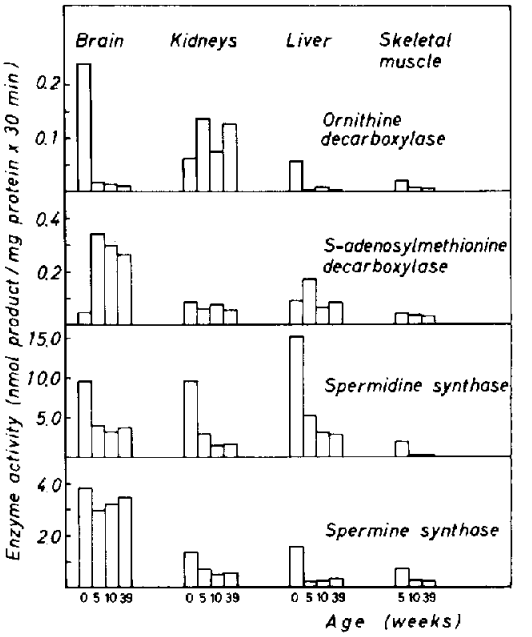

Fig.1. Effect of age on the activities of polyamine-synthesizing enzymes in some tissues of the rat. The values are means of 1 to 3 pooled samples, each obtained by combining 2–10 organs or tissues. For further details see the legend to table 1.

feasible to characterize these enzymes and to screen various compounds as inhibitors of polyamine synthesis. The inhibitors would be valuable in evaluating the suggested role of polyamines in growth processes [1].

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