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S-Adenosylmethionine Decarboxylase of Corn Seedlings[1]

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ABSTRACT

S-Adenosylmethionine decarboxylase (EC 4.1.1.50) has been purified 500-fold in 30% yield from the extract of etiolated corn seedlings (cv. Golden Crossbantam Bell). This preparation had a molecular weight of approximately 25,000. The K_m value was 5 micromolar for S-adenosylmethionine. Methylglyoxal bis(guanylhydrazone), hydroxylamine, and sulfhydryl reagents (such as p-hydroxymercuriphenylsulfonate and N-ethylmaleimide) were effective inhibitors of this enzyme. Germination of corn seed was accompanied by a rapid increase in enzyme activity and maximum activity occurred in 5-day-old seedlings.

The polyamines spermidine and spermine are distributed widely in organisms (2) and they are probably involved in the control of protein synthesis and growth through interaction with nucleic acids (3). Information on polyamine metabolism in higher plants is limited; however, a polyamine-specific oxidase from Gramineae has been characterized (10, 18). In animals and microorganisms (20), polyamines are synthesized by two separate enzymes, namely from S-adenosylmethionine by S-adenosylmethionine decarboxylase (EC 4.1.1.50) and by aminopropyltransferase (EC 2.5.1.16) from putrescine. These enzymes appear to function in sequence in animals and microorganisms in the synthesis of spermidine, as is shown in the following linked reactions:

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

$$\text{Decarboxylated AMe} + \text{putrescine} \rightarrow \text{spermidine} + \text{thiomethyladenosine}$$

where AMe is S-adenosylmethionine. Only limited information about these enzymes has previously been available in higher plants (19). S-Adenosylmethionine decarboxylase activity has been described in extracts of mung bean (6) and *Vinca rosea* (4). The principal difference noted between the crude enzyme preparations obtained from these species lies in the fact that the enzyme from mung bean is stimulated 2-fold by Mg^{2+} but not by putrescine, whereas the enzyme from *Vinca rosea* is unaffected by Mg^{2+} but putrescine enhances its activity 8-fold. This paper deals with the purification and properties of S-adenosylmethionine decarboxylase derived from corn seedlings.

MATERIALS AND METHODS

Plant Materials. Seedlings of corn (*Zea mays* L. cv. Golden Crossbantam Bell) were grown in moist vermiculite in plastic trays in the dark at 25 C. Following germination, the shoots were harvested at daily intervals.

Sources. The following materials were obtained from Sigma Chemical Co.: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, hyamine hydroxide, purtrescine dihydrochloride, sodium p-hydroxymercuriphenylsulfonate, N-ethylmaleimide, alcohol dehydrogenase (yeast), BSA, ovalbumin, trypsin inhibitor (chicken egg white), and Cyt c (horse heart). The following materials were obtained from Wako Pure Chemical Co.: 2,2'-dihydroxy-6,6'-dinaphthyl disulfide, 5,5'-dithiobis(2-nitrobenzoic acid), hydroxylamine hydrochloride, aminoguanidine bicarbonate, semicarbazide hydrochloride, and isonicotinic acid hydrazide. Other materials were obtained as follows: TMAP[2]-cellulose from Toyo Parupu Co. (Hiro-machi, Kure, Hiroshima 737-01, Japan); MGBG from Aldrich; Sephadex G-200 and CH-Sepharose 4B from Pharmacia; Bio-Gel P-150 from Bio-Rad Laboratories; L-glutamic acid decarboxylase from Kyowa Hakko Co.; and S-adenosyl-L-[carboxyl-^{14}C]methionine from Radiochemical Center, Amersham.

Preparation of MGBG-Sepharose 4B. MGBG was linked to Sepharose as described by Pegg (14) in the following way: CH-Sepharose 4B (7 g) was swollen overnight in 0.5 M NaCl (500 ml), washed with 0.5 M NaCl (2 liters), and then washed with deionized H_2O (2 liters). The washed gel was suspended in a total volume of 50 ml H_2O containing 0.6 g MGBG and the pH was adjusted to 5.5 with 1 M NaOH. The mixture was stirred slowly, 1.5 g 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide dissolved in 2.5 ml H_2O was added dropwise, and then the solution was shaken gently for 3 days at 5 C. The gel then was washed with 0.5 M NaCl (4 liters) and with 10 mM Tris-Cl (pH 7.5) containing 1 mM 2-mercaptoethanol and 0.1 mM EDTA and used as MGBG-Sepharose for affinity chromatography.

Assay of S-adenosylmethionine Decarboxylase Activity. S-Adenosylmethionine decarboxylase activity was assayed at 30 C by a modification of the method of Cohn *et al.* (5). The assay mixture contained 100 mM Tris-Cl buffer (pH 8.6), 1 mM 2-mercaptoethanol, 0.1 mM EDTA, and enzyme solution in a total volume of 0.4 ml. The reaction was carried out in a glass scintillation vial; to trap any $^{14}CO_2$ formed, the cap contained a quarter circle of Whatman 2.5-cm GF/C glass microfiber paper, impregnated with 20 μl 1 M hyamine hydroxide. After 5 min at 30 C, 0.5 nmol S-adenosyl-L-[carboxyl-^{14}C]methionine (61 mCi/mmol) was added to the assay mixture, and the vial was immediately capped. After 15 min, the reaction was stopped by the addition of 0.2 ml 1 M KH_2PO_4. The vial was immediately closed and shaken 60 min at 37 C to facilitate the release of CO_2. The paper containing the trapped $^{14}CO_2$ was transferred to another vial containing 5 ml toluene-base scintillation fluid and counted. One unit of enzyme activity is defined as the amount of enzyme catalyzing the formation of 1 μmol $^{14}CO_2$/min under the above assay conditions. Enzyme activity was a linear function of both incubation time and concentration under these conditions. The boiled enzyme preparations were used as the control.

Determination of Mol Wt. The mol wt of S-adenosylmethionine

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[2] Abbreviations: TMAP, trimethylamino-2-hydroxypropyl; MGBG, methylglyoxal bis(guanylhydrazone).

decarboxylase was determined on a Sephadex G-200 column (1.3 × 115 cm; flow rate, 4 ml/h) and a Biol-Gel P-150 column (2.0 × 50 cm; flow rate, 2 ml/h). Each column was equilibrated by the ascending method with 10 mM Tris-Cl buffer (pH 7.5) containing 0.1 or 2.0 M NaCl and 0.1 mM EDTA (1). The two columns were calibrated with Cyt *c* (mol wt = 13,000), trypsin inhibitor (mol wt = 28,000), egg albumin (mol wt = 45,000), BSA (mol wt = 68,000), and alcohol dehydrogenase (mol wt = 141,000) as markers of known mol wt.

Protein Determination. Protein content was determined by the method of Lowry *et al.* (11), with BSA as the standard.

RESULTS

Changes of *S*-Adenosylmethionine Decarboxylase Activity during Seedling Growth. Figure 1 shows the changes in *S*-adenosylmethionine decarboxylase activity during germination and early growth of corn. At the onset of germination, enzyme activity was nil or very low; thereafter, activity increased rapidly. The highest specific activity (activity per mg extractable protein or per seed) was found after about 5 days germination. Subsequently, enzymic activity declined as germination progressed. On the basis of protein content, a sharp decrease in activity occurred 6 days after the onset of germination but, on a per seed basis, the decrease was not dramatic.

Purification of *S*-Adenosylmethionine Decarboxylase from Corn Seedlings. A summary of the enzyme purification procedure is given in Table I. After surface-sterilizing, shoots (about 150 g) of 5-day-old seedlings for 5 min in 0.1% benzalkonium chloride solution, they were homogenized in a Waring Blendor with 300 ml 50 mM Tris-Cl (pH 7.5) containing 1 mM 2-mercaptoethanol and 0.1 mM EDTA. After the homogenate was filtered through four layers of gauze, the filtrate was centrifuged 15 min at 10,000*g*. The supernatant was fractionated stepwise with ammonium sulfate, and the fraction obtained between 20 and 50% saturation was collected by centrifugation for 15 min at 10,000*g*. The pellet containing the enzyme was dissolved in 100 ml Tris-Cl (pH 7.5) containing 1 mM 2-mercaptoethanol and 0.1 mM EDTA; the resulting solution was dialyzed overnight against the same buffer.

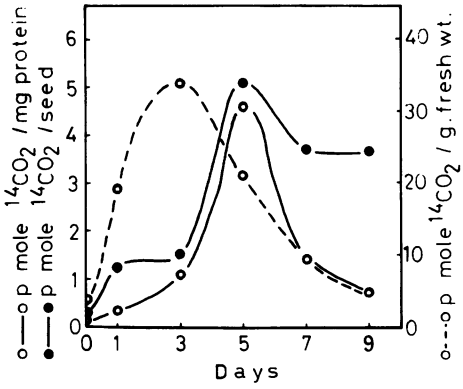

FIG. 1. Changes in *S*-adenosylmethionine decarboxylase activity in corn seedlings.

The dialysate was centrifuged for 15 min at 10,000*g* and applied to a column (1.1 × 30 cm) of TMAP-cellulose (about equivalent electric charge to triethylaminoethyl-cellulose) previously equilibrated with 10 mM Tris-Cl (pH 7.5) containing 1 mM 2-mercaptoethanol and 0.1 mM EDTA. The column was washed with the same buffer. Elution of the enzyme was achieved with 100 ml of a linear gradient of 0 to 0.5 M NaCl made up in the equilibration buffer. Active fractions were pooled (Fig. 2) and applied on a column of MGBG-Sepharose 4B (1.1 × 20 cm) previously equilibrated with 10 mM Tris-Cl (pH 7.5) containing 1 mM 2-mercaptoethanol and 0.1 mM EDTA. Elution was achieved with the same buffer (Fig. 3, curve A). Next, the column was washed with the equilibration buffer solution containing 0.3 M NaCl (Fig. 3, curve B). Finally, the enzyme was eluted with the equilibration buffer containing 0.3 M NaCl and 1 mM MGBG (Fig. 3, curve C). Each fraction of curve C then was dialyzed against the equilibration buffer thoroughly. Enzyme activity and total protein were determined in each dialyzed fraction. Active fractions were combined and concentrated to 5 ml in a collodion bag (Sartorius membrane filter SM 13,200). This solution was used as the purified enzyme preparation.

Effects of pH on Activity. Activity of the purified *S*-adenosylmethionine decarboxylase was determined with reaction mixtures maintained at pH values 7.1 to 9.5 by 0.2 M Tris-Cl buffers. The pH optimum for the enzyme was 8.6 (Fig. 4A). Samples of the enzyme were adjusted to various pH values and maintained at 5 C for 3 days before assaying at pH 8.6 and 30 C. The enzyme was stable in the range of pH 7 to 8, but was sharply reduced as alkalinity increased (Fig. 4B).

Kinetic Properties. The effect of concentration of *S*-adenosylmethionine on the reaction velocity was determined (Fig. 5). The double-reciprocal plots were linear for *S*-adenosylmethionine concentrations. The enzyme possessed the K_m value of 5 μM.

Mol Wt. *S*-Adenosylmethionine decarboxylase from corn seedlings had an approximate mol wt of 25,000 by comparing their elution volumes from gel filtrations of Sephadex G-200 (Fig. 6A) and Bio-Gel P-150 (Fig. 6B), respectively, with five proteins of known mol wt.

Effect of Inhibitors and Activators. Table II presents the effects of various substances on the enzyme activity. Mg^{2+} and putrescine which were activators of *S*-adenosylmethionine decarboxylase of mung bean (6) and *Vinca rosea* (4), respectively, were ineffective on corn enzyme when tested at the concentrations in the range of 0.1 to 25 mM. MGBG was a more effective inhibitor of corn enzyme than other carbonyl reagents, such as hydroxylamine, semicarbazide, $NaBH_4$, and KCN. Isonicotinic acid hydrazide, a specific inhibitor of some pyridoxal enzymes (7), was almost ineffective on corn enzyme. The sulfhydryl reagents such as *p*-hydroxymercuriphenylsulfonate, *N*-ethylmaleimide, 2,2'-dihydroxy-6,6'-dinaphthyl disulfide, and 5,5'-dithiobis(2-nitrobenzoic acid), at a concentration of 1 mM did inhibit corn *S*-adenosylmethionine decarboxylase activity. Very strong inhibition was obtained by *p*-hydroxymercuriphenylsulfonate and *N*-ethylmaleimide.

Table I. *Purification of Corn S-Adenosylmethionine Decarboxylase*

Step	Fraction	Total Volume	Total Activity	Protein	Specific Activity	Recovery
		ml	*units*	*mg/ml*	*μunits/mg protein*	%
1	Crude extract	310	279.0	13.1	0.069	100
2	$(NH_4)_2SO_4$ precipitate	120	169.2	7.8	0.181	60.6
3	TMAP-cellulose column eluates	100	115.0	2.4	0.479	41.3
4	MGBG-column eluates	20	85.2	0.12	35.5	30.5

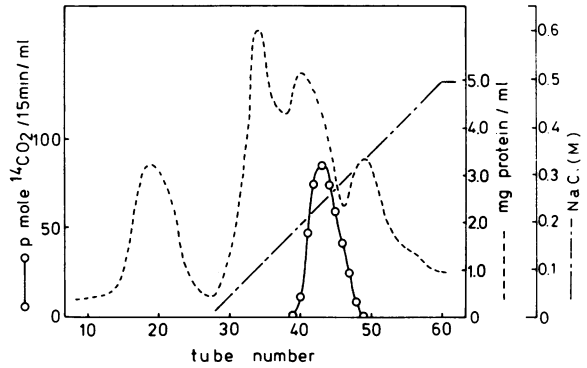

FIG. 2. TMAP-cellulose column chromatography of corn *S*-adenosylmethionine decarboxylase.

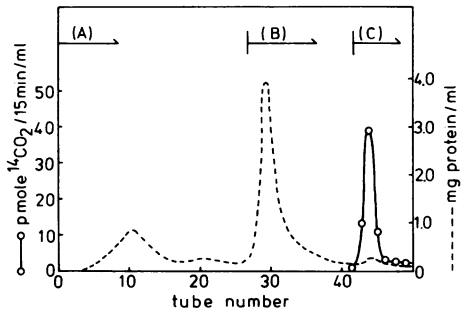

FIG. 3. MGBG-Sepharose 4B column chromatography of corn *S*-adenosylmethionine decarboxylase. For details, see "Purification of *S*-Adenosylmethionine Decarboxylase from Corn Seedlings" under "Results."

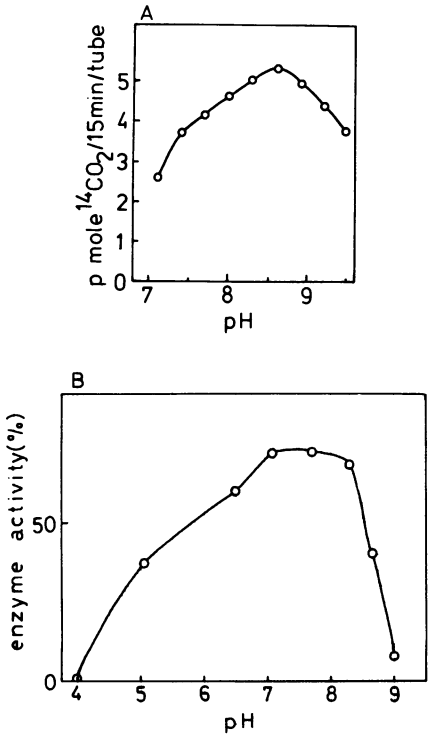

FIG. 4. A, effect of pH on corn *S*-adenosylmethionine decarboxylase. Assay conditions are given in the text. B, pH stability of corn *S*-adenosylmethionine decarboxylase. The following buffer solutions were used: acetate-sodium buffer for pH 4.0 and 5.1; Na/K-phosphate buffer for pH 6.5; Tris-Cl buffer for pH 7.1, 7.6, 8.3, 8.6, and 9.0. Assay conditions are given in the text.

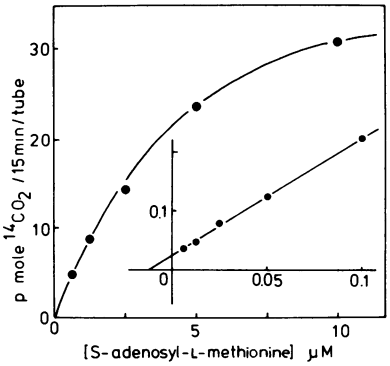

FIG. 5. Effect of substrate concentration on reaction rate. Standard assay conditions were employed. The inset is a Lineweaver-Burk plot of these same data.

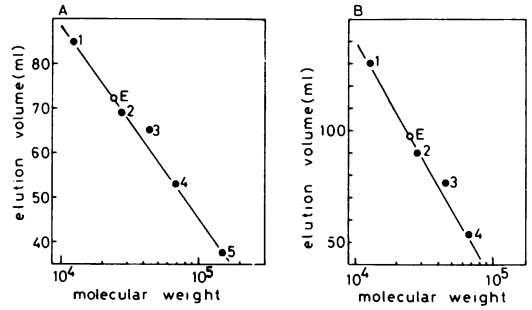

FIG. 6. A, determination of mol wt of corn *S*-adenosylmethionine decarboxylase by calibrated Sephadex G-200 gel filtration. 1, Cyt *c*; 2, trypsin inhibitor; 3, ovalbumin; 4, BSA; 5, alcohol dehydrogenase; E, *S*-adenosylmethionine decarboxylase. B, determination of mol wt of corn *S*-adenosylmethionine decarboxylase by calibrated Bio-Gel P-150 gel filtration. The numbers are the same as in A.

DISCUSSION

We have purified *S*-adenosylmethionine decarboxylase from corn seedlings approximately 500-fold. The apparent mol wt of native enzyme was determined to be 25,000 by two distinct gel filtrations on Sephadex G-200 and Bio-Gel P-150. This value is very small, compared with those of rat liver [mol wt = 155,000 (8) and 52,000 (9)], yeast [mol wt = 84,000 or 88,000 (5)], mouse mammary gland [mol wt = 68,000 (17)], mouse liver [mol wt = 74,000 (17)], and *Escherichia coli* [mol wt = 113,000 (21)]. The K_m for *S*-adenosylmethionine was determined as 5 μM. This is low when compared to those of *E. coli* [K_m = 90 μM (21)], bakers' yeast [K_m = 90 μM (15)], and rat liver [K_m = 36 μM (9) and 80 μM (8)]. A striking difference between *S*-adenosylmethionine decarboxylase from prokaryotic and eukaryotic organisms is the fact that the enzyme from all eukaryotes tested except mung bean (6) is stimulated by putrescine. Enzyme activity from *E. coli* (6), *Azotobactor* (6), and mung bean was enhanced by divalent cation such as Mg^{2+}. The purified enzyme from corn seedlings does not appear to be stimulated either by putrescine or Mg^{2+}, as it is in *Physarum polycephalum* (12) and *Tetrahymena pyriformis* (16). Corn enzyme activity was inhibited by carbonyl reagents, like hydroxylamine and semicarbazide, but not by isonicotonic acid hydrazide (Table II). The latter evidence, together with the ineffectiveness of added MGBG on *E. coli* glutamate decarboxylase, a pyridoxal phosphate-dependent enzyme (unpublished work), suggests that the catalytic activity of *S*-adenosylmethionine decarboxylase of corn seedlings requires for carbonyl groups something other than pyridoxal phosphate. The nature of the prosthetic group of the enzyme from corn seedlings, as well as in other higher plants, remains unsolved. There is no direct experimental evidence that

Table II. *Effects of Magnesium, Putrescine, and Some Inhibitors on Corn S-Adenosylmethionine Decarboxylase*

Compounds	Concn.	Activity
	mM	%
None		100
$MgCl_2$	0.1	99
	1.0	89
	10.0	71
	25.0	59
Putrescine	0.1	95
	1.0	76
	10.0	20
	25.0	14
Methylglyoxal bis(guanylhydrazone)	0.001	20
	0.01	0
p-Hydroxymercuriphenylsulfonate	0.1	0
	1.0	0
N-Ethylmaleimide	0.1	6
	1.0	2
5,5′-Dithiobis(2-nitrobenzoic acid)	0.1	36
	1.0	16
2,2′-Dihydroxy-6,6′-dinaphthyl disulfide	0.1	71
	1.0	52
Hydroxylamine	0.1	86 (60)[a]
	1.0	13 (5)
Semicarbazide	0.1	98
	1.0	51 (11)
Aminoguanidine	0.1	100
	1.0	100 (76)
Isonicotinic acid hydrazide	0.1	100
	1.0	90 (78)
	10.0	78
$NaBH_4$	10.0	25
KCN	10.0	63

[a] Values in parentheses show activity after preincubation for 2 h at 10 C.

either pyridoxal phosphate or any other type of carbonyl group functions as the prosthetic group. At the present time, the covalently bound pyruvate is thought to be a prosthetic group for *S*-adenosylmethionine decarboxylase in both prokaryotic and eukaryotic organisms (5, 8, 13, 21). The corn enzyme is strongly inhibited by typical sulfhydryl reagents, such as *p*-hydroxymercuriphenylsulfonate and *N*-ethylmaleimide, in a manner similar to that of enzymes from *E. coli* (21) and rat liver (9). Therefore, it is concluded corn *S*-adenosylmethionine decarboxylase contains carbonyl group(s) and sulfhydryl group(s) that are involved in enzymic activity.

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