Atrazine Resistance in a Velvetleaf (Abutilon theophrasti) Biotype Due to Enhanced Glutathione S-Transferase Activity

Michael P. Anderson and John W. Gronwald

Plant Science Research Unit, U.S. Department of Agriculture, Agricultural Research Service, and Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

We previously reported that a velvetleaf (Abutilon theophrasti Medic) biotype found in Maryland was resistant to atrazine because of an enhanced capacity to detoxify the herbicide via glutathione conjugation (JW Gronwald, Andersen RN, Yee C [1989] Pestic Biochem Physiol 34: 149–163). The biochemical basis for the enhanced atrazine conjugation capacity in this biotype was examined. Glutathione- and glutathione S-transferase activity were determined in extracts from the atrazine-resistant biotype and an atrazine-susceptible or "wild-type" velvetleaf biotype. In both biotypes, the highest concentration of glutathione (approximately 500 nanomoles per gram fresh weight) was found in leaf tissue. However, no significant differences were found in glutathione levels in roots, stems, or leaves of either biotype. In both biotypes, the highest concentration of glutathione S-transferase activity measured with 1-chloro-2,4-dinitrobenzene or atrazine as substrate was in leaf tissue. Glutathione S-transferase activity measured with 1-chloro-2,4-dinitrobenzene as substrate was 40 and 25% greater in leaf and stem tissue, respectively, of the susceptible biotype compared to the resistant biotype. In contrast, glutathione S-transferase activity measured with atrazine as substrate was 4.4- and 3.6-fold greater in leaf and stem tissue, respectively, of the resistant biotype. Kinetic analyses of glutathione S-transferase activity in leaf extracts from the resistant and susceptible biotypes were performed with the substrates glutathione, 1-chloro-2,4-dinitrobenzene, and atrazine. There was little or no change in apparent $K_m$ values for glutathione, atrazine, or 1-chloro-2,4-dinitrobenzene. However, the $V_{max}$ for glutathione and atrazine were approximately 3-fold higher in the resistant biotype than in the susceptible biotype. In contrast, the $V_{max}$ for 1-chloro-2,4-dinitrobenzene was 30% lower in the resistant biotype. Leaf glutathione S-transferase isozymes that exhibit activity with atrazine and 1-chloro-2,4-dinitrobenzene were separated by fast protein liquid (anion-exchange) chromatography. The susceptible biotype had three peaks exhibiting activity with atrazine and the resistant biotype had two. The two peaks of glutathione S-transferase activity with atrazine from the resistant biotype co-eluted with two of the peaks from the susceptible biotype, but peak height was three- to fourfold greater in the resistant biotype. In both biotypes, two of the peaks that exhibit glutathione S-transferase activity with atrazine also exhibited activity with 1-chloro-2,4-dinitrobenzene, with the peak height being greater in the susceptible biotype. The results indicate that atrazine resistance in the velvetleaf biotype from Maryland is due to enhanced glutathione S-transferase activity for atrazine in leaf and stem tissue which results in an enhanced capacity to detoxify the herbicide via glutathione conjugation.

Approximately 7 years ago, an atrazine-resistant velvetleaf biotype was discovered in a field in Maryland (24). Triazine herbicides, primarily atrazine, had been applied to the field for at least a decade and the field had been in no-till, continuous corn production for 5 years prior to the discovery of resistance. We (15) previously showed that the biotype from Maryland had a 10-fold greater tolerance to atrazine than an atrazine-susceptible or "wild-type" biotype from Minnesota. Resistance was not due to a modification at the herbicide-binding site, the 32-kD quinone-binding protein (15). Rather, the resistant biotype had an enhanced capacity to detoxify atrazine by conjugating it with GSH. Resistance was shown to be controlled by a single nuclear gene exhibiting partial dominance (2).

GSTs are multifunctional, dimeric proteins that catalyze the nucleophile attack of the thiolate anion of GSH with electrophilic groups on various substrates (22). These proteins, which are found in mammals (4, 21, 22), insects (3, 14), and plants (25), play a major role in the detoxification of xenobiotics. In plants, GSTs catalyze the detoxification of the s-triazine (5, 10, 12, 13, 15, 16, 18, 25), chloracneanilide (8, 10, 23, 25), and thiocarbamate (20, 25) herbicides. It is well established that the tolerance of maize and sorghum to atrazine is due to the high levels of GSH and GST (atrazine) in these species that facilitate the detoxification of the herbicide via GSH conjugation (13, 25, 28). In maize, GST (atrazine) activity is constitutively expressed in leaf and stem tissue (5, 11, 12, 13, 29). Maize roots contain little or no activity (12, 13, 29). Although GST (atrazine) is constitutively expressed in maize leaves, one report suggested that the levels of this enzyme are enhanced by pretreatment with atrazine. Jachetta

1 Cooperative investigation of the USDA-Agricultural Research Service and the Minnesota Agricultural Experiment Station. Paper No. 18,394, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN.
2 Present address: Department of Agronomy, Oklahoma State University, Stillwater, OK 74078.

Received for publication September 24, 1990
Accepted January 4, 1991
and Radosevich (18) reported that pretreating maize plants with atrazine resulted in an enhanced rate of atrazine metabolism via GSH conjugation in leaves during subsequent exposure to the herbicide. These authors suggested that atrazine pretreatment caused an increase in GSH and/or GST (atrazine) activity in maize leaves, although this was not determined.

The objective of the present study was to determine the biochemical basis for the enhanced capacity of the Maryland biotype to conjugate atrazine with GSH. It was hypothesized that the enhanced GSH conjugation capacity was due to enhanced GSH and/or GST (atrazine) activity. We found that resistance was not due to elevated GSH levels but rather to enhanced GST (atrazine) activity.

MATERIALS AND METHODS

Chemicals

[U-14C]Atrazine (16.8 μCi/mg, 98% pure) and technical grade atrazine (97% pure) were provided by CIBA-GEIGY Corporation (Greensboro, NC). GS-atrazine was prepared using the method of Crayford and Hutson (6). GSH was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). All other reagents were obtained from Sigma Chemical Company (St. Louis, MO).

Plant Materials

Seed of the atrazine-resistant biotype of velvetleaf (Abutilon theophrasti) was from a field near Westminster, MD (24). The atrazine-resistant seed used in this study was from one plant that survived a postemergence treatment of 10 kg/ha atrazine (2). Atrazine-susceptible or wild-type seed was obtained from a velvetleaf plant from Rosemount, MN. Seeds were placed on filter paper (Whatman No. 1) in Petri dishes and the filter paper was moistened with distilled water. After the seeds were incubated in the dark for 2 d at 4°C, they were boiled in distilled water for 1 min and rinsed for 2 h with tap water. The germinating (swollen) seeds were planted in moist vermiculite, covered with a layer of silica sand, and placed in a growth chamber maintained at 25°C and 55% RH with a 16-h photoperiod. The PAR was approximately 220 μE·m⁻²·s⁻¹. Seven days later (when in the one-leaf stage) the seedlings were removed from the vermiculite and transferred to an aerated nutrient solution described previously (15). At the five- to six-leaf stage, the plants were harvested and separated into leaves, stems (including petioles), and roots. Tissue fresh weights were obtained, and the tissue was quickly frozen with and stored in liquid N₂. Pretreatment with 30 μM atrazine was accomplished by adding technical grade atrazine, dissolved in ethanol, to the hydroponic solution of plants in the five- to six-leaf stage. The final ethanol concentration in the hydroponic solution was 0.3%. After 12 h, the plants were harvested as described above.

* Mention of a trademark, vendor, or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the University of Minnesota and does not imply its approval to the exclusion of other products or vendors which may also be suitable.

Extraction and Assay of GSH

Tissue that had been stored in liquid nitrogen for not more than 2 d was placed in a chilled mortar (4°C) and ground for 2 min in a nitrogen-flushed medium containing 5% (w/v) TCA, 10 mM EDTA, and acid-washed sand. The extract was centrifuged (2000g, 2.5 min) to remove precipitated protein and debris. The supernatant was then centrifuged (10,000g, 15 min), and the supernatant fractions were stored in liquid nitrogen for no more than 2 d before assay.

GSH was assayed using the procedure of Lamoureux and Rusness (19) with modifications. The assay medium contained 100 mM K⁺-phosphate buffer (pH 6.8), 10 mM EDTA, 1 mM CDNB, 1.0 unit equine GST, and 150 μL of extract in a final volume of 3.0 mL. The reaction was started by adding equine GST. Absorbance at 340 nm was recorded before commencing the reaction and after the reaction had run to completion (20 min). The amount of GSH in the tissue extract was proportional to the difference in absorbance before addition of the extract and after the reaction had run to completion. A calibration curve was made using GSH (0–80 nmol) as an internal standard. The data are expressed as the average of three extracts ± LSD (0.05). The experiment was repeated twice.

Extraction and Assay of GST

Frozen tissue was ground in a mortar (prechilled in liquid N₂) containing 100 mM K⁺-phosphate (pH 6.8), 2 mM EDTA, 20 mM 2-mercaptoethanol, 3.5% (w/v) PVPP, 2 mM PMSF, 10 mM GSH, 5% (w/v) BSA, and acid-washed sand. The homogenate was centrifuged at 20,000g for 15 min. The supernatant was centrifuged (100,000g, 60 min) and then desalted on a PD-10 column (Pharmacia) equilibrated in 100 mM K⁺-phosphate buffer (pH 6.8).

GST (atrazine) activity was measured using the procedure of Lamoureux and Rusness (19) with modifications. Enzyme activity was assayed at 30°C in a medium containing 10 mM GSH, 100 mM K⁺-phosphate (pH 6.8), 27, 40, or 200 μM [U-14C] atrazine, and a 160-μL aliquot (approximately 100 μg protein) of the desalted extract in a total volume of 200 μL. The reaction was started by adding GSH and stopped after 30 min by adding 200 μL of methylene chloride. The tubes containing the assay medium were vigorously shaken to partition atrazine into the organic phase and GS-atrazine into the aqueous phase. To facilitate partitioning of the layers, the tubes were centrifuged (10,000g, 5 min). A 40-μL aliquot was removed from the aqueous phase and radioactivity counted using liquid scintillation spectroscopy. Assays without enzyme were used to determine the nonenzymatic rate of GS-atrazine formation. The metabolite formed in the reaction was identified by co-chromatography with an authentic standard of GS-atrazine on Whatman TLC (LK6F) plates developed with butanol:acetic acid:water (12:3:5, v/v/v).

GST (CDNB) activity was assayed at 340 nm in a medium containing 100 mM K⁺-phosphate buffer (pH 6.8), 1 mM CDNB, 10 mM GSH, and 50 μL of tissue extract in a total volume of 1 mL (23). The reaction rate was calculated from the change in absorbance using the molar extinction coefficient of 9.6 mm⁻¹·cm⁻¹ (17). Nonenzymatic conjugation was determined with assay medium minus enzyme.
Kinetics

The kinetic constants for atrazine and CDNB were determined in the presence of 10 mM GSH. For GSH, the kinetic constants were determined in the presence of 200 μM atrazine. Results were analyzed on double reciprocal plots. Apparent Km values were obtained using linear regression. The experiment was repeated two or more times.

pH Optima

The pH optima for GST (atrazine) activity in leaf extracts were determined by assaying crude extracts over the pH range 5.5 to 8.5. Tissue was extracted as described above except that it was desalted into 20 mM Taps buffer at pH 8.4 instead of 100 mM K-phosphate buffer. The following buffer solutions (400 mM) containing 10 mM GSH were used to adjust the pH of the desalted extract: acetic acid, pH 5.0 and 5.5; succinic acid, pH 5.0 and 5.5; Mes, pH 6.0 and 6.5; phosphate, pH 6.5, 7.0, and 7.5; Mops, pH 7.0 and 7.5; Taps, pH 8.0 and 8.5; and Tris, pH 8.0 and 8.5. An aliquot (80 μL) of the desalted extract was added to 100 μL of the appropriate 400 mM buffer containing 10 mM GSH, and the assay was initiated by the addition of the 27 μM [14C]atrazine.

FPLC-Anion-Exchange Chromatography

GST isozymes from the resistant and susceptible biotypes were separated using FPLC anion-exchange chromatography. GST activity was extracted as described above with the exception that BSA was excluded and additional protectants, stabilizers, and protease inhibitors were added to the extraction buffer. GST activity from 2 g of leaf tissue was extracted in 30 mL of 100 mM Taps buffer (pH 8.5) containing 20 mM 2-mercaptoethanol, 3.5% (w/v) PVPP, 2 mM EDTA, 10 mM GSH, 10 μM antipain, 5 μg/mL pepstatin A, 1 mM PMSF (added immediately before extraction), 10 mM sodium metabisulfite, and 1.0 g of Amberlite XAD-4. The extract was centrifuged (100,000g, 1 h) and the supernatant was filtered through an Amberlite XAD-4 column (1.5 × 3.0 cm) to remove phenolics. The fraction containing GST activity was then loaded onto a DEAE-Sepharose column (1.5 × 2 cm) equilibrated in 50 mM Taps (pH 8.4). The column was flushed with 20 mL of buffer, and GST activity was eluted with a 50-mL NaCl gradient (0–400 mM) in Taps buffer. Active fractions were combined, diluted twofold and 4 mg of total protein was loaded onto a Mono Q (5/5) anion-exchange column (Pharmacia) equilibrated with 50 mM Taps buffer (pH 8.5) containing protease inhibitors (10 μM antipain, 6 μg/mL pepstatin). The column was flushed with 10 mL of buffer and GST (atrazine) activity was eluted at 0.75 mL/min using a 50-mL NaCl gradient (100 to 250 mM). Fractions (1 mL) were collected and assayed for GST (atrazine) activity (40 μM atrazine) and GST (CDNB) activity as described above.

RESULTS

GSH Levels

No significant differences were found in GSH levels in roots, stems, or leaves of the resistant and susceptible biotypes (Fig. 1). In both biotypes, the GSH levels were highest in the leaves. The concentration in the leaves was approximately threefold and 13-fold greater than that in the stems and roots, respectively. A 3-h pretreatment with 30 μM atrazine via hydroponic solution had no effect on GSH levels in the tissues of either the susceptible or resistant biotype (data not shown).

GST Extraction

In preliminary experiments, little or no GST (atrazine) activity was found when leaf tissue was extracted in 100 mM K-phosphate buffer, pH 6.8. The effects of adding various protectants to the extraction medium and desalting the extract were examined. Adding PVPP (5%, w/v), BSA (5% w/v), and GSH (10 mM) to the extraction medium and desalting the extract were necessary to obtain high activity. Removal of PVPP, BSA, or GSH from the extraction medium reduced activity by 16, 16, and 13%, respectively. If these protectants were included in the extraction medium, but the extract was not desalted prior to assay, enzyme activity was reduced by 45%.

GST (atrazine) activity was relatively stable when isolated as described above and stored in 100 mM K-phosphate buffer (pH 6.8) containing 3.5% BSA. Activity did not decline significantly (P = 0.05) when stored at 4°C for 7 d, −21°C for 2 weeks, or in liquid nitrogen (−196°C) for 41 d.

GST Activity

GST activity was measured in leaves, stems, and roots using atrazine and CDNB as substrates (Table I). In both biotypes, most of the GST (atrazine) activity (80–85% of the total on a per gram fresh weight basis) was in leaf tissue and the remainder in the stem. GST (atrazine) activity was not detectable in root tissue. Compared to the susceptible biotype, GST (atrazine) activity was 4.4- and 3.5-fold greater in leaf and stem extracts, respectively, of the resistant biotype. Pretreating both resistant and susceptible biotypes for 24 h with 30 μM atrazine via hydroponic solution had no effect on GST (atrazine) activity of leaf extracts compared to the untreated control (data not shown). CDNB is not a herbicide but is a substrate
Table 1. Glutathione S-Transferase Activity with Atrazine or CDNB as Substrate in Crude Extracts from Leaves, Stems, and Roots of Resistant and Susceptible Biotypes

<table>
<thead>
<tr>
<th>Organ</th>
<th>GST (Atrazine) Activity</th>
<th>GST (CDNB) Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td>nmol/min · g fresh wt</td>
<td>µmol/min · g fresh wt</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.77 ± 0.01</td>
<td>4.10 ± 0.33</td>
</tr>
<tr>
<td>Stem</td>
<td>0.17 ± 0.08</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td>Root</td>
<td>ND</td>
<td>0.50 ± 0.01</td>
</tr>
</tbody>
</table>

* Not detected.

frequently used to assay for GST activity in biological systems (17). GST (CDNB) activity was also highest in leaf tissue of both biotypes. In contrast to the findings with atrazine as substrate, GST (CDNB) activity was greater (1.3-fold) in leaves and stems of the susceptible biotype.

There have been conflicting reports of the pH optimum for GST (atrazine) activity in corn. Frear and Swanson (13) reported a pH optimum of approximately 6.6 to 6.8, whereas Guidewar and Dauterman (16) reported a pH optimum of approximately 8.0 to 8.5. The pH optimum for GST (atrazine) activity in velvetleaf was close to that reported by Frear and Swanson (13) (Fig. 2). The resistant biotype showed a broad pH optima between pH 6.0 and 6.5, and the susceptible biotype showed an even broader pH optima between pH 5.5 and 6.5.

The kinetic parameters for atrazine, CDNB, and GSH were determined in leaf extracts of resistant and susceptible biotypes. Lineweaver-Burke plots indicated that the apparent $K_m$ values for atrazine, CDNB, and GSH were similar between the resistant and susceptible biotypes (Table II). However, the $V_{max}$ for both atrazine and GSH were approximately threefold and 3.5-fold greater, respectively, in the resistant biotype. In contrast, with CDNB as a substrate, the $V_{max}$ was approximately 30% lower in the resistant biotype.

Anion-Exchange Chromatography

GSTs are known to exist as multiple forms (isozymes) in mammals (22), insects (7), and plants (29). Using FPLC-anion-exchange chromatography, we found that there were three peaks (A, B, and C) of GST (atrazine) activity in leaf extracts from the susceptible biotype and two peaks (B and C) in the resistant biotype (Fig. 3). The two peaks of GST (atrazine) activity in the resistant biotype co-eluted with two of the GST (atrazine) peaks in the susceptible biotype. However, the relative height of the two GST (atrazine) peaks (B and C) in the resistant biotype was three- and fourfold greater, respectively, than the corresponding peaks in the susceptible biotype. There were two major peaks of GST (CDNB) activity in both biotypes. Both peaks co-eluted with those for GST (atrazine) activity. Peak heights for GST (CDNB) activity were greater in the susceptible biotype.

Table II. Comparison of Glutathione S-Transferase Kinetic Parameters Between Atrazine-resistant and Susceptible Biotypes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Biotype</th>
<th>$R/S^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>Atrazine</td>
<td>160 ± 17</td>
<td>194 ± 9</td>
</tr>
<tr>
<td>GSH</td>
<td>2000 ± 340</td>
<td>2000 ± 700</td>
</tr>
<tr>
<td>CDNB</td>
<td>320 ± 14</td>
<td>310 ± 38</td>
</tr>
</tbody>
</table>

$V_{max}$ (nmol/min)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Biotype</th>
<th>$R/S^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>Atrazine</td>
<td>8.3 ± 1.1</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>GSH</td>
<td>5.2 ± 0.7</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>CDNB</td>
<td>2000 ± 170</td>
<td>2000 ± 170</td>
</tr>
</tbody>
</table>

* The ratio of kinetic parameters in resistant and susceptible biotypes.

DISCUSSION

Earlier we showed that a velvetleaf biotype from Maryland exhibited a 10-fold increase in tolerance to atrazine compared to a wild-type biotype found in Maryland (15). This biotype was resistant to atrazine because of its capacity to rapidly metabolize the herbicide via GSH conjugation. In this study, we showed that this enhanced capacity for GSH conjugation in the resistant biotype was due to increased GST (atrazine) activity in leaf and stem tissue. The 4.4-fold increase in GST (atrazine) activity in leaf tissue of the resistant biotype would account for the five- to sixfold increase in the rate of GS-atrazine formation measured in excised leaf discs (15).

Tissue GSH levels were the same in both biotypes (Fig. 1) and hence were not a factor contributing to enhanced GSH conjugation in the resistant biotype. The highest levels were found in leaf tissue, which is not surprising because chloroplasts are thought to play a major role in the synthesis of this

Figure 2. pH dependence of GST (atrazine) activity measured in crude extracts from leaves of resistant and susceptible biotypes. Values represent the means ± SE.
tripeptide (1). The level of GSH found in leaf tissue of both resistant and susceptible biotypes (approximately 500 nmol/g fresh wt) is comparable to that reported in developing maize leaves (19).

GST (atrazine) from velvetleaf was rather labile. A number of protectants were required in the homogenizing medium, and desalting was necessary to ensure high activity. Other investigators have also noted the lability of GST (atrazine) activity in plant extracts (13, 16, 28). Previous reports indicated the necessity of including BSA in the extraction medium (16) and desalting extracts (13) to protect against low molecular weight endogenous inhibitors in maize leaves. Timmerman (28) also indicated that 2-mercaptoethanol was required to stabilize maize GST (atrazine) activity during purification.

As determined by in vitro assays, most of the GST (atrazine) activity in velvetleaf is in leaf tissue. Low levels of activity were detected in stems and none in roots (Table I). This distribution of GST (atrazine) activity parallels that found in atrazine-tolerant grasses such as maize and wild proso millet (Panicum miliaceum L.). These grasses exhibit high levels of GST (atrazine) activity in leaves but contain little or no GST (atrazine) activity in roots (12, 13, 29). In maize (5), GST (atrazine) activity was reported to be lower in stem tissue (5.5 nmol GS-atrazine formed/mg protein-h) compared to leaf tissue (40.6 nmol GS-atrazine formed/mg protein-h).

There is an apparent discrepancy in GST (atrazine) activity in leaf and stem extracts of the resistant biotype (Table I) and the level of GS-atrazine conjugate found in these tissues after hydroponic treatment with [14C]atrazine (15). After a 3-h pretreatment with [14C]atrazine, the concentration of conjugate found in the stem was approximately 1.7-fold greater than that in leaf tissue (15). Yet, the results in Table I indicate that GST (atrazine) activity (on a per gram fresh weight basis) is 5.6-fold greater in leaf tissue extracts compared to stem extracts. The higher levels of conjugate in stem tissue probably relates to the method of treatment (i.e., hydroponic solution) in the earlier study (15) which resulted in greater availability of atrazine to stem than leaf tissue. When measured in leaf discs and 2-cm stem sections (split horizontally), the rates of conjugate formation in stem (0.38 nmol/g fresh weight/min) and leaf (2.0 nmol/g fresh weight/min) tissue were similar to the GST (atrazine) activity (expressed on a per gram fresh weight basis) measured in extracts from the two tissues (Table I).

There is increasing evidence that plants contain multiple forms of GST as is known to occur in mammals (22) and insects (7). In velvetleaf there are at least two isozymes of GST (atrazine) activity in the resistant biotype and three in the susceptible biotype. In maize leaves, two (28, 29) or three (11) GST isozymes exhibiting activity with atrazine have been reported.

The increase in relative peak height for GST (atrazine) activity in peaks B and C in the resistant biotype is associated with a decrease in relative peak height for GST (CDNB) activity (Fig. 3). One interpretation of these results is that each peak (B and C) contains one isozyme that exhibits cross-reactivity with CDNB and atrazine and that the relative specificity for the two substrates has been modified in the resistant biotype. However, this is not supported by the lack of change in apparent Km values for the two substrates (Table II). Based on the results obtained with kinetic analysis, the increase in GST (atrazine) activity in leaf tissue of the resistant biotype appears to be due to overproduction of two isozymes rather than a modification in affinity for CDNB or atrazine.

It is likely that the GST (atrazine) and GST (CDNB) activities in peaks B and C reflect the activity of different isozymes that co-elute. In maize, GST isozymes that exhibit activity with atrazine exhibit very little cross-reactivity with CDNB (12, 28, 29). Furthermore, one-dimensional, SDS-gel electrophoresis of the GSTs in each of the two peaks (B and C) indicates that there are at least three GST subunits in peak B and at least two in peak C (M. Anderson, unpublished results). If it is assumed that these subunits form only homodimers, then there are at least three isozymes in peak B and at least two in peak C. The resistant and susceptible biotypes used in this study are not isogenic lines, and differences in the GST (CDNB) elution profiles of the two biotypes may be unrelated to atrazine susceptibility or tolerance.

There are other cases in which overproduction of enzymes has resulted in herbicide resistance (9, 26, 27). However, in each case, the protein overproduced was the target site of the herbicide and resistant biotypes were selected in tissue culture. Resistance to phosphinothricin in alfalfa (Medicago sativa L.) suspension cells was due to the amplification of a gene coding for glutamine synthetase, the target site for the herbicide (9).

Resistance to glyphosate in cell cultures of Petunia hybrida (27) and Coridialis sempervirens (26) was the result of overproduction of 5-enolpyruvylshikimate-3-phosphate synthase.

Enhanced GST activity has contributed to pesticide or xenobiotic resistance in insects and mammalian cells. The development of resistance to organophosphorus insecticides in the housefly (Drosophila melanogaster L.) (7) and the light brown apple moth (Epiphyas posttuttara) (3) has been attributed, at least partially, to enhanced levels of GSTs that detoxify the insecticides. Resistance to permethrin and DTT in the mosquito (Aedes aegypti) was associated with higher activity of a particular GST isozyme (14). In mammals, enhanced GST
levels also play a role in the development of acquired drug resistance of tumor and nontumor cells. Batist et al. (4) reported a strong correlation between the degree of resistance to the anticancer drug Adriamycin in a breast cancer cell line and the elevation of GST activity. Resistance to nitrogen mustard alkylating agents in a Chinese hamster ovary line was attributed to amplification of a gene coding for a GST that detoxified the compounds (21).

As far as the authors are aware, this is the first study to show that enhanced GST activity can result in the development of acquired herbicide resistance in a weed species. Overproduction of two isozymes exhibiting activity with atrazine appears to be responsible for atrazine resistance in the velvetleaf biotype from Maryland. The mechanism of overproduction is not known, but earlier studies indicated that it is controlled by a single nuclear gene exhibiting partial dominance (2). The overproduction of the GST (atrazine) may be due to one of several mechanisms, including gene amplification, altered promoter strength of the GST (atrazine) gene, or altered expression of a regulatory gene.

ACKNOWLEDGMENT

We thank CIBA-GEIGY Corporation for providing [14C] atrazine and technical grade atrazine used in this study.

LITERATURE CITED


ENHANCED GST ACTIVITY IN VELVETLEAF 109

Copyright © 1991 American Society of Plant Biologists. All rights reserved.