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Cloning and molecular characterization of selenocysteine methyltransferase (AchSMT) cDNA from *Astragalus chrysochlorus*

Özgür Çakır[1*] and Şule Arı[1,2]

[1]Department of Molecular Biology and Genetic, Faculty of Science, Istanbul University, 34118 Vezneciler, Istanbul, Turkey
[2]Research and Application Center for Biotechnology and Genetic Engineering, 34118 Vezneciler, Istanbul, Turkey

***Corresponding author: ozgurckr@istanbul.edu.tr**

Abstract

Selenium (Se) is a dietary essential trace element in human nutrition and has been implicated in health, including cancer preventation. Methylselenocysteine (MeSeCys) is an effective chemopreventative compound. *Astragalus* sp. are known to accumulate Se with the majority of the selenoamino acids in the form of MeSeCys. The aim of this study was the cloning and charactherization of a cDNA encoding selenocysteine methyltransferase (SMT), the key enzyme responsible for MeSeCys formation, from *Astragalus chrysochlorus* using specific primers. Our results showed that *Astragalus chrysochlorus* SMT (AchSMT) is a 1020 bp (base pair) cDNA (GQ844862) with an open reading frame predicted to encode a 339 amino acid, 36.94 kDa protein. The predicted amino acid sequence of AchSMT (AEI53593) revealed 97% identity with *A. bisulcatus* selenocysteine methyltransferase (AbSMT). Bioinformatic analysis revealed that AchSMT lacks chloroplast and mitochondrial targeting sequences. The AchSMT possess a possible zinc-binding motif (GGCC) and a conserved cysteine residue upstream of this motif. AchSMT was expressed in *Escherichia coli*, then confirmed by DNA sequence analysis, western blotting and enzyme activity. Expression of AchSMT correlated with the presence of SMT enzyme activity in cell extracts. In *A. chrysochlrous* plants, analysis of AchSMT gene expression in response to selenate treatments showed that the AchSMT was constitutively expressed. The isolation of SMT gene will result in further studies for overproduction of valuable metabolite, methylselenocysteine.

Keywords: AchSMT, Cloning, Expression, MeSeCys, Selenium.
Abbrevations: AbSMT_*A. bisulcatus* selenocysteine methyltransferase; AchSMT_*A. chrysochlorus* selenocysteine methyltransferase; BoSMT_*Brassica oleracea* var. *italica* selenocysteine methyltransferase; Cys_Cysteine; HMT_Homocysteine S-methyltransferase; kD_kiloDalton; RT-PCR_Reverse transcription polymerase chain reaction; SeCys_Selenocysteine; MeSeCys_Se-methyl selenocysteine; Se_Selenium; SMT_Selenocysteine methyltransferase

Introduction

Selenium (Se) is an essential micronutrient for animals and humans, and a component of many proteins in variety of organisms (Birringer et al., 2002; Kryukov et al., 2003). In addition to requirements of Se as a nutrient, it also has health benefits potentials. Male fertility and immune function (McKenzie et al., 2001; Foresta et al. 2002), viral infection (Beck et al., 2003), and aging process (Soriano-Garcia, 2004) could be taken into account as health benefits of Se. Also, Se acts as a cancer preventive agent when used in appropiate amounts (Fleming et al., 2001; Ip et al., 2002; Whanger, 2004; McKenzie et al., 2009). Monomethylated form of Se, methylselenocysteine (MeSeCys), provides superior chemoprotective effects against cancer (Ip et al., 1991; Ganther, 1999; Ip et al., 2000; Whanger, 2002; McKenzie et al., 2009). An active anticancer agent, methyl selenol, is produced from MeSeCys, which has almost no toxicity effects at very low levels (Medina et al., 2001; Finley et al., 2001). Such properties make this compound especially beneficial as a chemopreventative agent. A lot of studies revealed the efficacy of MeSeCys in preventing animal mammary cancer (Ip and Ganther, 1992; Lu et al., 1996; Ip et al., 2000; Medina et al., 2001). MeSeCys is produced by plants including *Brassica* and *Allium* genera (Cai et al., 1995; Clark et al., 1996), and in Se-accumulating plants, such as *Astragalus bisulcatus* (Trelease et al., 1960; Nigam and McConnel, 1969).

A selenocysteine methyltransferase (SMT) with the capacity to use and turn S-methylmethionine to methylate SeCys (selenocysteine) and produce MeSeCys has been isolated from *A. bisulcatus*, a Se hyperaccumulator (Neuhierl and Böck, 1996; Neuhierl et al., 1999). SMT plays a key role to remove the toxic effect of selenium at higher levels in plants (Neuhierl and Böck, 1996). The gene encoding SMT is thought to confer Se tolerance to *A. bisulcatus*. Pickering et al. (2003) reported that SMT is constitutively expressed in roots and leaves of *A. bisulcatus*, and not induced by Se. In transgenic *Arabidopsis thaliana* expression of AbSMT, results in the production of MeSeCys and its derivative γ-glutamyl-Se-MeSeCys (Ellis et al., 2004). Accumulation of MeSeCys was similar in transgenic *Brassica juncea*, which expressed AbSMT (Leduc et al., 2004). SMT shares significant primary sequence homology with homocysteine S-methyltransferases (HMT) from *Arabidopsis thaliana* (Ranocha et al., 2000). Both SMT and HMT catalyze methyl transfer using S-methylmethionine as the methyl donor, but these enzymes exhibits different substrate preference as a methyl acceptor *in vitro* (Se-containing substrates for SMT and S-containing substrates for HMT) (Neuhierl and Böck,

1996; Ranocha et al., 2000). Se-enriched broccoli is very rich for MeSeCys (Cai et al., 1995) and MeSeCys is the primary form found in Se-enriched broccoli (Finley et al., 2001; Sugihara et al., 2004). Studies by Finley and coworkers (Finley et al., 2000, Finley and Davis, 2001; Davis et al., 2002) provide evidence for the role of Se-enriched broccoli in preventing cancer. Therefore, increasing the accumulation of MeSeCys in plants may promote its health beneficials (Finley, 2003; Çakır et al., 2012).

Very few plants, such as a Se hyperaccumulator *A. bisulcatus*, and secondary Se accumulators, broccoli (*Brassica oleracea* var. *italica*), *Camellia sinensis* and *Astragalus chrysochlorus* have been determined to accumulate high levels of Se (Cai et al., 1995; Pickering et al., 2003; Zhu et al., 2008; Ari et al., 2010). All of these plants have been shown to possess SMT gene. Sors et al. (2009) demonstrated that both Se hyperaccumulating (*A. racemosus* and *A. pectinatus*) and non-accumulating *Astragalus* species (*A. ceramicus, A. drummondii* and *A. leptocarpus*) produce an SMT-like enzyme. Sors et al. (2009) revealed that the SMT sequences derived from Se accumulators and non-accumulators are highly similar. *A. drummondii*, non-accumulator species, shows almost no selenocysteine methyltransferase activity *in vitro*. This explains the lack of ability to accumulate Se.

In the present study, we report the isolation and characterization of SMT cDNA from secondary Se accumulator *A. chrysochlorus*.

Results

Isolation of the SMT cDNA

A cDNA fragment was obtained by RT-PCR using specific primers designed according to conserved sequences among *Astragalus* species [*A. bisulcatus* SMT (CAA10368); *A. racemosus* SMT, (ACV03420.1), *A. pectinatus* SMT (ACV03421.1); *A. leptocarpus* (ACV03424.1), *A. ceramicus* (ACV03422.1)]. This cDNA was named as AchSMT (GenBank accession number: GQ844862). The AchSMT cDNA contained an open reading frame of 1020 bp that encodes 339 amino acid residues with a molecular mass of approximately 36.94 kD. AchSMT contains a consensus sequence of GGCC for a possible zinc-binding motif near the C-terminal and a conserved Cys residue upstream of the zinc-binding motif as other related methyltransferases (Ranocha et al., 2000). Nucleotide and deduced amino acid sequence BLAST analysis are shown in Table 1. It shares 97 % nucleotide identity with AbSMT. It was determined that AchSMT has no obvious chloroplast or mitochondrial targeting sequence. Comparison of deduced amino acid sequence of AchSMT with other SMTs is shown in Fig 1. The C-terminal end of the AchSMT protein contains three conserved motifs including G(I/V)NC starting at the amino acid position 242, YPNSGE at position 269 and GGCCR at position 310. Three conserved cysteine residues, Cys245, Cys312, Cys313 are thought to play role in creating a ternary structure believed to be responsible for zinc binding (Millian and Garrow, 1998; Peariso et al., 1998; Koutmos et al., 2008).

Expression analysis

To investigate AchSMT gene expression, *A.chrysochlorus* plants treated with different concentrations of selenate. RT-PCR reactions were performed to amplify cDNA of SMT. For the normalization of the SMT expression, actin gene was analysed. Agarose gels analyses showed that AchSMT from *A. chrysochlorus* is constitutively expressed (Fig 2).

Overproduction of AchSMT protein in E. coli

To confirm the identity of the protein encoded by SMT from *A. chrysochlorus* with SeCys-methyltransferase, the gene was cloned into plasmid pET100D-TOPO to be expressed in *E.coli*. The plasmid was cloned into *E. coli* strain BL21Star (DE3). Overexpression recombinant plasmid led to insoluble gene product (Fig 3). The amount of SMT protein expression increased when time prolonged. Its molecular mass was about 40 kDa as validated by SDS-PAGE, which is basically in agreement with an expected size of 40 kDa (36.94 kDa derived from the gene sequence and 3 kDa belongs to plasmid).

SMT enzyme activity assays and immunological detections

E. coli strain BL21 Star (DE3) cells containing pET100D-AchSMT plasmids were used in semi-quantitative assay of SMT activity and the selenocysteine and S-adenosylmethionine were used as substrates. As expected, fractions containing the plasmid yielded an additional spot (Rf = 0.58) indicated that AchSMT catalyses the methyl transfer to form MeSeCys. No SMT enzyme activity was detected in protein extracts from *E.coli* cells contained plasmid without SMT insert (Fig 4). The activity has been detected from the samples which were collected after 2, 4, and 6 hours.

The SDS lysates were prepared from bacteria containing pET100D-AchSMT plasmid and the proteins separated by SDS-PAGE and subjected to immunoblotting analysis (Fig 5). Immunobloting analysis showed that the cDNA which was isolated from *A. chrysochlorus* corresponds to selenocysteine methyltransferase and it encodes structurally and functionally accurate protein.

Discussion

MeSeCys is one of the most effective anticarcinogenic Se compounds (Ip et al., 1991; Ganther, 1999; Ip et al., 2000; Whanger, 2002; McKenzie et al., 2009). It is the major form of selenoamino acids found in *Astragalus* plants. In our previous study, *A. chrysochlorus* was evaluated to be a secondary accumulator of Se according to its capacity of Se accumulation and detected to possess cDNA fragment of SMT gene (Ari et al., 2010). Based on our work and the works conducted by others, it is very clear that the SMT gene is very important to determine the ability of Se tolerance and accumulation. Therefore, identification of *A. chrysochlorus* SMT permits a comprehensive study of gene expression in relation to MeSeCys production, Se tolerance and accumulation. The availability of such genetic material opens a practical avenue for the development of organisms with an enhanced ability to biosynthesis of MeSeCys for phytoremediation of Se contaminated land, and also provide a plant based source of the anticarcongenic compound, MeSeCys. Thus, identification of *A. chrysochlorus* cDNA encoding SMT, the key enzyme involved in the formation of MeSeCys, permits a comprehensive study of gene expression in relation to MeSeCys production in plants. AchSMT cDNA was successfully amplified from *A. chrysochlorus* using conserved SMT sequences. This AchSMT clone

Table 1. Aligments of nucleotide (GQ844862) and deduced amino acids (AEI53593) sequences of AchSMT

SMT Gene	% Identity with AchSMT	E value*
Astragalus bisulcatus SMT	97	0
Astragalus racemosus SMT	97	0
Astragalus pectinatus SMT	97	0
Astragalus drummondii SMT-Like gene	95	0
Astragalus ceramicus SMT	95	0
Astragalus leptocarpus SMT	95	0
Vitis vinifera hypothetical protein XM_002283008.1	54	2e-91
Vitis vinifera hypothetical protein XM_002283018.1	18	2e-31
Vitis vinifera contig VV87X163117.12	12	3e-20
Brassica rapa subsp. *pekinensis* clone	6	9e-15
SMT protein	% Identity with AchSMT	E value
Astragalus bisulcatus	97	e-176
Astragalus racemosus	97	e-179
Astragalus pectinatus	97	e-173
Astragalus drummondii	96	0
Astragalus ceramicus	96	e-179
Astragalus leptocarpus	96	e-179
Camellia sinensis	96	e-144
Brassica oleracea var. italica	93	e-131

*E-value: The Expect value (E) is the value that means the number of hits one can "expect" to see by chance when searching a database of a particular size.

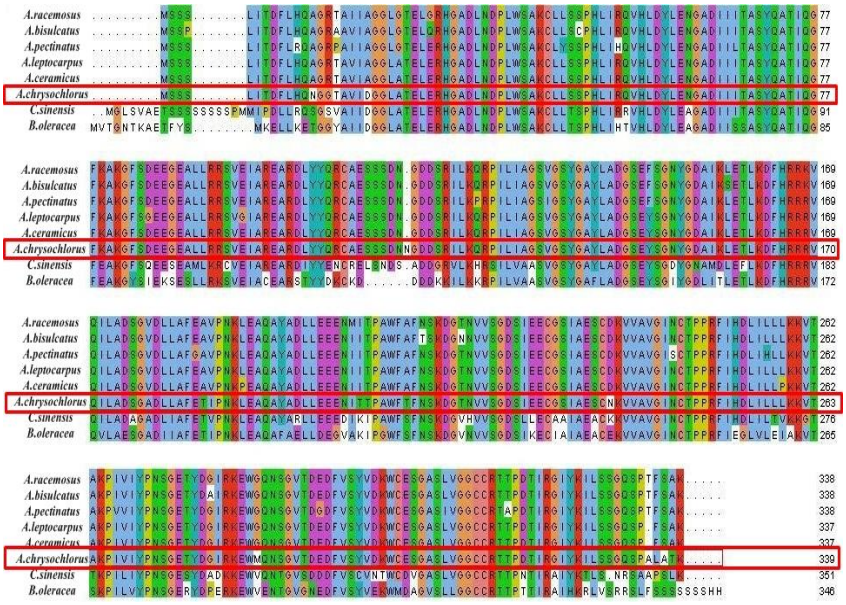

Fig 1. Alignment of the sequence of AchSMT (AEI53593) with those of related SMT proteins (*A. bisulcatus* SMT (CAA10368); *A. racemosus* SMT, (ACV03420.1), *A. pectinatus* SMT (ACV03421.1); *A. leptocarpus* (ACV03424.1), *A. ceramicus* (ACV03422.1), *C. sinensis* (ABF47292.1), *B. oleracea* (AAX20123.1). Conserved residues are shown in same colors. Red bar showes *A. chrysochlorus* SMT.

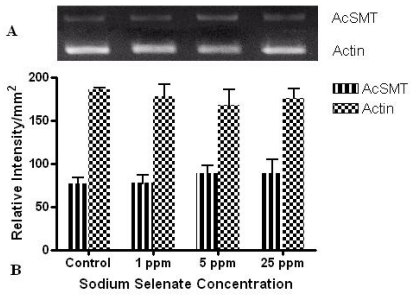

Fig 2. PCR products of *A. chrysochlorus* SMT gene and housekeeping actin gene (A), relative intensity graph of these PCR products (B).

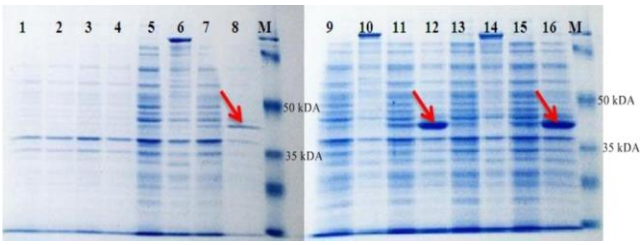

Fig 3. SDS-PAGE analysis of expressed proteins derived from SMT gene thereof at different inducing time. M: Marker (Fermentas, SM0441); *E.coli* BL21 Star (DE3), 1) pET100D-β-Galactosidase /0 h (-IPTG), 2) pET100D-β-Galactosidase /0 h (+IPTG), 3) pET100D-AchSMT/0 h (-IPTG), 4) pET100D-AchSMT/0 h (+IPTG), 5) pET100D-β-Galactosidase /2 h (-IPTG), 6) pET100D-β-Galactosidase /2 h (+IPTG), 7) pET100D-AchSMT/2 h (-IPTG), 8) pET100D-AchSMT/2 h (+IPTG), 9) pET100D-β-Galactosidase /4 h (-IPTG), 10) pET100D-β-Galactosidase /4 h (+IPTG), 11) pET100D-AchSMT/0 h (-IPTG), 12) pET100D-AchSMT/4 h (+IPTG), 13) pET100D-β-Galactosidase /6 h (-IPTG), 14) pET100D-β-Galactosidase /6 h (+IPTG), 15) pET100D-AchSMT/6 h (-IPTG), 16) pET100D-AchSMT/6 h (+IPTG), red arrows point to 40 kDA SMT protein (36.94 kDa derived from the gene sequence and 3 kDa belongs to plasmid).

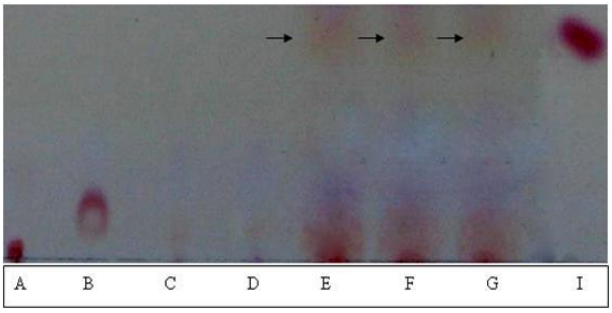

Fig 4. Semiquantitative assay for SMT activity by thin layer chromatography. A) Selenocysteine, B) S-adenosylmethionine, C) fractions after adding crude extracts of bacteria containing plasmid empty vector, fractions after adding crude extracts of bacteria containing the plasmid pET-AchSMT D) 0 h, E) 2 h, F) 4 h, G) 6 h, I) methylselenocysteine (MeSeCys). Arrows point to MeSeCys.

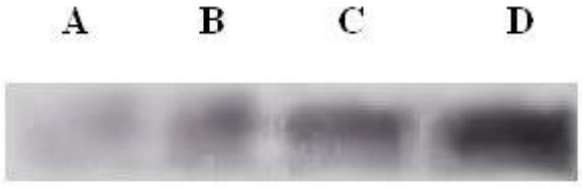

Fig 5. AchSMT protein is expressed in IPTG induced *E. coli* cells and detected from 0 h to 6 h. Immunoblot analysis of selenocysteine methyltransferase from *E. coli* BL21 Star (DE) containing pET100D-AchSMT, induced with IPTG for: A) 0 h, B) 2 h C) 4 h D) 6 h.

was functionally expressed in *E. coli* and its identity was confirmed by the positive SMT enzyme activity in catalyzing MeSeCys production. The deduced amino acid sequence of AchSMT shared high sequence identity with the AbSMT from *A. bisulcatus* (Neuhierl et al., 1999). For better understanding the metabolic function of SMT in Se metabolism, it is important to determine where the SMT enzyme is localized. In BLAST analysis, it was found that AchSMT do not possess chloroplast or mitochondrial targeting sequences. The cytosolic location of AchSMT has not been confirmed in the plant but it is consistent with the data which reveals that methylation of selenocysteine or selenomethionine takes place in the cytosol (Bourgis et al., 1999; Ranocha et al., 2000). The AchSMT protein resembles the related methyltransferases because it contains conserved Cys residue and a possible zinc binding motif (GGCC) near the C-terminal. This suggests that AchSMT may have a zinc co-factor for binding and/or activating the selenol group of selenocysteine. Although *A. chrysochlorus* is a secondary accumulator (Ari et al., 2010) like *C. sinensis* and Broccoli, it shows more sequence similarity with *Astragalus* species than *C. sinensis* or Broccoli. Thus, the BLAST searches revealed that SMT shares high sequence similarity in the same species genes.

SMT enzyme activity in non-accumulators is very low compared to accumulators (Sors et al., 2009). It is known that there is no correlation between enzyme activity of SMT and sequence homology. Different signature of conserved amino acid residues in the accumulator and nonaccumulator was found in SMTs. For example in the hyperaccumulators like *A. bisulcatus, A. pectinatus* and *A. rasemosus* phenylalanine residue at position 148 is replaced by tyrosine residue in non-accumulators, which are *A. drummondii, A. crassicarpus* and *A. leptocarpus* (Sors et al., 2009). Tyrosine residue was found at this position in AchSMT like *C. sinensis* and Broccoli which are other secondary accumulators. According to evidences that *A. chrysochlorus* is a member of secondary selenium accumulator (Ari et al., 2010), it was considered that the amino acid alteration at that point might not be related to function of enzyme. The substitution on this site may have modulated the structure of catalytic site of the enzyme because the recombinant AchSMT enzyme extracts prepared from *E. coli* contained significant SMT enzyme activity. Thus, expression of it in *E.coli* provided a means to explore substrate specificity of the enzyme *in vitro*.

AbSMT is constitutively expressed in roots and leaves of *A. bisulcatus* (Pickering et al., 2003). In contrast, Lyi et al. (2005) reported that BoSMT (Broccoli SMT) expression is extremely low or undetectable in plants which are not exposed to Se and up-regulated by selenate treatment. In leaf tissues, BoSMT expression has been detected to be under developmental control. Highest transcript level were found in very young leaves. Our results showed that AchSMT from *A. chrysochlorus* is constitutively expressed like AbSMT from hyperaccumulator *A. bisulcatus* in all selenate treatments. The different expression patterns between AbSMT, AchSMT and BoSMT may be due to the fact that *A. bisulcatus* is a native plant grown on seleniferous soils, while broccoli is not restricted to Se containing soils. It is thought that *A. chrysochlorus* may also grow on seleniferous soils, but there is no information for ecological Se status of the soils on endemic *A. chrysochlorus* grow.

Materials and methods

Plant materials

Plants were collected in June 2004 from Sertavul, Karaman, Turkey and were determined by Prof. Dr. Tuna Ekim (Istanbul University, Faculty of Science, Department of Botany). A voucher specimen was deposited in the herbarium ISTF no: 40006 (Istanbul University, Faculty of Science Herbarium).

Strains and plasmids

E.coli OneShot TOP10 strain (Invitrogen, Inc.) was used for general cloning purposes and SMT was expressed in *E. coli* strain BL21Star (DE3) (Invitrogen, Inc). Expression vector pET100D-TOPO was purchased from Invitrogen Inc.

Cloning and sequencing of Astragalus chrysochlorus SMT cDNA

Surface sterilized *A. chrysochlorus* seeds were incubated in growth-regulator-free MS (Murashige and Skoog, 1962) medium supplemented with 3 % (w/v) sucrose, 0, 1, 5 and 25 ppm sodium selenate and solidified with 0.8 % agar (w/v). Petri dishes were incubated in a growth chamber illuminated with fluorescent light (ca. 1400 µmol-2ms-1) over a 16h light, 8 h dark photoperiod at 25±2 ºC. 30-day-old plantlets which were grown in MS medium supplemented with 0, 1, 5 and 25 ppm sodium selenate were used for RNA isolation. Total RNA was extracted from *A. chrysochlorus* (TRIzol Reagent, Invitrogen, Carlsbad, CA), and reverse-transcribed to the first cDNA strand (SuperScript First-Strand Synthesis System, Invitrogen, 11904-018). RNA isolation and first strand cDNA synthesis were done according to the manufacturers instructions. The amplification of SMT was performed using a pair of primers P1 (5´ CACCATGTCGTCGTCATTGATAACCG 3´) and P2 (5´ TCACTTTGTGGCAAGAGCAGGAGATTGACC 3´). The primers designed according to the conserved sequences of selenocysteine methyltransferase genes found in NCBI database. The amplification reaction was carried out in a 25 µl volume containing 10–20 ng of DNA. The PCR mixture consisted of 1xPCR buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4), 1.5 mM $MgCl_2$, 10 mM dNTPs, 20 µM of each primer, and 3 units of Phusion DNA polymerase (New England Biolabs). Sterile distilled water was used to complete the volume to 25 µl and the reaction was performed using the Peltier Thermal Cycler (MJ Research, PTC- 200). The PCR mixture was incubated at 98 °C for 30 sec. prior to amplification for 30 cycles. Each cycle consisted of denaturation at 98 °C for 5 sec., annealing at 56 °C for 20 sec., and elongation at 72 °C for 20 sec. The PCR product was subjected to 1.2% agarose gel electrophoresis and observed under ultraviolet light. The PCR product (1020 bp DNA fragment) purified by using the Qiagen PCR purification kit (QIAGEN, Catalog no:28704) and was cloned into the pET100D-TOPO vector according to Champion™ pET Directional Expression Kit (Invitrogen, K100-01). The plasmid contained the PCR product was named pET100D-AchSMT and transformed into *E. coli* OneShot TOP10 strain according to the instructions and sequenced. DNA sequencing was achieved using the ABI PRISM 310 Genetic Analyser, Applied Biosystems (IONTEK).

Expression analysis by RT-PCR reaction

After total RNA isolation from plants grown in different concentrations of Se, the reverse-transcription was achieved as described above, PCR performed by designed SMT primers for *A. chrysochlorus*. PCR products were analysed on 1% agarose gel electrophoresis and observed under ultraviolet light. Agarose gels were imaged on densitometer (GS-800 Calibrated Densitometer, BIO-RAD) with 'Quantity One® SW' software. Experiments were done in triplicates.

Expression of SMT gene in E. coli

Plasmid pET100D-AchSMT was transferred into *E. coli* strain BL21Star (DE3) so that the corresponding gene could be overexpressed constitutively via T7 promoter. Cells were grown in 20 ml of LB medium containing 50 µg/ml ampicillin at 37°C and maximal aeration and grown until the cell reach a density of A_{600}= 0.5. Overproduction was started by the addition of isopropylthiogalactoside (IPTG, Sigma Cat no. I6758) to a final concentration of 1 mM. Incubation were continued and 0.5 ml medium were collected at 0, 2, 4, 6 h.

Preparation of a crude extract of E. coli

Bacteria cells were collected by centrifugation at 4,000 rpm for 30 sec. The supernatant was discarded. Cell pellets were resuspended by 2 ml PBS buffer and then 2 ml of 2x Sample Buffer containing 100 mM dithiothreitol, 2% SDS, 80 mM Tris/Cl, (pH 6.8), 0.006% bromophenol blue and 10% glycerol were added. The prepared samples were sonicated. Crude extracts from cultured cells of *E. coli* strain BL21Star (DE3) containing pET100D-AchSMT was centrifuged. The supernatant was used for gel electrophoresis. Proteins were denatured at 70°C for 3 min and these crude extracts were stored at –20°C. SDS-PAGE was performed according to Laemmli (Laemmli, 1970). Proteins were separated by SDS-PAGE and visualized by Coomassie staining.

SMT enzyme assay and immunological procedures

Bacteria cells were grown at 37°C like mentioned above to an absorbance of 1.0 measured at 650 nm. They were centrifuged at 4,000 rpm for 30 sec in 20 ml LB to get pellets. The pellets were resuspended in 2 ml extraction buffer consisted of 50 mM sodium citrate [(pH 6.0, 0.2 mM EDTA, 2 mM DTT, 10% (w/v) glycerol)]. Selenocysteine methyltransferase enzyme activity assays were conducted according to Neuhierl and Böck (1996), and Lyi et al. (2005). SMT activity yielded an additional spot corresponding to Se-methylselenocysteine (Methylselenocysteine standart was purchased from Fluka Cat no. 09974). Production of SMT in *E. coli* was also determined by immunoblotting following the methods Pickering et al. (2003). Seleno-Cys methyltransferase was visualized on the membrane using a polyclonal primary antibody raised against SMT in rabbits (Neuhierl et al., 1999), and a secondary anti-immunoglobulin G antibody raised in goat and conjugated to alkaline phosphatase. Blots were developed by the addition of nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate.

Statistical Analysis

Statistical analyses [analysis of variance (ANOVA)] were performed using the software package GraphPad Prism 4.0.

Conclusion

We have cloned a SMT gene from *A. chrysochlorus* that is responsible for the formation of MeSeCys. We demonstrated that the recombinant AchSMT extracts prepared from *E. coli* contained significant enzyme activity. The isolation of the *AchSMT* gene will allow its up-regulation in *A. chrysochlorus* to further enhance MeSeCys production for improving its health-promoting properties.

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