The same preparations promoted the synthesis of spermidine from decarboxylated AMe (prepared with the E. coli enzyme) at about four times the rate observed with AMe as substrate. All attempts by Pegg and Williams-Ashman (4) to separate the spermidine synthase system from the enzyme catalyzing decarboxylation of AMe were unfruitful. A number of lines of evidence, including consideration of affinities for AMe or exogenous decarboxylated AMe, hinted that spermidine (4) and spermine (5) were formed by partially purified soluble prostatic enzyme systems via mechanisms which did not involve free decarboxylated AMe as an intermediate.

This paper describes a procedure for a much more extensive purification of the putrescine-activated AMe decarboxylase of rat ventral prostate. The coupling between CO_2 release and production of spermidine (in the presence of putrescine), or of spermine (in the presence of spermidine), is broken at later stages of the isolation procedure, so that the more purified preparations exhibit high amine-enhanced decarboxylase activity with very little or no concomitant formation of polyamines.

Very recently, Raina and Hannonen (6) reported that various preparations obtained by ammonium sulfate fractionation of regenerating rat liver extracts did not exhibit constancy in the ratio of their activities in promoting spermidine and spermine synthesis from AMe as compared with decarboxylated AMe as propylamino group precursors, and that very low levels (0.02 mM) of putrescine induced small and variable increases in spermine synthesis in the presence of AMe, but not of decarboxylated AMe. Previously, higher concentrations of putrescine were found to depress spermine synthesis from AMe or decarboxylated AMe in regenerating liver (7), and rat prostate (5).

EXPERIMENTAL PROCEDURES

Unlabeled AMe was prepared with partially purified rat liver ATP:L-methionine S-adenosyl transferase as described previously (4). AMe-^{14}COOH was synthesized using DL-methionine-1-^{14}C (New England Nuclear, specific radioactivity 3.54 mCi/mmole).

Radioactive AMe-2-^{14}C was prepared similarly using DL-methionine-2-^{14}C (New England Nuclear, specific radioactivity 4.08 mCi/mmole). All AMe preparations were stored at pH 2 at -20°. The standard assay system for AMe decarboxylase activity contained the following ingredients in a final volume of 0.5 ml: 50 µmoles of sodium phosphate buffer of pH 7.2; 0.1 µmole of AMe-^{14}COOH; 2.5 µmoles of dithiothreitol; either 1.25 µmoles of putrescine or 2.5 µmoles of spermidine; and the enzyme solution. The $^{14}CO_2$ released was trapped into 0.1 ml of Hyamine hydroxide present in a polypropylene center well attached to the rubber stopper. The vessels were incubated for 30 min at 37°. The reaction was halted by injection of 0.5 ml of 40% (w/v) trichloroacetic acid through the rubber cap; the tubes were then incubated for a further 20 min at 25° in the closed system in order to ensure complete release of all bound CO_2. All counts were corrected for blanks containing no enzyme. Radioactivity was determined using a dioxane-based scintillation fluid (4).

When the formation of spermidine or spermine from AMe-2-^{14}C was estimated, the reactions were stopped with trichloroacetic acid as described above, and 0.5 µmoles of spermidine and spermine were added to the mixture after removal of the protein precipitate by centrifugation. A slurry (1 ml) of Dowex-50-H^+ (50% w/v in 1 N HCl) was added together with water to bring the total volume to about 5 ml. The tubes were mixed several times over a period of 30 min, and centrifuged. The ion-exchanger was washed 3-5 times with 0.5 N HCl. The polyamines were then eluted by two subsequent washings with 4 ml and 2 ml of 6 N HCl respectively. The combined eluates were evaporated to dryness under reduced pressure at 60°. The residues were dissolved in a specified volume (0.25-0.40 ml) of 0.01 N HCl. A small aliquot of this material was then spotted on paper, and subjected to electrophoresis in 0.1 M citric acid, pH 3.6 (8). The papers were sprayed with ninhydrin so as to visualize the polyamines; the appropriate spots were cut out and their radioactivity determined. The same paper electrophoretic

system was used to separate AMe from decarboxylated AMe, both of which were visualized with an ultraviolet lamp. An alternative procedure was sometimes employed for determination of spermine and its radioactivity. The reactions were halted with trichloroacetic acid; after centrifugation the samples were alkalinized and digested for 30 min at 100° (4) so as to degrade AMe. After cooling, the samples were cooled, acidified, and then processed with a Dowex-50-H^+ slurry as described above. The recovery of labeled spermidine and spermine by this method was greater than 85%. All other methods are described elsewhere (4, 5).

Purification of prostatic AMe decarboxylase. The ventral prostate glands from Sprague-Dawley rats weighing over 300 g were homogenized with two volumes of ice-cold 25 mM sodium phosphate of pH 7.2 containing 5 mM dithiothreitol and 0.1 mM EDTA. All operations were carried out at 0-2° . The homogenate was centrifuged at 25,000 x g_{max} for 10 min. The precipitate was washed with a small volume of the homogenization medium. The combined supernatant fluids were centrifuged at 45,000 x g_{max} for 90 min. The resulting soluble extract was passed through a plug of glass wool, and then fractionated by addition of solid ammonium sulfate. The proteins precipitating between 0.4 and 0.65 saturation contained practically all of the AMe decarboxylase activity of the crude extract. This ammonium sulfate precipitate (Fraction 2) was dissolved in 10 ml of homogenization medium and dialyzed overnight against 100 volumes of the same buffer. The dialyzed material was applied to a column of Serva DEAE cellulose (2.5 cm x 25 cm, previously equilibrated against 25 mM sodium phosphate of pH 7.2 containing 2.5 mM dithiothreitol and 0.1 mM EDTA). After washing the column with a further 150 ml of equilibrating solution, AMe decarboxylase was eluted with a linear gradient of 0.1 M to 0.4 M NaCl dissolved in the same solution, fractions of 10 ml being collected. The total volume of the gradient system was 400 ml. Four to five of the most active fractions were pooled (Fraction 3). This material was fractionated by addition of solid ammonium sulfate; the

proteins precipitating between 0.4 and 0.65 saturation were collected and dissolved in 5 ml of 25 mM sodium phosphate of pH 7.2 containing 2.5 mM dithiothreitol (Fraction 4). The latter fraction was chromatographed on a column of hydroxylapatite (Bio-Gel HT, Bio-Rad Laboratories, 2.5 cm x 10 cm, previously equilibrated against the buffer used to dissolve Fraction 4). Elution of AMe decarboxylase was accomplished with a linear gradient (0.1 M to 0.45 M) of sodium phosphate, the total volume of the gradient system being 200 ml. The three most active fractions of 10 ml volume were pooled (Fraction 5) and concentrated by precipitation with ammonium sulfate at 0.75 saturation. This material was immediately subject to gel filtration on a column of Bio-Gel P-200 (Bio-Rad Laboratories, 2.5 cm x 31 cm, previously equilibrated against 25 mM sodium phosphate of pH 7.2 containing 5 mM dithiothreitol and 0.1 mM EDTA), using flow adaptors and an upflow technique. The active material was pooled and designated as Fraction 6.

Table 1

Purification of AMe decarboxylase from rat prostate

Fraction	Total protein mg	AMe decarboxylase activity*		
		No amines added	Plus putrescine (2.5 mM)	Plus spermidine (5 mM)
1. Crude extract+	3 250	0.19	1.64	0.31
2. Ammonium sulfate 0.4 - 0.65 saturation	1 810	0.26	2.31	0.41
3. DEAE cellulose	111	2.3	15.6	3.4
4. Ammonium sulfate 0.4 - 0.65 saturation	34.8	4.6	24.2	5.8
5. Hydroxylapatite	5.3	19.0	112	29.3
6. Bio-Gel P-200	0.46	135	834	212

*nmoles CO_2 released/mg protein/30 min.

+Material obtained from 72 g of ventral prostate tissue

RESULTS AND DISCUSSION

Table 1 illustrates a typical purification of prostatic AMe decarboxylase. The final (Fraction 6) material represented a purification of about 700-fold (10% recovery) when activity was measured in the absence of added amines or in the presence of spermidine, and a purification of over 500-fold (7% recovery) when assayed in the presence of putrescine. The stimulation by putrescine dropped from 8,6-fold in Fraction 1 to 6.2-fold in Fraction 6; enhancement of CO_2 release by spermidine remained practically constant (1.5-fold) in all fractions. The Fraction 6 material lost less than 20% of its decarboxylase activity on storage at 2° for 2 weeks. Decarboxylation of AMe in the absence or presence of putrescine by Fraction 6 enzyme was unaffected by addition of pyridoxal-5-phosphate (0.2 mM), $MgCl_2$ (30 mM) or EDTA (10 mM).

Many experiments confirmed previous findings (4, 5) that with fairly impure enzyme preparations (Fractions 1, 2 and 3) there was a close correspondence between CO_2 release from AMe and the formation of spermidine (in the presence of putrescine) or of spermine (with spermidine added). However, after chromatography on hydroxylapatite (Fraction 5), the decarboxylase activity was still enhanced strongly by putrescine and spermidine, whereas hardly any concomitant synthesis of spermidine and spermine respectively was demonstrable. Fraction 6 preparations did not form any spermidine or spermine, and in the absence or presence of putrescine, they catalyzed a release of CO_2 that was practically stoichiometric with the formation of a substance which behaved like decarboxylated AMe on paper electrophoresis.

Table 2 shows that with the most purified preparations, stimulation of AMe decarboxylation by putrescine declined markedly when the pH was raised over the range of 5.8 to 8.7; maximal rates of CO_2 formation occurred at a higher pH in the absence as compared with the presence of putrescine. This confirms previous results (5) obtained with cruder enzyme preparations in which coupling between AMe decarboxylation and spermi-

Table 2

Effect of pH on AMe decarboxylase activity in the absence or presence of putrescine

pH	CO_2 released (nmoles/30 min)		Stimulation by putrescine (fold)
	No amines added	Plus putrescine (2.5 mM)	
5.8	0.006	0.16	26.7
7.0	0.25	1.90	7.6
7.8	0.47	1.65	3.5
8.7	0.41	0.93	2.3

Fraction 6 preparation (2.4 μg protein) was incubated under standard incubation conditions in the presence or absence of 2.5 mM putrescine. The pH of sodium phosphate buffer was varied as indicated. The pH values indicated represent experimentally determined values at the beginning of the incubation.

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dine synthesis was not disrupted. Under the conditions used in Table 2 the concentration of AMe (0.2 mM) is nearly a saturating one if putrescine is present; the experimentally determined K_m for AMe (with classical Michaelis-Menten kinetics) was 0.06 mM, a value which agrees closely with that obtained previously (4) with cruder preparations. In the absence of putrescine at pH 7.2, the kinetics of CO_2 release as a function of AMe concentration are complex, and the apparent K_m for AMe is 0.12 mM, so that the level of AMe in the standard test system minus putrescine as in Table 2 is not a saturating one.

The reason for the loss of coupling between AMe decarboxylation and the synthesis of spermidine or spermine at later stages of the purification procedure is not known. It could be related to removal of entirely separate propylamino transferase(s) of the type discovered by Tabor (9) in E. coli, or, on the contrary, to inactivation of sites or subunits necessary for polyamine synthesis that might be part of a single enzyme complex which also decarboxylates AMe (cf. 4).

We are extremely grateful to Dr. A. Raina for communicating to us the findings cited as reference 6 prior to their publication. This work was supported in part by a research grant (HD-04592) from the U. S. Public Health Service.

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