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Putrescine-Sensitive (Artifactual) and Insensitive (Biosynthetic) *S*-Adenosyl-L-Methionine Decarboxylase Activities of *Lathyrus sativus* Seedlings

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The crude extracts of 3-day-old etiolated seedlings of *Lathyrus sativus* contained two *S*-adenosyl-L-methionine decarboxylase activities. The artifactual putrescine-dependent activity was due to the H_2O_2 generated by diamine oxidase (EC 1.4.3.6) of this plant system and was inhibited by catalase. This observation was confirmed by using an electrophoretically and immunologically homogeneous preparation of *L. sativus* diamine oxidase. In the presence of putrescine, diamine oxidase, in addition to *S*-adenosylmethionine, decarboxylated L-lysine, L-arginine, L-ornithine, L-methionine and L-glutamic acid to varying degrees. The decarboxylation was not metal-ion dependent.

The biosynthetic *S*-adenosylmethionine decarboxylase (EC 4.1.1.21) was detected after removing diamine oxidase specifically from the crude extracts by employing an immunoaffinity column. This Mg^{2+}-dependent decarboxylase was not stimulated by putrescine or inhibited by catalase. The enzyme activity was inhibited by semicarbazide, 4-bromo-3-hydroxybenzoylamine dihydrogen phosphate and methylglyoxal-bis (guanylhydrazone). It was largely localized in the shoots of the etiolated seedlings and was purified 40-fold by employing a *p*-hydroxymercuribenzoate/AH-Sepharose affinity column, which also separated the decarboxylase activity from spermidine synthase.

S-Adenosyl-L-methionine decarboxylase, a key enzyme of polyamine biosynthesis, catalyses the decarboxylation of *S*-adenosylmethionine yielding *S*-methyladenosylhomocysteamine, which in turn donates its propylamine moiety for the stepwise biosynthesis of polyamines, spermidine and spermine. Contrary to earlier reports, it is now well established that the decarboxylation and the propylamine transfer reactions are catalysed by different enzymes [1]. Three types of *S*-adenosylmethionine decarboxylase activities were described: (a) prokaryotic enzymes, which were Mg^{2+}-dependent but putrescine insensitive; (b) lower eukaryotic enzymes, which were not influenced either by divalent cations or putrescine; and (c) the enzymes from animals and yeast, which were markedly stimulated by putrescine [2]. An evolutionary significance for the presence of the last-mentioned activity was attributed to the need for adequate levels of decarboxylated *S*-adenosylmethionine for enhanced polyamine biosynthesis [3]. Interestingly enough, in higher plants all the three types of activities were described [4].

Enzymes. *S*-Adenosyl-L-methionine decarboxylase or 5′-phosphoribosyl-5-amino-4-imidazolecarboxylate carboxy-lyase (EC 4.1.1.21); diamine oxidase or amine:oxygen oxidoreductase (deaminating) (EC 1.4.3.6); arginine decarboxylase or L-arginine carboxy-lyase (EC 4.1.1.19).

Arginine decarboxylase and a highly active diamine oxidase, two other enzymes of polyamine metabolism, were earlier purified and characterized from *Lathyrus sativus* seedlings [5,6] (and unpublished observations). In this paper we report the presence of two *S*-adenosylmethionine decarboxylase activities from etiolated seedlings of *L. sativus*, which were separated by immunoaffinity chromatography. One of these, being putrescine dependent but inhibited by catalase, was apparently artifactual. This artifactual activity was catalysed by the contaminating diamine oxidase activity. The other activity, besides being unaffected by putrescine and catalase, had an absolute requirement for Mg^{2+}. This biosynthetic decarboxylating activity was separated from the propylamine transferase (spermidine synthase) by affinity chromatography using *p*-hydroxymercuribenzoate/AH-Sepharose.

MATERIALS AND METHODS

Materials

S-Adenosyl-L-[1-^{14}C]methionine (spec. act. 60 Ci/mol), [1,4-^{14}C]putrescine dihydrochloride (spec. act. 54 Ci/mol) and L-[*methyl*-^{14}C]methionine (spec. act. 56 Ci/mol) were obtained from The Radiochemical

Centre (Amersham, U.K.). DL-[1-^{14}C]Ornithine (spec. act. 2–10 Ci/mol) and DL-[1-^{14}C]methionine (spec. act. 2–10 Ci/mol) were products of New England Nuclear (Boston, U.S.A.). L-[U-^{14}C]Lysine (spec. act. 120 Ci/mol), L-[U-^{14}C]arginine (spec. act. 66 Ci/mol) and L-[U-^{14}C]glutamic acid (spec. act. 55 Ci/mol) were procured from Bhabha Atomic Research Centre (Bombay, India). The sources of biochemicals used and growth of seedlings were referred to elsewhere [5].

Unlabelled *S*-adenosylmethionine, *S*-adenosyl-L-[*methyl*-^{14}C]methionine (spec. act. approx. 10–12 Ci/mol, 40% yield) were prepared enzymatically by using partially purified rabbit liver methionine-activating enzyme [8]. These preparations, as well as the commercially obtained *S*-adenosylmethionine were purified on Dowex 50W × 2 (100–200 mesh) columns prior to use and were repurified if stored for more than 4–6 weeks at −20 °C. Radiochemical purity was ascertained by paper electrophoresis [9].

Electrophoretically and immunologically homogenous *L. sativus* diamine oxidase was prepared from 5-day-old etiolated seedlings and the lyophilized preparations stored for 6–8 months at −20 °C were used in the study of the decarboxylation reactions. Pea seedling diamine oxidase was partially purified up to step 4 [10]. Rat ventral prostate *S*-adenosyl-L-methionine decarboxylase was isolated and assayed as described earlier [11].

Enzyme Assays

S-Adenosyl methionine decarboxylase activity was assayed in Warburg flasks as described for arginine decarboxylase from the same source [3]. A reaction mixture (0.5 ml) containing 20 nmol carboxy-labelled *S*-adenosylmethionine (0.25 μCi) for Mg^{2+}-dependent activity or 50 nmol in the case of putrescine-dependent decarboxylation, 2.5 μmol dithiothreitol, 2.5 μmol Mg^{2+}, 50 nmol pyridoxal phosphate, 100 μmol Tris-HCl buffer, pH 7.6, and 0.5–2 mg protein was incubated for 2 h at 37 °C in a Dubnoff metabolic shaker. The reaction was terminated by the addition of 0.6 ml of 2 M H_2SO_4 from the side-arm and radioactivity in CO_2 liberated was measured. 1 unit of enzyme activity is the amount of enzyme required to liberate 1 nmol $^{14}CO_2$/h at 37 °C. Specific activity is expressed as units/mg protein. The decarboxylation of the other amino acids was assayed in a similar manner adding 1 μmol of the substrate containing 0.25 μCi of the carboxy-labelled amino acid or 1 μCi of the uniformly labelled amino acid. In the case of L-glutamic acid, 5 μCi of the labelled amino acid was added. All assays were corrected for their respective non-enzymatic controls.

Propylamine transferase (spermidine synthase) activity was determined in the crude extracts following the removal of diamine oxidase activity by passing the extract through an immunoaffinity column as described below. The 1-ml reaction mixture for the assay of spermidine synthesis, containing 100 nmol unlabelled *S*-adenosylmethionine, 500 nmol putrescine (1 μCi), 5 μmol dithiothreitol, 5 μmol Mg^{2+}, 100 nmol Tris-HCl buffer, pH 7.6, and 0.5–2 mg protein, was incubated for 2 h at 37 °C. The reaction was terminated by the addition of 0.2 ml 1 M KOH. After 100 nmol each of carrier spermidine and spermine were added, the reaction mixture was heated at 100 °C for 30 min to degrade the sulfonium compounds, acidified with perchloric acid, stored overnight and the polyamines were isolated and separated by circular paper chromatography [12]. Polyamines and putrescine bands were located by spraying the chromatogram with ninhydrin and radioactivity was estimated in a toluene-based scintillating fluid. Corrections were made for the non-specific quenching on paper due to the ninhydrin reaction.

Diamine oxidase activity was assayed spectrophotometrically as described earlier [6].

Affinity Chromatography

p-Hydroxymercuribenzoate was coupled to AH-Sepharose as described earlier for aminoethyl-Sepharose [13]. The column after use was regenerated by passing 10 ml 100 mM $HgCl_2$ and washed thoroughly with equilibrating buffer (see legend to Fig. 3) prior to use. The stop-flow elution method of the enzyme was carried out essentially as described for the rat liver and sea-urchin enzyme [13].

The immunoaffinity column was prepared by coupling antibodies (immunoglobulin G) raised in rabbits against highly purified diamine oxidase of *L. sativus* seedlings, to CNBr-activated Sepharose 4B (unpublished results). The immunoaffinity column (8 × 1.2 cm) was equilibrated with 0.05 M Tris-HCl buffer, pH 7.6, containing 0.9% NaCl, 20 μM pyridoxal phosphate and 2 mM 2-mercaptoethanol. The crude extract of *L. sativus* seedlings was passed through this column twice at a flow rate of 20 ml/h to adsorb diamine oxidase specifically. The resultant effluent showed no detectable diamine oxidase activity.

Protein was estimated by the method of Lowry *et al.* [14] using bovine albumin as standard. Polyacrylamide gel electrophoresis was performed according to the method of Davis [15].

RESULTS

Co-purification of Putrescine-Stimulated S-*Adenosylmethionine Decarboxylase Activity with Diamine Oxidase*

Crude extracts of *L. sativus* seedlings exhibited high *S*-adenosylmethionine decarboxylase activity,

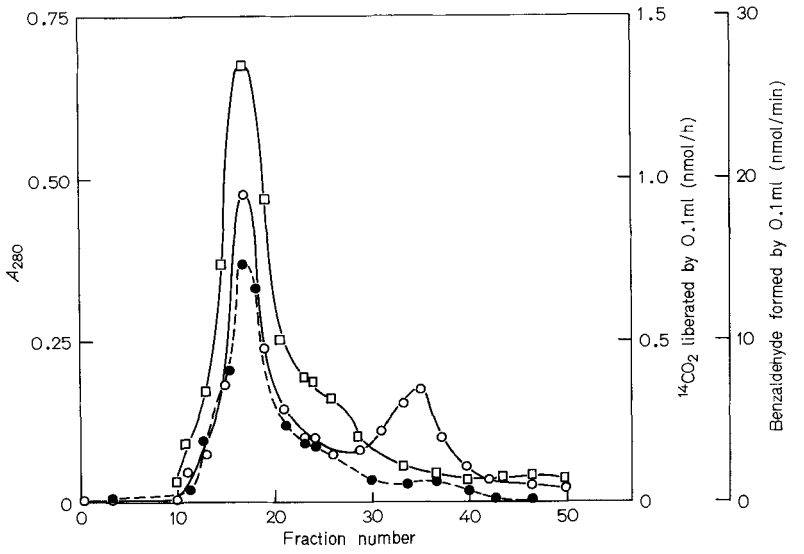

Fig. 1. *Co-purification of putrescine-dependent* S-*adenosyl-*L-*methionine decarboxylase and diamine oxidase from* L. sativus *seedlings on a Sephadex G-100 column.* For experimental details see text. Column dimensions: 35 × 2 cm, 2.5-ml fraction volume. (O——O) Absorbance at 280 nm, (●----●) *S*-adenosyl-L-methionine decarboxylase activity and (□—□) diamine oxidase activity

which was markedly dependent on the addition of exogenous putrescine. This activity was purified by $MnCl_2$ precipitation, $(NH_4)_2SO_4$ fractionation (25–60%) and negative adsorption on DEAE-Sephadex A-50. This purification procedure was essentially the same as that employed for purifying diamine oxidase of *L. sativus* [6], except for the inclusion of 2-mercaptoethanol and pyridoxal phosphate in the buffers. The DEAE-Sephadex fraction, which exhibited both the activities, contained one major and one minor protein component as revealed by analytical polyacrylamide gel electrophoresis at pH 8.3 [15]. This fraction was concentrated by aquacide and applied onto a Sephadex G-100 column. The two enzyme activities coincided with the major protein peak (Fig. 1, peak 1).

The ratio of the specific activities of the two activities was identical in all the steps of purification and in all the fractions of the column. It appeared, therefore, that diamine oxidase was mediating the decarboxylation of *S*-adenosylmethionine indirectly through the oxidative deamination of its substrate, putrescine. To substantiate this hypothesis, further experiments were conducted with a homogenous diamine oxidase preparation stored for 6–8 months at −20 °C and which was isolated under conditions that neither employed 2-mercaptoethanol nor pyridoxal phosphate [6] (and unpublished results). It is evident from Table 1 that even cadaverine (a good substrate for diamine oxidase [6]) but not lysine (a poor substrate) could effectively substitute for putrescine. The reaction was dependent on the amount of diamine oxidase added and this artifactual decarboxylation of *S*-adenosylmethionine was inhibited by the inclusion of beef liver catalase. Since H_2O_2 was one of the products of the diamine oxidase reaction, it was pertinent to investigate whether it could *per se* decarboxylate *S*-adenosylmethionine. This was found to be true, although a fivefold excess of H_2O_2 (assuming complete oxidation of putrescine) was required to bring about a comparable degree of decarboxylation.

Table 1. *Putrescine-dependent* S-*adenosyl-*L-*methionine decarboxylase activity of* L. sativus *diamine oxidase*
The 0.5-ml reaction mixture containing 2.5 µmol dithiothreitol, 100 nmol pyridoxal phosphate, 5 µmol putrescine, cadaverine or lysine, 50 nmol *S*-adenosyl-L-[1-^{14}C]methionine (0.25 µCi), 50 µmol Tris-HCl buffer, pH 7.6, and homogenous diamine oxidase was incubated for 2 h at 37 °C. The radioactivity in CO_2 was measured by liquid-scintillation counting

Addition to the reaction mixture	$^{14}CO_2$ liberated
	nmol/h
Putrescine	0
Putrescine + 50 µg diamine oxidase	0.526
Putrescine + 100 µg diamine oxidase	0.977
Putrescine + 100 µg diamine oxidase + 100 U catalase	0
Cadaverine + 50 µg diamine oxidase	0.343
Cadaverine + 50 µg diamine oxidase + 100 U catalase	0
Lysine + 50 µg diamine oxidase	0.042
10 mM H_2O_2	0.347
25 mM H_2O_2	0.957

Characterization of the Product of Diamine-Oxidase-Reaction-Dependent S-*Adenosylmethionine Decarboxylation*

A large-scale reaction was incubated with 1 mg pure diamine oxidase, 100 µmol putrescine and

500 nmol *S*-adenosylmethionine and 2 μCi of *S*-adenosyl-L-[*methyl*-^{14}C]methionine in 0.1 M Tris-HCl buffer, pH 7.6, for 4 h at 37 °C. The protein-free extract was loaded on to a Dowex 50 × 2 (100–200 mesh) column (5 × 1.2 cm) and the 4 M HCl fraction was concentrated and analysed for decarboxylated *S*-adenosylmethionine [8,9]. When an aliquot of the extract was subjected to paper electrophoresis at pH 3 in 0.1 M citrate/NaOH, a fast-moving radioactive spot, corresponding to *S*-adenosylhomocysteamine [9], was observed in addition to *S*-adenosylmethionine. Another aliquot of the solution was subjected to hydrolysis in 0.1 M NaOH at 90 °C for 30 min and products were resolved by paper chromatography using the solvent methylcellosolve/propionic acid/H_2O saturated with NaCl (70/15/15, v/v/v) [8]. Two distinct major bands of radioactivity with R_F of 0.6 (corresponding to methionine) and 0.88 (corresponding to 3-methylthiopropylamine) were observed.

Properties of the Diamine-Oxidase-Catalysed Decarboxylation

The data of Table 2 show that diamine-oxidase-mediated artifactual decarboxylation of *S*-adenosylmethionine was not dependent on either pyridoxal phosphate and/or dithiothreitol in contrast to the true biosynthetic *S*-adenosylmethionine decarboxylase activity to be described later. The omission of dithiothreitol significantly enhanced the decarboxylation rate. Addition of EDTA (1–5 mM) had no effect.

In view of the above observations, it was of interest, therefore, to investigate whether the putrescine-dependent decarboxylation is specific to *S*-adenosylmethionine or is a more general phenomenon. Table 3 shows the relative rates of decarboxylation of a few amino acids *vis-à-vis* that of *S*-adenosylmethionine. In all these cases the liberation of $^{14}CO_2$ was putrescine dependent and was abolished on addition of beef liver catalase. The decarboxylation of lysine occurred, albeit at a lower rate, even in the absence of putrescine [16] but was also inhibited by catalase. This observation can be explained by the fact that lysine is the only amino acid substrate for diamine oxidase [6]. All other amino acids tested were not decarboxylated in the absence of putrescine. When putrescine is present, basic amino acids, *viz.*, ornithine and arginine, were decarboxylated at relatively higher rates than a dicarboxylic acid like glutamic acid. Furthermore, the rate of *S*-adenosylmethionine decarboxylation was fivefold higher than that of methionine. Degradation of *S*-adenosylmethionine to free methionine did not occur under the assay conditions as revealed by paper chromatography employing methylcellosolve/propionic acid/H_2O saturated with NaCl (70/15/15, v/v/v) in agreement with the earlier observations on the stability of *S*-adenosylmethionine [17].

Table 2. *Necessary components of the putrescine-dependent decarboxylation of* S-*adenosyl-L-methionine by homogenous* L. sativus *diamine oxidase*
The components of the reaction mixture are as described in Table 1. 5 μmol putrescine and 50 μg diamine oxidase were used

Additions (+)/omissions (–) to the reaction mixture	$^{14}CO_2$ liberated
	nmol/h
None	0.55
+ Catalase, 100 U	0.0
+ EDTA 5 mM	0.56
– Pyridoxal phosphate	0.47
– Dithiothreitol	1.21

Table 3. *Relative rates of diamine-oxidase-reaction-dependent decarboxylation of some amino acids and* S-*adenosyl-L-methionine*
The 1-ml reaction mixture containing 1 μmol substrate with 0.1 μCi carboxy-labelled or 1 μCi uniformly labelled amino acid (5 μCi was used in case of glutamic acid), 10 μmol putrescine, 100 μg diamine oxidase and 100 μmol Tris-HCl buffer, pH 7.6, was incubated for 2 h at 37 °C

Substrate	$^{14}CO_2$ liberated	Relative rate
	nmol/h	%
L-Lysine	21.34	100
L-Lysine (putrescine omitted)	13.34	63
L-Ornithine	5.52	26
L-Arginine	3.12	15
L-Methionine	2.50	12
L-Glutamic acid	0.52	2
S-Adenosyl-L-methionine	12.59	59

We have observed that like the pig kidney diamine oxidase [18] the enzyme from *L. sativus* also generated the free radical, superoxide anion, $O_2^{\cdot-}$. This anion undergoes dismutation to generate H_2O_2. However, inclusion in the reaction of a model system consisting of NADH and phenazine methosulphate to generate $O_2^{\cdot-}$ *in situ* [19] failed to decarboxylate *S*-adenosylmethionine or lysine at both acidic and neutral pH values (data not given).

The H_2O_2-mediated decarboxylation of *S*-adenosylmethionine was considerably enhanced by the addition of 100 μg diamine oxidase protein, which, however, could not be substituted by an equivalent amount of bovine serum albumin (Table 4). This enhancement of decarboxylation by the enzyme protein alone in the presence of exogenous H_2O_2 was also observed with ornithine and glutamic acid (data not given).

Properties of the Biosynthetic S-*Adenosyl-L-Methionine Decarboxylase: Mg^{2+} Requirement and Putrescine Independence*

During the attempts to distinguish between the apparent putrescine-stimulated activity from the bio-

synthetic activity in the crude extracts of *L. sativus* seedlings, a Mg^{2+}-requiring weak *S*-adenosylmethionine decarboxylase activity was observed. This activity was not inhibited by catalase. The activity in the whole seedlings was very low and higher specific activity was obtained with the embryo axis portion (see below). The crude extracts of the embryo axis were passed through the immunoaffinity column to absorb diamine oxidase selectively and hence remove the artifactual decarboxylase activity. Putrescine addition to such an enzyme preparation failed to stimulate the decarboxylation of *S*-adenosylmethionine either in the presence or absence of 5 mM Mg^{2+} (data not given).

Distribution during Growth

To localize the enzyme and changes in its content during germination, the activity was assayed in the different parts of the seedling. Isolated cotyledons had negligible activity throughout the 10-day growth period. In contrast, the embryo axis showed the highest specific activity on the 3rd day of germination, declining thereafter (Fig. 2A). An essentially similar pattern but with a 3-fold higher specific activity was observed with the isolated shoots. By comparison, the root portion had feeble activity during the first few days of germination, increasing marginally thereafter. This was also true with the total activity in the root portion of the seedling. A major amount of the activity of the embryo axis was localized in the shoot portion, which rapidly increased during days 2–3 followed by a slow, yet steady increase subsequently (Fig. 2B).

Table 4. *Stimulation of H_2O_2-catalysed decarboxylation of* S-adenosyl-L-*methionine by diamine oxidase*
The 0.5-ml reaction mixture contained 50 nmol *S*-adenosyl-L-[1-^{14}C]methionine (0.25 μCi) and 5 μmol H_2O_2

Additions to reaction mixture	$^{14}CO_2$ liberated
	nmol/h
None	0.33
200 μg bovine serum albumin	0.32
200 μg diamine oxidase	0.72
200 μg diamine oxidase + 100 U catalase	0.0

Purification of the Enzyme

The isolated shoots of the 3-day-old etiolated seedlings were selected as starting material for the enzyme purification. This selection eliminated a major portion of the proteins of the seedling including diamine oxidase. Taking advantage of the sulfhydryl nature of several amino acid decarboxylases and particularly of rat liver and sea-urchin *S*-adenosylmethionine decarboxylase [13] and the absence of cysteine residues in diamine oxidase (unpublished) an affinity column of *p*-hydroxymercuribenzoate/AH-Sepharose was employed to separate and purify the biosynthetic enzyme. The elution profile of the enzyme from the affinity column is depicted in Fig. 3. The unadsorbed fractions contained all the diamine oxidase activity and the same could not be detected in the eluted fractions (not shown in Fig. 3). The enzyme eluted from this column was further fractionated batchwise using DEAE-Sephadex A-50. After washing the unadsorbed protein with 50 mM Tris-HCl, pH 7.6, containing 20 μM pyridoxal phosphate and 2 mM 2-mercaptoethanol, the ion-exchanger was washed successively with the above buffer containing 0.1 M, 0.3 M and 0.5 M KCl. The eluted protein fractions were concentrated by aquacide and dialysed against the same buffer. The 0.3 M KCl eluate had the *S*-adenosylmethionine decarboxylase activity while the 0.5 M KCl fraction showed arginine decarboxylase activity [5]. On polyacrylamide gel electrophoresis at

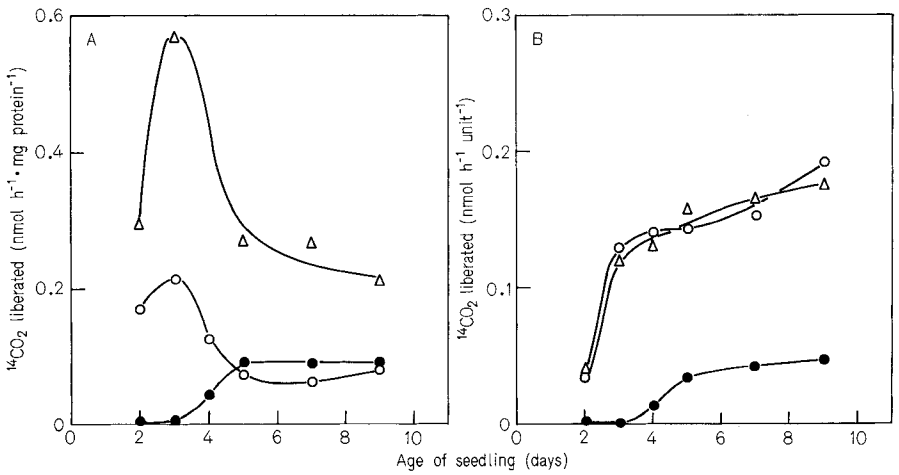

Fig. 2. *Distribution of Mg^{2+}-dependent* S-adenosyl-L-*methionine decarboxylase during growth of* L. sativus *seedlings.* 1 unit represents either an embryo axis, root or shoot. Data are the means of triplicate values. (A) Specific activity. (B) Total activity. (○——○) Embryo axis; (△——△) shoot; (●——●) root

pH 8.3 the 0.3 M KCl eluate showed two fast-moving protein bands of equal intensity. At this stage the enzyme was purified 42-fold with a yield of approximately 18% (Table 5). As the object of this investigation was to study the biosynthetic enzyme free from diamine oxidase contamination, the enzyme preparation after affinity chromatography was used in these studies.

pH and Temperature Optima

The enzyme exhibited a single pH optimum at pH 7.6 in 0.1 M Tris-HCl buffer containing 5 mM dithiothreitol, 100 μM pyridoxal phosphate and 5 mM Mg^{2+}. The activity was lower in 0.1 M sodium phosphate buffers. The enzyme at pH 7.6 in 0.1 M Tris-HCl exhibited maximum activity around 35–37 °C and had less than 30% of its activity at 45 °C.

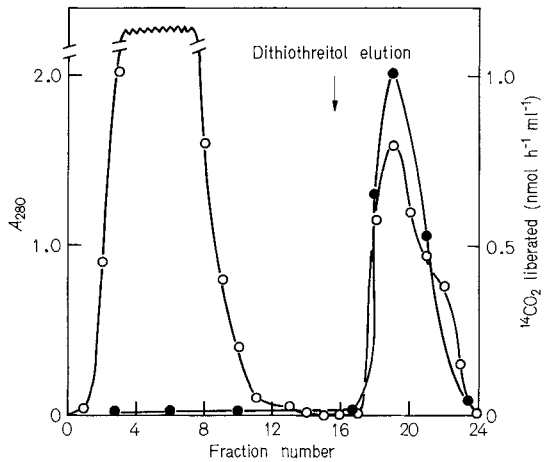

Fig. 3. *Affinity chromatography of Mg^{2+}-dependent* S-*adenosyl-*L-*methionine decarboxylase of* L. sativus *seedlings on a* p-*hydroxymercuribenzoate-Sepharose column.* The shoots or embryo axis were homogenized in 0.1 M Tris-HCl, pH 7.6, containing 5 mM dithiothreitol, 5 mM Mg^{2+} and 100 μM pyridoxal phosphate and centrifuged at 17000 × *g* for 30 min and supernatants were dialysed for 4–6 h against 10 mM Tris-HCl, pH 7.6, containing 10 μM pyridoxal phosphate. This fraction was loaded on to the *p*-hydroxymercuribenzoate-Sepharose column (4 × 0.8 cm) equilibrated with 10 mM Tris-HCl, pH 7.6, containing 10 μM pyridoxal phosphate and 0.1 mM dithiothreitol. The column was washed free of any unadsorbed proteins including diamine oxidase with 100 ml of equilibrating buffer and the enzyme eluted (arrow indicated) with 10 ml of 0.1 M Tris, pH 7.6, containing 50 mM dithiothreitol, 100 μM pyridoxal phosphate and 5 mM Mg^{2+}. The details of operation of the affinity column are as described for rat liver and sea-urchin enzyme [13]. (○——○) Absorbance at 280 nm; (●——●) *S*-adenosyl-L-methionine decarboxylase activity

Effect of Various Enzyme Inhibitors

The carbonyl reagent, semicarbazide, markedly inhibited the enzyme activity on preincubating the affinity fraction for 15 min before addition of other reactants. At 1 mM 80% inhibition was observed and complete inhibition at 10 mM. Even 1 mM 4-bromo-3-hydroxybenzoyloxyamine dihydrogen phosphate (an inhibitor of pyridoxal-phosphate-dependent enzymes [20]) and methylglyoxal-bis(guanylhydrazone) hydrochloride totally inhibited the enzyme activity. Only high concentrations (10 mM) of *p*-hydroxymercuribenzoate and EDTA could bring about 100% inhibition. This is understandable in view of the inclusion of 5 mM dithiothreitol and 5 mM Mg^{2+} in the assay mixture.

Resolution of S-*Adenosylmethionine Decarboxylase from Spermidine Synthase*

In view of the observation that different enzymes catalyse the decarboxylation of *S*-adenosylmethionine and subsequent propylamine transfer therefrom [1] it was of interest to resolve the two activities in this plant system also. The two aforementioned affinity columns were employed for this purpose. The crude extract of 3-day-old shoots was passed through the immunoaffinity column to remove diamine oxidase and hence to prevent oxidation of [^{14}C]putrescine and subsequent decarboxylation of *S*-adenosylmethionine. Such a crude extract was incubated with the components of the Mg^{2+}-dependent *S*-adenosylmethionine decarboxylase reaction (see Materials and Methods) along with the propylamine acceptor, *viz.*, [1,4-^{14}C]putrescine. When spermidine and spermine were analysed subsequently for radioactivity, a considerable amount of label was found in spermidine, indicating the presence in the crude extract of propylamine transferase activity capable of efficiently utilis-

Table 5. *Purification protocol of Mg^{2+}-dependent* S-*adenosyl-*L-*methionine decarboxylase from 3-day-old embryo axes of* L. sativus *seedlings*
HgOHBz = *p*-hydroxymercuribenzoate

Purification step	Total protein	Total activity	Specific activity	Purification	Yield
	mg	U	U/mg protein	-fold	%
1. Crude extract	100	12.8	0.128	1.00	100
2. HgOHBz-Sepharose affinity column	2.4	5.7	2.381	19	44.4
3. DEAE-Sephadex	0.4	2.3	5.5	42	18

Table 6. *Resolution of* S-*adenosyl-*L-*methionine decarboxylase from spermidine synthase activity*
Diamine-oxidase-free crude extract was obtained by employing an immunoaffinity column (see text). HgOHBz = *p*-hydroxymercuribenzoate

Enzyme fraction	$^{14}CO_2$ liberated (*a*)	[^{14}C]Spermidine formed (*b*)	Ratio *a*/*b*
	nmol/mg protein		
Diamine-oxidase-free crude extract	0.132	0.153	0.86
HgOHBz-Sepharose affinity fraction	2.818	0.028	100

ing *S*-adenosylhomocysteamine formed *in situ*. When the above enzyme extract was absorbed on a *p*-hydroxymercuribenzoate/AH-Sepharose column, as described earlier, the *S*-adenosylmethionine decarboxylase thus purified, when assayed for propylamine transferase, exhibited negligible label in the spermidine band despite the 20-fold purification of the decarboxylase (Table 6). Thus the ratios of the two activities in the two steps were drastically different. This suggests that *S*-adenosylmethionine decarboxylase activity is not coupled to the propylamine transferase reaction and that the two reactions are catalysed by different protein fractions. Further purification and characterization of the propylamine transferase is under progress employing decarboxylated *S*-adenosylmethionine and putrescine as substrates. During spermidine synthetase assay, only marginal incorporation of radioactivity was observed in the spermine band.

DISCUSSION

The most significant feature of the present investigation is the demonstration that *L. sativus* seedling extracts contain two distinct and separable *S*-adenosylmethionine decarboxylating activities. One of these is the sulfhydryl, Mg^{2+}-dependent biosynthetic decarboxylase which is not stimulated by the diamine, putrescine, while the other, although more active, is artifactual and is attributable to H_2O_2 liberated *in situ* during the oxidative deamination of putrescine catalysed by the contaminating diamine oxidase of this higher plant.

The biosynthetic *S*-adenosylmethionine decarboxylase has several common properties of amino acid decarboxylases in general and *S*-adenosylmethionine decarboxylases of other prokaryotic and eukaryotic systems in particular. The Mg^{2+} requirement and absence of activation by putrescine is similar to bacterial and plant enzymes described earlier [21]. The sulfhydryl nature of the *L. sativus* enzyme is evident by its binding to the organomercurial Sepharose column and subsequent elution by dithiothreitol, a procedure which was employed for the purification of rat liver and sea-urchin *S*-adenosylmethionine decarboxylase [13]. The inhibition brought about by a carbonyl reagent and a pyridoxal phosphate inhibitor is similar to that shown by other decarboxylases [5]. Methylglyoxal-bis(guanylhydrazone), a specific inhibitor of putrescine-dependent *S*-adenosylmethionine decarboxylase of mammalian tissues and yeast [22], inhibits this plant enzyme as well. The actively growing shoot had maximum amounts of the enzyme, as could be expected of the tissue which elaborates a major portion of the polyamines of the whole seedling [23]. Preliminary results suggest that *S*-adenosylmethionine decarboxylase of *L. sativus* could be separated from spermidine synthase.

The hypothesis that the artifactual decarboxylation of *S*-adenosylmethionine is due to the diamine oxidase reaction is supported by the observations that (a) putrescine-stimulated *S*-adenosylmethionine decarboxylase activity co-purified with *L. sativus* diamine oxidase, and this reaction was also mediated by the homogenous diamine oxidase; (b) the decarboxylation was inhibited by catalase and H_2O_2 *per se* decarboxylated *S*-adenosylmethionine and other amino acids; (c) cadaverine, also a good substrate for diamine oxidase [6], could effectively replace putrescine. The decarboxylation reaction is apparently non-enzymatic, although the presence of diamine oxidase protein greatly enhances the reaction rate (Table 4).

The non-enzymatic decarboxylation of amino acids, their derivatives and biologically important compounds has been extensively documented [21–31]. Among these models are (a) the Fenton's system ($H_2O_2 + Fe^{2+}$), which decarboxylated several aliphatic and aromatic amino acids [24–27]; (b) the decarboxylation of amino acids on heating with pyridoxal [28,29]; (c) photolytic decarboxylation of some dinitrophenylamino acids [30]; (d) pyridoxal phosphate and Mn^{2+}-dependent decarboxylation of several amino acids [31]; (e) decarboxylation of 3-oxo acids by primary amines and metal ions [32]; and in particular (f) decarboxylation of methionine catalysed by Fe^{2+} [33] or a combination of horse-radish peroxidase, pyridoxal phosphate and Mn^{2+} [34]. The present system represents yet another variant that is entirely dependent on H_2O_2 generated *in situ* and inhibited by catalase (Table 1). This is in direct contrast to the inhibition by H_2O_2 of the oxidative decarboxylation of amino acids catalysed by horse-radish peroxidase [34]. The present model is independent of added metal ion. The inhibitory influence of dithiothreitol, although not clear, is also observed with another non-enzymatic system [21]. Requirement of relatively higher concentrations of exogenous H_2O_2 (Table 1) to substitute for diamine-oxidase-catalysed putrescine-

stimulated decarboxylation of *S*-adenosylmethionine or lysine is not clear at present and may be due to the participation of nascent or an active species of H_2O_2 produced *in situ* and still bound to the active site (Table 4). Presumably the active site of diamine oxidase is involved in the enhanced rate of decarboxylation by bringing a proximity effect between H_2O_2 and the substrate. Thus, the different rates of decarboxylation observed with various amino acids and *S*-adenosylmethionine may be attributable both to differential affinities of the substrates and turnover of the enzyme. It is to be emphasized that methionine is decarboxylated at a distinctly lower rate than its activated derivative *S*-adenosylmethionine. The demonstration of *S*-methyladenosylhomocysteamine rules out the degradation of *S*-adenosylmethionine to free methionine and subsequent release of $^{14}CO_2$.

The presence of the artifactual putrescine-dependent *S*-adenosylmethionine decarboxylase activity calls for caution in interpreting data on putrescine-stimulated decarboxylation of *S*-adenosylmethionine in crude extracts of other systems. The partially purified pea seedling diamine oxidase (approx. 10 units), despite being immunologically cross-reactive with the *L. sativus* enzyme (unpublished results), did not exhibit this artifactual decarboxylase activity (data not given). A feeble catalase-sensitive lysine decarboxylase activity was observed when it was included in the glucose/glucose oxidase (approx. 80 units) system, which also generates H_2O_2 *in situ* [35]. Further, the absence of inhibition by catalase of the ventral prostate putrescine-dependent *S*-adenosylmethionine decarboxylase activity (data not given) suggested that this high artifactual activity observed with *L. sativus* diamine oxidase was not a very general phenomenon.

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