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Short communication

Glutathione S-Transferase Activities of S-Type and L-Type Thioltransferases from *Arabidopsis thaliana*

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The glutathione S-transferase (GST) activities of S-type and L-type thioltransferases (TTases), which are purified from the seeds and leaves of *Arabidopsis thaliana*, respectively, were identified and compared. The S-type and L-type TTases showed K_m values of 9.72 mM and 3.18 mM on 1-chloro-2,4-dinitrobenzene (CDNB), respectively, indicating the L-type TTase has higher affinity for CDNB. The GST activity of the L-type TTase was rapidly inactivated after being heated at 70°C or higher. The GST activity of the S-type TTase remains active in a range of 30-90°C. Hg^{2+} inhibited the GST activity of the S-type TTase, whereas Ca^{2+} and Cd^{2+} inhibited the GST activity of the L-type TTase. Our results suggest that the GST activities of two TTases of *Arabidopsis thaliana* may have different catalytic mechanisms. The importance of the co-existence of TTase and GST activities in one protein remains to be elucidated.

Keywords: *Arabidopsis thaliana*, Glutaredoxin, Glutathione S-transferase, Thioltransferase.

Introduction

Thioltransferase (TTase), also known as glutaredoxin (Grx), is generally a heat-stable protein that acts as a multifunctional glutathione-dependent disulfide oxidoreductase. TTase reductively cleaves a variety of disulfides, including protein disulfides and low-molecular-mass disulfides, in the presence of reduced glutathione (GSH). TTase (Grx), together with GSH and glutathione reductase, was shown to couple NADPH to the reduction of ribonucleotide (Holmgren, 1979), sulfate (Tsang, 1981), and methionine sulfoxide (Fuchs, 1977). TTase has also been identified with the ability to reduce non-disulfide substrates such as dehydroascrobate (Wells *et al.*, 1990) and alloxan (Washburn and Wells, 1997). TTase was detected within HIV-1, and shown to implicate in the regulation and/or maintenance of protease activity in HIV-1 infected cells (Davis *et al.*, 1997). Recently, the OxyR transcription factor, which is activated through the formation of a disulfide bond, was found to be deactivated by TTase (Zheng *et al.*, 1998).

TTases from mammalian, plant, and microbial cells are small proteins with the active site sequence of Cys-Pro-Phe(Tyr)-Cys-. TTases were isolated and characterized in a number of prokaryotic and animal species. Since plant TTase was identified from spinach (Morrel *et al.*, 1995), it was also purified and characterized from various other plant organisms, such as rice (Sha *et al.*, 1997), kale (Sa *et al.*, 1998a), Chinese cabbage (Cho *et al.*, 1998a; Cho *et al.*, 1999), and *Arabidopsis thaliana* Cho *et al.*, 1998b; Kim *et al.*, 1999a). Molecular sizes of plant TTases, especially isolated from kale, Chinese cabbage (a second form) and *Arabidopsis thaliana*, were found to be relatively large. The S-type TTase of *Arabidopsis thaliana* seed is a heat-stable protein with a molecular mass of 22 kD (Cho *et al.*, 1998b), whereas the L-type TTase of leaves has a molecular mass of 26.6 kD (Kim *et al.*, 1999a). *Arabidopsis thaliana* is the first plant in which the different forms of TTases are identified in the different plant organs.

The enzyme glutathione S-transferase (GST, EC 2.5.1.18) is a cytosolic enzyme found in mammals, insects, plants, and microbes that catalyzes the conjugation of glutathione with a large variety of hydrophobic and electrophilic compounds. This conjugation results in detoxification of these compounds and furthermore, facilitates their removal from biological tissue. In addition to catalytic functions, the GSTs can also bind covalently/noncovalently to a wide number of hydrophobic compounds, such as haem, drugs and carcinogens (Di Ilio *et al.*, 1991). GSTs also exhibit GSH peroxidase activity (Marrs, 1996). Multiple forms of GST were identified in a variety of plant species, such as chickpea, maize (*Zea mays*), wheat, and sorghum (Dean *et al.*, 1990; Hunaiti and Bassam, 1991; Irzyk and Fuerst, 1993; Riechers *et al.*, 1997). A diversity of xenobiotic chemicals and stresses

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induce GST in plants (Dean *et al.*, 1990). An auxin-inducible GST gene was isolated from *Arabidopsis thaliana* (van der Kop *et al.*, 1996).

In this communication, GST activities of the S-type and L-type TTases of *Arabidopsis thaliana* were identified, characterized, and compared.

Materials and Methods

Chemicals Bovine serum albumin (BSA), glutathione reductase (yeast), NADPH, 1-chloro-2,4-dinitrobenzene (CDNB), caffeic acid, and iodoacetamide were purchased from Sigma Chemical Co. (St. Louis, USA). 2-Hydroxyethyl disulfide (HED) was purchased from Aldrich Chemical Co. (Milwaukee, USA). All other chemicals and reagents were of highest grade commercially available.

Thioltransferase assay Since thioltransferase contained transhydrogenase activity, its activity was measured spectrophotometrically at 340 nm by the use of glutathione reductase (GR) as a coupling enzyme (Sa *et al.*, 1998b; Park *et al.*, 1999). In a total volume of 500 μl, two cuvettes, each containing 100 μg/ml of bovine serum albumin, 1 mM GSH, 1.5 mM HED, 6 μg/ml yeast glutathione reductase, 0.4 mM NADPH, and 0.1 M Tris-HCl, pH 8.0-2 mM EDTA, were monitored for several minutes to ensure that both cuvettes were balanced with respect to the nonenzymatic spontaneous reaction between GSH and HED. Enzyme was added to the sample cuvette and water to the control cuvette. The change in the absorbance was then monitored and the activity was expressed as μmol/min.

Glutathione S-transferase assay Glutathione S-transferase (GST) activity was determined spectrophotometrically, as described by Habig *et al.* (1974), with modifications. The reaction medium contained 100 mM potassium phosphate buffer (pH 6.5), 5.0 mM GSH, 2.0 mM CDNB, and enzyme source in a volume of 1.0 mL. The reaction, conducted at 25°C, was initiated by the addition of CDNB, and the change in the absorbance at 340 nm was monitored with a spectrophotometer. All initial rates were corrected for the background nonenzymatic reaction.

Results and Discussion

S-type and L-type TTases were purified from seeds and leaves of *Arabidopsis thaliana*, respectively (Cho *et al.*, 1998b; Kim *et al.*, 1999a). The two purified TTases were found to have different molecular masses and their migration was clearly different on native gel.

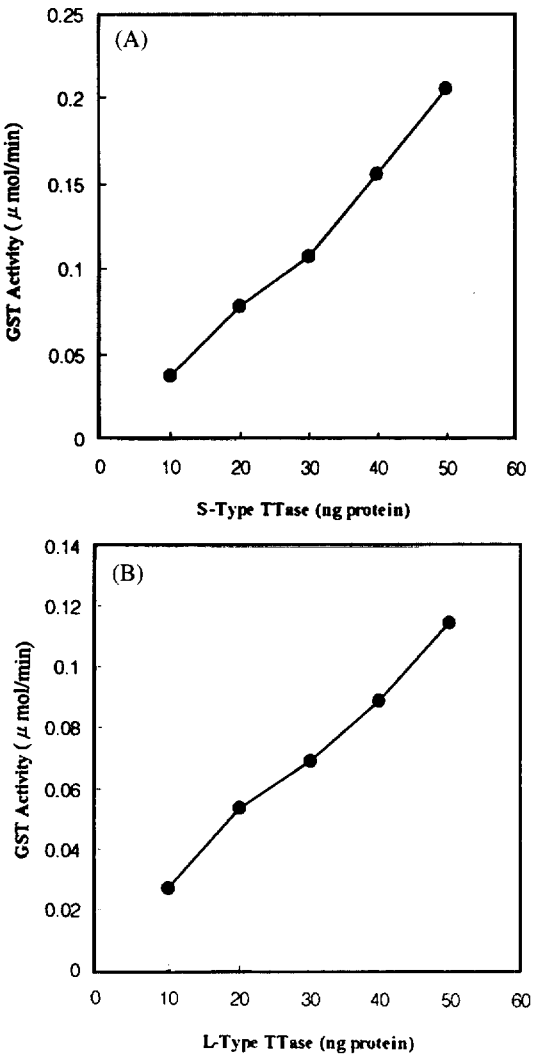

Fig. 1. Glutathione S-transferase (GST) activities of S-type (A) and L-type (B) thioltransferases purified from *Arabidopsis thaliana*. Various amounts of thioltransferases were used in the standard assay of GST activity described in 'Materials and Methods'.

Glutathione S-transferase activity The S-type and L-type TTases were found to contain significant GST activities, when CDNB was used as a substrate (Fig. 1). They showed the

Table 1. Kinetic parameters of gluathione S-tranferase activities of S-type and L-type thioltransferases purified from *Arabidopsis thaliana*

TTase	GST Activity[a]		TTase Activity[b]	
	Km (mM)	Vmax (μmoles/min)	Km (mM)	Vmax (pmoles/min)
S-type	9.72±0.32	1.08±0.15	3.70±0.28	5.14±0.20
L-type	3.18±0.21	0.92±0.18	22.20±0.34	3.22±0.13

[a]Glutathione S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate.
[b]Thioltransferase (TTase) activity was measured using 2-hydroxyethyl disulfide (HED) as a substrate.

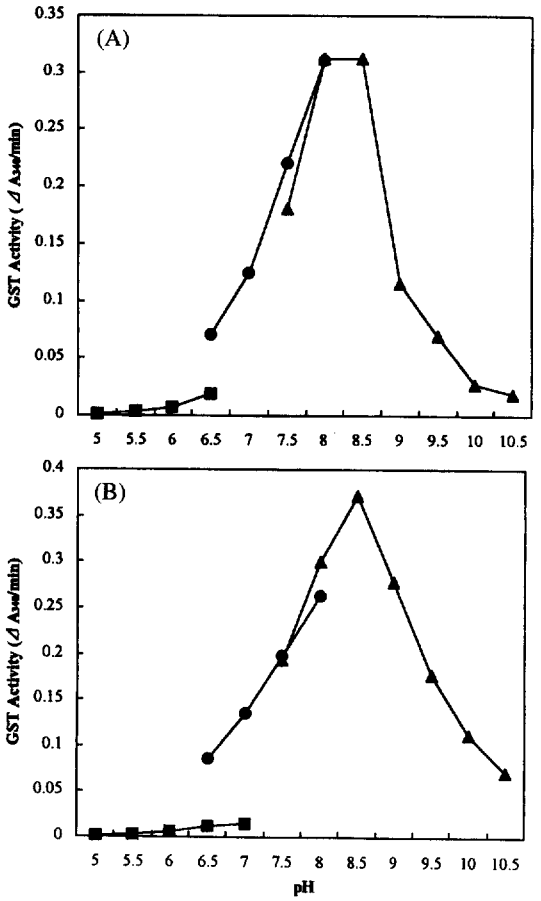

Fig. 2. Effect of pH on glutathione S-transferase (GST) activities of S-type (A) and L-type (B) thioltransferases purified from *Arabidopsis thaliana*. The initial velocities of GST reaction were monitored in the standard assay with a buffer system of 0.1 M sodium acetate (pH 5.0-7.0, -■-), 0.1 M sodium phosphate (pH 6.5-8.0, -●-), or 0.1 M Tris-HCl (pH 7.5-10.5, -▲-).

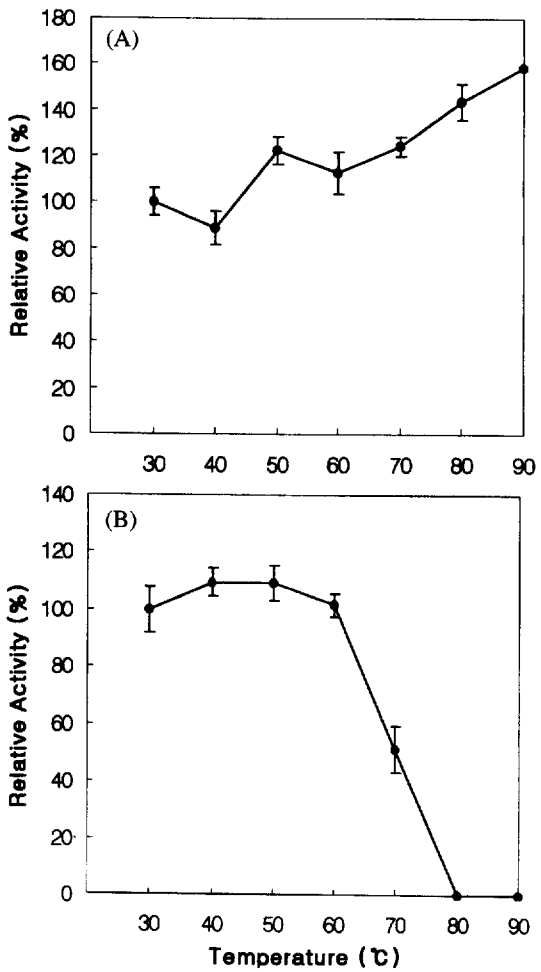

Fig. 3. Heat stability of glutathione S-transferase (GST) activities of S-type (A) and L-type (B) thioltransferases purified from *Arabidopsis thaliana*. The TTases were incubated in a water bath for 30 minutes with a temperature range from 30-90°C. After rapid cooling in an icebox, the heat-treated samples were added to an assay mixture to measure GST activity.

linear relationships between GST activities and the amount of TTase in a range of 10-50 ng. The S-type TTase gave a reaction rate of 3.71 μmol/min/mg protein, whereas the L-type TTase had a reaction rate of 2.71 μmol/min/mg protein. It indicates that the S-type has a higher catalytic activity than the L-type TTase. These results confirm that the S-type and L-type TTases have apparent GST activities. However, the physiological meaning remains elusive. Previously, it was found that a second TTase of *Schizosaccharomyces pombe* also contained GST activity (Kim *et al.*, 1999b).

Kinetic properties The S-type TTase showed a Km value of 9.72 mM on CDNB (Table 1). The Km value of the L-type TTase appeared to be 3.18 mM (Table 1). It indicates that the L-type TTase contains the higher affinity for CDNB. The S-type TTase has a higher affinity on HED than on CDNB, whereas the L-type TTase has higher affinity on CDNB than on HED (Table 1). The Vmax values for the GST activities of S-type and L-type TTases were found to be similar. The results indicate that the GST activities of the two *A. thaliana* TTases have similar kinetic properties.

pH optima The GST activities of the *A. thaliana* TTases, as a function of pH, were determined by using the standard assay mixture. Fig. 2 shows that the GST activities of the S-type and L-type TTases contain the optimal pH of 8-8.5 and 8.5, respectively. They also have similar pH optimum values for TTase activity using HED as a substrate. The results suggest that the GST activities of the two *A. thaliana* TTases are maximal at basic pH.

Heat stability The purified enzymes were incubated in a water bath with a temperature range from 30-90°C for 30 minutes. After rapid cooling in an ice bath, the heat-treated enzyme was added to a standard assay mixture with CDNB as a substrate. Fig. 3 shows that the GST activity of the L-type

Table 2. Relative effects of metal ions on glutathione S-transferase activities of S-type and L-type thioltransfeases purified from *Arabidopsis thaliana.*

Metal ions	GST activities[a]	
	S-type	L-type
None	100.0	100.0
$AlCl_3$	95.9	90.3
$CaCl_2$	96.4	76.2
$CdCl_2$	97.5	67.6
$HgCl_2$	76.7	97.7
$MgCl_2$	97.2	97.1
$MnCl_2$	100.0	87.1
$ZnCl_2$	97.3	100.0

[a]The purified S-type and L-type thioltransferases were incubated with 0.5 mM metal ions for 20 minutes at 30°C, and then, the GST activity was assayed under the standard conditions. The GST activities were represented as relative values.

TTase was rapidly inactivated after being heated at 70°C or higher. However, the GST activity of the S-type TTase remains active in a range of 30-90°C. The TTase activity of the S-type TTase was partially inactivated after being heated at 80°C or higher (Cho *et al.*, 1998b). Therefore, at 80°C or higher, the TTase activity of the S-type TTase becomes inactive, but its GST activity remains active. This fact may suggest that the TTase and GST activities of the S-type TTase carry different active centers.

Effects of metal ions Effects of metal ions on the GST activities of the S-type and L-type TTases were examined in the presence of 0.5 mM metal ions (Table 2). Hg^{2+} was found to inhibit the GST activity of the S-type TTase, whereas Ca^{2+} and Cd^{2+} appeared to inhibit the GST activity of the L-type TTase. The results indicate that the GST activities of the two TTases contain different molecular mechanisms.

Effects of iodoacetamide and caffeic acid Effects of iodoacetamide and caffeic acid on the GST activities of the two TTases were examined. Iodoacetamide (1 mM) did not affect the GST activities of the two TTases. Caffeic acid (0.2 mM) reduced the GST activities of S-type and L-type TTases to 79.4% and 87.8% of the control values, respectively. These indicate that the GST activities of the two TTases are slightly inhibited by caffeic acid. Previously, it was reported that rat liver GST was inhibited by caffeic acid (Das *et al.*, 1984).

Here, we described the GST activities, which are carried out by the S-type and L-type TTases of *Arabidopsis thaliana.* Their GST activities show slight differences in kinetic properties, heat stability, and metal ion effect. Physiological meaning of GST activity, contained in the TTases, remains unknown. It is also unknown which of TTase and GST activity of the proteins is major. There have been at least 11 thioltransferase homologue sequences of *Arabidopsis thaliana* stored in the GenBank data base. However, until recently, no thioltransferase gene was cloned from the genome of *Arabidopsis thaliana.* Further investigation is needed to identify the physiological roles of TTase and GST activities of *A. thaliana* TTases.

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