Methylglyoxal (MG) is one of the aldehydes that accumulate in plants under environmental stress. Glutathione S-transferases (GSTs) play important roles, including detoxification, in the stress tolerance systems of plants. To determine the effects of MG, we characterized recombinant GST. MG decreased GST activity and thiol contents with increasing $K_m$. GST can serve as a target of MG modification, which is suppressed by application of reduced glutathione.

**Key words:** methylglyoxal; glutathione S-transferase; glutathione; thiol; 1-chloro-2,4-dinitrobenzene

Methylglyoxal (MG) is a highly cytotoxic $\alpha$-oxoaldehyde formed mainly through glycolysis at the triose-phosphate step. Levels of MG increase upon the exposure of plants to abiotic and heavy metal stresses. MG accumulation results in a number of adverse effects, such as increases in the degradation of proteins by modification of arginine, lysine, and cysteine residues, adduction with guanyl nucleotide in DNA, and inactivation of antioxidant defense systems. In plants, the MG is detoxified mainly by the maintenance of glutathione homeostasis via glyoxalase system. Reduced glutathione (GSH) is the most abundant low molecular weight thiol in plants. It protects plants against a range of peroxides, xenobiotics and heavy metals. MG decreases total thiol and GSH levels, whereas exogenous GSH decreases MG levels.

Glutathione S-transferases (GSTs) are ubiquitous proteins that catalyze the conjugation of toxic xenobiotics and electrophilic and hydrophobic substrates with GSH. Plant GSTs are induced by a wide variety of biotic and abiotic stresses. Some GST isozymes have glutathione peroxidases that play major roles in oxidative stress tolerance, reducing organic hydroperoxides. Overexpression of the tobacco $parB$ gene encoding GST confers resistance to various oxidative stresses, but the interaction between GST and MG remains to be clarified.

GSTs have been purified and characterized from a wide variety of plant species. In this study, we prepared and characterized recombinant *Nicotiana tabacum* GST (rNtGST, phi class GST), and investigated the effects of MG on rNtGST as well as the protective effect of GSH in the inhibition of rNtGST by MG.

Tobacco (*N. tabacum* L. cv. Bright Yellow-2) cells were subcultured weekly into a standard medium (modified LS medium), and were maintained as described previously. Total RNA isolated from 4-d-old cells was reverse-transcribed into first-strand cDNA using oligo (dT) primer and Moloney murine leukemia virus reverse transcriptase (Takara, Ohtsu, Japan). A 642-bp fragment of *NtGST* cDNA was amplified by polymerase chain reaction (PCR) using forward primer 5'-CATATGGCGATCAAAGTCCATGGTAGGCC-3' (NdeI restriction site underlined) and reverse primer 5'-CTCGAGATTATTTTGAGCTTCTCCATCC-3' (XhoI restriction site underlined). The PCR cycling conditions were 30 cycles of 10 s at 98 °C, 15 s at 55 °C, and 60 s at 68 °C. The PCR products were cloned into pGEM-T easy vector (Promega, Madison, WI). The resulting plasmid was transformed into *Escherichia coli* DH5$\alpha$ cells and verified by DNA sequencing. The plasmid was digested with restriction enzymes Ndel and XhoI, and ligated into similarly digested expression plasmid pET-24b(+). The pET-NtGST construct was transformed into *E. coli* Rosetta (DE3) pLysS cells. The cells were cultured in Luria-Bertani medium supplemented with 50 μg/ml of kanamycin and 34 μg/ml of chloramphenicol by Overnight Express™ Autoinduction System 1 (Novagen, Darmstadt, Germany) at 30 °C at 200 rpm in the dark for 20 h. The cells were harvested by centrifugation, re-suspended in phosphate-buffered saline, and disrupted by sonication. The supernatant was collected by centrifugation and the cell pellet was dissolved in phosphate-buffered saline. Proteins were separated on 10% SDS–PAGE gels and visualized by Coomassie Brilliant Blue R-250 staining. The supernatant without purification was used for the determination of the biochemical and kinetic properties of the rNtGST. GST activity and the thiol and disulfide contents were assayed as described previously. The reaction mixture of GST contained 100 mM Na-phosphate buffer (pH 6.5), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 1 mM GSH, and the supernatant. The activity was calculated from the increase in absorbance at
340 nm for 1 min using an extinction coefficient of 9.6 mM$^{-1}$ cm$^{-1}$. The apparent $K_m$ value for CDNB was determined using CDNB concentrations ranging from 0.25 to 1.5 mM and a fixed GSH concentration of 1 mM. The apparent $K_m$ value for GSH was determined using GSH concentrations ranging from 0.25 to 1.5 mM and a fixed CDNB concentration of 1 mM. The $K_m$ values were estimated from a Lineweaver-Burk plot. The protein content was measured using BSA as standard.12)

The auxin-regulated parB gene encoding GST (GenBank accession no. P30109)13) was amplified from the cDNA of N. tabacum. This NtGST cDNA encodes a peptide of 213 amino acids with a calculated molecular mass of 24.0 kDa. An approximately 20-kDa band was detected in the rNtGST protein by SDS–PAGE (Fig. 1A). No band of 20 kDa was visible in the protein extracted from pET-24b(+) (Fig. 1A). GSTs protect plants against a wide variety of stresses by detoxifying xenobiotics and reactive electrophilic compounds.6,7)

The rNtGST exhibited a higher GST activity (0.626 µmol/min/mg of protein), while GST activity was negligible in the protein extracted from pET-24b(+) (data not shown). As compared to the tobacco-cultured cells,11) rNtGST showed approximately 7-fold higher GST activity. Higher GST activity in the overexpression of parB transgenic plants provides tolerance by detoxifying toxic radicals generated in response to various oxidative stresses.8)

MG has recently been found in higher plants.2–4) The MG level has been found to increase from 40 to 370 µM in plants under various stresses.3,4) The cytotoxic effect of MG on animals is well established, but its effect on plants is yet to be understood. We examined the effect of MG on the rNtGST protein. Significant inhibition of GST activity was observed when rNtGST protein was treated with 1 and 10 mM MG (Fig. 1B). Besides detoxification of ROS,5) GSH is required as a cofactor in several metabolic processes, including MG detoxification. A protective effect of exogenous GSH against MG toxicity has been reported in plants.1,3) The application of GSH significantly ameliorated the inhibition of GST due to 10 mM MG (Fig. 1B), but it failed to recover to the original level.

The $K_m$ values for GSH and CDNB were 0.98 ± 0.04 mM and 1.47 ± 0.08 mM respectively under the control condition (Table 1). A relatively low $K_m$ value (0.83 mM) for GSH was observed for PtGSTU1.14) Different plant GSTs have different affinities for CDNB.14) The
low $K_m$ value (0.47 mM) of PtGSTU1 for CDNB suggests important roles in detoxification and buffering against environmental stresses.\textsuperscript{14} The MG treatments caused increases in the $K_m$ values for both CDNB and GSH (Table 1), indicating a lower affinity of NtGST for its substrate, CDNB or GSH. The increases in the $K_m$ values of the rNtGST protein due to MG treatment were probably due to modification of certain amino acid residues, including cysteine.

Thiol, a highly reactive constituent of protein molecules, participates in the detoxification of xenobiotics and toxic substances. MG has been found to decrease the total thiol and GSH contents in plants.\textsuperscript{1,3} MG decreased the thiol contents and increased the disulfide contents in rNtGST (Fig. 1C). Modification of cysteine residues might be one of the reasons for the decrease in thiol, because MG can react with cysteine. Collectively, the present results suggest that MG might cause toxic effects in cells by depleting the thiol and GSH contents and altering the redox homeostasis.

In conclusion, GST can serve as a target of the MG modification, which is suppressed by application of GSH. Exogenous GSH might confer resistance to MG and other environmental factors via inhibition of protein modification and the maintenance of redox homeostasis.

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### References


#### Table 1. Effects of MG on $K_m$ Values for rNtGST Using GSH and CDNB

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH $K_m$ (mM)</th>
<th>CDNB $K_m$ (mM)</th>
</tr>
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<tbody>
<tr>
<td>0 mM MG</td>
<td>0.98 ± 0.04</td>
<td>1.47 ± 0.08</td>
</tr>
<tr>
<td>0.5 mM MG</td>
<td>1.00 ± 0.07</td>
<td>2.32 ± 0.09*</td>
</tr>
<tr>
<td>1 mM MG</td>
<td>1.17 ± 0.22</td>
<td>2.86 ± 0.29*</td>
</tr>
<tr>
<td>10 mM MG</td>
<td>1.85 ± 0.47*</td>
<td>2.10 ± 0.23*</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD for three independent experiments. The supernatant was incubated with 0 to 10 mM MG for 1 h at 20 °C, and then GST activity was measured. Control represents no incubation. * indicates significant difference from 0 mM MG ($p < 0.05$) by Student’s t-test.