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Biochemical Characteristics of *S*-adenosylmethionine Decarboxylase from Carnation (*Dianthus caryophyllus* L.) Petals

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We have partially purified S-adenosylmethionine decarboxylase (EC 4.1.1.50, SAMDC) from carnation (*Dianthus caryophyllus* L.) petals and generated polyclonal antibodies against CSDC 16 protein (Lee *et al.*, 1996) overexpressed in *E. coli*. The protein has been purified approximately 126.8 fold through the steps involving ammonium sulfate fractionation, DEAE-Sepharose column chromatography and Sephacryl S-300 gel filtration. Its molecular mass was 42 kDa in native form and we could also detect a band of 32 kDa molecular mass on SDS-PAGE in western blot analysis using the polyclonal antibodies. The Km value of this enzyme for *S*-adenosylmethionine was 26.3 μM. The optimum temperature and pH for *S*-adenosylmethionine decarboxylase activity were 35°C and pH 8.0, respectively. Putrescine and Mg^{2+} had no effects on the activation of the enzyme activity. Mg^{2+} did not have any significant effects on the enzyme activity. SAMDC activity was inhibited by putrescine, spermidine and spermine. Methylglyoxal bis-(guanylhydrazone) (MGBG), carbonyl reagents such as hydroxylamine and phenylhydrazine, and sulfhydryl reagent such as 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) were effective inhibitors of the enzyme. However, isonicotinic acid hydrazide known as an inhibitor of 5'-pyridoxal phosphate (PLP) dependent enzyme activity had no significant effect on the enzyme activity. These results and our previously reported results (Lee *et al.*, 1997b) suggest that *S*-adenosylmethionine decarboxylase is a heterodimer, αβ, and some carbonyl group and sulfhydryl group are involved in the catalytic activity.

Key words: SAMDC, biochemical characteristic, molecular mass, antibody, processing

Polyamines, positively charged low molecular weight compounds, occur ubiquitously in both eukaryotic and prokaryotic cells (Pegg and McCann, 1982). Their roles have been suggested in a variety of cellular structures and processes (Marton and Morris, 1987). It is generally agreed that they are essential for cell growth and division (Evans and Malmberg, 1989). Also, exogenously applied polyamines delayed senescence in leaf-disc and cut carnation flower (Fuhrer *et al.*, 1982; Lee *et al.*, 1997a).

Their biosynthetic pathway is well established (Tabor and Tabor, 1984). In plants, arginine and ornithine are converted into putrescine, which is mediated by arginine decarboxylase (ADC) and ornithine decarboxylase (ODC), respectively. Putrescine is converted successively into spermidine and spermine through transfer of a aminopropyl group from decarboxylated *S*-adenosylmethionine (dcSAM). dcSAM is produced from *S*-adenosylmethionine (SAM) by the catalytic reaction of *S*-adenosylmethionine decarboxylase (SAMDC). The concentration of dcSAM is very low under physiological conditions and the turn-over rate of SAMDC enzyme activity is very rapid (about 1 hour in mammalian tissues). Enzyme activity is highly modulated by various factors such as mitogens tumor promoters and hormones (Tabor and Tabor, 1984; Marić *et al.*, 1992). These characteristics suggest that SAMDC constitutes one of the rate-limiting factors as its activity may actually control the rate of polyamine formation (Grillo *et al.*, 1981; Evans and Malmberg, 1989; Park and Lee, 1990).

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Three different classes of SAMDC have been described with respect to activation of the enzyme by putrescine or Mg^{2+}. While the mammalian enzyme is activated by putrescine, it does not require Mg^{2+} in activation (Pegg, 1974). The enzyme from *E. coli* is activated by Mg^{2+} but not by putrescine (Markham *et al.*, 1982). The third class of the enzyme is activated by neither putrescine nor Mg^{2+}. Mammalian SAMDC is synthesized as an inactive proenzyme and processed into nonidentical two subunits to form an active dimeric enzyme (Pajunen *et al.*, 1988; Stanley *et al.*, 1994). Putrescine has been also suggested to promote this processing of SAMDC in plants as well as in mammalian system (Stanley *et al.*, 1994).

In plants, SAMDC has been found and purified from mung bean (Coppoc *et al.*, 1971), *Vinca rosea* (Baxter and Coscia, 1973), *Lathyrus sativus* (Suresh and Adiga, 1977), corn (Suzuki and Hirasawa, 1980), chinese cabbage (Yamanoha and Cohen, 1985), tobacco (Hiatt *et al.*, 1986) and soybean (Yang and Cho, 1991; Choi and Cho, 1994). Each of these plant enzymes is different in the aspect of the activation by putrescine and Mg^{2+}. All three classes of the enzyme are found in plants. Their molecular masses and characters are diverse even though SAMDCs from mammalian have similar characters among them (Tabor and Tabor, 1984). Recently, cDNAs encoding SAMDC have been isolated and studied in plants (Taylor *et al.*, 1992; Bolle *et al.*, 1995; Schröder and Schröder, 1995; Lee *et al.*, 1996). Their amino acid sequences are highly homologous from which we could assume that plant SAMDCs might have similar characters between plant species even though their diverse characteristics have been reported.

In this paper, we investigated the characteristics of SAMDC using the protein purified partially from carnation petals and polyclonal antibody against SAMDC expressed in *E. coli* using pMAL-c2 (NEB, USA) vector system.

MATERIALS AND METHODS

Plant material

Carnation (*Dianthus caryophyllus* L.) flowers were harvested from plants grown under green house conditions. Petals were collected and stored at -80°C until extraction.

Enzyme assay

SAMDC activity of carnation was determined as previously described (Park and Lee, 1994). Petals were homogenized in extraction buffer (25 mM Tris-Cl, pH 7.6, 1 mM EDTA, 15 mM β-mercaptoethanol, and 5% glycerol (v/v)). The reaction buffer was the same with the extraction buffer except that glycerol was eliminated. 0.1 μCi [carboxyl-^{14}C] SAM (Amersham, 60 mCi/mmol) was used as the substrate for SAMDC activity assay.

Purification of SAMDC

Carnation petals were homogenized in the extraction buffer. The homogenate was clarified successively by centrifugation at 5,000 rpm for 20 min and 15,000 rpm for 30 min. Ammonium sulfate was added to the supernatant and a pellet precipitated by 20-50% saturation was collected. The pellet was resuspended in the extraction buffer and dialyzed against the same buffer for overnight. Insoluble materials were removed by centrifugation at 15,000 rpm for 30 min. This fraction was loaded on DEAE-Sepharose column (Sigma, USA) preequilibrated with extraction buffer and washed with the same buffer containing 0.05 M NaCl, then eluted with a linear gradient of 0.05 M-0.15 M NaCl. The active fractions were pooled, concentrated and dialyzed against gel filtration buffer (50 mM Tris-Cl, pH 7.6, 1 mM EDTA and 15 mM β-mercaptoethanol) for overnight. These were loaded on Sephacryl S-300 column (Sigma, USA) and eluted with the same buffer at a flow rate of 12 ml/h. The active fractions were pooled, concentrated and dialyzed against the extraction buffer overnight. This fraction was used as a proenzyme source. All steps are processed at 4°C.

Protein quantification

Protein content was determined by the method of Bradford (1976) with bovine serum albumin (BSA) as the standard.

Determination of molecular mass

The molecular mass of native SAMDC was determined on a Sephacryl S-300 column (2×130 cm) preequilibrated with the gel filtration buffer. The column was calibrated with β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), BSA (66 kDa), and carbonic anhydrase (29 kDa) as markers of known molecular mass.

Carbonyl and sulfhydryl group modification

The partially purified SAMDC was incubated with carbonyl reagents at 30°C for 30 min, dialyzed against the extraction buffer for 16 h and then the enzyme activity was measured. The control was also preincubated without the reagents and dialyzed in a same

manner. Before the treatment of sulfhydryl reagent, DTNB, β-mercaptoethanol was eliminated from the enzyme source through the dialysis. Then the enzyme was incubated with DTNB at 30°C for 30 min, dialyzed against β-mercaptoethanol-eliminated extraction buffer and the enzyme activity was measured.

Generation of the polyclonal antibodies from fusion proteins

Portion of the SAMDC cDNA, CSDC16, which were used in *E. coli* expression vectors to generate antigens for polyclonal antibodies, was amplified by polymerase chain reaction using pCSDC16 (Lee *et al.*, 1996) as a template. Two primers used are shown in Fig. 1a. Amplified products were digested with *Bam*HI and *Xba*I, and ligated into pMAL-c2 vector (New England BioLab, USA) linearized with *Bam*HI and *Xba*I. These are diagrammed in Fig. 1b. This construct was introduced into *E. coli* and expressed with IPTG induction. Overexpressed protein was purified using amylose affinity column chromatography as manufacturer's manual and then immunized mice.

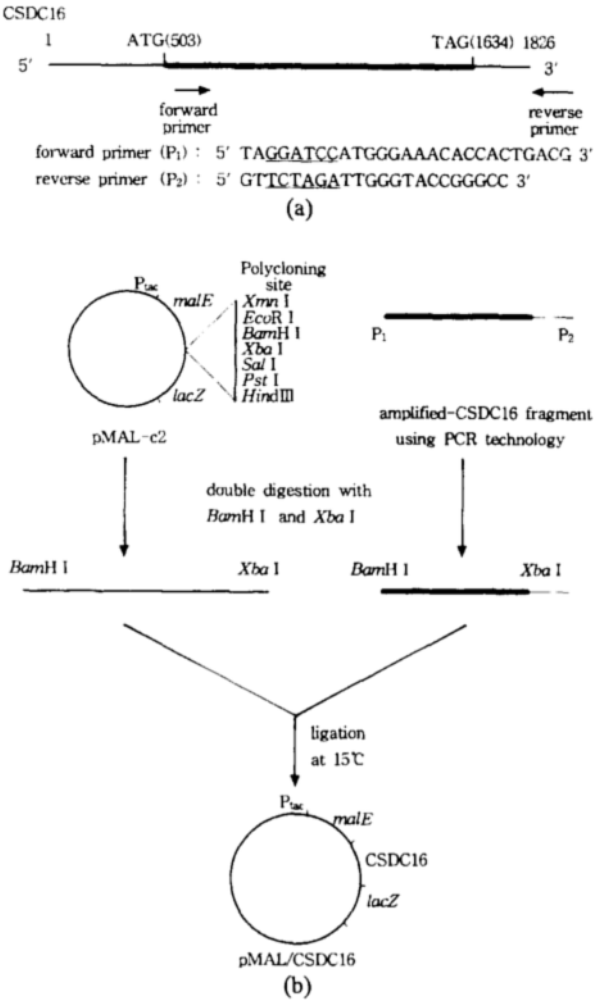

Fig. 1. Diagram of the construction of pMAL/CSDC16. a) PCR primers for the amplification of SAMDC gene. Forward primer starts at the position 518th nucleotide and reverse primer is located at the vector outside of *CSDC16*. b) Polymerase chain reaction was performed for thirty-five cycles of denaturation (95°C, 1 min), annealing (55°C, 1.5 min) and polymerization (72°C, 3 min) after predenaturation (95°C, 4 min) using pCSDC16 as a template. The resulting product was purified, digested with *Bam*HI and *Xba*I, and ligated into linearized pMAL-c2 with *Bam*HI and *Xba*I using T4 DNA ligase.

Western blot analysis

Western blots were performed essentially as described by Harlow and Lane (1988). After the gel was transferred to nitrocellulose membrane, the membrane was washed with phosphate-buffered saline (PBS) solution. Then the blot was soaked in ponceau-S solution for 5 min and washed with distilled water to visualize the bands of molecular weight markers. The membrane was blocked in PBS solution containing 3% BSA overnight at 4°C, washed once for 15 min and twice for 5 min with PBS-T solution containing 1% Tween-20. Primary antibody was applied at a 3000-fold dilution with gentle shaking for 40 min at room temperature and the membrane was washed four times each for 15 min with PBS-T solution. Secondary antibody, anti-IgG HRP (Sigma, USA) was applied for 1 h at room temperature and the membrane washed as above. Chemiluminescence method was used for detection with ECL (Amersham, USA)

RESULTS

Partial purification of SAMDC

A crude extract was made from 500 g petals in 1.5 L of extraction buffer. SAMDC activity was recovered almost in the 20-50% ammonium sulfate fractionation. When the fraction was applied to DEAE-Sepharose column (Sigma, USA) and eluted with a linear gradient of 0.05 M-0.15 M NaCl, we could detect SAMDC activity in the fractions eluted at about 0.14 M NaCl (Fig. 2a). However, the enzyme activity was recovered with only 39.1% from DEAE-Sepharose column chromatography. These active fractions were forwarded to Sephacryl S-300 (Sigma, USA) gel filtration chromatography (Fig. 2b) and we could recover SAMDC activity with 37.6% recovery and 126.8 purification fold. We used these active fractions as enzyme source in further experiments. These results

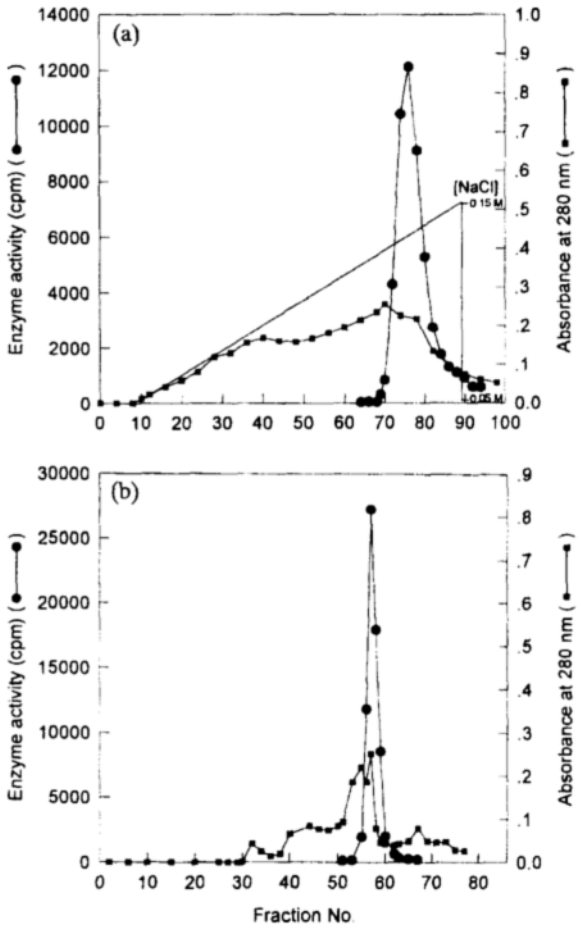

Fig. 2. Elution patterns of SAMDC on a DEAE-Sepharose column (a) and on a Sephacryl S-300 column (b). Enzyme activity was assayed as described in materials and methods.

are summarized in Table 1.

Determination of SAMDC molecular mass

SAMDC from carnation had an approximate native molecular mass of 42 kDa by comparing their elution volumes from gel filtration of Sephacryl S-300 (Fig. 3a) with four proteins of known molecular mass. In western blot analysis using polyclonal antibodies raised against overexpressed SAMDC in *E. coli*, we could detect a band which had an approximate molecular mass of 32 kDa (Fig. 3b, lane 6). This increpancy in size implied that SAMDC was composed with at least two subunits. Preimmune serum and antibodies against maltose binding protein did not react with any protein in the crude extract of petals (Fig. 3b, lane 2, 4).

Biochemical characteristics of SAMDC

SAMDC activity showed its highest activity at the temperature of 35°C among the tested temperatures (Fig. 4a). The optimal pH for SAMDC activity was pH 8.0 (Fig. 4b) and the Km value of the enzyme for SAM was 26.3 μM (Fig. 5). This Km value is lower than those of the mammalian SAMDCs, 50-100 μM (Demetriou *et al.*, 1978; Pösö and Pegg, 1982), and *E. coli* SAMDC, 60 μM (Markham *et al.*, 1982). However, this K_m value was somewhat higher among plant SAMDCs: those of corn SAMDC, soybean SAMDC I and SAMDC II were 5 μM (Suzuki and Hirasawa, 1980), 8.1 μM (Yang and Cho, 1991), 16 μM (Choi and Cho, 1994) respectively. In chinese cabbage, the Km value of SAMDC was 38 μM (Yamonoha and Cohen, 1985) which was higher than that of carnation SAMDC.

Effects of amines and ions

We investigated the effects of polyamines on SAMDC activity (Fig. 6). The enzyme activity was inhibited by putrescine, spermidine and spermine with concentration-dependent manner. The degree of inhibition, however, was different with the types of each amine. Spermidine was the strongest inhibitor at the low concentration (<0.1 mM) among these polyamines, but in the range of 0.1 mM to 10 mM spermine had slightly more inhibitory effect. These inhibitions by polyamines were also reported in the soybean SAMDC (Yang and Cho, 1991). In the soy-

Table 1. Purification of SAMDC from carnation petals. SAMDC activity was assayed as described in materials and methods. One unit for enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 nmole CO_2 for 1 h.

	Total protein (mg)	Total activity (nmole/h)	Specific activity (nmole/h/mg protein)	Recovery (%)	Purification (fold)
Crude extract	1,044	1.16824	0.001119	100	1
Ammonium sulfate fractionation	299.5	1.15158	0.003845	98.5	3.44
DEAE-Sepharose chromatography	15.34	0.4569	0.029782	39.1	26.6
Sephacryl S-300 gel filtration	3.1	0.44011	0.14197	37.6	126.8

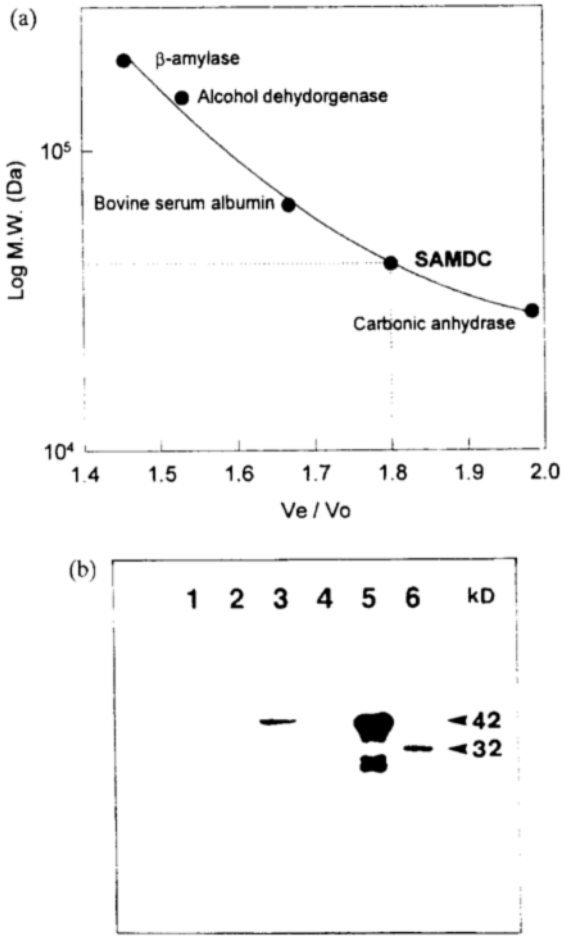

Fig. 3. Determination of SAMDC molecular mass by calibrated Sephacryl S-300 gel filtration (a) and western blot analysis (b). a) Plot of the ratio of elution volume (Ve) to void volume (Vo) against molecular mass for proteins on Sephacryl S-300 column. β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa) and carbonic anhydrase (29 kDa) were used as molecular weight markers. b) Immunoblots with anti-SAMDC serum of the cleaved products of MBP/SAMDC fusion protein and crude extracts of carnation petals. Lane 1, 3 and 5, the cleaved products of MBP/SAMDC fusion protein; Lane 2, 4 and 6, crude extracts of carnation petals; Lane 1 and 2, immunoblots with preimmune serum; Lane 3 and 4, immunoblots with anti-MBP serum; Lane 5 and 6, immunoblots with anti-SAMDC serum.

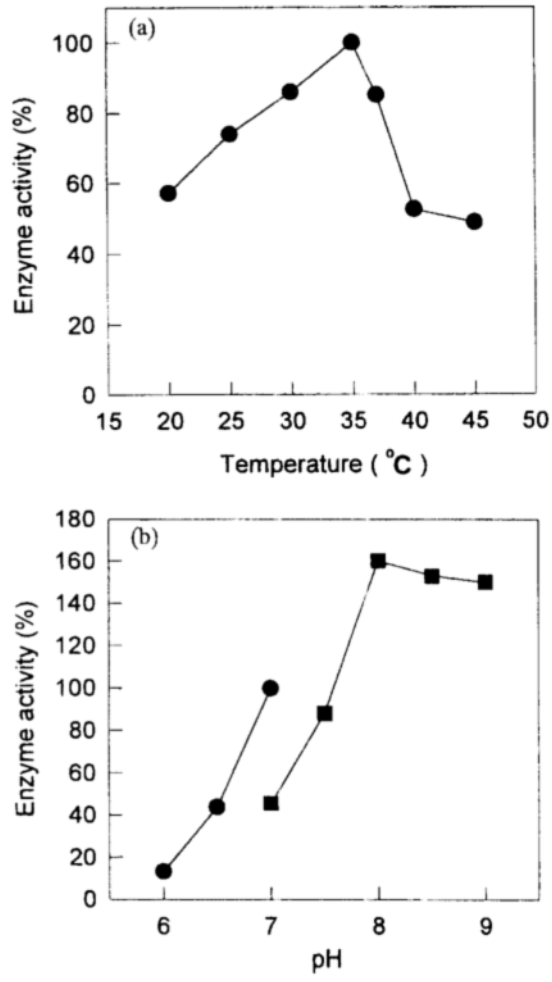

Fig. 4. Effects of temperature (a) and pH (b) on the activity of the partially purified SAMDC. Enzyme activity was assayed as described in materials and methods except for the temperature (a) or the pH (b). The reaction buffer used from pH 6.0 to pH 7.0 was 25 mM potassium phosphate buffer containing 1 mM EDTA and 15 mM β-mercaptoethanol (● ●). The reaction buffer used from pH 7.0 to pH 9.0 was 25 mM Tris-Cl buffer instead of 25 mM potassium phosphate (■ ■). The values are the means of three independent experiments.

bean SAMDC, however, spermidine and spermine caused the stronger inhibition than in the carnation SAMDC. At the 1 mM they inhibited SAMDC activity to the 28% and 23% relative activity respectively.

Table 2 presents the effects of several cations on the SAMDC activity. Mg^{2+} which was activators of SAMDC of mung bean (Coppoc *et al.*, 1971) and prokaryote (Markham *et al.*, 1982) did not activate carnation SAMDC at the concentration of 0.1 mM and 1 mM. In our system, Mg^{2+} inhibited the enzyme activity slightly. This slight inhibition by Mg^{2+} has been generally observed in plant SAMDCs. The other cations which were tested had no significant effects on SAMDC activity at the concentration of 0.1 mM, and inhibited the activity with 10-30% degree of inhibition at the concentration of 1 mM except of Co^{2+} and Cu^{2+}. 1 mM of Co^{2+} and Cu^{2+} caused the inhibition by 41% and 52%, respectively.

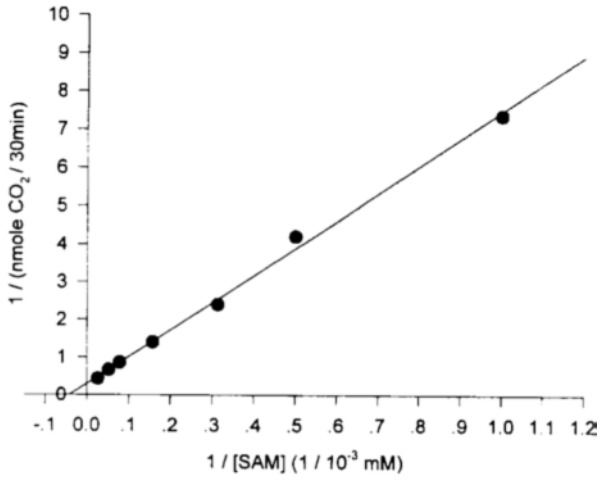

Fig. 5. Dependence of SAMDC activity on the substrate concentration (Lineweaver-Burk plot). The activities of SAMDC under different substrate concentrations were measured by the method described in materials and methods. The K_m value for the substrate, *S*-adenosylmethionine, is 26.3 μM.

Table 2. Effect of some cations on SAMDC activity. SAMDC activity was assayed as described in materials and methods. Enzyme activity was represented as a percentage over the control value. The values are the means of three independent experiments

	Concentration (mM)	Relative activity (%)
Control		100
Mg^{2+}	0.1	96
	1	86
Ca^{2+}	0.1	94
	1	70
Mn^{2+}	0.1	90
	1	78
Co^{2+}	0.1	84
	1	59
Cu^{2+}	0.1	86
	1	48
Na^{+}	0.1	101
	1	92
K^{+}	0.1	87
	1	77

Effects of several inhibitors on SAMDC activity

MGBG which had been known as an inhibitor of SAMDC inhibited carnation SAMDC activity at the concentration of 1 μM to the 14% and 10 μM to the 1.7% relative activity respectively (Table 3). The carbonyl group-modifying reagents, phenylhydrazine and hydroxylamine, potentially inhibited the enzyme activity at the concentration of 1 mM to the 4.4% and 6.2% relative activity, respectively. However, isonicotinic acid hydrazide, a specific inhibitor of some PLP-dependent enzymes, had no significant effects on the enzyme activity at the concentration of 1 mM. A typical sulfhydryl reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), also inhibited the enzyme activity of SAMDC (Table 4). DTNB modifies cystein residue through the reaction with sulfhydryl group.

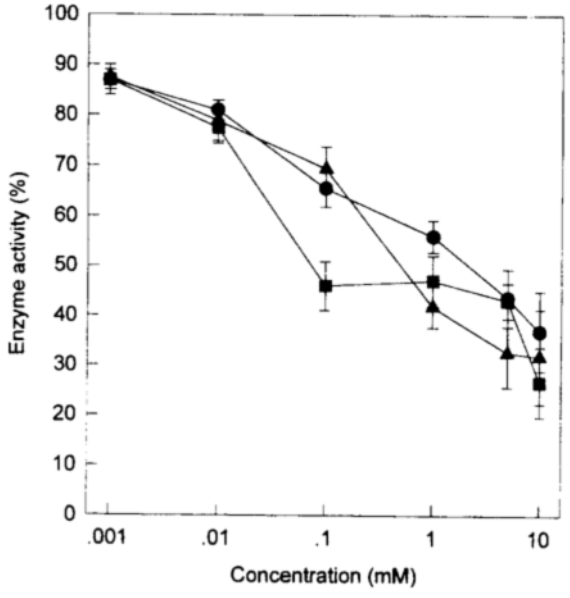

Fig. 6. Effects of polyamines on SAMDC activity. The activities of SAMDC were assayed as described in materials and methods using the reaction buffer containing various concentrations of putrescine (●···●), spermidine (■—■) and spermine (▲—▲). Error bar represents standard deviation.

Table 3. Effect of various inhibitors on SAMDC activity. SAMDC activity was assayed as described in materials and methods. Enzyme activity was represented as a percentage over the control value. The values are the means of three independent experiments

	Concentration (mM)	Relative activity (%)
Control		100
Phenylhydrazine*	1	4.4
	10	4.4
Hydroxylamine*	1	6.2
	10	3
MGBG	0.001	14
	0.01	1.7
Isonicotinic acid hydrazide	1	98

*SAMDC was preincubated with phenylhydrazine or hydroxylamine at 30°C for 30 min, dialyzed against extraction buffer for 16 h, and then assayed. Control was also processed in the same condition without the reagent.

Table 4. Effect of DTNB on SAMDC activity. SAMDC activity was assayed as described in materials and methods. Enzyme activity was represented as a percentage over the control value. Parentheses values means the percentage over the control 2. The values are the means of three independent experiments. -ME means that β-mercaptoethanol was removed from SAMDC with dialysis. -MER means that reaction buffer used did not contain β-mercaptoethanol. +MER means that reaction buffer used contains 15 mM β-mercaptoethanol. Control 1 contained -mercaptoethanol in the enzyme source. Control 1 and control 2 were also preincubated without DTNB, and then dialyzed against the extraction buffer and β-mercaptoethanol-omitted extraction buffer, respectively

Treatment	Relative activity (%)
Control 1 (+MER)	100
-β-mercaptoethanol (-ME), +MER	102
Control 2 (-ME, -MER)	25 (100)
-ME, -MER, +1 mM DTNB	6.8 (27)
-ME, -MER, +10 mM DTNB	0 (0)

Mercaptoethanol prevents cystein from making disulfide linkage. When 2-mercaptoethanol was eliminated from the reaction buffer and the enzyme source (control 2), the enzyme activity was greatly reduced to 25% of the control 1. Treatment of 1 mM DTNB caused inhibition of the enzyme activity to 27% of the control 2 and treatment of 10 mM DTNB diminished the enzyme activity perfectly.

DISCUSSION

We have purified SAMDC partially from carnation petals by 126.8 fold (Table 1). The apparent molecular mass of native form was determined to be approximately 42 kDa by the gel filtration on Sephacryl S-300. The molecular masses of plant SAMDCs which have been reported are variable. The molecular masses are 25 kDa for corn (Suzuki and Hirasawa, 1980), 35 kDa for chinese cabbage (Yamanoha and Cohen, 1985), and 66 kDa and 110 kDa for SAMDC I and SAMDC II, respectively from soybean (Yang and Cho, 1991; Choi and Cho, 1994).

Mammalian SAMDC is synthesized as a proenzyme with approximate size of 38 kDa (Pajunen *et al.*, 1988). This proenzyme is processed into nonidentical two polypeptides (32 kDa and 6 kDa) with a autocatalytic reaction and these two subunits forms a mature enzyme with approximate size of 76 kDa in a form of heterodimer ($\alpha_2\beta_2$). But it has been thought that this might not be true in plant SAMDCs because monomeric as well as dimeric form of SAMDC had been reported and their size were variable. The subunit molecular mass of plant SAMDC was found by SDS-PAGE to be 35 kDa in tobacco (Hiatt *et al.*, 1986), 66 kDa in soybean SAMDC I (Yang and Cho, 1991), and 66 kDa and 58 kDa in soybean SAMDC II (Choi and Cho, 1994). cDNAs encoding SAMDC have been cloned and sequenced recently from potato (Taylor *et al.*, 1992), spinach (Bolle *et al.*, 1995), periwinkle (Schröder and Schröder, 1995) and carnation (Lee *et al.*, 1996). Their deduced amino acid sequences were highly homologous among them, which implicated that plant SAMDCs might have similar characteristics. While the overall similarities between plant SAMDC cDNAs are apparent through the entire coding region, there are no significant homologies between plant SAMDC cDNA and animal SAMDC cDNA (Lee *et al.*, 1997b). However, several regions including the proenzyme processing site, LSESSLFV (Stanley *et al.*, 1994) were highly conserved in both plants and animals. This implied that plant SAMDC might be synthesized as a proenzyme and processed into two distinct subunits as the similar manner with the animal SAMDCs. We showed recently that two carnation SAMDCs were synthesized as a proenzyme which was processed into two subunits (ca. 33 kDa and 9 kDa) (Lee *et al.*, 1997b). The molecular mass of native enzyme, 42 kDa (Fig. 3a), is well matched with the calculated size from the cDNAs of carnation SAMDC, which suggested that carnation SAMDC might be processed into two subunits and formed a mature enzyme in a form of heterodimer, $\alpha\beta$, instead of $\alpha_2\beta_2$.

In western blot analysis we could detect a band of a size of 32 kDa, and this band might represent a large subunit generated through the processing. The reason we could not detect a small subunit by western blot analysis is not certain. However, we could surmise that the antibodies which could react with a small subunit had not been raised.

Putrescine is an activator of SAMDC from mammalian (Pegg *et al.*, 1974; Tabor and Tabor, 1984), *Vinca rosea* (Baxter and Coscia, 1973) and *Lathyrus sativus* (Suresh and Adiga, 1977). Glutamic acid residue responsible for the activation by putrescine was reported (Stanley *et al.*, 1994) and the residue is well conserved in our two SAMDC genes (Lee *et al.*, 1997b). Thus, SAMDC from carnation was expected to be activated by putrescine. However, carnation SAMDC was not activated by putrescine but inhibited (Fig. 6). Inhibition of SAMDC activity by putrescine has been reported in soybean (Yang and

Cho, 1991). On the contrary, SAMDC activity of chinese cabbage was neither stimulated nor inhibited by putrescine (Yamanoha and Cohen, 1985).

SAMDC from *E. coli* requires Mg^{2+} for activity (Markham *et al.*, 1982) and from mung bean is activated by Mg^{2+} (Coppoc *et al.*, 1971). Mn^{2+} and Ca^{2+} result in approximately equal activation with Mg^{2+} in *E. coli*. However, carnation SAMDC could not be activated by Mg^{2+} and some other divalent and monovalent cations (Table 2). Therefore, carnation SAMDC as well as soybean SAMDC (Yang and Cho, 1991) mighy be classified into the third group in the aspects of the lack of activation by putrescine and Mg^{2+}. The third group, however, should be classified more minutely whether putrescine acts as an inhibitor or not. While putrescine had any significant effects on the activity of chinese cabbage SAMDC, the activity of carnation and soybean SAMDCs was inhibited by putrescine.

SAMDC is known to be processed with a autocatalytic cleavage reaction that generates the pyruvate at the amino-terminal of the large subunit in mammalian (Stanley *et al.*, 1989) and prokaryotes (Markham *et al.*, 1982; Kashiwagi *et al.*, 1990). Plant SAMDCs are also known to contain a covalently linked pyruvate which is known to be involved in the catalytic activity (Suzuki and Hirasawa, 1980; Yamanoha and Cohen, 1985; Choi and Cho, 1994). Carnation SAMDC activity was inhibited by carbonyl reagents, such as hydroxylamine and phenylhydrazine, but not by isonicotinic acid hydrazide (Table 3). The former suggests that a certain carbonyl group is needed for the carnation SAMDC activity. The latter evidence suggests that the carbonyl groups other than pyridoxal phosphate which is known to be a cofactor of many decarboxylases is required in the catalytic activity of carnation SAMDC. Thus, from the results which have been reported, we can think that carnation SAMDC has the covalently bound pyruvate as a prosthetic group at the amino-terminal of the large subunit. Carnation SAMDC activity was reduced greatly by the elimination of β-mercaptoethanol and inhibited strongly by a typical sulfhydryl reagent, DTNB (Table 4), in a similar manner to other SAMDCs (Suzuki and Hirasawa, 1980; Choi and Cho, 1994), which implied that the sulfhydryl group of cystein was important for the catalytic activity of carnation SAMDC.

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