SEPARATION OF TWO PROTEINS REQUIRED FOR SYNTHESIS OF SPERMIDINE FROM S-ADENOSYL-L-METHIONINE AND PUTRESCINE IN RAT PROSTATE

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Received January 18, 1971

Abstract: An enzyme catalyzing transfer of a propylamino group from exogenous decarboxylated S-adenosyl methionine to putrescine to form spermidine (spermidine synthase) has been separated from the putrescine-activated S-adenosyl methionine decarboxylase in soluble extracts of rat ventral prostate. Neither enzyme alone catalyzes the formation of significant quantities of spermidine from S-adenosyl methionine and putrescine, a reaction which proceeds readily on addition of suitable proportions of the two separable enzymes. Aspects of the interaction of these two proteins in the biosynthesis of spermidine by rat prostate are discussed.

Pegg and Williams-Ashman (1) showed that partially purified, soluble enzyme preparations from rat ventral prostate catalyzed the following reactions: (a) Decarboxylation of S-adenosyl methionine (AMe) which was stimulated many fold and in a specific fashion by putrescine; (b) Stoichiometric formation of spermidine, CO_2 and 5'-methylthioadenosine (MTA) from putrescine and AMe; (c) Synthesis of spermidine from putrescine and exogenous decarboxylated AMe, the latter compound being prepared with the aid of the specific decarboxylase discovered in Escherichia coli by Tabor (2). These findings indicated that the over-all synthesis of spermidine by rat prostate involved the following linked reactions:

$$\text{AMe} \longrightarrow \text{decarboxylated AMe} + CO_2$$

$$\text{decarboxylated AMe} + \text{putrescine} \longrightarrow \text{spermidine} + CO_2 + \text{MTA} + H^+$$

Sum: $$\text{AMe} + \text{putrescine} \longrightarrow \text{spermidine} + CO_2 + \text{MTA} + H^+$$

Previously, Tabor et al. (3) provided evidence that these reactions accounted for the formation of spermidine in E. coli. Two entirely separate enzymes from E. coli catalyze the two intermediary reactions: (a) An AMe decarboxylase, which is activated by Mg^{++} but not by putrescine, and which contains enzyme-bound pyruvate as a prosthetic group (4); (b) A propylamino transferase (spermidine synthase) which utilizes decarboxylated AMe so formed to donate a propylamino moiety to putrescine to form spermidine and MTA (5). However, Pegg and Williams-Ashman (1) were unable to separate the rat ventral prostate putrescine-activated AMe decarboxylase from enzyme(s) catalyzing the synthesis of spermidine with either AMe or exogenous decarboxylated AMe as the propylamino group donor. This eventuality, as well as considerations of affinities of the prostatic enzyme system for AMe, decarboxylated AMe, and putrescine in regard to the kinetics of spermidine production suggested that free decarboxylated AMe might not be an intermediate in spermidine biosynthesis by the prostate enzyme system (1).

Jänne and Williams-Ashman (6) recently purified the putrescine-activated AMe decarboxylase of rat prostate to a very high degree. Although evolution of CO_2 from AMe was enhanced many fold by putrescine and spermidine by enzyme preparations obtained at every step of the purification procedure, the more highly purified preparations failed to catalyze any formation of spermidine from AMe and putrescine. We will show here that a prostatic enzyme catalyzing synthesis of spermidine from added decarboxylated AMe and putrescine can be separated from the putrescine-activated AMe decarboxylase. Coupling between spermidine synthesis from AMe and the decarboxylation of AMe can be re-established by addition of the two enzyme preparations in suitable proportions. Pertinent to our findings is a recent preliminary report of Raina and Hannonen (7) indicating that, in regenerating rat liver, enzyme(s) catalyzing the synthesis of spermidine and spermine from added decarboxylated AMe are distinguishable from putrescine-activated AMe decarboxylase.

EXPERIMENTAL PROCEDURES

AMe in the unlabeled form, or prepared from DL-methionine containing ^{14}C on carbon-1 (AMe-^{14}COOH) or carbon 2 (AMe-2-^{14}C) were synthesized enzymically as described previously (1). Decarboxylated AMe was prepared with the aid of the E. coli AMe decarboxylase, purified from frozen cells through the ammonium sulfate fraction described by Wickner et al. (4). The decarboxylated AMe was purified by chromatography on Amberlite CG 50 type 2 ion-exchange resins (cf. 2 for the elution conditions). The purity of the preparations of decarboxylated AMe was checked by paper electrophoresis using 0.1 M citric acid of pH 3.0 as buffer (cf. 6). The preparations of decarboxylated AMe were entirely free of the AMe used as starting material.

The activity of putrescine-activated decarboxylation of AMe-^{14}COOH was assayed as described earlier (6). The standard assay system for spermidine synthase activity contained the following ingredients in a final volume of 0.25 ml: 25 µmoles of sodium phosphate of pH 7.2; 0.025 µmole of unlabeled decarboxylated AMe; 0.25 µmole of putrescine-1,4-^{14}C (400,000 cpm); 1.25 µmoles of dithiothreitol, and the enzyme solution. The reactions were initiated by addition of enzyme, and halted, after an incubation period of 30 min at 37°, with 1 ml of 10% (w/v) trichloroacetic acid. After centrifugation, 0.5 µmole of carrier spermidine was added to each tube. Radioisotope incorporated into spermidine was determined after isolation of the latter free from putrescine by a procedure involving a Dowex ion-exchanger and final paper electrophoresis (6).

Putrescine-activated AMe decarboxylase was purified from rat ventral prostate through the fraction 6 described by Jänne and Williams-Ashman (6). Separation of spermidine synthase from AMe decarboxylase activity was achieved as follows. The initial stages of the purification procedure involving fractionation with ammonium sulfate and then on DEAE-cellulose columns were as previously described (6). However, the two enzyme activities were partially separated by chromatography on DEAE-cellulose, the spermidine synthase activity being eluted earlier than the AMe decar-

boxylase when a linear gradient of 0.1 M to 0.4 M NaCl was applied to the protein adsorbed on the column (cf. 6). Practically complete separation of spermidine synthase activity from AMe decarboxylase was achieved by application of the fraction from the DEAE cellulose column containing the former enzyme to a column of hydroxylapatite (Bio-Gel HT, Bio Rad Laboratories, 2.5 x 15 cm) previously equilibrated with 25 mM sodium phosphate, pH 7.2, containing 2.5 mM dithiothreitol. Four of the most active fractions containing spermidine synthase activity obtained from the DEAE-cellulose column were pooled and concentrated by precipitation with ammonium sulfate at 0.8 saturation at 0°. The resulting proteins were dissolved in 5 ml of the equilibration buffer. The sample was applied to the column at hydrostatic pressure and the top of the column was washed with a few ml of the buffer. The column was then connected to the fraction collector, and eluted with a linear gradient of potassium phosphate from 0.05 M to 0.5 M in the above buffer at a flow rate of 125 ml/h. The total volume of the gradient was 200 ml and fractions of 10 ml were collected. The spermidine synthase activity was hardly adsorbed to the column, and was eluted very close to the void volume. However, in addition to this major peak of activity, an additional minor peak of spermidine synthase activity eluted later. The residual AMe decarboxylase activity was eluted at about 0.2 - 0.25 M potassium phosphate, and was clearly separated from the synthase activity. The three most active fractions of the major peak containing spermidine synthase activity were pooled, concentrated by ammonium sulfate precipitation at 0.8 saturation, and dialyzed overnight against 20 vol of 25 mM sodium phosphate, pH 7.2, containing 5 mM dithiothreitol and 0.1 mM EDTA. The final spermidine synthase preparation was stored at 2°. All enzyme fractionations were carried out at 0-2°.

One unit of enzyme activity was defined as the amount of enzyme causing release of 1 nmole of CO_2 from AMe-^{14}COOH, or the formation of 1 nmole of spermidine from ^{14}C-putrescine and decarboxylated AMe in 30 min at 37° under the conditions described either above or in the protocols.

RESULTS AND DISCUSSION

A previous communication (6) described the complete separation of putrescine-enhanced prostatic AMe decarboxylase from enzyme(s) catalyzing the over-all synthesis of spermidine from AMe and putrescine. Table 1 illustrates the virtually complete separation of an enzyme in rat ventral prostate catalyzing formation of spermidine from decarboxylated AMe and putrescine (spermidine synthase) from putrescine-activated AMe decarboxylase. The ability of Fraction 3 preparations to promote spermidine synthesis was stable to storage at 2° for several days. Under the experimental conditions summarized in Table 1, the formation of spermidine from added decarboxylated AMe exhibited a broad pH optimum at around pH 8. This is in contrast to the pH optimum of about 7 for the prostatic AMe decarboxylase when the latter enzyme was assayed in the presence of an excess of putrescine (6). Paper electrophoretic analyses (8) showed that,

Table 1

Partial purification of a prostatic enzyme catalyzing synthesis of spermidine from decarboxylated S-adenosyl methionine and putrescine (spermidine synthase)

Fraction	Enzyme activity, units/mg protein: Spermidine synthase	AMe decarboxylase
1. Ammonium sulfate fraction 0.40-0.65 saturation	2.1	2.8
2. DEAE cellulose	42	2.1
3. Hydroxylapatite	175	0.8

AMe decarboxylase activity was assayed by liberation of CO_2 from AMe^{14}COOH in the presence of 2.5 mM putrescine, and spermidine synthase activity was determined by formation of spermidine from ^{14}C-putrescine and decarboxylated AMe, as described in the text.

under the conditions in Table I, no radioactivity whatsoever was incorporated into spermine when spermidine was synthesized from decarboxylated AMe and putrescine. The

apparent K_m for decarboxylated AMe in the presence of putrescine (1 mM) at pH 7.2 was determined to be about 0.05 mM in experiments with Fraction 3 preparations of the spermidine synthase.

Table 2 shows that the stoichiometric formation of CO_2 and spermidine from AMe and putrescine can be re-established by mixing suitable amounts of preparations of prostatic AMe decarboxylase and spermidine synthase whose activities are virtually uncontaminated by one another. At the end of the 30 min incubation in the experiment depicted in Table 2, the final concentration of decarboxylated AMe formed from AMe in the presence of the two enzymes necessary for spermidine synthesis from AMe and putrescine would be expected to be about 0.0015 mM if no coupling between AMe decarboxylation and spermidine synthesis had occurred, since we have shown previously (6) that the purified AMe decarboxylase alone forms CO_2 and decarboxylated AMe in equimolar proportions. This value for the AMe concentration (0.0015 mM) is about 3% of the K_m for decarboxylated AMe in the spermidine synthase reaction. It would be

Table 2

Requirement for two enzyme fractions for the synthesis of spermidine from S-adenosyl methionine and putrescine in rat prostate

Enzyme or combination	Product formed (nmoles/30 min)	
	CO_2 from AMe-^{14}COOH	Spermidine from AMe-2-^{14}C
AMe decarboxylase	1.38	0.02
Spermidine synthase	0.15	0.19
AMe decarboxylase plus spermidine synthase	1.53	1.47

The incubation mixture contained, in a final volume of 1.0 ml: 100 µmoles sodium phosphate of pH 7.2; 2.5 µmoles putrescine; 0.2 µmole of AMe-^{14}COOH or AMe-2-^{14}C as indicated; 5 µmoles of dithiothreitol; purified prostatic AMe decarboxylase of activity shown and/or prostatic spermidine synthase (30 units of enzyme obtained from hydroxylapatite column, as in Table 1). The tubes were incubated for 30 min at 37°.

of obvious interest to examine whether addition of a large pool of unlabeled decarboxylated AMe would affect the entry of radioactivity from AMe-2-^{14}C into spermidine when the two enzyme preparations were incubated together in the presence of putrescine, as this might help to decide whether enzyme-bound rather than free decarboxylated AMe is an intermediate in the over-all synthesis of spermidine from AMe and putrescine. Unfortunately, such experiments are difficult to design unequivocally, because the release of CO_2 and formation of decarboxylated AMe catalyzed by the highly purified preparations of prostatic AMe decarboxylase is very powerfully inhibited by added unlabeled decarboxylated AMe in either the presence or absence of putrescine (more than 70% inhibition by 0.1 mM decarboxylated AMe when the initial concentration of AMe-^{14}COOH was 0.2 mM).

We thank Dr. A. Raina for communicating to us the findings described in reference No. 7 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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