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## Studies on S-adenosylmethionine decarboxylase from human prostate

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**Biochimica.** — *Studies on S-adenosylmethionine decarboxylase from human prostate* (*). Nota di VINCENZO ZAPPIA, MARIA CARTENÌ e GAETANO IRACE, presentata (**) dal Socio F. CEDRANGOLO.

RIASSUNTO. — Sono riportati risultati relativi alla presenza dell'enzima S-adenosilmetionina decarbossilasi in estratti di prostata umana. Per dosare l'attività enzimatica è stato messo a punto un metodo di saggio che impiega S-adenosilmetionina marcata nel carbossile e che comporta l'isolamento ed il dosaggio della $CO_2$ marcata formatasi dalla reazione.

L'enzima è stato parzialmente caratterizzato ed è stata evidenziata una specifica attivazione pH-dipendente da parte della putrescina. Altre poliammine, quali spermina e spermidina, non modificano sensibilmente la velocità di reazione.

Reagenti carbonilici esercitano un variabile grado di inibizione sulla attività enzimatica dimostrando la presenza di gruppi carbonilici essenziali per l'attività catalitica. La reazione viene anche inibita in misura notevole da reagenti dei gruppi SH quali etilmaleimide, iodoacetato, etc.

È stata, infine, messa in evidenza una decarbossilazione non enzimatica dell'adenosilmetionina ad opera del piridossalfosfato.

## INTRODUCTION

Spermine and spermidine are normal constituents of several mammalian tissues and bacteria [1, 2]. In mammals the prostate gland and seminal fluid are particularly rich in such polyamines [3, 4]. During the last decade several functions have been attributed to spermine and spermidine [5], and the interaction mechanisms between these polycations and polynucleotides or cell membranes have been partly elucidated [6, 7]. In addition the polyamines have been found to be growth factors for bacteria [8], insects [9], animal and plant cells in culture [10]. Marked differences in polyamines content in various regions of human brain have also been observed [11] and their functions in the central nervous system have been investigated recently [12]. However, despite the large number of reports, relatively little is known about the specific physiological functions of these compounds, especially in man.

The enzymic reactions involved in polyamine biosynthesis have been elucidated in rat ventral prostate by Pegg and Williams-Ashman [13, 14] and in E. Coli by Tabor and collaborators [15]. In both systems decarboxylation of S-adenosylmethionine (Ado-Met), followed by the transfer of the propylamine moiety to putrescine [16], represents the first step of the biosynthetic pathway. However, no work on the enzymes involved in polyamine

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biosynthesis in human tissues has been reported. In order to investigate the control mechanisms of polyamines biosynthesis in the human reproductive system, we have initiated a systematic study of the enzymes of this pathway in human prostate.

The present paper describes some properties of an enzyme preparation from human prostate gland which catalyzes the decarboxylation of Ado-Met to S–adenosyl (5′)–3–methylthiopropylamine and $CO_2$.

## EXPERIMENTAL PROCEDURE

*Materials*: Sodium carbonate–$^{14}C$ (15 mCi per mmole) was supplied by New England Nuclear, Boston; L–methionine–1–$^{14}C$ (15.8 mCi per mmole) and S–adenosyl–L–methionine (carboxyl–$^{14}C$), 47 mCi per mmole, were obtained from the Radiochemical Centre, Amersham; in some experiments S–adenosyl–L–methionine (carboxyl–$^{14}C$) was prepared from L–methionine–$^{14}C$ by biosynthesis with yeast and isolated by ion exchange chromatography according to Shapiro and Ehninger [17]. Since commercial non-radioactive Ado-Met was contaminated by numerous impurities, it was always synthesized by the above procedure with minor modifications [18]. The purity of the sulfonium compound was checked by thin layer chromatography [18] and it was stored at —20° to avoid decomposition. EDTA was supplied by Merck; isonicotinic acid hydrazide by Calbiochem; pyridoxal phosphate by Hoffmann–La Roche; putrescine dihydrochloride, spermine tetrahydrochloride and spermidine trihydrochloride, hydroxylamine, hydrazine, penicillamine, N–ethylmaleimide, iodacetic acid were supplied by Sigma; hyamine hydroxide was furnished by Packard Instrument Company. All other chemicals were obtained from commercial sources.

*Enzymatic assay*: The reaction was carried out in modified Warburg flasks which contained a removable center well. After incubation, 0.5 ml of 3 M $H_2SO_4$ was added from the side arm into the reaction mixture. The $^{14}CO_2$ produced by the reaction was trapped by 0.3 ml of hyamine hydroxide in the center well. After 15 min of vigorous agitation the absorption of $^{14}CO_2$ was quantitative, as has been demonstrated by recovery experiments with sodium carbonate–$^{14}C$. The center well was then removed and dropped into a counting vial containing scintillation solution [19].

*Determination of radioactivity*: Radioactivity was measured in a Tri-Carb liquid scintillation spectrometer (Packard), model 3380 equipped with an absolute activity analyzer. A 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.

*Preparation of tissue extracts*: Freshly excised prostate glands from patients with prostatic hypertrophy were immediately frozen at —20° after surgery. All the remaining steps were performed at 2°. The frozen glands

were rinsed with 0.3 M sucrose containing 0.3 mM EDTA pH 7.0, minced in small pieces and homogenized for 4 min in a Waring Blendor, with 4 volumes of the same buffer. The extract was homogenized again for 4 min with a Potter Elvehjem apparatus with a teflon pestle. The homogenate was then centrifuged for 2 hrs at 20,000 × g and the supernatant was used for the assay.

Protein was determined by the micro Lowry procedure [20] with bovine serum albumine as standard.

## RESULTS

Preliminary experiments showed that prostatic extracts, incubated in presence of Ado-Met-$^{14}$COOH, catalyzed the release of $^{14}CO_2$ from the sulfonium compound. Fig. 1 shows the effect of enzyme concentration on

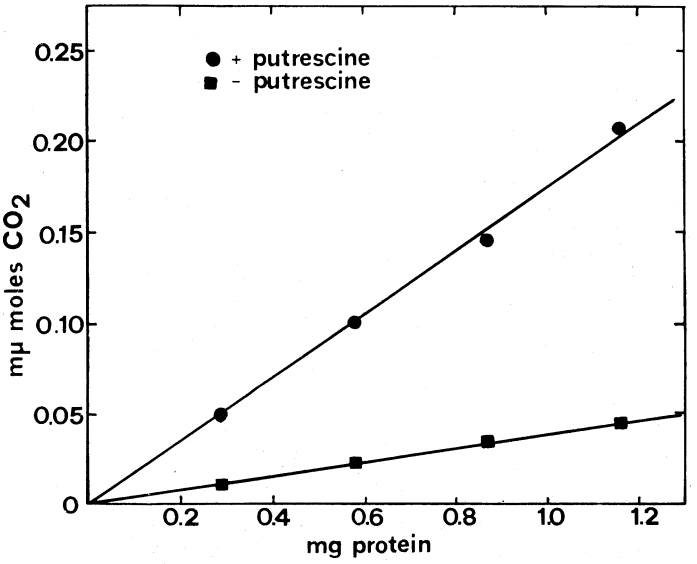


Fig. 1. – *Effect of enzyme concentration on Ado-Met decarboxylation.*
The incubation mixture contained: 50 μmoles phosphate buffer pH 7.5, 5 mμmoles of Ado-Met-$^{14}$COOH (170,000 cpm), 2.5 μmoles of putrescine or no putrescine, the indicated amounts of enzyme in a final volume of 400 μl. Incubation was carried out for 60 minutes at 37°. $^{14}CO_2$ was measured as reported under "Materials and Methods".

Ado-Met decarboxylation: the reaction is linear with respect to the enzyme concentration over a 4 fold range. In addition the decarboxylation is markedly stimulated by putrescine, added in large excess compared to the concentration of the substrate; an average of 5-fold activation can be observed.

In fig. 2 is reported the time course of decarboxylation. The enzyme activity is almost linear for the first 30 minutes, then declines and after 60 minutes a plateau is reached.

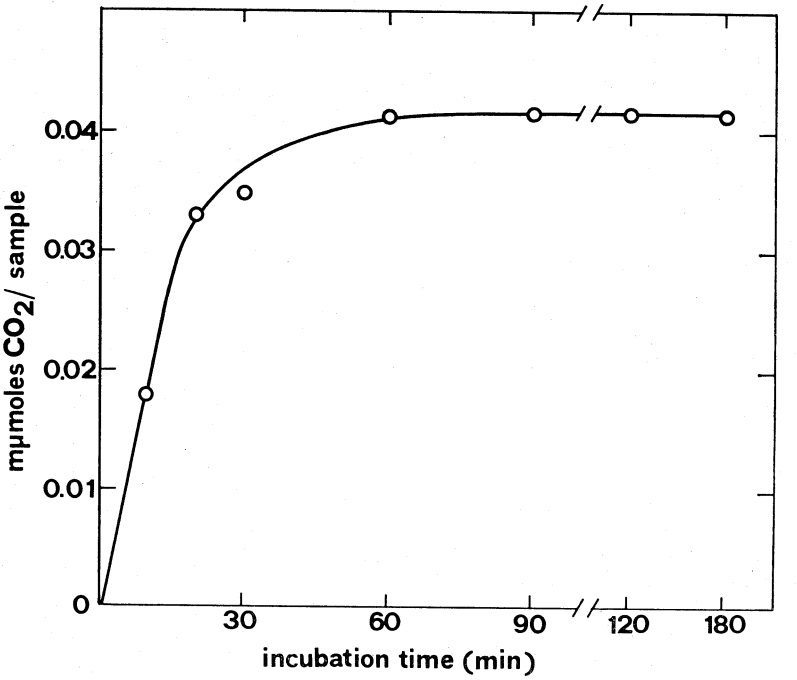


Fig. 2. – *Time course of Ado–Met decarboxylation.*
The incubation mixture contained: 50 μmoles phosphate buffer pH 7.5, 2.5 μmoles of putrescine, 5 mμmoles of Ado-Met-$^{14}$COOH (170,000 cpm), 0.3 mg protein in a final volume of 400 μl. The incubation was carried out for the indicated times at 37°. $^{14}CO_2$ was measured as reported under "Materials and Methods".

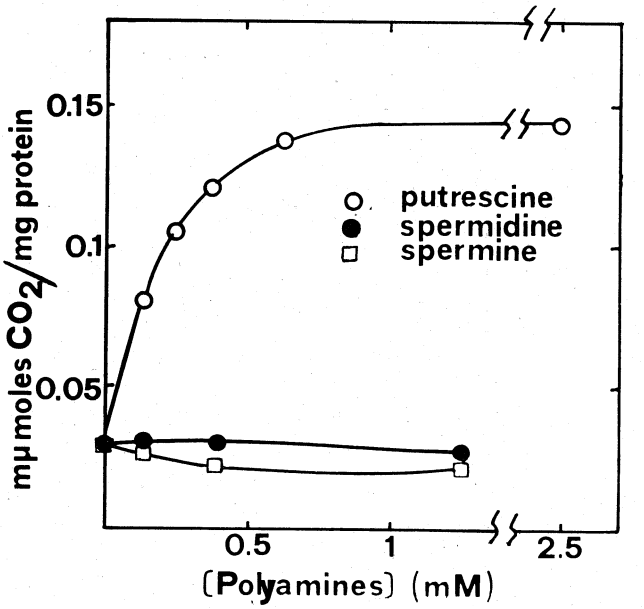


Fig. 3. – *Effect of polyamines on Ado–Met decarboxylation.*
The composition of the assay medium is reported in fig. 2 except that the listed polyamines were added as indicated in this figure and that 1.16 mg of enzyme sample were used. The incubation was carried out for 60 min at 37°.

TABLE I.

*S–Adenosylmethionine decarboxylation as a function of* pH *in the absence or presence of putrescine.*

The incubation mixture contained: 5 mμmoles of Ado–Met–$^{14}$COOH (170,000 cpm), 50 μmoles of sodium phosphate buffer at the indicated pH, 1.16 mg of protein, in a final volume of 400 μl. Reaction mixture without enzyme were prepared as controls. The incubation was carried out for 30 min at 37° as indicated under "Materials and Methods".

| pH | $CO_2$ released (mμmoles/mg protein/hr) no additions | plus putrescine (2.5 mM) | Activation by putrescine (fold) |
|---|---|---|---|
| 5.7 | not detectable | 0.032 | ∞ |
| 6.3 | 0.0014 | 0.06 | 42.8 |
| 6.9 | 0.02 | 0.17 | 8.5 |
| 7.4 | 0.04 | 0.22 | 5.5 |
| 8.0 | 0.01 | 0.14 | 14.0 |

Table I illustrates the effect of pH on the enzyme activity. Either in the absence or in the presence of putrescine the optimal pH is about 7.4, with one-half of the maximal activity occurring at pH 6.9 in absence of putrescine. It can also be observed that the stimulatory effect of putrescine is pH dependent: the stimulation is maximal at low pH values.

The effects of polyamines putrescine, spermidine and spermine is reported in fig. 3. A maximal rate of decarboxylation is reached with 0.7 mM putrescine, whereas the same levels of spermidine and spermine did not show any appreciable effect.

TABLE II.

*Inhibition of Ado-Met decarboxylase by isonicotinic acid hydrazide and carbonyl reagents.*

Assay was performed in presence of putrescine at pH 7.5 as described in Table I, except that the listed compounds were added to the incubation mixture in the indicated concentrations.

| Additions | Concn (mM) | Activity (mμmoles $CO_2$ released/mg protein/hr) | Relative activity (%) |
|---|---|---|---|
| None | — | 0.14 | 100 |
| Hydroxylamine | 1 | 0.0 | 0.0 |
| Hydrazine | 1 | 0.0 | 0.0 |
| D–Penicillamine | 1 | 0.175 | 125 |
| Isonicotinic acid Hydrazide | 1 | 0.129 | 92 |
| Isonicotinic acid Hydrazide | 10 | 0.072 | 51 |

Table II shows the effect of known inhibitors of pyridoxal phosphate enzymes [21] on Ado-Met decarboxylation. Hydroxylamine and hydrazine were the most effective inhibitors tested; also isonicotinic acid hydrazide inhibited significantly at higher concentrations; D–penicillamine, on the contrary, exerted a marked activation. This result suggests a chelating effect of the penicillamine on inhibitor ions present in the crude extract.

TABLE III.

*Non-enzymatic decarboxylation of methionine and Ado-Met by Pyridoxal phosphate.*

The release of $^{14}CO_2$ was measured as indicated under "Materials and Methods". The assay flasks contained: 5 mμmoles of Ado–Met–$^{14}$COOH (170,000 cpm) or 15.8 mμmoles of L–Methionine–1–$^{14}$COOH (200,000 cpm), 50 μmoles of phosphate buffer pH 7.5, 2.5 μmoles of putrescine, Pyridoxal–5′–phosphate in the amounts indicated, in a final volume of 1.2 ml. The incubation was carried out for 90 min at 37°.

| Substrate | Pyridoxal–5′–Phosphate concentration (mM) | mμmoles $CO_2$ released/90min |
|---|---|---|
| Ado–Met . . . . . | — | not detectable |
| Ado–Met . . . . . | 2 | 0.998 |
| Ado–Met . . . . . | $2 \times 10^{-1}$ | 0.07 |
| Ado–Met . . . . . | $2 \times 10^{-2}$ | 0.004 |
| Ado–Met . . . . . | $2 \times 10^{-3}$ | not detectable |
| L–methionine . . . . . | — | not detectable |
| L–methionine . . . . . | 2 | 0.09 |

To further investigate the nature of the coenzyme involved in the decarboxylation, pyridoxal phosphate (P–5–P) has been added to the standard reaction mixture: even at relatively high concentrations of P–5–P ($10^{-3}$M) no appreciable activation of the enzyme could be evidenced. On the other hand P–5–P, in absence of prostate extracts, catalyzes non-enzymatically Ado-Met decarboxylation. The results are reported in Table III: about 20 % of substrate added is converted by 2 mM P–5–P in 90 minutes. When L–methionine was used as substrate, only 0,7 % was converted in the same experimental conditions.

A partial inactivation of the enzyme by oxygen was observed in preliminary experiments, suggesting the presence of essential thiol groups. Table IV reports the effect of mercaptoethanol and several SH–inhibitors on the decarboxylation. $10^{-3}$M N–Ethylmaleimide and $10^{-4}$M $HgCl_2$ caused a 100 % inhibition; whereas $10^{-3}$M iodoacetate exerted only 7% inhibition. An activation by mercaptoethanol is also evident.

TABLE IV.

*Effect of β–mercaptoethanol and sulphydryl inhibitors on Ado-Met decarboxylase.*

Complete system contained: 50 μmoles phosphate buffer pH 7.5, 2.5 μmoles putrescine, 5 mμmoles of Ado–Met–$^{14}$COOH (170,000 cpm), 1.2 mg protein and the listed compounds in the indicated concentrations in a final volume of 400 μl. The incubation was carried out for 30 minutes at 37°

| Addition | Concn (mM) | Activity (mμmoles $CO_2$ released/mg protein/hr) | Relative activity (%) |
|---|---|---|---|
| None | — | 0.14 | 100 |
| N–Ethylmaleimide | 1 | 0.0 | 0.0 |
| Iodoacetate | 1 | 0.13 | 92.8 |
| Iodoacetate | 10 | 0.12 | 85.7 |
| $HgCl_2$ | 0.1 | 0.0 | 0.0 |
| β–Mercaptoethanol | 1 | 0.16 | 114.2 |

## DISCUSSION

The reported experiments demonstrate the presence in the human prostate gland of an enzyme(s) decarboxylating Ado-Met, suggesting that polyamines biosynthesis in man occurs with reactions similar to those demonstrated in rat tissues and E. Coli [13–15].

In analogy to the rat prostate decarboxylase [13] the activity is strongly and specifically stimulated by putrescine; this activation is pH–dependent, as reported in Table I. The mechanism of this stimulation has not been elucidated: putrescine could merely remove the reaction product if spermidine synthetase were present in the preparation. In fact this enzyme catalyzes the propylamine transfer from S–adenosyl–(5′)–3–methylthiopropylamine to putrescine forming spermidine and methylthioadenosine [13]. An allosteric–type mechanism can also be postulated even if there is no evidence of sigmoid kinetics. Recently Jänne and Williams–Ashman [22] demonstrated that the putrescine-activated decarboxylation occurs also in purified preparations where spermidine synthetase is absent.

The inhibition by sulphydryl reagents (Table IV) suggests an involvement of thiol groups in the catalytic process. The inhibition in the presence of carbonyl reagents is indicative for the presence of either pyridoxal phosphate or pyruvate as coenzyme. Pyruvate has been demonstrated to be the carbonyl moiety involved in Ado-Met decarboxylase, from E. Coli [15].

The observation that Ado-Met is non-enzymatically decarboxylated by pyridoxal-phosphate at 37° is of interest and experiments are in progress

to investigate in more details this model reaction. It worth to noting that concentrations of pyridoxal phosphate promoting Ado-Met decarboxylation are similar to the physiological ones [21]. The strongly cationic properties of the sulfonium compound could explain its different reactivity if compared to methionine: in fact the sulfonium pole may stabilize the carbanion formed from the loss of $CO_2$ by accommodating its negative charge [23].

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