Purification and Characterization of Corn Glutathione *S*-Transferase†

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ABSTRACT: Two glutathione *S*-transferase (GST) activities have been identified and purified from etiolated corn tissue. The first, designated GST I enzyme, is constitutively present in corn tissue, and the second, designated GST II enzyme, is present only in tissue which has been treated with chemical antidotes which protect corn against chloroacetanilide herbicides. The total activity constitutes approximately 2% of the soluble protein in these tissues. The native forms of these enzymes have molecular weights of approximately 50 000 as determined by Sephadex G-100 chromatography. On sodium dodecyl sulfate–polyacrylamide gels, GST I enzyme migrates primarily as a single band of molecular weight 29 000, and GST II enzyme migrates as primarily two bands of molecular weight 29 000 and 27 000. Both enzymes catalyze the formation of a glutathione–herbicide conjugate in vitro when the herbicide alachlor is used as a substrate. This conjugation results in elimination of the biological activity of the herbicide.

The enzyme glutathione *S*-transferase (GST)[1] catalyzes the conjugation of glutathione with a large number of hydrophobic, electrophilic compounds. This conjugation results in detoxification of these compounds and, furthermore, facilitates their removal from biological tissue. Some of these compounds such as bilirubin, a product of heme breakdown, are of biological origin (Litwak et al., 1971), and others, such as the herbicide atrazine, are of synthetic origin (Shimabukuro et al., 1971). In certain cases, as with carcinogenic azo dyes, the reaction results in covalent linkage of the enzyme with the substrate, thereby protecting more important biological functions from being impaired (Ketterer et al., 1967; Litwak et al., 1971). Although glutathione is the sole reducing compound used by these enzymes, the specificity of the hydrophobic compounds which are acted upon is very broad (Jakoby, 1978).

GST enzyme activity has been identified in many eukaryotic organisms including plants, insects (Clark et al., 1973), and animals (Jakoby, 1978). Isozymes have been purified from human (Kamisaka et al., 1975) and rat livers (Habig et al., 1974; Bhargava et al., 1980). Each of the purified isozymes has a distinctive pattern of reactivity depending on the carbon skeleton or the reactive group of the substrate. However, in a given tissue, these enzymes collectively have an enormous range of catalytic capability. In general, the transferases are dimers and have molecular weights between 45 000 and 50 000 (Habig et al., 1974; Kamisaka et al., 1975).

We have been interested in the role of GST in protecting plants against toxic chemicals. We report here the purification of two distinguishable GST activities from etiolated corn tissue. The first, designated GST I, is constitutively present in corn tissue. The second, designated GST II, appears only in etiolated tissue derived from seeds that have been treated with herbicide antidotes, chemical agents also termed "safeners", which increase the tolerance of corn to chloroacetanilide and thiocarbamate herbicides. Both GST I and II catalyze the in vitro conjugation and hence detoxification of the herbicide alachlor with glutathione. This is the first demonstration that a plant enzyme can detoxify this class of herbicides and that a novel enzyme species responsible for herbicide detoxification is induced by antidote treatment.

Materials and Methods

Materials

In all experiments, corn hybrid 3708A from Pioneer Hi-bred International was used. The Pharmacia affinity column matrices were obtained from Sigma Chemical Co. All chemical seed antidotes and the [^{14}C]phenyl-labeled alachlor were provided by Monsanto Agricultural Products Co.

Methods

Safener Treatment of Corn. For the initial testing of the effect of various protecting agents on GST activity, 10 g of dry corn seeds was coated with a 1-mL solution of methylene chloride containing 10–15 mg of antidote. The methylene chloride solvent was evaporated with bursts of compressed air, and the seeds were imbibed on moist filter paper for 5–6 days. For enzyme isolations, 300 g of seeds was used which were coated with a 5-mL solution of methylene chloride containing 450 mg of antidote.

Enzyme Assay. Enzyme activity was measured spectrophotometrically at 340 nm by using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Booth et al., 1961). The concentrations of reagents in a 1-mL assay were 0.1 M potassium phosphate buffer, pH 6.5, containing 5 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene. Enzyme activity using the herbicide alachlor as a substrate was measured by using a radioactive assay. Enzyme was incubated in 60-μL samples including 1 mM herbicide, 10 nCi of ^{14}C-labeled herbicide, and 10 mM glutathione in 0.1 M potassium phosphate, pH 6.5. After 1 h of incubation at 37 °C, the reaction was diluted into 0.75 mL of H_2O and extracted 2 times with chloroform and once with ether. The aqueous phase containing the reaction product was counted to determine the amount of activity present. When this assay was used, little nonenzymatic activity could be detected when boiled extracts were used or when glutathione was omitted from the assay.

Preparation of Extracts. Tissue was frozen under liquid N_2 and ground to a powder in a Waring blender. The powder was suspended into 0.2 M Tris-HCl, pH 7.8, containing 1 mM EDTA and 7.5% w/v polyvinylpolypyrrolidone. The suspension was centrifuged at 10 000 rpm for 10 min to obtain the crude extract. For bacterial or yeast extracts, the cells were ground in a mortar and pestle using fine ground glass as an abrasive.

Purification. Nucleic acids were precipitated by addition of 0.1 volume of 1.4% protamine sulfate. A 30–70% $(NH_4)_2SO_4$ precipitate was obtained from the resulting supernatant

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[1] Abbreviations: alachlor (LASSO), 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide; CDNB, 1-chloro-2,4-dinitrobenzene; GST, glutathione *S*-transferase; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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Table I: Relative Specific Activities for Glutathione *S*-Transferase in Crude Extracts[a]

extract	rel sp act.
green corn leaves	1.0
etiolated corn leaves	1.2
green alfalfa leaves	0.19
etiolated alfalfa tissue	0.19
alfalfa tissue callus	0.32
rat liver	0.79
Escherichia coli	0.004
Saccharomyces cerevisiae	0.002

[a] The relative specific activities of GST as measured in various crude extracts are shown. The substrate of the reaction, 1-chloro-2,4-dinitrobenzene, was used at a concentration of 1 mM. The specific activity of the corn leaf extract was 0.9 μmol min^{-1} mg^{-1}.

and suspended into 0.01 M potassium phosphate buffer, pH 7.3. After dialysis overnight vs. this buffer, this fraction was applied to a 40-mL DEAE-Sepharose column which had been equilibrated with 0.01 M potassium phosphate, pH 7.3. After the sample had been loaded, a 400-mL linear potassium phosphate, pH 7.3, gradient from 10 to 250 mM was applied to the column. Two peaks of enzyme activity were obtained. GST I eluted at a buffer concentration of 50 mM and GST II at 100 mM.

Preparation of the Affinity Column. Reduced glutathione and bromosulfophthalein were conjugated in an overnight reaction in basic solution (Clark et al., 1977). The conjugate was partially purified by acetone precipitation and paper chromatography (Whelan et al., 1970). This complex was attached to a Sepharose matrix by reacting the conjugate with either cyanogen bromide activated Sepharose or 6-aminohexanoic acid activated Sepharose as directed by the manufacturer.

Affinity Chromatography. (*A*) *Low pH.* Fractions from the DEAE-Sepharose column chromatography containing GST I enzyme were combined and applied to a 10-mL affinity column which had been equilibrated with 0.05 M potassium phosphate, pH 7.3. After the column was washed with 5 volumes of the buffer, the bound GST activity was eluted with a buffer containing 5 mM reduced glutathione.

(*B*) *High pH.* A similar procedure was used except that GST II enzyme was used, and the column buffer was 0.05 M Tris-HCl, pH 8.0.

Sephadex G-100 Chromatography and HPLC. Approximately 1.5-mL samples of either enzyme or the molecular weight standards blue dextran, bovine serum albumin, ovalbumin, trypsinogen, and lysozyme were applied to a 150-mL Sephadex G-100 column. Elution profiles were determined by monitoring either the absorbance at 280 nm or the enzyme activity. A standard curve was constructed by plotting the log of the molecular weight vs. elution volume. High-pressure liquid chromatography (HPLC) was performed by using TSK-Gel SW-type and TW-type columns (Beckman Instruments) and an elution buffer of 0.1 M potassium phosphate, pH 6.5.

$NaDodSO_4$–polyacrylamide gel electrophoresis was performed essentially by the method of Weber & Osborn (1969). The acrylamide to bis(acrylamide) ratio was 30:0.174, and the gel concentrations for the separating and stacking gels were 17.5% and 5%, respectively.

Results

The specific activity of GST was measured in crude extracts prepared from a variety of sources (Table I). Of these tissues, crude extracts from green corn leaves and, in particular, etiolated corn leaves contain the highest levels. These levels of specific activity are even higher than those found in rat livers where the transferase activities have been reported to constitute between 1 and 5% of the soluble cellular protein (Fleischner et al., 1972). Crude extracts from alfalfa tissue have 20% of the specific activity of corn extracts, and the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae* have much lower levels. Such low levels have also been reported in the bacterium *Salmonella typhimurium* (Summer et al., 1980).

Table II: Effect of Various Antidotes on GST Activity in Corn[a]

chemical	crop treated with antidote	rel sp act. root tissue	rel sp act. shoot tissue
none	none	1.0	1.0
3-(2,5-dimethoxyphenacyl)-phthalide	rice	1.4, 1.0	1.1, 1.1
O,O-diethyl *O*-*m*-tolyl phosphorothioate	wheat	1.0, 1.2	1.5, 2.0
5-(2,4-dichlorophenyl)-4-isoxazolecarboxylic acid, ethyl ester	rice, wheat, corn, sorghum	1.5, 1.3	1.4, 1.3
α-[(cyanomethoxy)imino]-benzeneacetonitrile	sorghum, corn	1.8, 1.4	1.6, 2.1
naphthalic anhydride	sorghum, corn	1.9, 1.5	1.7, 2.1
2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylic acid, benzyl ester	sorghum, corn	2.1, 2.0	2.0, 2.3
N,N-diallyl-2,2-dichloro-acetamide	corn	2.2, 2.4	2.8, 2.6

[a] A list of the various antidotes and the relative specific activities of GST found in crude extracts of etiolated corn tissues treated with these antidotes compared to controls (none) which were not treated or which were treated with methylene chloride alone is shown. The specific activities found in crude extracts of untreated roots and shoots were 1.6 and 0.6 μmol min^{-1} mg^{-1}, respectively.

Treatment of various tissues with certain alkylating agents has been shown to cause a rise in the level of GST activity (Lay & Casida, 1976; Reyes et al., 1969). In particular, treatment of corn with antidotes to certain herbicides has been shown to increase the total level of GST activity in corn tissue by 2–3-fold (Lay & Casida, 1976). Etiolated tissue was prepared from corn seeds pretreated with various antidotes as listed in Table II to further test this result. Crude extracts were prepared from the roots and shoots of these tissues and assayed for GST activity. Since the specific activity of transferase is 2.5–3-fold higher in root tissue than in shoot tissue, these tissues were separated to eliminate problems resulting from mixing of unequal amounts of the two tissues.

The effects of the antidote treatments on GST activity are listed in Table II. In general, the corn antidotes raised enzyme levels between 1.5- and 2.5-fold in both roots and shoots. However, the rice and wheat antidotes had little effect on enzyme levels. The untreated control tissues were prepared either with no pretreatment or by pretreatment with methylene chloride which, by itself, has no effect on enzyme levels. These data demonstrate a correlation between tissue-specific protection by antidotes against certain herbicides and the antidote-induced rise in levels of this enzyme.

The GST enzyme activities present were purified to further characterize this elevation of GST levels in antidote-treated tissue. Ammonium sulfate precipitated extracts were prepared from etiolated corn tissue treated with the corn antidote 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylic acid, benzyl ester, or from untreated corn tissue. These extracts were chromatographed on parallel DEAE-Sepharose columns as

Table III: Summary of Purification

	volume (mL)	[protein] (mg)	sp act. (μmol min^{-1} mg^{-1})	x-fold purification	yield (%)
crude extract	450	1400	1.1	(1.0)	(100)
ammonium sulfate precipitate	45	1100	1.2	1.1	88
GST I, DEAE-Sepharose	22.5	74	6.1	5.6	29
GST II, DEAE-Sepharose	45	110	2.6	2.4	19
GST I through affinity column	8.0	5.2	66	60	22
GST II through affinity column	9.0	4.4	21	19	9

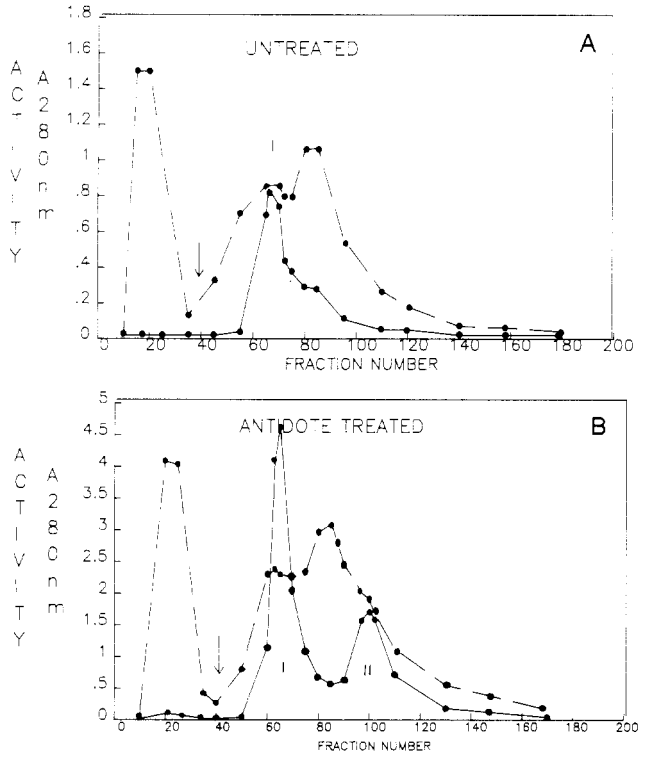

FIGURE 1: DEAE-Sepharose column chromatography of ammonium sulfate precipitated fractions from untreated (A) and antidote-treated (B) etiolated corn tissue. Enzyme activity (ΔA_{340nm} per minute) is presented by solid lines and the UV absorbance at 280 nm by dashed lines. The arrows indicate where the gradient elution was initiated.

shown in Figure 1A,B. In untreated tissue, a single peak of activity with a small trailing edge eluted at a buffer concentration of approximately 50 mM (GST I enzyme). In antidote-treated tissue, a similar peak of activity eluted at 50 mM buffer, but, in addition, a second peak of activity (GST II) eluted at a buffer concentration of approximately 100 mM. Similar results to these were obtained when the corn antidote *N,N*-diallyl-2,2-dichloroacetamide was used.

GST I from both these DEAE-Sepharose columns was chromatographed over an affinity column for GST (Figure 2). Quantitative binding to the enzyme activity occurred when 0.05 M potassium phosphate, pH 7.3, was used, and nearly quantitative elution of the enzyme activity was achieved by adding 5 mM reduced glutathione to this buffer. Little additional enzyme activity eluted when 0.05 M Tris-HCl buffer, pH 8.0, containing 5 mM glutathione was added to the column. These data suggest that the GST I enzyme activities from the antidote-treated and untreated tissue are the same.

However, when GST II of the DEAE-Sepharose column was chromatographed on the affinity column, a different elution profile was observed (Figure 3). Only a small portion of enzyme activity eluted with the 0.05 M potassium phosphate, pH 7.3, containing 5 mM glutathione. Substantially more activity eluted when 0.05 M Tris-HCl, pH 8.0, containing 5 mM glutathione was applied to the column. Also, unlike

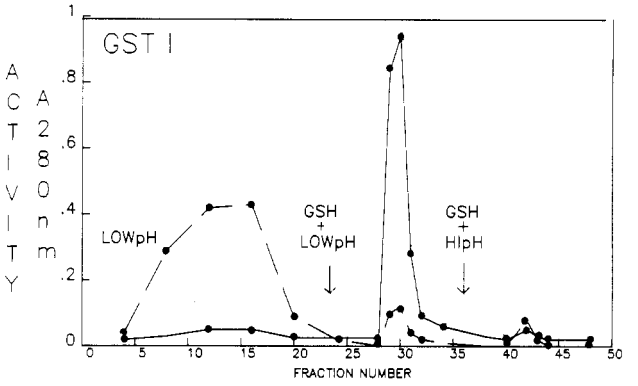

FIGURE 2: Affinity column chromatography of GST I enzyme from the DEAE-Sepharose column. The initial buffer (low pH) was 0.5 M potassium phosphate, pH 7.3. The first enzyme elution used this buffer containing 5 mM reduced glutathione (low pH, GSH), and the second elution used 0.05 M Tris-HCl buffer, pH 8.0, containing 5 mM reduced glutathione (high pH, GSH). The elution of absorbance at 280 nm is shown by a dashed line and the elution of enzyme activity (ΔA_{340} per minute) by a solid line.

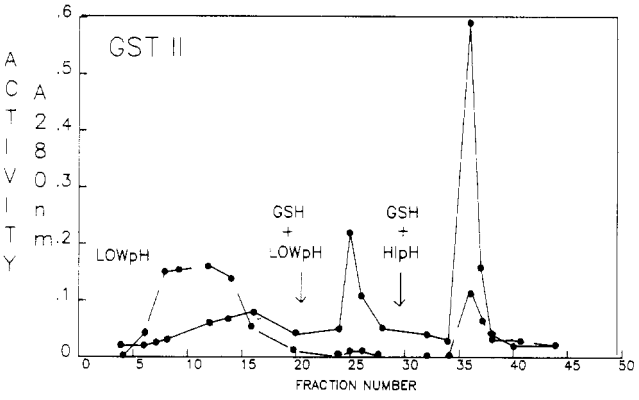

FIGURE 3: Affinity chromatography of GST II enzyme from the DEAE-Sepharose column using conditions as described in Figure 2.

the activity which is associated with GST I, quantative binding to the affinity column of this GST II enzyme activity occurred when chromatography using 0.05 M Tris-HCl, pH 8.0, was used as well as the potassium phosphate, pH 7.3 (data not shown). Therefore, GST I enzyme can be purified on this affinity column by using the pH 7.3 buffer, and GST II enzyme can be purified by using the pH 8.0 buffer.

A summary of the purification of the two GST enzymes from antidote-treated etiolated corn tissue is presented in Table III. Together, the two enzymes constitute approximately 2% of the soluble protein in etiolated corn tissue. When the results from several purifications are compared, purified GST I enzyme consistently had a specific activity 2–3-fold higher than that for purified GST II enzyme in the CDNB assay.

These two purified enzymes were separated on the basis of their molecular weight by $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 4) by using a high acrylamide to bis(acrylamide) ratio (30:0.174). GST I enzyme consisted predominantly (greater than 95% by densitometer analysis)

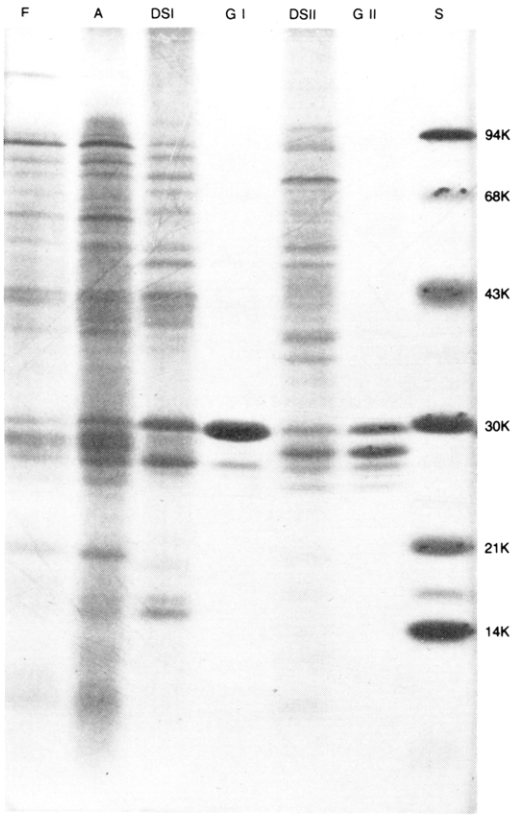

FIGURE 4: $NaDodSO_4$–polyacrylamide gel electrophoresis of various fractions during the purification of the two GST activities from antidote-treated etiolated corn tissue. The fractions are crude extract (E), ammonium sulfate precipitate (A), GST I region of the DEAE-Sepharose column (DSI), purified GST I enzyme (G I), GST II region of the DEAE-Sepharose column (DSII), purified GST II enzyme (G II), and various protein standards whose molecular weights are shown on the side (S).

of a single polypeptide of molecular weight 29 000, and GST II enzyme consisted predominantly (90% by densitometer analysis) of two equal staining polypeptides or molecular weights 29 000 and 27 000. These two polypeptides barely resolved on $NaDodSO_4$–polyacrylamide gels when a more typical acrylamide:bis(acrylamide) ratio of 30:0.8 was used. The smaller molecular weight bands of 25 000 in GST I preparations and 25 000 and 23 000 in GST II preparations were consistently present in small quantities in both preparations, but their identity has not been determined. However, the relative amounts of these bands compared to the two major peptides as determined by staining on gels decline through the two affinity purification steps, suggesting that they are at least not major forms of GST enzyme if, in fact, they do have GST activity.

The primary 27 000 molecular weight band of GST II enzyme and the M_r 23 000 polypeptide present in reduced amounts only appeared in extracts prepared from antidote-treated tissue and were not present in extracts from untreated tissue (data not shown). This indicates that their presence was directly related to treatment of the tissues with corn antidotes.

The two purified enzymes were further chromatographed on a Sephadex G-100 column and subsequently by HPLC. Single, uniform peaks were observed for both enzyme preparations following each procedure. By comparison with the chromatographic behavior of protein markers of known molecular weight, GST I enzyme and GST II enzyme were found to have molecular weights near 50 000. This indicates that both enzymes exist as dimers in their native form, a property shared by most GST proteins thus far studied. Monomer bands of 25 000 or 23 000 daltons corresponding to the minor species in these preparations were not observed by either procedure, suggesting that they too exist as dimers in the native state.

A comparison of the specific activities of these two purified enzymes on CDNB and on the chloroacetanilide herbicide alachlor shows that both have much higher specific activities on the substrate CDNB than on the herbicide. The specific activities of GST I using CDNB and alachlor are 74 and 0.023 μmol min^{-1} mg^{-1}, respectively, and the specific activities of GST II using CDNB and alachlor are 26 and 0.038 μmol min^{-1} mg^{-1}, respectively. However, the antidote–induced GST II enzyme has a significantly higher specific activity on the herbicide than does GST I enzyme and significantly lower on the substrate CDNB. Specifically, a comparison of the ratios of the relative activities of the enzymes on alachlor and the substrate CDNB indicates that GST II has a 5-fold higher ratio than does GST I.

Both GST enzyme activities have an unusual salt dependence. At potassium phosphate concentrations less than 20 mM, no detectable GST activity on CDNB when either enzyme is used can be observed. Maximal activity is obtained at potassium phosphate concentrations in excess of 50 mM. A similar profile based on ionic strength is obtained by adjusting the salt concentration with sodium chloride, suggesting that this effect is based on the total ionic strength and not on a specific salt.

Since GST I and II exhibit different relative activities on CDNB and alachlor, approximate K_m and V_{max} values were determined for each enzyme by Lineweaver–Burk analysis to determine whether they exhibit different substrate binding specificities. In fact, the K_m's of the two enzymes for each substrate are about equal. When CDNB is used as the substrate, GST I and II have K_m's of 3.0 and 2.8 mM, respectively, and when alachlor is the substrate, both enzymes have K_m's of 0.6 mM. However, the V_{max} (1.8 μmol/min) for CDNB of GST I is twice that of the V_{max} for GST II (0.84 μmol/min). When alachlor is used as a substrate, on the other hand, the V_{max}'s are about equal (1.0 nmol/min). These data confirm the results presented above in terms of specific activities of the enzymes on the two substrates. The similarities in these results are not too surprising since both enzyme preparations contain the same 29 000 molecular weight polypeptide (as discussed below) whose kinetic properties (particularly K_m) may obscure contributions made by the induced 27 000 molecular weight subunit.

As is apparent from Figure 4, both GST I and II contain a polypeptide of molecular weight 29 000. These polypeptides were electroeluted from a $NaDodSO_4$–polyacrylamide gel, and partial amino-terminal sequences were obtained for each. In both cases, the sequence X-Pro-Cys-Lys-Leu-Tyr-Gly-Ala-Val was obtained, indicating that these polypeptides are the same. The amino-terminal amino acid X has not been identified for either polypeptide. The 27 000-dalton polypeptide unique to GST II was also purified by electroelution, but sequence analysis of the amino terminus was unsuccessful apparently due to the presence of a blocked amino-terminal amino acid. This is additional evidence that the 27 000 and 29 000 molecular weight polypeptides present in GST II preparations are different.

Discussion

Glutathione *S*-transferase (GST) constitutes approximately 1% of the soluble protein in untreated etiolated corn tissue. This activity, designated as GST I, is a dimer apparently consisting of two 29 000-dalton subunits. Its physical properties

are similar to those of the rat liver ligandin which is also a glutathione transferase (Habig et al., 1974). Moreover, its physical properties and behavior on DEAE-Sepharose suggest that it is identical with the GST partially purified by Guddewar & Dauterman (1979) and reported to be responsible for atrazine resistance in corn (Shimabukuro et al., 1971).

Atrazine resistance results from GST's ability to detoxify atrazine by catalyzing the formation of a water-soluble atrazine–glutathione complex. This conjugation has been shown to occur in vitro and in vivo (Guddewar & Dauterman, 1979; Shimabukuro et al., 1970; Chang et al., 1974). Furthermore, it has been shown that crude extracts made from various atrazine-resistant corn lines possess elevated levels of GST whereas an atrazine-sensitive corn line (GT112) has less than 1% of the GST activity found in the resistant lines (Shimabukuro et al., 1971).

Corn herbicide antidotes are compounds which selectively protect corn from injury by a herbicide. The antidotes themselves are not phytotoxic and do not cause any obvious phenotype alterations or dramatic alterations in protein synthesis. Several possible mechanisms of action of these antidotes have been proposed including direct competition between the antidote and herbicide at the site of action, decreased uptake, and increased metabolism of the herbicide. In the case of the herbicide EPTC (ethyl *N*,*N*-di-*n*-propylthiocarbamate), increased, rather than decreased, uptake has been reported as a result of application of the antidote *N*,*N*-diallyl-2,2-dichloroacetamide, indicating that decreased uptake of this herbicide is an unlikely mechanism (Chang et al., 1974). Other studies have indicated that increased metabolism in vivo of both thiocarbamate and chloroacetanilide herbicide does occur, but the mechanism of increased acetanilide metabolism was not understood (Chang et al., 1974; Leavitt & Penner, 1979; Lay & Casida, 1976). Finally, increased levels of glutathione and a glutathione *S*-transferase activity active on the herbicide EPTC as result of antidote treatment have been reported (Lay & Casida, 1976).

The level of GST has been shown to be chemically inducible in both plant and animal systems. Such increases have been proposed to be important mechanisms for increasing the resistance of a given system to otherwise toxic chemicals. In rats, the administration of the drug phenobarbitone results in an elevation of GST in the liver by a factor of 2–3 (Reyes et al., 1969). Of more relevance here is the fact that Lay & Casida, (1976) have shown that the antidote *N*,*N*-diallyl-2,2-dichloroacetamide increases the level of GST in corn also by a factor of 2–3. However, our findings indicate that, in corn, not only is GST activity increased but also, in fact, a novel GST species is induced. This novel species that we designate GST II has a native molecular weight similar to that of GST I, but our preparations of GST II contain apparently equal amounts of 29 000- and 27 000-dalton polypeptides, suggesting that GST II may be a dimer of nonidentical subunits. Preliminary protein sequencing data of the amino terminus indicate that the 29 000-dalton polypeptides in both GST preparations are identical and suggest that the 27 000-dalton subunit is responsible for GST's altered physical and catalytic properties.

We find that both GST I and II catalyze the conjugation of glutathione with the herbicide alachlor to form a water-soluble nontoxic compound. Alachlor is a chloroacetanilide herbicide sold under the trade name LASSO and used widely for weed control in corn fields. However, GST II, which is specifically induced by agents that protect corn from chloroacetanilide herbicides, interestingly exhibits greater relative activity on these herbicides than the constitutive GST I. It has been suggested that conjugation of a variety of compounds with glutathione can occur in vivo strictly by virtue of a chemical reaction and that enzymatic detoxification is not significant (Leavitt & Penner, 1979). However, our findings, particularly the antidote induction of a novel GST with greater specificity for chloroacetanilide herbicides, indicate that enzymatic detoxification is an important mechanism by which detoxification of this class of herbicide occurs in vivo.

Acknowledgments

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Registry No. CDNB, 97-00-7; alachlor, 15972-60-8; 5-(2,4-dichlorophenyl)-4-isoxazolecarboxylic acid ethyl ester, 76344-80-4; α-[(cyanomethoxy)imino]benzeneacetonitrile, 63278-33-1; naphthalic anhydride, 81-84-5; 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylic acid benzyl ester, 72850-64-7; *N*,*N*-diallyl-2,2-dichloroacetamide, 37764-25-3; GST, 50812-37-8.

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