Purification and partial characterization of human polyamine synthases

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Spermidine synthase was purified to apparent homogeneity from human spleens (8700-fold) by affinity chromatography. The native enzyme was composed of two subunits of identical M_r (35000) and showed an apparent M_r of 62000 in pore-gradient gel electrophoresis. Its pI was 5.1. Spermine synthase was purified to apparent homogeneity from placenta (5300-fold) and from kidney (4600-fold). The native enzyme was composed of two subunits of identical M_r (45000) and showed an apparent M_r of 78000 in pore-gradient gel electrophoresis. In isoelectric focusing it revealed two bands, with pI values of 4.9 and 5.0. Both synthases were present in all human tissues studied, but revealed a clear tissue-specific pattern. Mouse antisera against spermidine synthase revealed only one band, of M_r 35000, in all purified enzyme preparations and in crude human tissue extracts in immunoblotting. Antisera against spermine synthase showed an immunoreactive band corresponding to the M_r of the subunit of spermine synthase. These antisera did not indicate any cross-reactivity in immunoblotting. Thus spermine synthase and spermidine synthase do not share homologous antigenic sites and are totally different proteins.

INTRODUCTION

The polyamines spermidine and spermine are natural polycations present in all mammalian cells. They interact with negatively charged cellular elements such as cell membranes and DNA, and are considered essential for cell growth and differentiation (Tabor & Tabor, 1984; Pegg, 1986; Porter & Sufrin, 1986). Inhibition of polyamine synthesis results in cessation of cell growth, and is cytotoxic in some cell models (Jänne *et al.*, 1978; Marton *et al.*, 1985). In the pathway for polyamine biosynthesis, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase catalyse the synthesis of their respective precursors, putrescine and decarboxylated *S*-adenosylmethionine. The two decarboxylases have been thoroughly studied, e.g. purified to homogeneity from several sources and characterized with antibodies, and their genes have been cloned [Hickok *et al.* (1987); Tabor & Tabor (1987); for other references see Tabor & Tabor (1984) and Pegg (1986)]. The enzymes synthesizing spermidine and spermine, i.e. the aminopropyltransferases spermidine synthase and spermine synthase respectively, are less well characterized. Previously, spermidine synthase has been purified from rodent (Samejima & Yamanoha, 1982; Yamanoha & Samejima, 1984) and bovine sources (Raina *et al.*, 1984), and spermine synthase only from bovine brain (Pajula *et al.*, 1979). The synthases have not been analysed or purified from human tissues previously. In the present paper we describe purification of the human synthases, their tissue distributions, some of their properties and their immunological characterization with polyclonal antibodies.

EXPERIMENTAL

Chemicals

1,4-Diaminobutane (putrescine), spermidine and spermine as their hydrochloride salts were obtained from Calbiochem, Los Angeles, CA, U.S.A. These amines were purified as previously described (Pajula *et al.*, 1979). *S*-Adenosyl-(5′)-3-thiopropylamine linked to Sepharose and the dihydrogen sulphate of *S*-adenosyl-(5′)-3-methylthiopropylamine (decarboxylated adenosylmethionine) were generously given by Dr. Keijiro Samejima, Josai University, Sakado, Japan.

DL-[2-¹⁴C]Methionine (sp. radioactivity 46.8 mCi/mmol) was supplied by New England Nuclear Corp., Boston, MA, U.S.A. Radioactive *S*-adenosylmethionine was synthesized from DL-[2-¹⁴C]methionine essentially as described by Pegg & Williams-Ashman (1969). Radioactive *S*-adenosyl-(5′)-3-methylthiopropylamine was enzymically prepared from *S*-adenosylmethionine labelled at C-2 of the methionine moiety (Pajula *et al.*, 1979), by using adenosylmethionine decarboxylase extracted from *Escherichia coli* strain HT 383/p SPD1 (Markham *et al.*, 1983). Unlabelled decarboxylated adenosylmethionine was prepared in a similar way. The products were purified as described previously (Raina *et al.*, 1984).

Preparation of spermine-Sepharose has been described previously in detail (Pajula *et al.*, 1978).

Dithiothreitol was purchased from Calbiochem, and (NH₄)₂SO₄ and sucrose (both special enzyme grade) were from Schwarz/Mann, Spring Valley, NY, U.S.A. Whatman phosphocellulose paper P81 was from Reeve

Abbreviation used: PMSF, phenylmethanesulphonyl fluoride (Sigma P-7626).

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Angel and Co., London E.C.4, U.K. CH-Sepharose (6-aminohexanoic acid linked to Sepharose) 4B was from Pharmacia Fine Chemicals, Uppsala, Sweden.

All other chemicals used were reagent-grade materials from various commercial sources.

Enzyme assays

The activity assays of spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22) were performed as previously described in detail (Raina *et al.*, 1976, 1983b; Pajula *et al.*, 1979). Under standard assay conditions the reaction mixture contained, in a total volume of 0.1 ml, 0.1 M-potassium phosphate buffer, pH 7.4, 5 mM-dithiothreitol, 1 mM-putrescine or -spermidine respectively, 0.01 mM-decarboxylated *S*-adenosylmethionine labelled at C-1 of the propylamine group (7 mCi/mmol) and enzyme protein. The reaction was stopped after incubation for 10–30 min at 37 °C by adding 0.01 ml of 1 M-KOH. The tubes were thoroughly stirred and incubated for 30 min at 100 °C. After cooling, 0.02 ml of 1 M-HClO₄ was added, and the acid supernatant was separated by centrifugation at 3000 *g* for 5 min. An 0.02 ml portion of the supernatant was applied on a disc of phosphocellulose ion-exchange paper (Whatman P81). The numbered discs were then washed in a beaker with 10 mM-Tris/HCl buffer, pH 8.0, containing 100 mM-NaCl, by using mechanical stirring and three buffer changes at 10 min intervals.

Tissue preparation

Tissue samples for assaying synthase activities were obtained from forensic autopsies (sudden accidents as the cause of death) performed within 2 days from death, and the samples were stored at –80 °C until used. The tissues were homogenized in 3–4 vol. of 0.25 M-sucrose containing 0.1 mM-EDTA, 2 mM-2-mercaptoethanol and 0.2 mM-PSMF (Sigma Chemical Co.). The homogenates were centrifuged at 100 000 *g*_{av} for 45 min at +4 °C. The supernatant fractions were filtered through glass wool, applied to a Sephadex G-25 column (Pharmacia PD10) to remove excess salts, and eluted by 25 mM-Tris/HCl buffer, pH 7.4, containing 2 mM-2-mercaptoethanol. The eluted fractions were used for the enzyme assays.

Analytical electrophoretic techniques

Pore-gradient gel electrophoresis was performed by using PAA 4/30 gradient gels from Pharmacia as described by Kajander & Raina (1981). To localize enzyme activities in the gel, duplicate samples were run. One lane was stained for protein, and the other was cut into 1 mm sections that were crushed and dissolved in the enzyme-activity assay mixture, and thereafter the usual activity assays were performed.

SDS/polyacrylamide-gel electrophoresis was performed in slabs of 10% (w/v) acrylamide with an overlay of 3% acrylamide and the buffer system of Laemmli (1970). The separated proteins were stained either with Coomassie Brilliant Blue or with AgNO₃ (Wray *et al.*, 1981).

Isoelectric focusing was performed with PhastGel IEF 3-9 (Pharmacia), according to the manufacturer's instructions. The pH profiles of the gels were established by using the pI calibration-kit proteins from Pharmacia.

Protein determination

Protein was measured by the method of Bradford

(1976) or by that of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard.

Immunization

Antibodies against the purified enzymes were prepared in mice (spermidine synthase) or in rabbits (spermine synthase). Enzyme protein (20 µg) was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously at several sites. Boosters of 15 µg of enzyme were mixed with Freund's incomplete adjuvant and administered three times at 2-week intervals. A week after the last injection, 10 µg of enzyme in phosphate-buffered saline (116 mM-NaCl, 20.8 mM-Na₂HPO₄, 2.9 mM-KH₂PO₄) was given intraperitoneally, and after 3 days blood was collected by cardiac puncture. The antisera were stored at –20 °C.

Immunoblotting

Samples containing 1–250 µg of protein were loaded on to each slot in a standard SDS/polyacrylamide slab gel. After electrophoresis, the proteins were electrophoretically transferred to nitrocellulose with a Trans-Blot apparatus (Bio-Rad). After transfer, the nitrocellulose was processed as in Billings *et al.* (1983) by saturating for 1 h in blocking solution (2% powdered milk solution in phosphate-buffered saline), followed by reaction with 1:200 diluted mouse (spermidine synthase) or rabbit (spermine synthase) antiserum in the blocking solution overnight at +4 °C. The filters were then washed and incubated with a 1:500 dilution of alkaline phosphatase-conjugated rabbit anti-(mouse immunoglobulins) serum (Dakopatts, Copenhagen, Denmark) or goat anti-(rabbit IgG) serum (Bio-Yeda, Rehovot, Israel) in blocking solution for 2 h. After washes in phosphate-buffered saline, filters were developed with 1 mg of 5-bromo-4-chloro-indol-3-yl phosphate (Sigma Chemical Co.)/ml in 0.15 M-2-methyl-2-aminopropan-1-ol-buffer containing 1 mM-MgCl₂, washed in distilled water and air-dried.

Purification of spermidine synthase from human spleen

Four human spleens obtained from autopsies performed within 2 days from death were stored at –80 °C until used. The spleens (650 g) were homogenized in 3 vol. of 0.25 M-sucrose containing 0.1 M-EDTA, 2 mM-2-mercaptoethanol and 0.2 mM-PMSF, with an Omni-Mixer (Sorvall). The homogenate was centrifuged at 14 000 *g*_{av} for 30 min at +4 °C. The supernatant fraction was filtered through glass wool. The crude supernatant (1585 ml) was fractionated with solid (NH₄)₂SO₄. The proteins precipitated between 35% and 55% saturation with (NH₄)₂SO₄ were collected by centrifugation at 14 000 *g*_{av} for 30 min, dissolved in 160 ml of 10 mM-Tris/HCl buffer, pH 7.1 (at 20 °C), containing 0.08 M-NaCl and 4 mM-2-mercaptoethanol (buffer A) and dialysed at +4 °C overnight against 60 vol. of the same buffer, the buffer being changed twice. The dialysed fraction was centrifuged at 14 000 *g*_{av} for 20 min at +4 °C to remove any precipitated protein.

The dialysed (NH₄)₂SO₄ fraction (233 ml) was applied to a DEAE-cellulose (Whatman DE-52) column (8.5 cm × 34 cm) equilibrated with buffer A. The column was washed with 1000 ml of buffer A containing 1 mM-dithiothreitol and connected to a linear gradient of 0.08–0.4 M-NaCl in buffer A (total gradient volume was 4000 ml). Spermidine synthase activity was eluted

between 0.19 M- and 0.26 M-NaCl. The active fractions (900 ml) were pooled, 2-mercaptoethanol and EDTA were added to 5 mM and 1 mM respectively, and the fractions were adjusted to 70% saturation with $(\text{NH}_4)_2\text{SO}_4$ to concentrate the enzyme. The precipitate was dissolved in 55 ml of 50 mM-Tris/HCl buffer, pH 7.1 (at 20 °C), containing 0.15 M-NaCl, 0.1 mM-EDTA and 1 mM-dithiothreitol (buffer B), dialysed overnight against 180 vol. of the same buffer and centrifuged at 14000 $g_{av.}$ for 10 min.

The dialysed DEAE-cellulose fraction (75 ml) was applied to a spermine-Sepharose column (1.6 cm $\times$ 12 cm), equilibrated with buffer B. The column was washed with 30 ml of buffer B. Spermidine synthase was eluted with 200 ml of the same buffer containing 0.3 M-NaCl. The active fractions were pooled and concentrated by 70% saturation with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was dissolved in 25 ml of 20 mM-potassium phosphate buffer, pH 7.2, containing 0.3 M-NaCl, 0.1 mM-EDTA and 0.1 mM-dithiothreitol (buffer C), dialysed overnight against 150 vol. of the same buffer, the buffer being changed twice during dialysis, and centrifuged at 14000 $g_{av.}$ for 10 min.

The dialysed spermine-Sepharose fraction (32 ml) was applied to a *S*-adenosyl-(5')-3-thiopropylamine-Sepharose column (1 cm $\times$ 6 cm), equilibrated with buffer C, at a flow rate of 0.2 ml/min. The column was washed with 40 ml of buffer C, followed by buffer C containing 0.6 M-NaCl (100 ml). Spermidine synthase was then eluted with buffer C containing 0.4 mM-decarboxylated adenosylmethionine synthesized chemically (Samejima *et al.*, 1978). The active fractions (24 ml) were pooled and concentrated to a final volume of 0.55 ml by ultrafiltration in a Centrifo CF 25 cone (Amicon Corp., Danvers, MA, U.S.A.). The enzyme preparation was stored at -80 °C in 20% glycerol.

Spermidine synthase from bovine brain and spleen was purified as described by Raina *et al.* (1984).

Purification of spermine synthase from human placenta

Human full-term placentas were obtained fresh after normal births from a local hospital and stored at -80 °C until used. The placentas (1.5 kg) were homogenized in 3 vol. of 0.25 M-sucrose containing 0.1 mM-EDTA, 2 mM-2-mercaptoethanol and 0.2 mM-PMSF, with an Omni-Mixer, and the homogenate was centrifuged at 14000 $g_{av.}$ for 30 min. After filtration through glass wool the crude supernatant (3330 ml) was fractionated with solid $(\text{NH}_4)_2\text{SO}_4$. The proteins precipitated between 50% and 75% saturation of $(\text{NH}_4)_2\text{SO}_4$ were collected by centrifugation at 14000 $g_{av.}$ for 30 min, dissolved in 500 ml of 10 mM-Tris/HCl buffer, pH 7.1 (at 20 °C), containing 0.08 M-NaCl and 0.5 mM-2-mercaptoethanol (buffer A), and dialysed overnight against 20 vol. of the same buffer, the buffer being changed twice during dialysis. The dialysed fraction was centrifuged at 14000 $g_{av.}$ for 20 min.

The dialysed $(\text{NH}_4)_2\text{SO}_4$ fraction (740 ml) was applied to a DEAE-cellulose (Whatman DE-52) column (8.5 cm $\times$ 30 cm) equilibrated with buffer A containing 1 mM-2-mercaptoethanol. The column was washed with 900 ml of buffer A containing 5 mM-2-mercaptoethanol and connected to a linear gradient of 0.08–0.5 M-NaCl in buffer A (total gradient volume was 4000 ml). Spermine synthase activity was eluted between 0.23 M- and 0.34 M-NaCl. The active fractions (790 ml) were pooled, 2-

mercaptoethanol was added up to 5 mM, and the solution was then brought to 80% saturation with $(\text{NH}_4)_2\text{SO}_4$ to concentrate the enzyme. The precipitate was dissolved in 82 ml of 50 mM-Tris/HCl buffer, pH 7.1 (at 20 °C), containing 0.15 M-NaCl, 0.1 mM-EDTA and 0.5 mM-2-mercaptoethanol (buffer B), dialysed overnight against 120 vol. of the same buffer and centrifuged at 14000 $g_{av.}$ for 10 min. Dithiothreitol was added up to 5 mM. The dialysed fraction (108 ml) was then applied to a spermine-Sepharose (1.6 cm $\times$ 11 cm) column, equilibrated with buffer B. The column was washed with 30 ml of buffer B containing 1 mM-dithiothreitol and with 150 ml of the same buffer containing 0.3 M-NaCl. Spermine synthase activity was eluted with 60 ml of buffer B containing 0.3 M-NaCl and 1 mM-spermidine. The active fractions were pooled (48 ml), concentrated by ultrafiltration in a Centrifo CF/25 cone to a final volume of 5.0 ml and dialysed overnight against 3 $\times$ 2 litres of the equilibration buffer, containing 5 mM-2-mercaptoethanol, to remove excess salt and spermidine.

The second and third affinity chromatographies were performed on a spermine-Sepharose column (1 cm $\times$ 5 cm) equilibrated as described above. The dialysed enzyme preparation (5.2 and 2.5 ml respectively) was applied to the column. The washing and elution were carried out with the same buffers (6, 16, and 18 ml respectively) as in the first affinity chromatography. The active fractions were concentrated by ultrafiltration in a Centrifo cone. The final volume was 1.27 ml. Glycerol was added up to 20% concentration, and the enzyme preparation was stored at -80 °C.

Spermine synthase from bovine brain and spleen was purified as described by Pajula *et al.* (1979).

RESULTS

Distribution of spermidine synthase and spermine synthase

No data about the distributions of spermidine synthase and spermine synthase in human tissues have been previously published. We surveyed the distributions of the synthase activities in order to find most suitable

Table 1. Spermidine synthase and spermine synthase activities in human tissues

Placentas were obtained from normal births, and brain samples were from medical autopsies. All other tissue samples were obtained from forensic autopsies. Tissue preparations were carried out as described in the Experimental section. Enzyme activities were measured from the 100000 $g_{av.}$ -supernatant fractions after removal of salts with a Sephadex G-25 column. Enzyme activities are expressed as pmol/min per mg of soluble protein and are means $\pm$ S.E.M. of 7–8 tissue preparations.

Tissue	Spermidine synthase	Spermine synthase
Liver (<i>n</i> = 8)	25 $\pm$ 2.7	8 $\pm$ 2.4
Spleen (<i>n</i> = 8)	37 $\pm$ 6.6	10 $\pm$ 3.4
Kidney (<i>n</i> = 8)	21 $\pm$ 1.4	77 $\pm$ 9.1
Pancreas (<i>n</i> = 8)	203 $\pm$ 45	8 $\pm$ 1.5
Lung (<i>n</i> = 8)	10 $\pm$ 2.7	6 $\pm$ 2.1
Full-term placenta (<i>n</i> = 7)	10 $\pm$ 1.5	49 $\pm$ 6.2
Brain cortex, frontal lobe (<i>n</i> = 7)	108 $\pm$ 2.2	38 $\pm$ 5.6

Table 2. Purification of spermidine synthase from human spleen

The crude extract was prepared from 650 g of spleen tissue and processed as described in the Experimental section. Spermidine synthase activity was determined under standard assay conditions.

Purification step	Total protein (mg)	Specific catalytic activity (nmol/min per mg of protein)	Total activity (nmol/min)	Yield (%)	Purification factor (fold)
Crude supernatant	39 000	0.076	2970	100	1
(NH ₄) ₂ SO ₄ fractionation	4340	0.353	1530	51.7	4.6
DEAE-cellulose chromatography	627	2.22	1390	47.0	29.2
Spermine-Sepharose	290	3.23	938	31.6	42.5
Adenosylthiopropylamine-Sepharose	0.35	660	231	7.8	8680

starting material for the enzyme purification. Table 1 shows the synthase activities from desalted 100 000 g_{av.} supernatant fractions prepared from seven different human tissues. Both synthases were present in all tissues studied, but large differences in the enzyme activities were observed. Spermidine synthase activity surpassed that of spermine synthase in all other tissues except the kidney and placenta.

The spleen was chosen as the raw material for the purification of spermidine synthase, since this synthase activity was most stable in the spleen tissue or its preparations during storage and freeze-thawings. Activity in the pancreas was lost rapidly during storage of the tissue homogenate (results not shown). Spermine synthase activity was well preserved in the homogenates of the kidney and the placenta (results not shown).

Purification of spermidine synthase and spermine synthase

Purification data of spermidine synthase from human spleens are shown in Table 2. Purification involved (NH₄)₂SO₄ fractionation, DEAE-cellulose chromatography and two affinity-chromatographic steps. Spermine-Sepharose chromatography was used to absorb spermine synthase activity from the enzyme preparation. Thereafter, spermidine synthase bound on to *S*-adenosyl-(5')-3-thiopropylamine linked to Sepharose was eluted biospecifically with decarboxylated *S*-adenosylmethionine. The final preparation had a specific catalytic activity about 8700-fold that of the crude supernatant enzyme.

Purification data of spermine synthase from human placentas are shown in Table 3. Purification involved (NH₄)₂SO₄ fractionation, DEAE-cellulose chromatography and affinity chromatography on spermine-Sepharose. Spermine synthase bound on spermine-Sepharose was eluted biospecifically with spermidine. This enzyme preparation still contained contaminating proteins. The preparation was dialysed and concentrated and then subjected to repeated chromatography on spermine-Sepharose. After the third spermine-Sepharose chromatography, the final preparation had a specific catalytic activity of about 5300-fold that of the crude supernatant enzyme. Spermine synthase was also purified from human kidneys 4600-fold by the same procedures (results not shown).

Properties of the purified spermidine synthase

Purified human spleen spermidine synthase (10 µg) was subjected to SDS/polyacrylamide-gel electrophoresis. Silver staining of the gel revealed one band, corresponding to an *M_r* of 35 000. Scanning the gel indicated that at least 98 % of all stained material was in that band. When subjected to pore-gradient gel electrophoresis the native enzyme showed a minor microheterogeneity, as shown in Fig. 1, lane 1. The enzyme activity co-migrated with the protein band (results not shown). The apparent *M_r* for the human spleen spermidine synthase was 62 000. This was identical with that of the bovine brain enzyme shown as a barely

Table 3. Purification of spermine synthase from human full-term placenta

The crude extract was prepared from 1500 g of placenta and processed as described in the Experimental section. Enzyme activity was determined under standard assay conditions.

Purification step	Total protein (mg)	Specific catalytic activity (nmol/min per mg of protein)	Total activity (nmol/min)	Yield (%)	Purification factor (fold)
Crude supernatant	56 100	0.0857	4800	100	1
(NH ₄) ₂ SO ₄ fractionation	32 700	0.107	3510	73.1	1.2
DEAE-cellulose chromatography	1100	0.785	862	17.9	9.2
Spermine-Sepharose I	4.80	127	609	12.7	1480
Spermine-Sepharose III	1.40	455	632	13.2	5310

Fig. 1. Pore gradient-gel electrophoresis of native spermidine synthase and spermine synthase

Samples (4 μ g) of purified human enzymes were subjected to PAA 4/30 (Pharmacia) gradient-gel electrophoresis for 16 h at 120 V together with the marker proteins and the bovine enzymes and stained with Coomassie Brilliant Blue. Lanes: 1, spermidine synthase from human spleen; 2, spermine synthase from human kidney; 3, spermidine synthase from bovine brain; 4, spermine synthase from bovine spleen. Markers: a, thyroglobulin (M_r 669 000); b, ferritin (M_r 440 000); c, catalase (M_r 232 000); d, lactate dehydrogenase (M_r 140 000); e, bovine serum albumin (M_r 67 000).

visible band in Fig. 1, lane 3. It appears that the native enzyme is composed of two subunits of equal molecular masses. In pore-gradient electrophoresis, proteins of different sizes are sieved out by the smallness of the pores according to the smallest dimension (shape). Thus the native spermidine synthase is probably not a spherical protein. Isoelectric focusing revealed one band corresponding to a pI of 5.1 (Fig. 2, lane 1), identical with that of the bovine brain enzyme (results not shown).

Properties of the purified spermine synthase

Purified human kidney spermine synthase (10 μ g) was subjected to SDS/polyacrylamide-gel electrophoresis. Coomassie Blue staining revealed one major band corresponding to an M_r of 45 000. Scanning the gel indicated that about 95 % of all stained material was in that band. The placental enzyme revealed one band, corresponding to an identical M_r of 45 000. When subjected to pore-gradient gel electrophoresis the native kidney enzyme migrated as a sharp band corresponding to an apparent M_r of 78 000 (Fig. 1, lane 2). The enzyme activity co-

Fig. 2. Isoelectric focusing of spermidine synthase and spermine synthase purified from human tissues

Samples (20 ng) of spleen spermidine synthase (1) and placental spermine synthase (2) were subjected to isoelectric focusing on Phast Gel IEF 3-9 (Pharmacia). After focusing, the gel was stained with AgNO_3 , and the pI values were deduced with the aid of pI-calibration-kit proteins (Pharmacia).

migrated with the protein band (results not shown). Bovine spleen enzyme (Fig. 1, lane 4) showed a major band corresponding to a M_r of 85 000. The high- M_r bands visible in the bovine preparation were shown to have enzyme activity (results not shown), and are thus likely to be aggregates produced by long storage of the enzyme. Spermine synthase appears to be composed of two subunits of identical molecular masses. The native enzyme does not seem to have a spherical shape. In isoelectric focusing the placental spermine synthase revealed microheterogeneity, with two bands corresponding to pI values of 4.9 and 5.0 (Fig. 2, lane 2). Identical patterns were seen with the human kidney and bovine spleen enzymes (results not shown).

Enzyme kinetic properties

The purified spermidine synthase preparation did not catalyse any spermine formation, and under standard assay conditions the purified spermine synthase preparation did not catalyse spermidine formation. Kinetic analysis of both synthase reactions was carried out with crude enzyme preparations because the by-product, methylthioadenosine, inhibits spermine synthase, and to a lesser extent spermidine synthase (Pajula & Raina, 1979). Crude enzyme preparations contain the

Fig. 3. Immunoblots of spermidine synthase and spermine synthase

Samples were run on SDS/10% (w/v)-polyacrylamide gel and treated with mouse antiserum against spermidine synthase (X) or with rabbit antiserum against spermine synthase (Z), as described in the Experimental section. Lanes: 1, DEAE-cellulose-chromatographic fraction of human spleen preparation containing 160 μ g of protein, including both spermidine synthase and spermine synthase; 2, DEAE-cellulose-chromatographic fraction of bovine spleen preparation containing 120 μ g of protein including both spermidine synthase and spermine synthase; TPI, 50 μ g of prestained triosephosphate isomerase (Sigma no. T-9400), M_r 26600; 3, 1 μ g of homogeneous spermidine synthase from bovine spleen; 4, 1 μ g of purified spermine synthase from human placenta.

phosphorylase that splits methylthioadenosine. Both synthase reactions followed Michaelis-Menten kinetics.

Spermidine synthase in crude spleen extracts had K_m values of 0.08 mM for putrescine and 7 μ M for decarboxylated *S*-adenosylmethionine. The corresponding K_m values of crude spermine synthase from human placenta were 0.2 mM for spermidine and 1 μ M for decarboxylated *S*-adenosylmethionine.

Immunological characterization of the synthases

Mouse immune sera were prepared separately against both synthases. The polyclonal antibody against human spleen spermidine synthase detected purified human and bovine spermidine synthases (M_r 35000 band), but not spermine synthase. Anti-(spermine synthase) sera detected both human kidney and placental spermine synthases (M_r 45000 band) as well as the bovine spleen and brain enzymes, but not spermidine synthase (Fig. 3). The M_r 35000 and M_r 45000 bands were identified by the specific antisera for all purification steps by the immunoblotting technique.

As shown in Fig. 4, when crude extracts of several human tissues were subjected to Western-blot analysis

with the antiserum to spermidine synthase, bands corresponding to a M_r of 35000 were specifically detected. Differences in the intensities of the bands were approximately proportional to the differences in the enzyme activities, except for the pancreas extract. This is probably due to the very high protease activity in pancreas extracts. Also, the spermidine synthase activity was lost in the pancreas extracts during even short periods of storage. Immunoreactive enzyme protein was most abundant in spleen and kidney extracts. The malignant human B-lymphocyte WI-L2 cell extract gave a strong band of equal M_r . The enzyme activity in these extracts was 165 pmol/min per mg of soluble protein.

DISCUSSION

Mammalian cells have separate spermidine synthase and spermine synthase activities for the synthesis of the polyamines. In this paper we describe for the first time the tissue distribution, purification and partial characterization of the human polyamine-synthesizing enzymes.

The purification procedure for spermidine synthase

Fig. 4. Distribution of immunoreactive spermidine synthase in human tissues

Samples of crude, gel-filtered supernatant fractions of tissue homogenates, each containing 250 μ g of protein (lanes 1–5 and 8), were run on SDS/10% (w/v)-polyacrylamide gels, transferred to a nitrocellulose membrane, treated with mouse anti-(spermidine synthase) serum, followed by alkaline phosphatase-conjugated anti-mouse IgG, and were then stained with bromochloro-indolylphosphate, as described in the Experimental section. Lanes: 1, liver; 2, spleen; 3, kidney; 4, pancreas; 5, lung; 6, 3 μ g of spermidine synthase purified from human spleen; 7 and 8, human B-lymphocyte WI-L2 cell extract (50 and 250 μ g of protein).

was modified from that used previously by Samejima and co-workers (Samejima & Yamanoha, 1982; Samejima *et al.*, 1983) and Raina *et al.* (1984) for the purification of rat prostate and bovine brain enzyme respectively. The modifications included adjustment of $(\text{NH}_4)_2\text{SO}_4$ precipitation, use of spermine-Sepharose and elimination of the gel filtration and the hydroxyapatite steps. Re-evaluation of the affinity chromatography using *S*-adenosyl-(5')-3-thiopropylamine linked to Sepharose revealed that both spermidine synthase and spermine synthase were bound on the column, the latter enzyme even more tightly (T. O. Eloranta, unpublished work), and could not be well separated from each other. Spermine-Sepharose was effective in removing spermine synthase from the preparation before the final affinity purification. The purification factor and catalytic activity of the spermidine synthase from human spleen (8700-fold, specific activity 660 nmol/min per mg) are in the same range as those published for the enzymes isolated from bovine brain [7100-fold, 712 nmol/min per mg (Samejima *et al.*, 1983); 11000-fold, 1121 nmol/min per mg (Raina *et al.*, 1984)] and rat prostate [4500-fold, 1300 nmol/min per mg (Samejima *et al.*, 1983)]. The apparent M_r , subunit composition, pI and enzyme-kinetic data of human spleen spermidine synthase are very similar to those of the enzyme isolated from rat prostate (Samejima & Yamanoha, 1982) and bovine brain (Raina *et al.*, 1984).

Spermine synthase was purified by using spermine

coupled to Sepharose (Pajula *et al.*, 1979). After coupling, this ligand resembles spermidine, the substrate of spermidine synthase. Affinity chromatography with this positively charged ligand is complicated by non-specific ionic binding and by apparently specific binding with other proteins present in the tissue preparations. Both placenta and kidney extracts contain proteins that bind spermidine tightly (E. O. Kajander, unpublished work). By carefully adjusting the chromatographic conditions and repeating the spermine-Sepharose step three times, it was possible to obtain practically homogeneous spermine synthase preparation. The purification factor and catalytic activity of the human placental spermine synthase (5300-fold, specific activity 455 nmol/min per mg) are fairly similar to those of the enzymes purified from bovine brain [6000-fold, 396 nmol/min per mg (Pajula *et al.*, 1979)] and from rat prostate [2300-fold, 206 nmol/min per mg (Raina *et al.*, 1983a)]. The apparent M_r , subunit composition and isoelectric-focusing data are identical with those previously reported for the bovine brain enzyme (Pajula *et al.*, 1979). K_m values for decarboxylated *S*-adenosylmethionine and spermidine of the crude human enzyme preparations were 3–5-fold higher than those of crude bovine enzyme preparations or those from other sources (Hannonen *et al.*, 1972b; Pegg *et al.*, 1981). However, kinetic analyses in the crude extracts are complicated because human placenta and kidney contain proteins that bind spermidine tightly. Thus the obtained K_m value for spermidine is probably too high.

Antisera to human spleen spermidine synthase detected the enzyme from human and bovine sources. Only one band was obtained, which suggests the presence of a single form of spermidine synthase in these species. Similarly, antisera to human placental spermine synthase detected a single band from the purified human and bovine enzyme preparations. There was no cross-reactivity in Western blots between spermidine synthase and spermine synthase. Lack of homologous antigenic sites suggests that these proteins have unique structures. Human and bovine spermidine synthases are apparently closely related. The same seems to be true for spermine synthases in these mammalian species. No indication of any tissue- or species-specific isoenzymic forms of either enzyme was found.

Both synthase activities were present in all human tissues studied. Very high spermidine synthase activity was present in the pancreas, where spermidine synthase surpassed spermine synthase activity 25-fold. In the rat pancreas this value was 66-fold (Raina *et al.*, 1983a). Human kidney contained the highest spermine synthase activity, and in both the kidney and the placenta spermine synthase surpassed spermidine synthase activity 3- and 5-fold respectively. However, in the rat kidney spermidine synthase surpassed spermine synthase activity (Raina *et al.*, 1983a) 3-fold. Thus there are large differences in both synthase activities in various tissues. Variations between species do occur in this tissue-specific activity pattern. Since both synthases compete for the same rate-limiting substrate (decarboxylated *S*-adenosylmethionine), their relative activities may be important factors in the regulation of cellular pool fractions of spermidine and spermine, especially ratios of the 'nascent' pools. We have shown (Kajander *et al.*, 1988) that in some malignant human tumours and cultured malignant cells spermidine synthase activity is elevated. In the rat, spermidine

synthase, but not spermine synthase, is known to be induced after partial hepatectomy (Hannonen *et al.*, 1972a) or testosterone stimulation of the seminal vesicle (Käpyaho *et al.*, 1980). Further work is warranted to verify whether the synthase activities do affect the relative polyamine pools, and whether spermidine, spermine or their relative ratio perform specific functions in differentiated cells.

This work was supported by grants from the National Research Councils for Medical and Natural Sciences, Finland. The skilful technical assistance of Miss Heli Martikainen and Miss Eija Korhonen is gratefully acknowledged.

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Received 17 November 1988/18 January 1989; accepted 18 January 1989