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The specificity of S-Adenosylmethionine Decarboxylase

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Biochimica. — *The specificity of S-Adenosylmethionine Decarboxylase* (*). Nota di VINCENZO ZAPPIA (**), RICCARDO CORTESE, CYNTHIA R. ZYDEK-CWICK, e FRITZ SCHLENK, presentata (***) dal Corrisp. F. CEDRANGOLO.

RIASSUNTO. — L'adenosilmetionina decarbossilasi è stata purificata 60 volte dall'*Escherichia coli* ed un nuovo micrometodo di saggio dell'enzima viene riportato. Numerosi derivati ed analoghi dell'adenosilmetionina sono stati saggiati quali substrati: adenosilomocisteina, inosilomocisteina, inosilmetionina ed acido adenosil-(2-idrossi-4-metiltio)-butirrico. L'enzima è altamente specifico verso l'adenosilmetionina e dai risultati ottenuti è possibile postulare tre « binding sites » tra enzima e substrato.

È stata inoltre presa in esame la relazione tra configurazione sterica del polo di solfonio e reattività della adenosilmetionina, comparando l'attività della forma (—) con l'analogo racemo. Dai risultati ottenuti si può concludere che lo stereoisomero (—) è l'unica forma attiva nel sistema investigato.

The role of S-adenosylmethionine (S—AM) in the biosynthesis of spermidine has been established by Tabor *et al* [1, 2]. First, the sulfonium compound is decarboxylated; thereafter, the propylamino moiety is transferred to putrescine, according to the following equations:

(*a*) S-Adenosylmethionine → S-adenosyl-(5′)-3-methylthiopropylamine + CO_2;

(*b*) S-Adenosyl-(5′)-3-methylthiopropylamine + putrescine → spermidine + 5′-methylthioadenosine.

S—AM decarboxylase, which catalyzes the reaction (*a*), has been purified from *Escherichia coli* [3] and has been found also in mammalian tissues [4]. In the present study, the specificity of this enzyme has been investigated by using various analogues and derivatives of S—AM as substrate. The relation between the steric configuration of the sulfonium pole and the reactivity of S—AM as substrate of the decarboxylase has also been examined. Several binding sites between S—AM and the enzyme protein are postulated on the basis of the results.

MATERIALS AND METHODS.

Carboxyl-labeled S—AM was synthesized from L-methionine-(^{14}COOH) by biosynthesis with yeast [5] and isolated by ion exchange chromatography [6]. Carboxyl-labeled S-adenosyl-L-homocysteine was obtained by enzymatic

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synthesis from adenosine and L–homocysteine–(^{14}COOH) [6, 7]. The authors are grateful to Dr. S. K. Shapiro for furnishing part of this material. S–Inosylhomocysteine was obtained from S–adenosyl–L–homocysteine by deamination with adenosine deaminase from *Aspergillus oryzae* [8]. S–Inosyl–L–methionine was prepared by methylation of S–inosyl–L–homocysteine with methyl iodide, and S–adenosyl–L–(2–hydroxy–4–methylthio)butyric acid was obtained by deamination of S—AM with nitrous acid [9]. (±)S–Adenosyl–L–methionine was synthesized by methylation of S–adenosyl–L–homocysteine. The compounds were radiochemically pure as judged by thin-layer chromatography and paper ionophoresis [9].

S—AM decarboxylase was purified from *Escherichia coli* according to the procedure of Tabor [3]. The source material was obtained from Miles Laboratories, Elkhart, Indiana. Heating of the preparation at 60° for 5 minutes gave additional purification by coagulation of inert protein, and a sixtyfold increase of activity compared with the starting cell material was achieved.

TABLE I.

Substrate Specificity of S–Adenosylmethionine Decarboxylase.

Purified enzyme (2.1 mg protein), 25 mμmoles of Mg^{2+}, 20 μmoles of Tris–HCl buffer, pH 7.2, and the labeled substrates in the concentrations listed were incubated at 37° for 1 hour in a volume of 200 μl. The $^{14}CO_2$ released was determined as described under Materials and Methods.

Substrate (^{14}C–carboxyl–labeled)	Concentration	Specific radioactivity	$^{14}CO_2$ released
	(mM)	(cpm/μmole) × 10^3	(cpm/sample)
S–Adenosyl–L–methionine	1.1	160	20,840
S–Adenosyl–L–homocysteine	1.4	200	64
S–Inosyl–L–homocysteine	1.7	97	61
S–Inosyl–L–methionine	1.2	110	54
S–Adenosyl–L–(2–hydroxy–4–methylthio)butyric acid	1.1	144	81
L–Methionine	1.4	160	54
L–Homocysteine	1.2	160	51

Enzymatic assay. The decarboxylation was carried out at 37° in Warburg flasks with a removable center well. After incubation of the reaction mixture, 0.4 ml of 1.5 N perchloric acid was added from the side arm, and the labeled CO_2 was trapped by 0.1 ml of hyamine hydroxide. After 5 minutes

of agitation the absorption of $^{14}CO_2$ was complete. The central well was removed, dropped into a counting vial, and the radioactivity was measured by scintillation spectrometry. A 0.4% solution of 2,5-diphenyloxazole in a mixture of equal volumes of toluene and ethanol was used as scintillation fluid.

RESULTS AND DISCUSSION.

Table I lists the results of experiments concerning the substrate specificity of S—AM decarboxylase. The enzyme appears to be highly specific for S—AM. Related thioethers and sulfonium derivatives were found inert; methionine and homocysteine likewise were not acted upon by the decarboxylase. The lack of activity of S-adenosyl-L-homocysteine is of importance because it is the biological catabolite formed from S—AM when the latter donates the methyl group to specific acceptors in transmethylations.

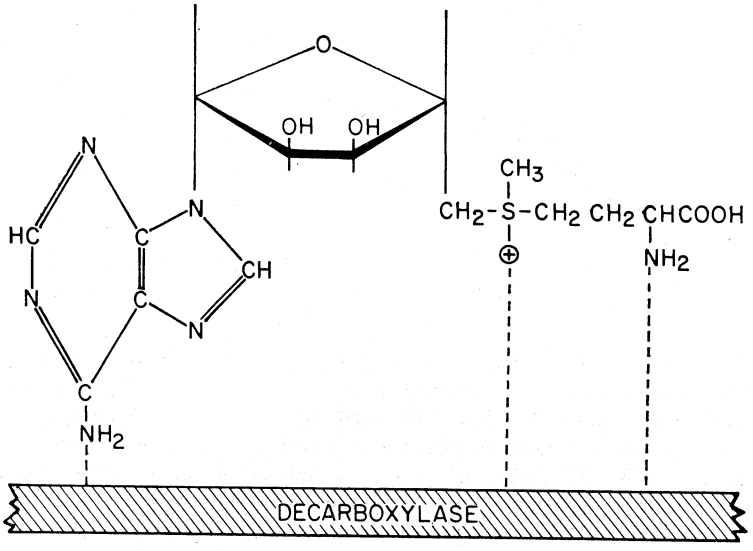

Fig. 1. – Proposed binding sites of S-adenosyl-L-methionine to its specific decarboxylase.

The inactivity of the S-inosyl- and 2-hydroxy analogues of S—AM suggests that both amino groups are attached at specific binding sites of the enzymatic protein. Furthermore, the inactivity of the thioethers indicates that the sulfonium configuration is a prerequisite for the utilization of the substrate. Thus, as least three binding sites as indicated in fig. 1, can be postulated. Similar binding sites have been demonstrated earlier for S—AM and methyl transfer enzymes [9]. However, the specificity of the latter class of enzymes is less stringent than that of S—AM decarboxylase.

De la Haba *et al.* have demonstrated that in some enzymatic systems the (—) sulfonium isomer of S—AM is the only active form. The enzymes

tested showed a high degree of specificity toward the sulfonium configuration [10]. In a similar fashion, we have compared the (—) and the (±) stereoisomers as substrates of S—AM decarboxylase. A comparison of the natural sulfonium compound, (—)S—AM, with the racemic analogue is shown in fig. 2. The progress of the reaction with the racemic compound is virtually the same as that observed with half the concentration of the (—) form. This shows that the decarboxylase is selective with respect to the configuration of the sulfonium center.

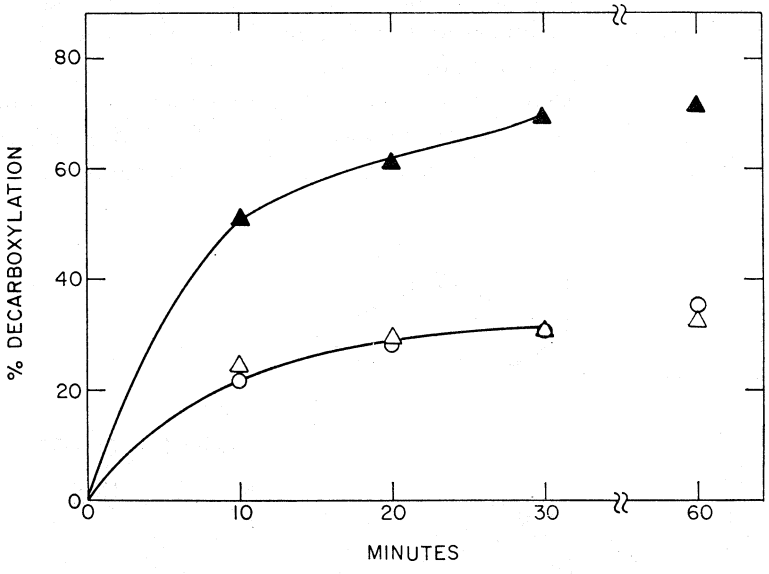

Fig. 2. – Utilization of the sulfonium diastereoisomers of S–adenosyl–L–methionine by decarboxylase from *E. coli*. ^{14}C carboxyl–labeled (—)S–adenosyl–L–methionine, 210 mμmoles (▲–▲) or 105 mμmoles (△–△), and ^{14}C carboxyl–labeled (±)S–adenosyl–L–methionine, 210 mμmoles (o–o), were incubated with 5.3 mg of purified enzyme, 20 mμmoles of Mg^{2+}, and 20 μmoles of Tris–HCl buffer, pH 7.2, in a volume of 200 μl. The specific activity of the sulfonium compounds was 1.6×10^5 cpm/μmole. The assay of decarboxylation was performed as specified under Materials and Methods.

The center of asymmetry at carbon atom 2 of the methionine moiety of S—AM was not considered in the present specificity studies; the amino acid part of the compounds used was always in the L–form.

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