J. Biochem. Mol. Biol. Vol. 28, No. 2 pp. 100~106 March 31, 1995

Purification and Characterization of S-adenosylmethionine Synthetase from Soybean (*Glycine max*) Axes

Dae Gun Kim, Tae Jin Park, Jong Yeol Kim and Young Dong Cho*

Department of Biochemistry, College of Science, Yonsei University, Seoul 120-749, Korea
(Received August 6, 1994)

Abstract: S-adenosylmethionine (SAM) synthetase was purified to homogeneity from soybean (*Glycine max*) axes. The enzyme was purified 216-fold with a 1.5% yield by ammonium sulfate fractionation, acetone fractionation, ion exchange chromatography with DEAE-sephacel, gel filtration with Sephacryl S-300, and afffinity chromatography with ATP-agarose. The enzyme activity reached a maximum 3 days after germination. SAM synthetase had a subunit molecular weight of 57,000 daltons from a silver stained single band on SDS-PAGE. The molecular weight of the enzyme was 110,000 daltons from Sephacryl S-300 gel filtration. The enzyme was composed of two identical subunits. The K_m values of the enzyme for L-methionine and ATP were 1.81 and 1.53 mM, respectively. The enzymatic activity was not affected by polyamines, agmatine, or SAM analogues, but was inhibited by SAM. The inhibition pattern was showed non-competitive for L-methionine and uncompetitive for ATP. The activity of SAM synthetase was inhibited by thiol-blocking reagents. The enzyme was induced by treatment with 10^{-3} M putrescine at germination. Experimental data revealed a possible novel regulation mechanism of polyamine biosynthesis through several endogenous intermediates.

Key words: *Gycine max*, induction, product inhibition, putrescine, S-adenosylmethionine synthetase.

S-adenosylmethionine (SAM) has been known to occupy a central metabolic position in both eukaryotes and prokaryotes since it serves as the major methyl group donor in the transmethylation reaction involved in the synthesis of numerous secondary metabolites (Tabor and Tabor 1984). Furthermore, SAM is involved in polyamine biosynthesis as an aminopropyl group donor following conversion of SAM to decarboxylated SAM (dcSAM) by the action of SAM decarboxylase (SAMDC), and in the ethylene biosynthesis of higher plants (Adams and Yang 1977; Yang and Hoffman 1984; Adams and Yang 1979). Methylthioadenosine (MTA) is formed from dcSAM after donation of an aminopropyl group to putrescine (Put) or spermidine (Spd). The formation of SAM is catalyzed by S-adenosylmethionine synthetase (ATP: L-methionine S-adenosyl transferease, EC 2.8.1.5), with ATP and methionine in the presence of Mg^{2+} and K^+.

This enzyme has been studied extensively in bacteria, yeast, and mammals, and purified from yeast, rat liver, rat kidney, bovine brain, human lymphocytes, and *E. coli* (Cantoni 1953; Mudd and Cantoni 1958; Chiang and Cantoni 1977; Cherest *et al.*, 1978; Thomas *et al.*, 1987; Markham *et al.*, 1980; Okada *et al.*, 1981; Suma *et al.*, 1986; Mitsui *et al.*, 1988; Cabrero *et al.*, 1987; Abe *et al.*, 1980). Two multiple forms of SAM synthetase, α and β (mol. wt. 100,000 and 200,000 daltons) have been reported from rat liver. The cDNA clones of SAM synthetase have been prepared from rat liver tissue. The rat liver SAM synthetase gene exhibited 68% homology with the yeast enzyme gene and 52% similarity with the Met K gene of *E. coli*. However, no attempt has been made to purify SAM synthetase from higher plants and, consequently, there have been no reports on its molecular weight, subunit structure and kinetic properties. Recently, there has been one report on purification of this enzyme from germinated wheat embryos (Mathur *et al.*, 1991). Two isozymes of SAM synthetase were separated by DEAE cellulose chromatography from yeast cells. The expression of the two genes of this enzyme (SAM I and SAM II) showed a differential regulatory response to methionine. Except for the limited information on the wheat germ enzyme, virtually nothing is known about the regulation of SAM synthetase in plant systems.

A possible scheme for regulation of SAMDC by agmatine (Agm), Spd and spermine (Spm) in soybean has been proposed with the suggestion that such inhibition might control polyamine biosynthesis through re-

*To whom correspondence should be addressed.
Tel: 82-2-361-2697, Fax: 82-2-313-1608.

ducing the concentration of dcSAM (Yang and Cho 1991). To make this regulation scheme of SAMDC biochemically meaningful it is essential to know if he level of the substrate, SAM, can be controlled by any endogeneous metabolites. In connection with this proposed scheme (Yang and Cho 1991), a biochemically relevant model for study of the regulation of SAM biosynthesis by Put, SAM, and MTA is described herein. Additionally, the properties of soybean SAM synthetase are described. The data presented here shows for the first time the inducer of SAM synthetase and that SAM and MTA are inhibitors of SAM synthetase in plant systems. This suggests that the SAM concentration can be effectively regulated by these inhibitors and, in turn, polyamine biosynthesis can also be regulated through SAM synthetase and SAMDC activities.

Materials and Methods

Chemicals

L-[1-^{14}C]Methionine was supplied by Amersham International (Bucks, UK). L-methionine, ATP, putrescine, spermidine, spermine, Tris-Cl, bovine serum albumin, blue dextran, Coomassie R-250, and ATP-agarose were purchased from Sigma Chemical Co. (St Louis, USA). Sephacryl S-300 was purchased from Phamarcia (Uppsala, Sweden). DEAE-Sephacel was obtained from Bio-Rad (Richmond, USA). Phosphocellulose (P-81) was supplied by Whatman (Kent, UK). All other chemicals were of the purest available commercial grade.

Plant materials

Soybean seeds (*Glycine max*) were grown at 25℃ in the dark and harvested as described by Kang and Cho (1990).

SAM synthetase assay

SAM synthetase activity was assayed at 30℃ by a modification of the method of Geller *et al.* (1986). An assay mixture containing 100 mM Tris-Cl (pH 8.0), 100 mM KCl, 20 mM $MgCl_2$, 10 mM ATP, 2 mM methionine, 0.05 μCi L-[1-^{14}C] methionine, and enzyme solution in a total volume of 40 μl was placed in an eppendorf tube. After 1 h incubation the reaction was stopped by adding 40 μl of 0.5 M $HClO_4$ and the reaction mixture was spotted on P-81 phosphocellulose paper (1.5×1.5 cm). The paper was then dried for 1 h under a hood. The paper was washed with 5 mM potassium phosphate buffer (pH 7.0), 50 ml per disk three times to remove unincorporated L-[1-^{14}C] methionine, then washed with 200 ml of ethanol and 100 ml of ether. After further drying the paper was transferred to vials containing 10 ml of scintillation fluid (PPO 4.0 g, POPOP 0.1 g, 1000 ml of toluene), and was counted. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 nmol of SAM per 1 h. Specific activity was expressed as units/mg protein. SAM synthetase activity was a linear function of both incubation time and concentration of substrates under these conditions.

Tripolyphosphatase assay

Phosphate was assayed by a modification of the method of Heinonen and Lahti (1981). A 0.2 ml sample containing 5~40 nmol of phosphate was mixed with 0.8 ml of freshly prepared acid-acetone-molybdate solution [5 N H_2SO_4/acetone/10 mM ammonium molybdate (1/2/1)] followed by addition of 0.08 ml of 1.0 M citric acid. The absorbance at 335 nm was immediately measured. The pyrophosphate amount was determined by an adaption of the method of Heinonen *et al.* (1981). A sample containing 2~20 nmol of pyrophosphate in 0.1 ml was mixed with 0.3 ml of 0.67 N H_2SO_4. Additionally, 0.2 ml of a freshly prepared mixtutre of 40 mM ammonium molybdate/5 N H_2SO_4 /triehylamine (80/20/1) was added to a 1.5 ml plastic Eppendorf tube and allowed to stand at 22℃ for 20 min before being centrifuged for 30s at 12,800×g in a microcentrifuge. The supernatant was transferred to another tube and mixed with 0.1 ml of 1 N H_2SO_4 and 0.03 ml of 1 M β-mercaptoethanol. After 20 min the absorbance at 700 nm was measured.

Enzyme purification

Three day old-soybean axes (200 g) were blended in a chilled electric mixer with 250 ml of 50 mM Tris-HCl (pH 8.0), containing 10% (w/v) glycerol, 15 mM β-mercaptoethanol and 1 mM EDTA (buffer A). The homogenate was filtered through four layers of gauze and clarified by centrifugation (13,000×g, 30 min). The supernatant was adjusted to 60% saturation with solid $(NH_4)_2SO_4$ and stirred at 4℃ for 5 h. The solution was centrifuged and the pellet was discarded. The supernatant was brought to 70% saturation with solid $(NH_4)_2SO_4$ and treated as above, except that the pellet was retained. The pellet was resuspended in 10 ml of buffer A. The suspension was brought to 40% saturation with precooled (−20℃) acetone and stirred for 10 min. After centrifugation at 5,000×g for 10 min, the resulting supernatant was brought to 80% saturation with additional precooled acetone and stirred for 10 min. Following centrifugation at 5,000×g for 10 min, the pellet was resuspended in buffer A. This solution was dialyzed against buffer A. After centrifugation (13,000×g, 10 min), the supernatant was applied to a DEAE-Sephacel column (2.8×5 cm) previously equili-

brated with buffer A. The column was washed with 50 mM Tris-HCl (pH 8.0), containing 50 mM KCl, 10% (w/v) glycerol, 15 mM β-mercaptoethanol and 1 mM EDTA (buffer B). Elution of the enzyme was achieved with 300 ml of a linear gradient of 0.05 M to 0.3 M KCl made up in buffer A. The flow rate was 30 ml per hour. Active fractions were pooled, concentrated by ultrafiltration (Amicon's stirred cell 8050 with YM-10 membrane), and dialyzed with 50 mM Tris-HCl (pH 8.0), containing 10% (w/v) glycerol, 0.1 M KCl, 15 mM β-mercaptoethanol, and 1 mM EDTA (buffer C). The dialyzed enzyme was applied to a Sephacryl S-300 column (1.3×150 cm) which had been pre-equilibrated with buffer C. The flow rate was 15 ml per hour. Active fractions from Sephacryl S-300 filtration were pooled and dialyzed with 50 mM Tris-HCl (pH 8.0), containing 10% (w/v) glycerol, 10 mM $MgCl_2$, 15 mM β-mercaptoethanol, and 1 mM EDTA (buffer D). The enzyme was applied to a ATP-agarose column which had been pre-equilibrated with buffer D. The column was washed with buffer D, then washed with the same buffer containing 4 mM ATP. The enzyme was eluted with the same buffer containing 8 mM ATP. The flow rate was 10 ml per hour. Active fractions were pooled and dialyzed against buffer A. All steps of this procedure were carried out at 4℃ except for acetone fractionation, which was performed at −5℃.

Substrate specificity

Several ATP analogues were used to determine the substrate specificity for the SAM synthetase of Glycine max. Each ATP analogue was added to a reaction mixture instead of ATP. The final volume was fixed at 100 ml. After preincubation for 10 min at 37℃, the reaction was started. Subsequently, the relative Vmax value was calculated.

Protein determination

Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard protein.

Polyacrylamide gel electrophoresis

SDS gel (12.8%) electrophoresis was performed as described by Laemmli (1970). Visulalization of proteins on gel was accomplished by the silver staining method (Merril *et al.*, 1981). Samples were treated as described by Kang and Cho (1990).

Determination of molecular weight

The native molecular weight of purified soybean SAM synthetase was estimated by gel filtration through a Sephacryl S-300 column (1.3×150 cm) according to the method of Andrews (1964). The flow rate was 10ml per hour. Blue dextran was used to measure the void volume. The column was calibrated with thyroglobulin (670,000 daltons), γ-globulin (158,000 daltons), ovalbumin (44,000 daltons), myoglobin (17,000 daltons) and vitamin B-12 (1,350 daltons) as markers of known molecular weight. The subunit molecular weight of purified soybean SAM synthetase was determined by 12% SDS-PAGE with phosphorylase B (97,400 daltons), bovine serum albumin (66,200 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (31,000 daltons), and trypsin inhibitor (21,500 daltons) serving as molecular weight standard markers.

Results and Discussion

Change in SAM synthetase activity at soybean seed germination and the role of putrescine in induction of SAM synthetase

Fig. 1 shows changes in SAM synthetase activity during germination and the early growth period of the soybeans. At the onset of germination, enzyme activity was relatively low compared to germinated wheat embryos (Mathur *et al.*, 1991). Thereafter, enzyme activity reached a maximum value three days after germination and declined steadily as germination progressed.

Since the substrate of the enzyme, methionine, controls the expression of the SAM synthetase gene in yeast and wheat embryos (Mudd and Cantoni 1958; Mathur *et al.*, 1991), an attempt was made to test the effect of L-methionine on the regulation of this enzyme in soybean axes. For this purpose soybean seeds were

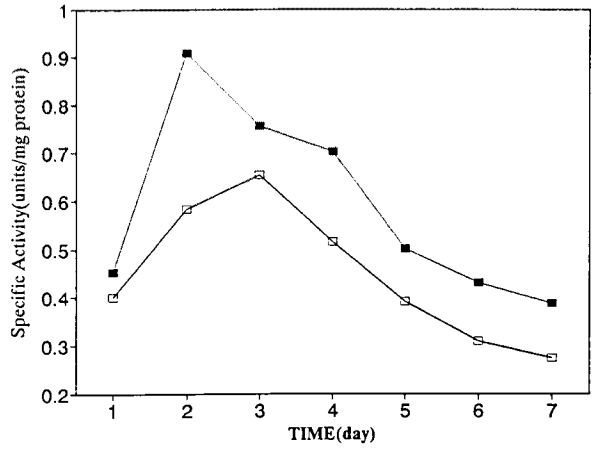

Fig. 1. Change in SAM synthetase activities in soybean (*Glycine max*) axes after treatment with putrescine at seed germination. Soybean seeds were germinated in water containing 10^{-3} M of putrescine and the axes were collected at sebsequent days and homogenized. The SAM synthetase activity was assyed at 30℃ as described in the Materials and Methods. Specific activity was expressed as U/mg protein. □–□ : Control, ■–■ : Putrescine.

germinated in water containing different concentrations of L-methionine (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} M). There was, however, no evidence of a change in the activity of soybean SAM synthetase due to L-methionine (Table 1). SAM is involved in polyamine biosynthesis after converson to dcSAM by SAMDC. Accordingly soybean seeds were germinated in a medium containing Put, Spd, and Spm (from 10^{-2} M to 10^{-5} M) in order to see if there was any feedback effect on SAM synthetase. Enzyme activity was increased up to 90% following treatment with 10^{-3} M putrescine for two days after the germination (Fig. 1). Other concentrations were less effective. Spd and Spm had no effect on the enzyme activity (Table 1). The enhanced activity of SAM synthetase was inhibited by the cycloheximide (10 μg/ml), strongly suggesting a requirement for de novo protein synthesis for the enhanced activity dut to putrescine. As shown in Fig. 1, the soybean SAM synthetase activity of the control was a maximum at three days after germination, but in the case of putrescine treatment, maximum activity was observed two days after germination. Incubation of purified SAM synthetase with Put failed to enhance enzyme activity. Such cumulative results strongly demonstrate that Put acts as an inducer of SAM synthetase in *Glycine max*.

Table 1. Effects of L-methionine and polyamines in SAM synthetase activity from soybean axes. Soybean seeds were germinated in water containing different concentrations of these compounds. The SAM synthetase activity was assyed at 30℃ as described in the Materials and Methods. Specific activity was expressed as U/mg protein.

Addition		Relative activity (%)
	None	100
L-Methionine	10^{-2} M	92
	10^{-3} M	102
	10^{-4} M	96
	10^{-5} M	84
Putrescine	10^{-2} M	92
	10^{-3} M	153
	10^{-4} M	83
	10^{-5} M	78
Spermidine	10^{-2} M	79
	10^{-3} M	78
	10^{-4} M	87
	10^{-5} M	95
Spermine	10^{-2} M	76
	10^{-3} M	80
	10^{-4} M	88
	10^{-5} M	92

SAM synthetase purification and stability

The typical purification procedure for soybean SAM synthetase is summarized in Table 2. Although the activity of the enzyme has been reported in higher plants, such as barley and peas (Mathur *et al.*, 1991), the enzyme has not been purified and, consequently, properties of the enzyme are unknown. However, the enzyme has recently been purified from germinated wheat embryos and three isozymes have been isolated by DE-52 ion-exchange column chromatography during purification (Mathur *et al.*, 1991). In the case of soybean, there is no evidence of the presence of isozymes from the purification steps. In addition, there are other differences between the enzymes from soybean and wheat embryo. The soybean enzyme did not bind to Phenyl-Sepharose, to which wheat SAM synthetase bounded during purification. Also, soybean SAM synthetase precipitated at a 60~70% $(NH_4)_2SO_4$, whereas the wheat enzyme precipitated at 30~60%. Acetone fractionation (40~80%) was a critical purification step because of the drastic elimination of other proteins. The enzyme was finally purified using ATP-agarose affinity chromatography. After these steps the specific activity of the final preparation was 158 U per mg of protein, which was 216-fold higher than the crude enzyme.

Soybean SAM synthetase activity was unstable in crude extracts and partially purified preparations. More than 70% of the original activity was lost within three days at 4℃. However, in the presence of 10% glycerol, both purified and partially purified enzymes could be

Table 2. Purification procedure of SAM synthetase from soybean (*Glycine max*) axes. One unit of activity is defined as the amount of enzyme catalyzing the the production of 1 nmol SAM from ATP and methionine per 1 h.

Fraction	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)	Purification (fold)
Crude	1140	834.4	0.732	100.0	1
$(NH_4)_2SO_4$ (60~70%)	192	480.7	2.50	57.6	3.41
Acetone (40~80%)	37	184.3	4.98	22.1	6.80
DEAE-Sephacel	6	120.7	20.11	14.0	27.47
Sephacryl S-300	2.4	80.1	33.38	9.6	45.59
ATP-agarose	0.08	12.7	158.75	1.5	216.87

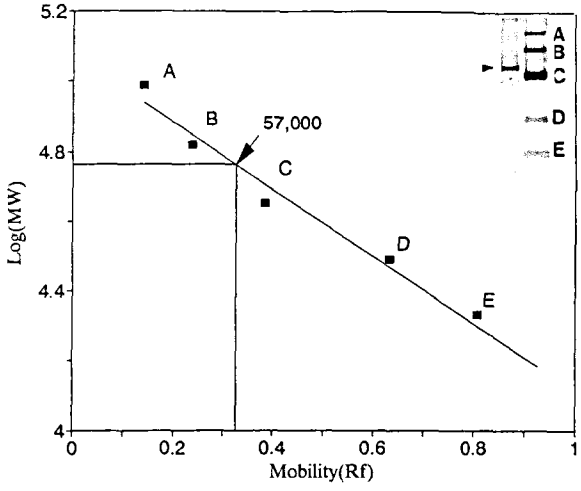

Fig. 2. Determination of molecular weight of SAM synthetase by SDS-polyacrylamide gel electrophoresis. A, phosphorylase b (97,400); B, bovine serum albumin (66,200); C, ovalbumin (45,000); D, carbonic anhydrase (31,000); E, trypsin inhibitor (21,500).

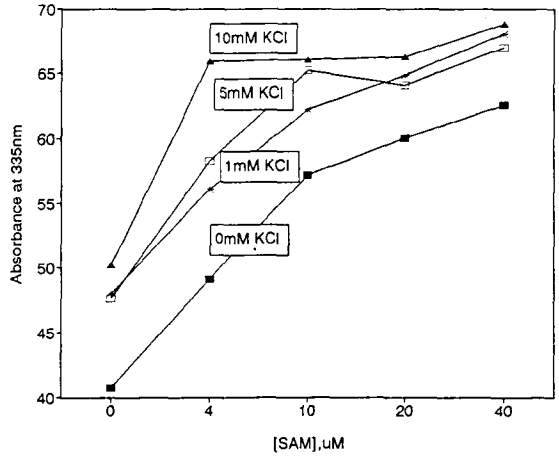

Fig. 3. Dependence of tripolyphosphatase activity of SAM synthetase on KCl and SAM. The tripolyphosphatase activity at various conentrations of SAM and different fixed KCl was assayed as described in the Materials and Methods and the absorbance at 335 nm was measured.

Table 3. Substrate specificity for SAM synthetase All experiments measured [methyl-^{14}C] methionine incorporation into SAM and were performed in 0.1 M Tris-chloride, pH 8.0, containing 20 mM $MgCl_2$, 0.1 M KCl, 10 mM ATP, 0.025 μCi [methyl-^{14}C]methionine at 30℃. Rates are normalized to the maximal rate with ATP equal to 100.

Compound	Relative V_{max}
ATP	100
GTP	24
3'deoxy ATP	58
2'deoxy ATP	17
AMPPNP	21
8-BromoATP	14
ADP	24
ATPγS	2

Table 4. Effects of thiol-blocking reagents on SAM synthetase.

Addition		Relative activity (%)
	None	100
NEM	0.5 mM	71
	1.0 mM	57
	2.8 mM	42
DTNB	0.5 mM	50
	1.0 mM	42
	2.8 mM	31
PCMB	0.5 mM	80
	1.0 mM	75
	2.8 mM	56

Purified enzyme was used. After preincubation for 30 min at 30℃, reaction was carried out.

stored without loss of the activity for up to two weeks at −20℃.

Electrophoretic homogeneity and subunit structure

Purified SAM synthetase showed a single silver stained band on SDS-PAGE (12.8%, Fig. 2). All electrophoresis patterns were reproducible and were seen in every preparation of the purified enzyme which was tested. The molecular weight of the subunit of SAM synthetase was 57,000 daltons, as determined from the relative mobility of the standard protein markers. The molecular weight of the native enzyme was estimated to be 110,000 daltons by molecular seiving on a calibrated Sephacryl S-300 column. These results suggest that the SAM synthetase of soybean axes is composed of two identical subunits.

Specificity, metal effects and modification

ATP analogues showed much lower relative V_{max} values when compared to substantial substrate ATP (Table 3), and 3'deoxy ATP showed the highest substrate specificity, in agreement with Markham *et al.* (1980). Like SAM synthetase from other sources (Chiang and Cantoni 1977; Mudd, 1963), the enzyme from soybean axes also showed tripolyphosphatase activity. Tripolyphosphatase activity was activated by SAM, the product of the SAM synthetase reaction (Fig. 3). This activity was proportional to the K% concentration. The soybean enzyme had an optimum pH and temperature of 8.3 and 30℃, respectively, similar to the enzyme from wheat (Mathur *et al.*, 1991). SAM synthetases from other sources reported to require Mg^{2+} and K^+ for their activities (Suma *et al.*, 1986; Mathur

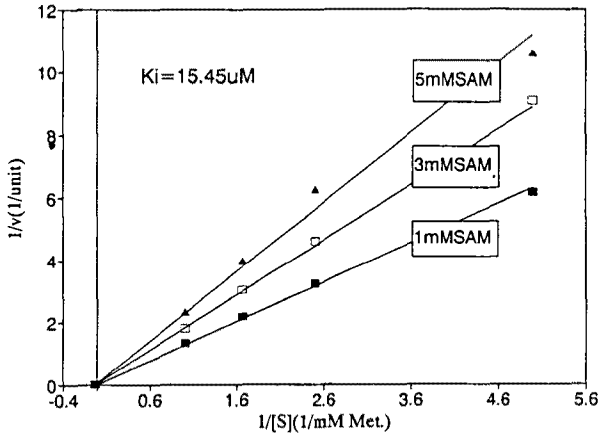

Fig. 4. Lineweaver-Burk plot showing effects of varying methionine concentration at different fixed SAM concentration and a constant ATP concentration.

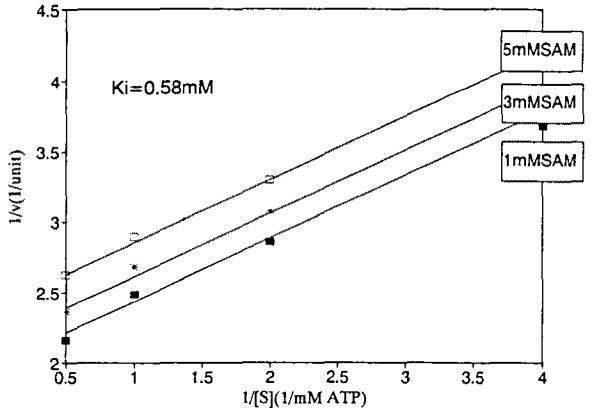

Fig. 5. Lineweaver-Burk plot showing effects of varying ATP concentration at different fixed SAM concentration and a constant methionine concentration.

et al., 1991; Chiang and Cantoni 1977). Both metal ions are essential for the soybean enzyme. Li^+ can substitute for K^+ in the case of the soybean enzyme, but Na^+ and NH_4^+ cannot substitute. Soybean SAM synthetase does require reducing agents for activity, and is inhibited by mercaptide forming or oxidizing thiol reagents in a wide range of concentrations (Table 4). DTNB is the most potent inhibitor, followed by NEM and PCMB. DTNB at 2.8 mM inhibited the enzyme up to 70%. These results suggest that the soybean enzyme is sulfhydryl dependent, as is the enzyme from *E. coli* (Markham and Satishchandran 1988).

Kinetics and a propose scheme of inhibition

Considering that dimeric enzyme consist of identical subunits, the soybean enzyme was expected to follow sigmoidal kinetics. However, the enzyme followed typical Michaelis-Menten kinetics with K_m values for L-methionine and ATP of 0.53 mM and 1.81 mM, respectively. These values are much larger than those from *E. coli*, yeast, and animal cell enzymes (Markham *et al.*, 1980; Suma *et al.*, 1986; Chiang and Cantoni 1977). Both double-reciprocal plots showed good linearity, indicating the absence of cooperativity, which is in agreement with the data reported for the enzyme from *E. coli* (Markham *et al.*, 1980), and human lymphocytes (Kotb Kredich 1985).

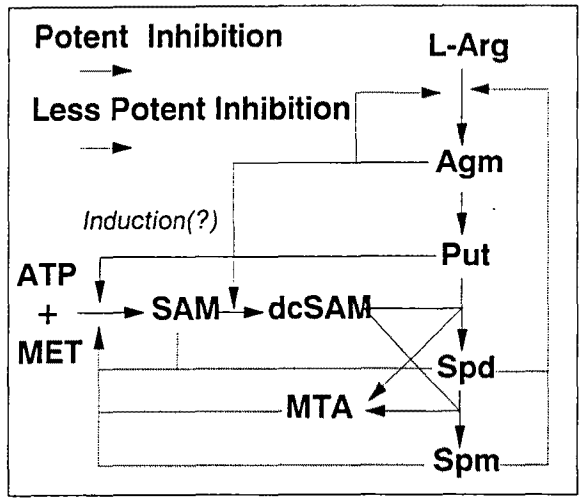

Fig. 6. Possible scheme of the regulation of polyamine biosynthesis by agmatine, polyamine and MTA.

The product of SAM synthetase, SAM, inhibited the enzyme non-competitively and uncompetitively with respect to methionine and ATP, respectively. K_i values, determined by the method of Dixon and Webb *et al.* (1964), were 15.45 μM and 0.58 mM for L-methionine and ATP, respectively. Similar results were obtained for in Sulfolobus solfataricus, *E. coli*, and human lymphocyte enzymes (Markham *et al.*, 1980; Pocelli *et al.*, 1988; Kotb and Kredich 1985). However, purified SAM synthetase was also inhibited more than 30% by 0.5 mM methylthioadenosine (MTA) which is formed from decarboxylated SAM (dcSAM) during the synthesis of Spd and Spm. Recently, a MTA nucleosidase was purified which could remove MTA from the soybean axes (unpublished data). Therefore, these data suggest that SAM synthetase activity is closely related to MTA nucleosidase activity.

In our previous report, SAMDC was isolated and characterized from soybean axes (Yang and Cho, 1991). Recently, a new SAMDC was identified from the same source (Choi and Cho, 1994). All results suggest a more extensive scheme (Fig. 4). The results of data analysis further supports previous findings (Yang and Cho 1991) and therefore should help in our limited understanding of polyamine biosynthesis in higher plants.

Acknowledgement

This article is offered to commemorate the retirement of professor Chung-No Joo from the Department of Biochemistry, College of Science, Yonsei University.

We are pleased to acknowledge the support of the Korea Science and Engineering Foundation (91050005).

References

Abe, T., Yamano, H., Teraoka, H. and Tsukada, K. (1980) *FEBS Lett.* **121**, 29.

Adams, D. O. and Yang, F. S. (1977) *Plant Physiol.* **60**, 892.

Adams, D. O. and Yang, F. S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 170.

Andrews, P. (1964) *Biochem. J.* **91**, 221.

Cabrero, C., Puerta, J. and Alemany, S. (1987) *Eur. J. Biochem.* **170**, 299.

Cantoni, G. L. (1953) *J. Biol. Chem.* **201**, 403.

Cherest, H., Surdin-kerjan, Y., Exinger, F. and Lacroute, F. (1978) *Mol. Gen. Genet.* **163**, 153.

Choi, Y. S. and Cho, Y. D. (1994) *Biochim. Biophys. Acta.* **1201**, 466.

Chiang, P. K. and Cantoni, G. L. (1977) *J. Biol. Chem.* **252**, 4056.

Dixon, M. and Webb, E. C. (1964) *Enzymes*, 2nd ed. p. 330, Longmans Green and Co. London and Academic Press, New York.

Geller, A. M., Kotb, M. Y., Jernigan Jr., H. M. and Kredich, N. M. (1986) *Exp. Eye Res.* **43**, 997.

Heinonen, J. K., Honkasalo, S. H. and Kukko, E. I. (1981) *Anal. Biochem.* **117**, 293.

Heinonen, J. K. and Lahti, R. J. (1981) *Anal. Biochem.* **113**, 313.

Kang, J. H. and Cho, Y. D. (1990) *Plant Physiol.* **93**, 1230.

Kotb, M. and Kredich, N. M. (1985) *J. Biol. Chem.* **260**, 3923.

Laemmli, U. K. (1970) *Nature*, **227**, 680.

Lowry, O. H., Rosebrough, J. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.

Markham, G. D., Hafner, E. W., Tabor, C. W. and Tabor, H. (1980) *J. Biol. Chem.* **255**, 9082.

Markham, G. D. and Satishchandran, C. (1988) *J. Biol. Chem.* **263**, 8666.

Mathur, M., Saluja, D. and Sachar, R. C. (1991) *Biochim. Biophys. Acta.* **1078**, 161.

Merril, C. R., Goldman, D., Sedman, S. A. and Ebert, M. H. (1981) *Science* **211**, 1437.

Mitsui, K., Teraoka, H. and Tsukada, K. (1988) *J. Biol. Chem.* **263**, 11211.

Mudd, S. H. (1963) *J. Biol. Chem.* **238**, 2156.

Mudd, S. H. and Cantoni, G. L. (1958) *J. Biol. Chem.* **231**, 481.

Okada, G., Teraoka, H. and Tsukada, K. (1981) *Biochemistry.* **20**, 934.

Pocelli, M., Cacciapuoti, G., Carteni-farina, M. and Ganbacorta, A. (1988) *Eur. J. Biochem.* **177**, 273.

Suma, Y., Shumizu, K. and Tsukada, K. (1986) *J. Biochem.* (Tokyo) **100**, 67.

Tabor, C. W. and Tabor, H. (1984) *Adv. Enzymol.* **56**, 251.

Thomas, D. and Surdin-kerjan, Y. (1987) *J. Biol. Chem.* **262**, 16704.

Yang, S. F. and Hoffman, N. E. (1984) *Annu. Rev. Plant. Physiol.* **35**, 155.

Yang, Y. G. and Cho, Y. D. (1991) *Biochem. Biophys. Res. Comm.* **181**, 1181.