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S-Adenosylmethionine Decarboxylase from Human Prostate

ACTIVATION BY PUTRESCINE

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1. The presence of *S*-adenosylmethionine decarboxylase in human prostate gland is reported. A satisfactory radiochemical enzymic assay was developed and the enzyme was partially characterized. 2. Putrescine stimulates the reaction rate by up to 6-fold at pH 7.5: the apparent activation constant was estimated to be 0.13 mM. The stimulation is pH-dependent and a maximal effect is observed at acid pH values. 3. Putrescine activation is rather specific: other polyamines, such as spermidine and spermine, did not show any appreciable effect. 4. The apparent K_m for the substrate is 4×10^{-5} M. The calculated *S*-adenosylmethionine content of human prostate (0.18 μmol/g wet wt. of tissue) demonstrates that the cellular amounts of sulphonium compound are saturating with respect to the enzyme. 5. The enzyme is moderately stable at 0°C and is rapidly inactivated at 40°C. The optimum pH is about 7.5, with one-half of the maximal activity occurring at pH 6.6. 6. Several *carboxy*-^{14}C-labelled analogues and derivatives of *S*-adenosylmethionine were tested as substrates. The enzyme appears to be highly specific: the replacement of the 6′-amino group of the sulphonium compound alone results in a complete loss of activity. 7. Inhibition of the enzyme activity by several carbonyl reagents suggests an involvement of either pyridoxal phosphate or pyruvate in the catalytic process. 8. The inhibitory effect of thiol reagents indicates the presence of 'essential' thiol groups.

Polyamines are distributed in a rather ubiquitous way in several bacterial cells, in plants and most mammalian tissues (Tabor & Tabor, 1964; Raina *et al.*, 1966); among them, prostate gland and seminal fluid are particularly rich in spermine and spermidine (Mann, 1964; Rhodes & Williams-Ashman, 1964; Williams-Ashman, 1965).

During the last decade the physiological role(s) of the polyamines spermine and spermidine has been widely investigated (Herbst & Bachrach, 1970). Several functions have been attributed to these polycations: they have been found to stabilize ribosomal structure (Cohen & Lichtenstein, 1960; Norton *et al.*, 1968), stimulate aminoacyl-tRNA synthetases (Doctor *et al.*, 1970), and activate RNA synthesis by DNA-dependent RNA polymerase (Abraham, 1968). The nature of interactions between polyamines and polynucleotides or cell membranes has also been elucidated (Liquori *et al.*, 1967; Gabbay *et al.*, 1970; Silver *et al.*, 1970). Moreover, a 'general' regulatory role of polyamines in the rate of transcription has been proposed (Raina & Jänne, 1970).

Polyamine biosynthesis was first investigated in *Escherichia coli* mainly by Tabor and his colleagues (Tabor *et al.*, 1958; Tabor, 1962*a*,*b*) and more recently in rat ventral prostate (Pegg & Williams-Ashman, 1969, 1970). In both systems decarboxylation of *S*-adenosyl-L-methionine, followed by the transfer of the propylamine moiety to putrescine, represents the first step of the overall biosynthesis. Spermidine formed from the latter reaction is in turn converted into spermine by reacting with a second molecule of *S*-adenosyl(5′)-3-methylthiopropylamine (decarboxylated *S*-adenosyl-L-methionine).

S-Adenosyl-L-methionine decarboxylase has been extensively purified from *E. coli* and pyruvate has been identified as the prosthetic group (Wickner *et al.*, 1970). The enzyme has also been partially characterized in *Saccharomyces cerevisiae* (Jänne *et al.*, 1971*b*) and in rat liver (Feldman *et al.*, 1971, 1972; Hannonen *et al.*, 1972). However, despite the large number of reports, no results on polyamine biosynthesis in human tissues have been reported in the literature so far. Since the concentrations of polyamines in prostate are very high, an investigation of their biosynthetic pathway in human prostate and of the role of spermidine and spermine in human reproductive tract appeared of particular interest. In the present paper the occurrence of putrescine-dependent *S*-adenosyl-L-methionine decarboxylase in human prostate gland is reported for the first time and some regulatory properties of this enzyme are described. Preliminary results of this work have been reported (Zappia *et al.*, 1971*a*; Zappia & Cartenì, 1971).

Materials and Methods

Chemicals

S-Adenosyl-L-methionine was prepared from cultures of *Saccharomyces cerevisiae* (Schlenk & De Palma, 1957) and isolated by ion-exchange chromatography (Shapiro & Ehninger, 1966); L-[1-^{14}C]-methionine, *S*-adenosyl-L-[*carboxy*-^{14}C]methionine and *S*-adenosyl-L-[*methyl*-^{14}C]methionine were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.; DL-[1-^{14}C]homocysteine thiolactone was obtained from Cal-Atomic (Los Angeles, Calif., U.S.A.); in some experiments *S*-adenosyl-L-[*carboxy*-^{14}C]methionine was prepared from labelled methionine by the procedure of Schlenk & De Palma (1957) with some modifications (Zappia *et al.*, 1969*b*). *S*-Adenosyl-L-[*carboxy*-^{14}C]homocysteine was prepared biosynthetically from labelled L-homocysteine and adenosine in the presence of the condensing enzyme (EC 3.3.1.1) from rat liver (Duerre, 1962). *S*-Inosyl-L-[*carboxy*-^{14}C]methionine was obtained by deamination of *S*-adenosyl-L-[*carboxy*-^{14}C]homocysteine in the presence of adenosine deaminase (EC 3.5.4.4) from *Aspergillus oryzae* and subsequent methylation of the inosyl thioether with methyl iodide (Zappia *et al.*, 1969*b*). *S*-Pentosyl-[*carboxy*-^{14}C]methionine was produced by alkaline hydrolysis of labelled *S*-adenosyl-L-methionine (Parks & Schlenk, 1958; Zappia *et al.*, 1968). *S*-Methyl-L-[*carboxy*-^{14}C]methionine sulphonium salt was obtained in the iodide form as described by Toennies & Kolb (1951). The chemical and radiochemical purity of the above compounds was checked by t.l.c. and paper electrophoresis; the sulphonium compounds were stored at −20°C at pH3.5 to prevent decomposition.

Putrescine dihydrochloride, spermine tetrahydrochloride, spermidine trihydrochloride, hydroxylamine, hydrazine and *N*-ethylmaleimide were supplied by Sigma Chemical Co., St. Louis, Mo., U.S.A. The purity of the polyamines was checked by high-voltage electrophoresis. Pyridoxal phosphate was obtained from Hoffmann-La Roche, Nutley, N.J., U.S.A., Hyamine hydroxide was purchased from Packard Instruments Co., Downers Grove, Ill., U.S.A. All other chemicals were the purest available grades from standard commercial sources.

Preparation of tissue extracts

Prostate glands from patients with prostatic hypertrophy were frozen at −20°C immediately after surgery. The diagnosis of prostate adenoma was checked histologically and carcinomatous glands were discarded. The frozen glands, rinsed with 0.3M-sucrose–0.3mM-EDTA buffer, pH7.0, were minced in small pieces and homogenized for 4min in a Waring Blendor with 4vol. of the same buffer. A second homogenization of the extract was performed with a Potter–Elvehjem apparatus for 5min. The homogenate was then centrifuged for 2h at 20000*g* and the resulting supernatant was used as the enzyme source.

Enzyme assay

The reaction was followed by measurement of $^{14}CO_2$ released from *S*-adenosyl[*carboxy*-^{14}C]methionine or other *carboxy*-^{14}C-labelled analogues. The assay was performed in modified Warburg flasks with removable centre wells that contained 0.3ml of Hyamine hydroxide (Zappia *et al.*, 1969*a*). After incubation 0.5ml of 3M-H_2SO_4 was added from the side arm into the reaction mixture to stop the reaction and release $^{14}CO_2$. A further incubation with vigorous shaking for 30min at 37°C ensured a complete trapping of $^{14}CO_2$ by Hyamine hydroxide. Quantitative recovery of CO_2 in these experimental conditions was tested by using labelled sodium carbonate.

Determination of radioactivity

Radioactivity was measured in a Tri-Carb liquid-scintillation spectrometer (Packard), model 3380, equipped with an absolute radioactivity analyser; 20ml of an 0.4% solution of 2,5-diphenyloxazole in a mixture of equal volumes of toluene and ethanol was employed. The quenching was corrected by external standardization.

Protein determination

Protein concentrations, unless otherwise stated, were determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin (Sigma Chemical Co.) as standard.

Results

Preliminary experiments (Zappia & Cartenì, 1971) showed the ability of human prostate extracts to decarboxylate *S*-adenosyl-L-methionine. Some 95% of the enzyme activity was retained in the supernatant after centrifugation at 20000*g*, indicating that the enzyme is not associated with the cellular particulate fraction.

Fig. 1 shows the effect of putrescine, spermine and spermidine on the enzyme activity at an initial substrate concentration of 12μM: putrescine markedly enhanced the reaction rate, with a maximal activation (5-fold) at a concentration of 0.7mM. On further increase in the putrescine concentration, the decarboxylation rate was constant up to 2.5mM, then declined, and no activation was observed with 70mM-

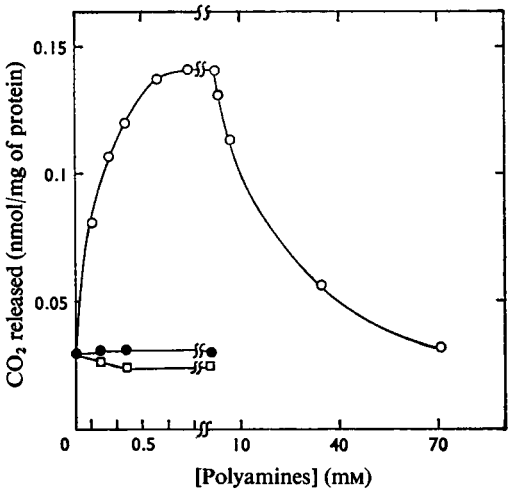

Fig. 1. *Effect of polyamines on S-adenosylmethionine decarboxylation*

The incubation mixture contained 50μmol of sodium phosphate buffer, pH7.5, 5nmol of *S*-adenosyl-L-[*carboxy*-^{14}C]methionine (170000c.p.m.), and enzyme (1.16mg of protein) in a final volume of 400μl. The polyamines were added as indicated in the figure. The incubation was done for 60min at 37°C. $^{14}CO_2$ was measured as described in the Materials and Methods section. ○, Putrescine; ●, spermidine; □, spermine.

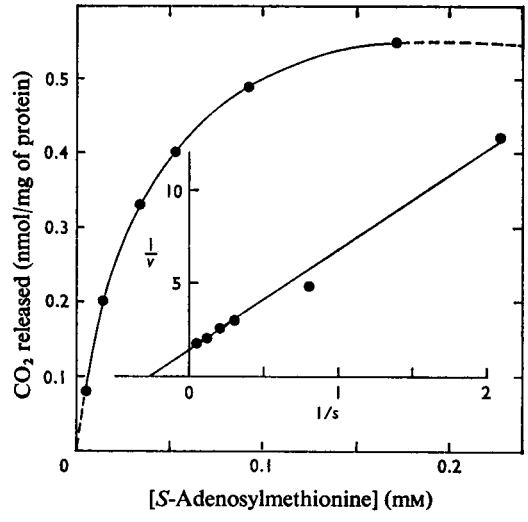

Fig. 2. *Effect of substrate concentration on the reaction rate*

The assay medium contained 50μmol of phosphate buffer, pH7.5, 2.5μmol of putrescine, and enzyme (1.16mg of protein); *S*-adenosyl-L-[*carboxy*-^{14}C]-methionine was added at the concentrations given. The total volume of the assay medium was 400μl and the incubation was done at 37°C for 60min. The K_m, from the double-reciprocal plot, was 4×10^{-5}M.

putrescine. Spermine and spermidine failed to have any appreciable effect up to 2.5mM.

The effect of substrate concentration on the reaction rate in the presence of 2.5mM-putrescine is shown in Fig. 2. A maximal rate of decarboxylation was obtained with 0.15mM-*S*-adenosyl-L-methionine. From the double-reciprocal plot in the insert, a K_m of 4×10^{-5}M was calculated. To investigate if the enzyme is saturated *in vivo* by *S*-adenosyl-L-methionine the concentration of sulphonium compound in the prostate was measured by an isotope-dilution technique (Salvatore *et al.*, 1971; Zappia & Ayala, 1972). An average of 184μmol of sulphonium compound/g wet wt. of prostate was calculated from two separate determinations.

The affinity between putrescine and *S*-adenosyl-L-methionine decarboxylase was investigated in the presence of 12μM-*S*-adenosyl-L-methionine: from the Hofstee plot shown in Fig. 3 an apparent activation constant of 0.131mM was obtained.

Fig. 4 illustrates the effect of pH on enzyme activity in the presence of putrescine: the optimum pH of decarboxylation was about 7.4 in 0.12M-sodium phosphate buffer, with one-half of the maximal activity occurring at pH6.6. Tris–HCl buffer had a

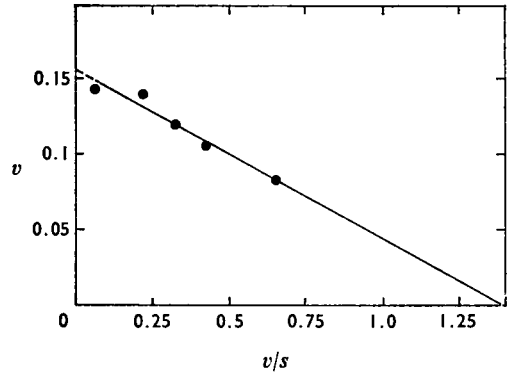

Fig. 3. *Effect of putrescine concentration on the enzyme activity*

The assay was performed as described in Fig. 1 except that putrescine was added to the incubation mixture at the indicated concentrations. The concentration of putrescine (s) is in mM; enzyme velocity (v) is expressed as nmol of $^{14}CO_2$ released/h per mg of protein. $V_{max.} = 0.156$ and $K_m = 0.131$mM.

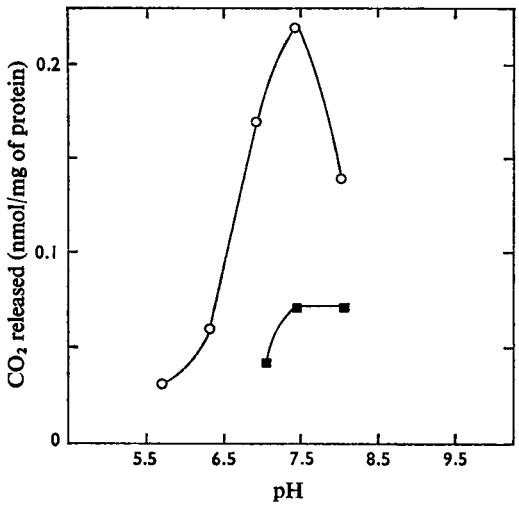

Fig. 4. *Effect of pH on S-adenosyl-L-methionine decarboxylase*

The incubation mixture contained 50μmol of sodium phosphate buffer (o) or 50μmol of tris–HCl buffer (■), at the indicated pH, 2.5μmol of putrescine, 5nmol of *S*-adenosyl-L-[*carboxy*-^{14}C]methionine (170000c.p.m.), and enzyme (1.16mg of protein) in a final volume of 400μl. The incubation was done for 60min at 37°C.

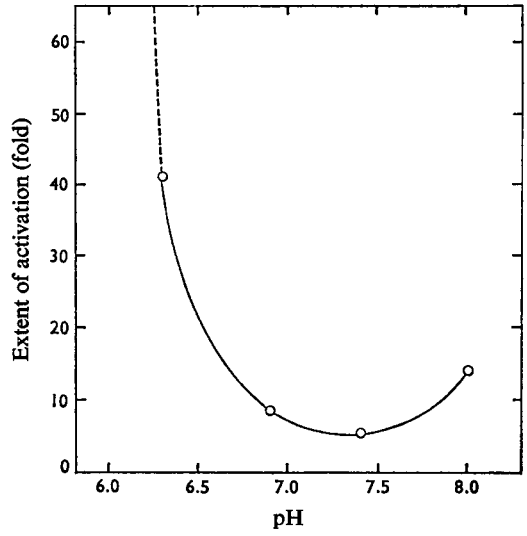

Fig. 5. *Putrescine activation of S-adenosyl-L-methionine decarboxylase as a function of pH*

The assay was performed in the presence and absence (control) of 2.5mM-putrescine in 0.12M-sodium phosphate buffer at various pH values as reported in Fig. 4.

significant inhibitory effect: 70% inhibition was observed at pH7.5. Tris inhibition was also reported for *S*-adenosyl-L-methionine decarboxylase from rat ventral prostate (Pegg & Williams-Ashman, 1969).

Putrescine activation is pH-dependent: Fig. 5 shows that polyamine stimulation was maximal at low pH values, whereas at the optimum pH for enzyme assay (see Fig. 4) the extent of activation decreases; at higher pH values an increase of activation occurs.

To investigate the specificity of the enzyme, various *carboxy*-^{14}C-labelled analogues and derivatives of *S*-adenosyl-L-methionine were tested as substrates: the results are reported in Table 1. The enzyme appears to be highly specific for *S*-adenosyl-L-methionine: replacement only of the 6′-amino group of the sulphonium compound resulted in a complete loss of activity. Also other related sulphur compounds including *S*-methylmethionine, a biological methyl-group donor (Schlenk, 1965), were totally inactive. The lack of activity of *S*-adenosylhomocysteine is noteworthy since the thioether is a catabolite of *S*-adenosylmethionine: it is formed when the latter donates the methyl group to specific acceptors in transmethylations (Mudd & Cantoni, 1964).

The effects of isonicotinic acid hydrazide and of some carbonyl reagents on the reaction are shown in Table 2. Hydroxylamine, hydrazine and phenylhydrazine were very effective inhibitors at 1mM. Isonicotinic acid hydrazide, a well-known inhibitor of pyridoxal phosphate enzymes (Braunstein, 1960), exerted only a moderate inhibition at higher concentrations.

Table 3 illustrates the action of several bivalent cations on enzyme activity: Hg^{2+} and Cu^{2+} were strongly inhibitory. The same concentrations of Zn^{2+} and Co^{2+} exerted only a partial inhibition; Mg^{2+}, which is required for the activity of *S*-adenosyl-L-methionine decarboxylase from *E. coli* (Wickner *et al.*, 1970), did not have any effect on the reaction rate with our system.

The enzyme was stable to freeze–thawing and was rapidly inactivated by exposure to high temperatures (Fig. 6). In preliminary experiments (Zappia *et al.*, 1971*a*) a partial inactivation of the enzyme by exposure to oxygen was observed, suggesting the presence of essential thiol groups. The effect of thiol inhibitors, i.e. *N*-ethylmaleimide, iodoacetic acid and $HgCl_2$, was therefore tested; 1mM-*N*-ethylmaleimide and 0.1mM-$HgCl_2$ caused a 100% inhibition, whereas 1mM-iodoacetate exerted only a 7% inhibition. Further a moderate stimulation of the enzyme activity occurred in the presence of 1mM-2-mercaptoethanol.

Table 1. *Substrate specificity of S-adenosylmethionine decarboxylase*

The incubation mixture contained: 50μmol of sodium phosphate buffer, pH7.5, 2.5μmol of putrescine, labelled substrates at the concentrations listed, and enzyme (1.2mg of protein) in a final volume of 400μl. Reaction mixtures without enzyme were prepared as controls. The incubation was done for 60min at 37°C as indicated in the Materials and Methods section.

Substrate (*carboxy*-^{14}C-labelled)	Concn. (μM)	Specific radioactivity (c.p.m./nmol)	Enzyme activity (nmol of CO_2 released/h per mg of protein)
S-Adenosyl-L-methionine	10	34000	0.14
S-Pentosyl-L-methionine	10	34000	<0.005
S-Methyl-L-methionine	81	6000	<0.005
L-Methionine	26	19000	<0.005
S-Inosyl-L-methionine	28	20000	<0.005
S-Adenosyl-L-homocysteine	28	20000	<0.005

Table 2. *Inhibition of S-adenosylmethionine decarboxylase by isonicotinic acid hydrazide and carbonyl reagents*

The assay was performed with 10μM-*S*-adenosyl-L-[*carboxy*-^{14}C]methionine as described in Table 1, except that the listed compounds were added to the incubation mixture at the indicated concentrations.

Additions	Concn. (mM)	Enzyme activity (nmol of CO_2 released/h per mg of protein)	Relative activity (%)
None	—	0.14	100
Hydroxylamine	1	0.0	0.0
Hydrazine	1	0.0	0.0
Phenylhydrazine	1	0.03	21.4
Isonicotinic acid	1	0.129	92
hydrazide	10	0.072	51

Table 3. *Effect of bivalent cations on the enzyme activity*

The assay was performed as described in Table 1, except that the listed cations were added to the incubation mixture at the indicated concentrations.

Cations	Concn. (mM)	Enzyme activity (nmol of CO_2/h per mg of protein)	Relative activity (%)
None	—	0.14	100
Hg^{2+}	0.1	0.0	0.0
Cu^{2+}	0.01	0.14	100
Cu^{2+}	0.1	0.053	37.9
Cu^{2+}	1	0.0	0.0
Zn^{2+}	1	0.062	44.4
Co^{2+}	1	0.085	61.05
Mg^{2+}	1	0.138	98.06
Ca^{2+}	1	0.146	106

Discussion

In the present paper the occurrence of *S*-adenosyl-L-methionine decarboxylase in human prostate gland is reported for the first time. Therefore in human tissues, like other systems, *S*-adenosylmethionine presumably has to be decarboxylated before transferring its propylamine moiety to putrescine in the biosynthesis of polyamine.

The enzyme resembles in many properties the analogous decarboxylase of rat ventral prostate (Pegg

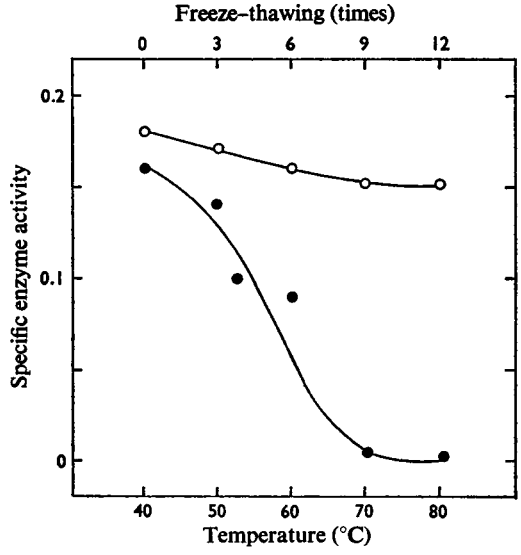

Fig. 6. *Stability of S-adenosyl-L-methionine decarboxylase*

The enzyme was preincubated at the indicated temperatures for 5min (●) and freeze–thawing was performed within 3min (○). The incubation mixture contained 50μmol of phosphate buffer, pH7.5, 2.5μmol of putrescine, 5nmol of *S*-adenosyl-L-[*carboxy*-^{14}C]methionine (170000c.p.m.), and enzyme (1.16mg of protein) in a final volume of 400μl. The incubation was done for 60min at 37°C. $^{14}CO_2$ was measured as described in the Materials and Methods section. Specific enzyme activity is expressed as nmol of CO_2 released/h per mg of protein.

& Williams-Ashman, 1969). It does not require Mg^{2+}, which is essential for the *S*-adenosylmethionine decarboxylase purified from *E. coli* (Wickner *et al.*, 1970) and, like rat prostate decarboxylase (Pegg & Williams-Ashman, 1969), is activated by putrescine and inhibited by tris. The stimulation by putrescine is rather specific: other polyamines, such as spermidine and spermine, did not exert any measurable effect on the reaction rate (see Fig. 1). Therefore enzyme activation in human prostate seems strictly related to the chain length of the polycation. With rat prostate decarboxylase, on the contrary, spermidine also significantly enhances the reaction rate.

It is noteworthy that the putrescine concentration in mammalian prostate gland (Mann, 1964) is similar to the apparent activation constant of the enzyme (0.13mM): this finding gives more support to the physiological regulatory role of putrescine activation. At putrescine concentrations higher than the physiological ones the enzyme activation is no longer observable (see Fig. 1). This result resembles the inhibition by excess of substrate, suggesting the involvement of both amino groups of putrescine as binding sites with the enzyme.

At present the mechanism of putrescine stimulation is not clear. The polyamine could merely remove the reaction product [*S*-adenosyl(5′)-3-methylthiopropylamine] if propylamine transferase was present in the preparation. This hypothesis can be excluded, since the activation is observable also in more purified preparations from human prostate (V. Zappia, unpublished work), from rat prostate (Jänne *et al.*, 1971*a*) and from rat liver (Hannonen *et al.*, 1972), where propylamine transferase is not detectable. The absence of sigmoid kinetics makes a classical allosteric-type mechanism improbable. Moreover the pH-dependence of putrescine activation (see Fig. 5) suggests a protective effect of the diamine towards pH-dependent configurational changes of the enzyme.

An unambiguous interpretation of the activation mechanism would require more detailed experiments with homogeneous preparations of the enzyme: promising results have been obtained in preliminary experiments on purification by affinity chromatography (Zappia *et al.*, 1971*b*).

The enzyme has the same affinity for *S*-adenosylmethionine as the rat prostate decarboxylase; the K_m was 4×10^{-5}M (see Fig. 2). The calculated *S*-adenosylmethionine concentration in human prostate (0.184μmol/g wet wt. of tissue) is 4 times the average value found in several mammalian tissues (0.05μmol/g wet wt. of tissue) (Salvatore *et al.*, 1971). Compared with the K_m of the decarboxylase, it appears that in prostate gland the cellular amounts of sulphonium compound are saturating with respect to the enzyme.

The inhibition by Zn^{2+} is noteworthy also, because of the unusually high concentration of this cation in human prostate (69.2mg/100g dry wt.) (Mawson & Fischer, 1953).

The inhibition by thiol reagents suggests an involvement of thiol groups in the catalytic process, although only a moderate activation by reducing agents (mercaptoethanol) has been demonstrated.

Like the decarboxylase from rat ventral prostate and *E. coli*, the enzyme is inhibited by carbonyl reagents. Pyruvic acid was identified as the carbonyl moiety involved in the enzyme catalysis in *S*-adenosylmethionine decarboxylase from *E. coli* (Wickner *et al.*, 1970), whereas pyridoxal phosphate is probably the coenzyme in rat prostate decarboxylase (Pegg & Williams-Ashman, 1969) and in rat liver decarboxylase (Feldman *et al.*, 1972).

The enzyme is highly specific, like the *S*-adenosylmethionine decarboxylase from *E. coli* (Zappia *et al.*, 1969*a*). Sulphonium analogues and related thioethers tested as substrates (see Table 1) were inert. The inactivity of *S*-inosyl derivatives and of pentosylmethionine suggests that the 6′-amino group of the adenine moiety is a specific binding site. Further, the importance of the -onium pole of *S*-adenosylmethion-

ine in the interaction with the enzyme is inferred by the inactivity of S-adenosylhomocysteine. These two binding sites are also involved in the interactions between S-adenosylmethionine and methyl-transfer enzymes (Zappia *et al.*, 1969*b*). It has also been demonstrated (Pegg, 1969) that replacement of the methyl group of S-adenosylmethionine by an ethyl one results in a sharp decrease of biological activity in spermidine biosynthesis. Also the S-adenosylmethionine derivatives, in which the methionine moiety has been substituted by 2-hydroxy-4-methylthiobutyric acid (Zappia *et al.*, 1969*a*), is completely inactive as substrate of the decarboxylase from *E. coli*. These results, together with the present results, suggest that any modification at the three organic substituents of the -onium pole is not compatible with a biological activity of the sulphonium compound in spermidine biosynthesis. Also the steric configuration of the -onium pole of the molecule affects S-adenosylmethionine reactivity with *E. coli* decarboxylase (Zappia *et al.*, 1969*c*). The presence of sulphur seems not to be essential, since S-adenosylselenomethionine is a good precursor of spermidine (Pegg, 1969).

Finally, the inactivity of S-adenosylhomocysteine, the product of one methyl-transfer reaction, indicates that in cellular metabolism transmethylases compete with S-adenosylmethionine decarboxylase for the utilization of the same substrate. The interrelation between these two classes of reactions was demonstrated by Zappia *et al.* (1969*b*), since S-adenosyl-(5')-3-methylthiopropylamine is an inhibitor of methyl transferases.

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