Available online at www.sciencedirect.com
SCIENCE DIRECT
Phytochemistry 67 (2006) 668–674
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Molecular and biochemical characterization of a serine racemase from *Arabidopsis thaliana* ☆

Yoshiyuki Fujitani [a], Nobuyoshi Nakajima [b], Koji Ishihara [c], Tadao Oikawa [d], Kazutoshi Ito [e], Manabu Sugimoto [a,*]

[a] *Research Institute for Bioresources, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan*
[b] *Collaborative Research Center, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan*
[c] *Department of Life Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*
[d] *Department of Biotechnology, Faculty of Engineering, Kansai University, 3-3-35 Yamate-cho, Suita, Osaka 564-8680, Japan*
[e] *Plant Bioengineering Research Laboratories, Sapporo Breweries Ltd., 37-1 Nitta-Kizaki-cho, Ohta, Gunma 370-0393, Japan*

Received 23 August 2005; received in revised form 21 November 2005
Available online 17 February 2006

Abstract

A cDNA encoding a homolog of mammalian serine racemase, a unique enzyme in eukaryotes, was isolated from *Arabidopsis thaliana* and expressed in *Escherichia coli* cells. The gene product, of which the amino acid residues for binding pyridoxal 5′-phosphate (PLP) are conserved in this as well as mammalian serine racemases, catalyzes not only serine racemization but also dehydration of serine to pyruvate. The enzyme is a homodimer and requires PLP and divalent cations, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, or Ni^{2+}, at alkaline pH for both activities. The racemization process is highly specific toward L-serine, whereas L-alanine, L-arginine, and L-glutamine were poor substrates. The V_{max}/K_m values for racemase activity of L- and D-serine are 2.0 and 1.4 nmol/mg/min/mM, respectively, and those values for L- and D-serine on dehydratase activity are 13 and 5.3 nmol/mg/min/mM, i.e. consistent with the theory of racemization reaction and the specificity of dehydration toward L-serine. Hybridization analysis showed that the serine racemase gene was expressed in various organs of *A. thaliana*.

Keywords: *Arabidopsis thaliana*; Serine racemase; D-Amino acid; Pyridoxal 5′-phosphate

1. Introduction

D-Amino acids such as D-alanine and D-glutamate have been recognized as the components of peptidoglycans in bacterial cell walls, although they have been isolated not only from microorganisms but also from animals and plants as well (Robinson, 1976; Hashimoto et al., 1992, 1993; Bruckner and Weshauser, 2003). Recently, it has been reported that D-serine acts as an agonist at the glycine site of a *N*-methyl-D-aspartate receptor and D-aspartate serves as an agonist at its glutamate site in the mammalian nervous system (Dunlop et al., 1986; Nagata et al., 1989, 1994; Hashimoto et al., 1992, 1993, 1995), showing that D-amino acids play an important role in eukaryotes. D-Amino acids are synthesized by amino acid racemases that catalyze the racemization of L- and D-amino acids, and a number of the enzymes such as alanine, aspartate, glutamate, serine, and phenylalanine racemase have been isolated and their genes cloned from microorganisms (Esaki et al., 2002). Serine racemase is a unique enzyme

Abbreviations: PLP, pyridoxal 5′-phosphate; PCR, polymerase chain reaction; IPTG, isopropyl-b-D-thiogalactopyranoside; AtSR, *Arabidopsis thaliana* serine racemase.

☆ The sequence reported in this paper has been deposited in the DDBJ, EMBL, and GenBank data banks under the Accession No. AB206823.

* Corresponding author. Tel.: +81 86 424 1661; fax: +81 86 434 1249.
E-mail address: manabus@rib.okayama-u.ac.jp (M. Sugimoto).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.01.003

discovered in eukaryotes, which requires pyridoxal 5′-phosphate (PLP) as a coenzyme (Uo et al., 1998; Wolosker et al., 1999a). Interestingly, the amino acid sequence of human and mouse brain serine racemases showed ca. 30% identity to that of bacterial biosynthetic threonine dehydratase (Wolosker et al., 1999b; De Miranda et al., 2000). Moreover, mouse serine racemase catalyzes not only serine racemization but also dehydration of serine to pyruvate in the presence of divalent metal ions (De Miranda et al., 2002; Strisovsky et al., 2003; Foltyn et al., 2005). Thus, mammalian serine racemases are distinct from other amino acid racemases. In plants, a number of D-amino acids such as D-alanine, D-aspartate, and D-glutamate were detected in pea seedlings (Ogawa et al., 1977), barley grain, blossoms of hops (Erbe and Bruckner, 2000), and tobacco leaves (Kullman et al., 1999); D-alanine was also identified as a dipeptide such as in D-alanylglycine and D-alanyl-D-alanine in wild rice (Manabe and Ohira, 1983; Manabe, 1985). The genomic DNA of *Arabidopsis thaliana* formerly registered on the database as a putative serine/threonine dehydratase gene of *Escherichia coli* displays 46% amino acid sequence identity to the human and rat serine racemases (Wolosker et al., 1999b); however, there is no evidence whether the homolog has serine racemase activity or some other amino acid racemase activity.

In this paper, we cloned the cDNA encoding the homolog of mammalian serine racemases from *A. thaliana* and characterized the gene product. The expressed protein is a bifunctional PLP-dependent enzyme catalyzing racemization of serine and dehydration of serine to pyruvate in the same way as mammalian serine racemases.

2. Results and discussion

2.1. Sequence analysis of A. thaliana serine racemase

The putative open reading frame of the gene, predicted from the genomic DNA sequence of *A. thaliana*, was a homolog of mammalian serine racemase, and was amplified by RT-PCR. However, the resulting nucleotide sequence and its deduced amino acid sequence were 45 bases and 15 amino acids shorter than the predicted sequences, respectively. The deduced amino acid sequence of *A. thaliana* serine racemase (AtSR) was compared with that of human and mouse serine racemases (Wolosker et al., 1999b; De Miranda et al., 2000), a homolog of mouse serine racemase encoded in the YKL218c gene of *Saccharomyces cerevisiae*, and a catalytic N-terminal domain of *E. coli* L-threonine dehydratase (Gallagher et al., 1998), which belong to the fold type II of PLP enzymes (Grishin et al., 1995). AtSR showed 46%, 45%, 39%, and 30% identity with human and mouse serine racemases, the homolog of *S. cerevisiae*, and the catalytic domain of *E. coli* L-threonine dehydratase, respectively. Alignment analysis of its amino acid sequence with the CLUSTAL W algorithm (Tompson et al., 1994) showed that the PLP-bound Lys62/Phe61/Gly241 which sandwiches the PLP ring, Ser315 of which the side-chain is hydrogen-bonded to the pyridium nitrogen of PLP, Asn89 which stabilizes the 3′ oxygen of PLP by a hydrogen bond of *E. coli* threonine dehydratase (Gallagher et al., 1998) correspond to Lys59, Phe58, Gly238, Ser314, and Asn86 of AtSR, respectively, and all of these residues were conserved in human and mouse serine racemases (Wolosker et al., 1999b; De Miranda et al., 2000). The glycine-rich group which coordinates the phosphate group of PLP is constituted by a triglycine loop (Gly 186–188) in AtSR, whereas it is a tetraglycine loop in human and mouse serine racemases (Fig. 1). Amino acid sequence alignment shows that AtSR belongs to fold type II as for mammalian serine racemases and bacterial serine/threonine dehydratase, of which the Schiff base lysine is near the N-terminus and the glycine-rich loop is closer to the C-terminus; by contrast, bacterial and fungal alanine racemases belong to fold types III and I, respectively (Grishin et al., 1995). These results indicate that eukaryotic serine racemases represent a distinct group in the overall amino acid racemase family.

2.2. Purification of recombinant A. thaliana serine racemase

E. coli cells harboring pAtSR produced an extra protein whose expression level attained ca. 9% of the total protein after 6 h of IPTG induction. Purification of the recombinant AtSR with Ni-NTA column chromatography yielded ~95% pure preparation (Fig. 2). The molecular mass of AtSR estimated by SDS–PAGE was ca. 35 kDa, which matches to that of 35 kDa calculated from the amino acid sequence, and ca. 60 kDa by gel filtration, suggesting that AtSR is a homodimer protein.

The purified AtSR was incubated with L-serine and $CaCl_2$ that activates mammalian serine racemases (De Miranda et al., 2002; Strisovsky et al., 2003), and the production of D-serine and pyruvate was determined. Specific activities of racemization of L- to D-serine and dehydration of L-serine to pyruvate were 4.1 and 86 nmol/min/mg, respectively.

2.3. Enzymatic characterization of recombinant A. thaliana serine racemase

The absorption spectrum of the purified AtSR showed maxima at 280 and 415 nm. After dialysis against 500 volumes of the dialyzing buffer containing 10 mM sodium borohydride, the absorption at 415 nm disappeared and an increase in absorbance at 330 nm was observed, with loss of enzyme activity (Fig. 3A). Furthermore, the racemase activity of AtSR was inhibited completely by hydroxylamine (Fig. 3B), indicating that AtSR depends on PLP and is bound with it through a Schiff base (Grishin et al., 1995).

The optimum pH for both L-serine racemization and dehydration activities were examined in the reaction mixture containing 1 mM $CaCl_2$. AtSR has maximum

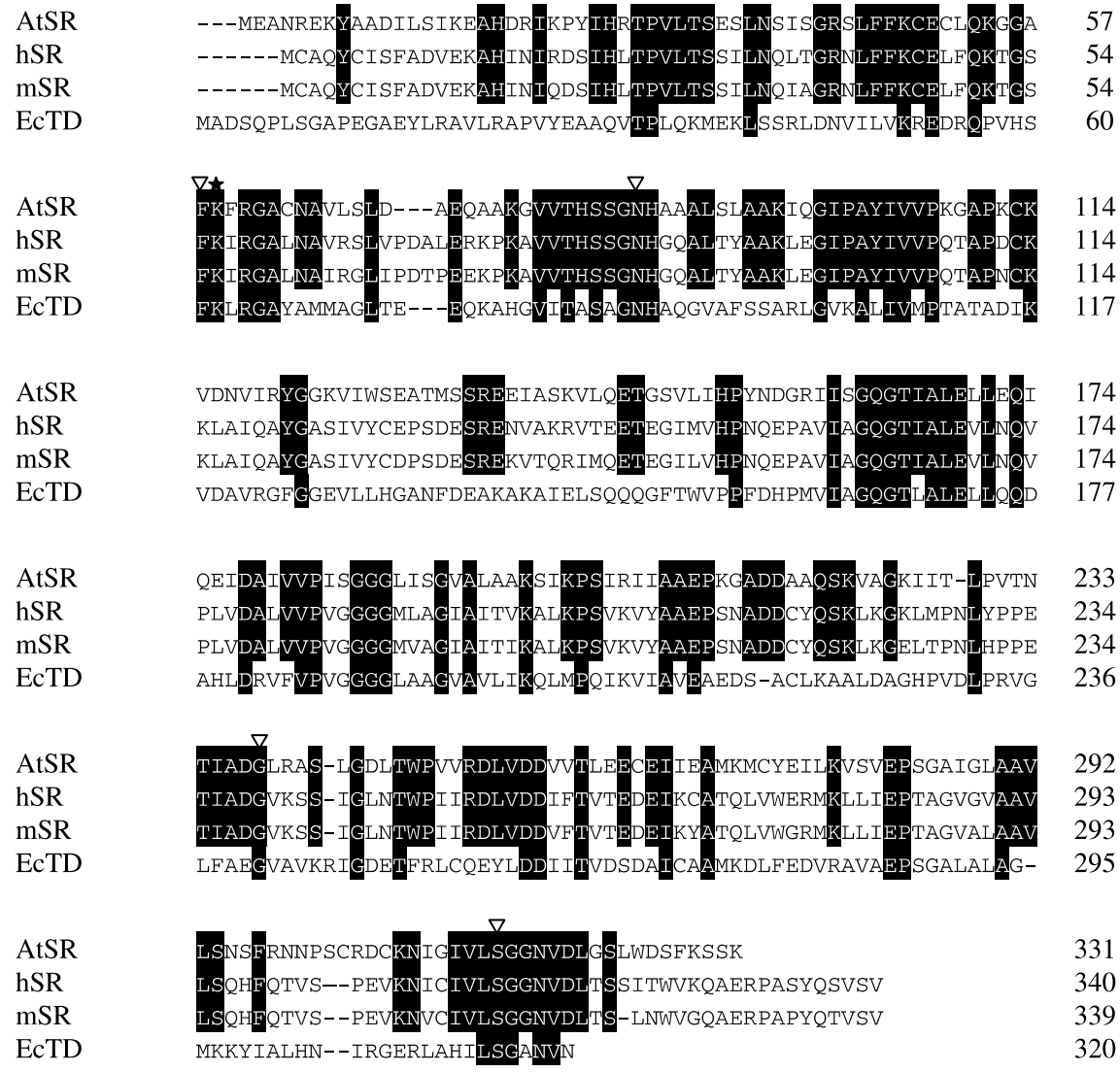

Fig. 1. Alignment of the deduced amino acid sequence of *A. thaliana* serine racemase with those of human and mouse serine racemases, *S. cerevisiae* L-*threo*-3-hydroxyaspartate dehydratase, and *E. coli* biosynthetic L-threonine dehydratase. *A. thaliana* serine racemase (AtSR) corresponds to GenBank Accession No. AB206823, human serine racemase (hSR) corresponds to AF169974, mouse serine racemase (mSR) corresponds to AF148321, and *E. coli* biosynthetic L-threonine dehydratase (EcTD) corresponds to Swiss-Plot No. P04968. Gaps, indicated by dashes, are introduced in the sequences to maximize the homology. Identical amino acid residues among serine racemases or all of four enzymes are represented by black boxes. An asterisk and arrowheads indicate the Lys matching to the PLP-binding Lys and amino acid residues matching to those interacting with PLP in *E. coli* biosynthetic L-threonine dehydratase, respectively.

activities for racemization and dehydration at pH 8.5 and 9.5, and has high activities from pH 8.0 to 8.5 and from pH 8.5 to 9.5, respectively.

The effects of divalent cations on L-serine racemization and dehydration activities were analyzed by the addition of 1 mM divalent ion to the reaction mixture at pH 8.5. Racemase activity increased 3.6, 2.9, and 3.0 times with Ca^{2+}, Mg^{2+}, and Mn^{2+}, respectively, when compared to that in the presence of EDTA, whereas Fe^{2+} had a more limited effect to increase 1.4 times and both Ni^{2+} and Zn^{2+} inhibited activity. Addition of Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, and Ni^{2+} increased 6.8, 7.5, 9.3, 2.8, and 2.7 times dehydratase activity, respectively, and Zn^{2+} had no effect on activity (Fig. 4). It has been shown that mouse serine racemase was activated by Ca^{2+}, Mg^{2+}, Mn^{2+}, and ATP, especially when Ca^{2+} was bound to the enzyme directly (Cook et al., 2002). Moreover, since there is a synergy between $Mg^{2+}$and ATP on activity (De Miranda et al., 2002), we evaluated the optimum concentration of Ca^{2+} and the effect of ATP on AtSR activity. AtSR activity increased with increasing amounts of Ca^{2+} reaching maximum activity at 1 mM Ca^{2+}. However, AtSR was not activated by 1 mM ATP and the enzyme activity in the presence of 1 mM $Mg^{2+}$and 1 mM ATP was no different from that in the presence of 1 mM Mg^{2+}.

The racemase activities converting various L- to D-amino acids were examined in the reaction mixture containing 1 mM $CaCl_2$ at pH 8.5. AtSR showed high substrate

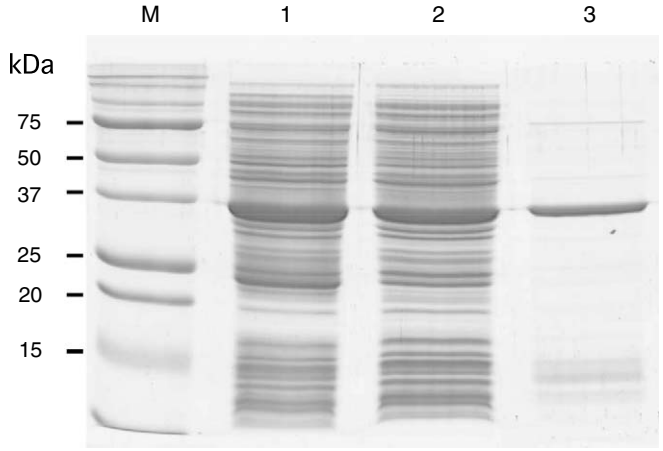

Fig. 2. Analysis of the expression of AtSR in *E. coli* by SDS–polyacrylamide gel. *E. coli* cells harboring pAtSR were harvested after IPTG induction at 25 °C for 6 h. Total *E. coli* lysate (lane 1), the soluble protein solution (lane 2), and the purified AtSR with Ni-NTA column (lane 3) were subjected to 15% SDS–PAGE with molecular mass marker (lane M) followed by Coomassie Brilliant Blue R-250 staining.

specificity for L-serine, whereas L-alanine, L-arginine, and L-glutamine were poor substrates for AtSR, having activities of ~13%, 7.0%, and 4.5% of that for L-serine, respectively.

Alanine racemases from *Salmonella typhimurium* and *Schizosaccharomyces pombe* could catalyze the racemization of L-serine, but the activities on L-serine were 15% and 3.7% of those on L-alanine, respectively (Esaki and Walsh, 1986; Uo et al., 2001). An amino acid racemase with low substrate specificity from *Pseudomonas putida* also showed racemase activity with L-serine; however, the activity was much lower than those of basic and aliphatic straight-chain amino acids such as L-arginine and L-glutamine, respectively (Soda and Osumi, 1969). These results show that the enzymatic properties of AtSR are apparently different from those of microbial amino acid racemases, except for the optimum pH at 8.0 to 8.5; bacterial alanine racemases have maximum activity at alkaline pH (Esaki et al., 2002).

2.4. Kinetic characterization of recombinant *A. thaliana* serine racemase

The K_m and V_{max} values were estimated in the reaction mixture containing 1 mM $CaCl_2$, 10 μM PLP at pH 8.5. The K_m and V_{max} values of racemase activity converting L- to D-serine were 2.5 mM and 5.0 nmol/mg/min, and those converting D- to L-serine were 0.77 mM and 1.1 nmol/mg/min. The V_{max}/K_m values for the conversion from L- to D-serine and from D- to L-serine were 2.0 and 1.4 nmol/mg/min/mM. The K_m and V_{max} values of dehydratase activity converting L-serine to pyruvate were 20 mM and 250 nmol/mg/min, and those eliminating D-serine to pyruvate were 5.0 mM and 26 nmol/mg/min. The V_{max}/K_m values for L- and D-serine elimination were 13 and 5.3 nmol/mg/min/mM. The K_m values for L-serine of rat and mouse serine racemases are 9.8 and 30 mM, and those for D-serine are 60 and 49 mM. The V_{max} values for L-serine of rat and mouse serine racemases are 83 and

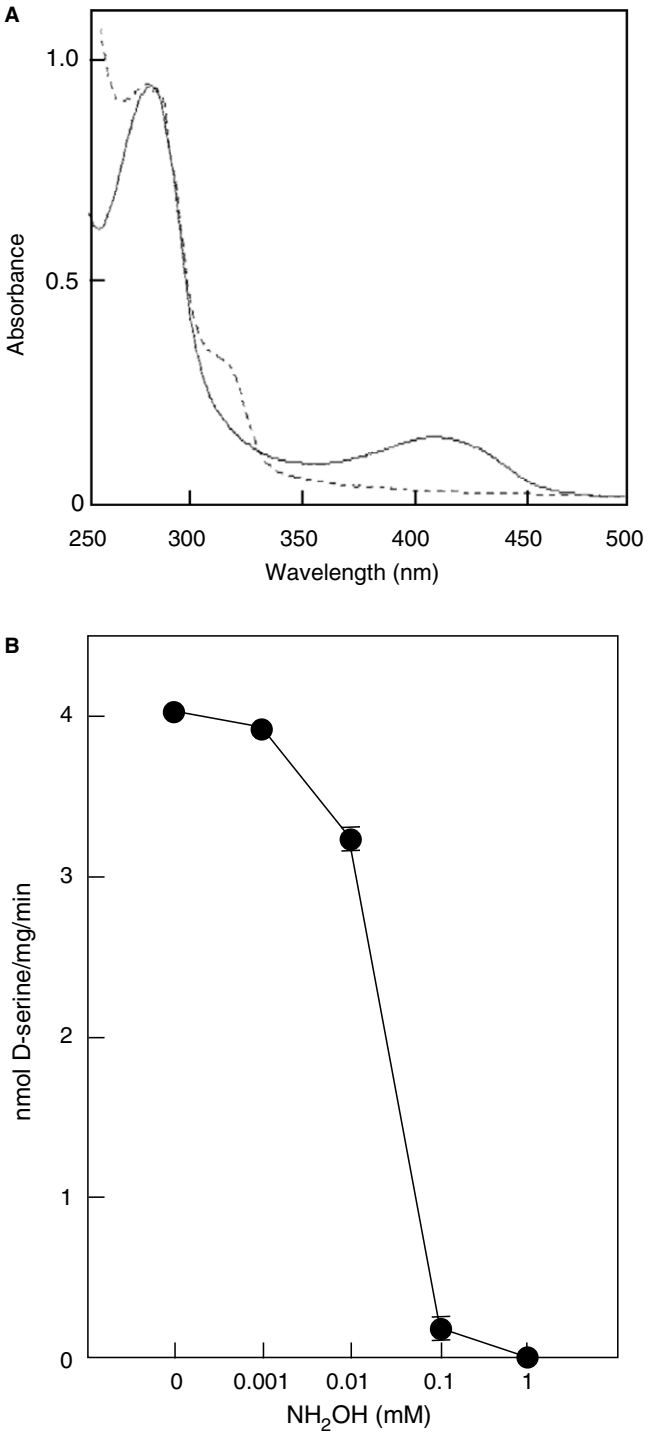

Fig. 3. Absorption spectra of AtSR (A) and inhibition of AtSR by hydroxylamine (B). A: The spectra of the purified AtSR (1 mg/ml) before and after dialyzing against 500 volumes of 20 mM Tris–HCl (pH 7.5) buffer containing 1 mM DTT, 10 μM PLP and 10 mM sodium borohydride are shown as a solid line and a dashed line, respectively. B: Racemase activity of the purified AtSR converting L- to D-serine was assayed under the presence of different concentrations of hydroxylamine. The results represent the average ± SE of three experiments.

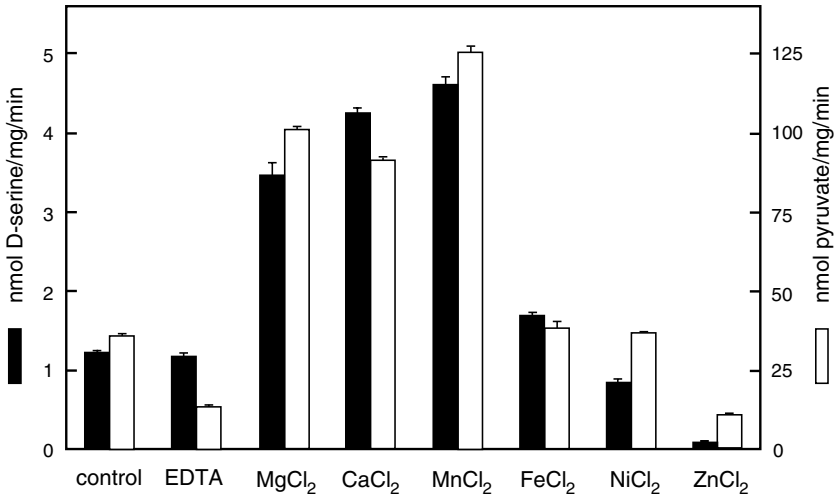

Fig. 4. Effect of divalent cations on racemase and dehydratase activities of AtSR. Racemase (black columns) and dehydratase (white columns) activities of the purified AtSR were assayed under the presence of 1 mM metal chloride or 1 mM EDTA. The results represent the average ± SE of three experiments.

110 nmol/min/mg, and those for D-serine are 370 and 130 nmol/min/mg (Wolosker et al., 1999a; Strisovsky et al., 2003). The K_m and V_{max} values for both L- and D-serine of AtSR are thus one to two orders lower than those of mammalian serine racemases, showing that AtSR has higher affinity for L- and D-serine and lower activity than mammalian serine racemases. When comparing the V_{max}/K_m values for L- and D-serine of AtSR to those of mammalian serine racemases, all enzymes indicate the same ratio of the V_{max}/K_m for L- to D-serine, 1:0.7, which is consistent with the theoretical value for the racemization reaction (Briggs and Haldane, 1925). On the dehydratase activity, AtSR has higher affinity and activity than mouse serine racemase, of which the K_m values for L- and D-serine are 75 and 75 mM and the V_{max} values for L- and D-serine are 77 and 4.3 nmol/min/mg (Strisovsky et al., 2003). The V_{max} values for L-serine of both enzymes are one order higher than those for D-serine, showing that AtSR and mouse serine racemase have the specificity of dehydration toward L-serine. The ratios of the V_{max}/K_m for L- to D-serine of AtSR and rat serine racemase, 1:0.4 and 1:0.06, suggest that AtSR has lower specificity to enantiomer than rat serine racemase on the dehydratase activity.

2.5. Localization of *A. thaliana* serine racemase

The expression level of the AtSR gene in the plant parts was analyzed by hybridization with the cDNA of AtSR as a probe (Fig. 5). Our blot detected a single 1.1-kb band in the shoots, roots, rosette, and inflorescence of *A. thaliana*. A number of D-amino acids have been identified in plants, however, plant biosynthetic mechanisms for D-amino acids have not been found. We have thus elucidated that *A. thaliana* has a serine racemase and that its gene is expressed in various organs, implying that plant cells require D-amino acids or D-amino acid-containing peptides as the physiologically functional component and have a D-amino acid metabolic pathway.

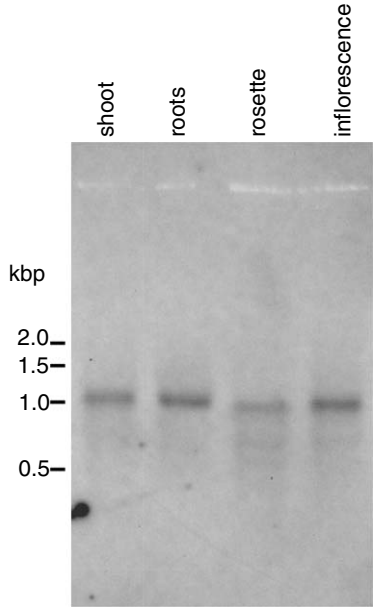

Fig. 5. Expression profile of AtSR gene in various organs of *A. thaliana*. Three microgram of each cDNA sample prepared from the shoots, roots, rosette, and inflorescence were electrophoresed on 1.2% agarose gel and transferred to a nylon membrane. The membrane was hybridized with a fragment of AtSR cDNA.

3. Experimental

3.1. Reagents

Ultrapure L-serine was purchased from Fluka and D-serine, L-alanine, L-arginine, and L-glutamine were from Sigma. Pyridoxal 5′-phosphate (PLP), flavin adenine dinucleotide (FAD), *o*-phenylenediamine (OPA), 2,4-dinitrophenylhydrazine (2,4-DNP), pyruvate, and hydroxylamine were obtained from Nacalai Tesque (Japan). D-Amino acid oxidase from porcine kidney, L-amino acid oxidase from *Crotalus adamanteus*, peroxidase from

horseradish, and catalase from bovine liver were obtained from Sigma. Other reagents were of analytical grade.

3.2. Plant material

Plants of *Arabidopsis thaliana*, ecotype Columbia, were grown in a growth chamber in a light/dark cycle of 16 h/8 h at 23 °C.

3.3. Cloning of A. thaliana serine racemase gene

Based on the putative open reading frame of the *A. thaliana* serine racemase gene (GenBank Accession Nos. AL161532 and AL049500), two primers were designed as follows: sense primer, 5′-CCCATATGGAAGCAAATAGAGAGAAGTA-3′, which creates a *Nde*I site (indicated by an *underline*), and antisense primer, 5′-CCCTCGAGTTTTGAACTCTTAAATGAAT-3′, which creates an *Xho*I site (indicated by an *underline*). The open reading frame was amplified by reverse-transcription PCR (RT-PCR) using the OneStep RT-PCR kit (Qiagen) with total RNA from 2-week-old shoots of *A. thaliana*. The PCR product of 1008 bp was cloned into a pGEM-T vector (Promega) and subjected to sequencing using an ABI PRISM 310 DNA sequencer (Applied Biosystems). The *Nde*I- and *Xho*I-digested fragment of the plasmid was cut out and subcloned into a *Nde*I- and *Xho*I-digested pET20b(+) vector (Novagen), in which the endogenous stop codon of the serine racemase gene was substituted by a polyhistidine tag gene. The resulting the plasmid, pAtSR, was transformed into *E. coli* BL21(DE3) pLysS cells.

3.4. Expression and purification of serine racemase

E. coli cells harboring pAtSR were grown at 25 °C in an LB medium containing 50 μg/ml of ampicillin. When OD_{600} became 0.5, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the culture at a final concentration of 0.5 mM. After cultivation at 25 °C for 6 h, cells were harvested by centrifugation and frozen at −80 °C at least for 2 h. The frozen cell pellet was suspended in a Bug-Buster™ HT protein extraction reagent (Novagen) according to the manufacturer's instruction and the soluble protein solution was purified using a Ni-NTA column (Qiagen) equilibrated with 20 mM Tris–HCl buffer (pH 7.9) containing 0.5 M NaCl and 5 mM imidazole. The column was washed with the same buffer followed by 60 mM imidazole in the same buffer, and the absorbed protein was eluted with 1 M imidazole in the same buffer. The enzyme solution was collected, dialyzed against 20 mM Tris–HCl buffer (pH 7.5) containing 1 mM DTT and 10 μM PLP, and concentrated by Ultracent-30 (Tohso, Japan). The purity and the molecular mass of the enzyme were estimated by SDS–PAGE and gel filtration with a Superdex 200 HR column (Amersham Biosciences) equilibrated with 50 mM sodium phosphate buffer (pH 7.0) containing 0.15 M NaCl and 10 μM PLP. The protein concentration was quantified according to the method of Bradford (1976) with bovine serum albumin as the standard.

3.5. Enzyme assays

Serine racemase activity was assayed according to the method described by Cook et al. (2002) with some modification. The activity in the direction from L- to D-serine was determined with a reaction mixture (500 μl) containing 20 mM Tris–HCl (pH 8.5), 10 μM PLP, 10 mM L-serine, 1 mM $CaCl_2$, 1 nM FAD, 50 μg/ml OPA, 3 U/ml D-amino acid oxidase, 3 U/ml peroxide, and enzyme. The reaction was incubated at 37 °C for 1 h and absorbance at 411 nm was measured to calculate the amount of D-serine. D-Serine and L-amino acid oxidase instead of L-serine and D-serine oxidase were used to determine the activity in the direction from D- to L-serine. The calibration curve was made with 0 to 100 μM L- and D-serine showing a linear range.

Serine dehydratase activity was assayed according to the method described by Uo et al. (2002). The reaction mixture (500 μl) consisted of 20 mM Tris–HCl (pH 8.5), 10 μM PLP, 10 mM L- or D-serine, 1 mM $CaCl_2$, and the enzyme was incubated at 37 °C for 30 min and stopped by addition of 2 M HCl (0.5 ml) containing 0.03% (w/v) 2,4-DNP. After incubation at 4 °C for 5 min, 2 M NaOH (1 ml) was added and absorbance at 520 nm was measured to calculate the amount of pyruvate. The calibration curve was made with 0–500 μM pyruvate showing a linear range.

L-Serine solution used in the enzyme assay was pre-treated with D-amino acid oxidase and catalase in order to remove contaminating D-serine according to the method described by De Miranda et al. (2002). L-Amino acid oxidase was used to remove contaminating L-serine in D-serine solution.

3.6. cDNA preparation and hybridization

cDNA was synthesized and amplified using the SMART PCR cDNA Synthesis Kit (Clontech). Total RNA isolated from shoots (2-week-old), roots, rosette, and inflorescence (6–8-week-old) was reverse-transcribed at 42 °C for 1 h with the cDNA synthesis primer and the SMART II oligonucleotide. The reaction mixture was amplified with the polymerase chain reaction primer supplied in the kit according to the manufacturer's instruction. Three microgram of cDNA was electrophoresed on 1.2% agarose gel and transferred onto Hybond-N^+ nylon membrane (Amersham Biosciences). The membrane was hybridized with a fragment of the AtSR cDNA labeled with digoxigenin-11-dUTP using a PCR DIG Labeling Kit (Boehringer Mannheim) with primers 5′-TCAGGTATGGTGGTAAGGTTATATGGA-3′ and 5′-TCCACTTGGTTCCACAGAGACCTTCA G-3′. Hybridization was performed at 42 °C for 16 h in 50% formamide, 5× sodium saline citrate (SSC), 0.25% non-fat milk powder, 0.1% sodium dodecyl sulfate (SDS), 5× Denhardt's reagent. The membrane was

washed twice with 1× SSC and 0.1% SDS at room temperature, and twice with 0.1× SSC and 0.1% SDS at 68 °C.

Acknowledgment

This research was supported in part by the Ohara Foundation in Kurashiki.

References

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Briggs, G.E., Haldane, J.B.S., 1925. Note on the kinetics of enzyme action. Biochem. J. 19, 338–339.

Bruckner, H., Weshauser, T., 2003. Chromatographic determination of L- and D-amino acids in plants. Amino Acids 24, 43–55.

Cook, S.P., Galve-Roperh, I., Martinez, d.P., Rodriguez-Crespo, I., 2002. Direct calcium binding results in activation of brain serine racemase. J. Biol. Chem. 277, 27782–27792.

De Miranda, J., Santoro, A., Engelender, S., Wolosker, H., 2000. Human serine racemase: molecular cloning, genomic organization and functional analysis. Gene 256, 183–188.

De Miranda, J., Panizzutti, R., Foltyn, V.N., Wolosker, H., 2002. Cofactors of serine racemase that physiologically stimulate the synthesis of the *N*-methyl-D-aspartate (NMDA) receptor coagonist D-serine. Proc. Natl. Acad. Sci. USA 99, 14542–14547.

Dunlop, D.S., Neidle, A., McHale, D., Dunlop, D.M., Lajtha, A., 1986. The presence of free D-aspartic acid in rodents and man. Biochem. Biophys. Res. Commun. 141, 27–32.

Erbe, T., Bruckner, H., 2000. Chromatographic determination of amino acid enantiomers in beers and raw materials used for their manufacture. J. Chromatogr. A 881, 81–91.

Esaki, N., Walsh, C.T., 1986. Biosynthetic alanine racemase of *Salmonella typhimurium*: purification and characterization of the enzyme encoded by the *alr* gene. Biochemistry 25, 3261–3267.

Esaki, N., Kurihara, T., Soda, K., 2002. Isomerizations, second ed. In: Drauz, K., Waldmann, H. (Eds.), Enzyme Catalysis in Organic Synthesis, vol. 3 Wiley-VCH, Weinheim, pp. 1281–1333.

Foltyn, V.N., Bendikov, I., De Miranda, J., Panizzutti, R., Dumin, E., Shleper, M., Lu, P., Toney, M.D., Kartvelishvily, E., Wolosker, H., 2005. Serine racemase modulates intracellular D-serine levels through an α,β-elimination activity. J. Biol. Chem. 280, 1754–1763.

Gallagher, D.T., Gilliland, G.L., Xiao, G., Zondlo, J., Fisher, K.E., Chinchilla, D., Eisenstein, E., 1998. Structure and control of pyridoxal phosphate dependent allosteric threonine deaminase. Structure 6, 465–475.

Grishin, N.V., Phillips, M.A., Goldsmith, E.J., 1995. Modeling of the spatial structure of eukaryotic ornithine decarboxylases. Protein Sci. 4, 1291–1304.

Hashimoto, A., Nishikawa, T., Hayashi, T., Fujii, N., Oka, T., Takahashi, K., 1992. The presence of free D-serine in rat brain. FEBS Lett. 296, 33–36.

Hashimoto, A., Kumashiro, S., Nishikawa, T., Oka, T., Takahashi, K., Mito, T., Takashima, S., Doi, N., Mizutani, Y., Yamazaki, T., Kaneko, T., Ootomo, E., 1993. Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. J. Neurochem. 61, 783–786.

Hashimoto, A., Oka, T., Nishikawa, T., 1995. Anatomical distribution and postnatal changes in endogenous free D-aspartate and D-serine in rat brain and periphery. Eur. J. Neurosci. 7, 1657–1663.

Kullman, J.P., Chen, X., Armstrong, D.W., 1999. Evaluation of the enantiomeric composition of amino acids in tobacco. Chirality 11, 669–673.

Manabe, H., 1985. Occurrence of D-alanyl-D-alanine in *Oryza australiensis*. Agric. Biol. Chem. 49, 1203–1204.

Manabe, H., Ohira, K., 1983. Effect of light irradiation on the D-alanylglycine content in rice leaf blades. Plant Cell Pysiol. 24, 1137–1142.

Nagata, Y., Konno, R., Yasumura, Y., Akino, T., 1989. Involvement of D-amino acid oxidase in elimination of free D-amino acids in mice. Biochem. J. 257, 291–292.

Nagata, Y., Horiike, K., Maeda, T., 1994. Distribution of free D-serine in vertebrate brains. Brain Res. 634, 291–295.

Ogawa, T., Bando, N., Sasaoka, K., 1977. Occurrence of D-α-amino-*n*-butyric acid in legume seedlings. Agric. Biol. Chem. 41, 1811–1812.

Robinson, T., 1976. D-Amino acids in higher plants. Life Sci. 19, 1097–1102.

Soda, K., Osumi, T., 1969. Crystalline amino acid racemase with low substrate specificity. Biochem. Biophys. Res. Commun. 35, 363–368.

Strisovsky, K., Jiraskova, J., Barinka, C., Majer, P., Rojas, C., Slusher, B.S., Konvalinka, J., 2003. Mouse brain serine racemase catalyzes specific elimination of L-serine to pyruvate. FEBS Lett. 535, 44–48.

Tompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Uo, T., Yoshimura, T., Shimizu, S., Esaki, N., 1998. Occurrence of pyridoxal 5′-phosphate-dependent serine racemase in silkworm, *Bombyx mori*. Biochem. Biophys. Res. Commun. 246, 31–34.

Uo, T., Yoshimura, T., Tanaka, N., Takegawa, K., Esaki, N., 2001. Functional characterization of alanine racemase from *Schizosaccharomyces pombe*: a eucaryotic counterpart to bacterial alanine racemase. J. Bacteriol. 183, 2226–2233.

Uo, T., Yoshimura, T., Nishiyama, T., Esaki, N., 2002. Gene cloning, purification, and characterization of 2,3-diaminopropionate ammonia-lyase from *Escherichia coli*. Biosci. Biotechnol. Biochem. 66, 2639–2644.

Wolosker, H., Sheth, K.N., Takahashi, M., Mothet, J.P., Brady Jr., R.O., Ferris, C.D., Snyder, S.H., 1999a. Purification of serine racemase: biosynthesis of the neuromodulator D-serine. Proc. Natl. Acad. Sci. USA 96, 721–725.

Wolosker, H., Blackshaw, S., Snyder, S.H., 1999b. Serine racemase, a glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission. Proc. Natl. Acad. Sci. USA 96, 13409–13414.