Purification and Characterization of Glutathione *S*-Transferases of *Eoreuma loftini* and *Diatraea saccharalis* (Lepidoptera: Pyralidae)

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ABSTRACT Glutathione *S*-transferases (GST) from larvae of the sugarcane borer, *Diatraea saccharalis* (F.) and the Mexican rice borer, *Eoreuma loftini* (Dyar), were characterized. Apparently homogeneous GST isoenzymes from the larvae of both species were purified with GSH-affinity chromatography and isoelectric focusing. *E. loftini* had higher GST activity (31.12 units/g wet weight of tissue) compared with *D. saccharalis* (24.8 units/g wet weight of tissue). The GST isoenzyme of *D. saccharalis* with a pI value of 9.3 was a dimer of two identical subunits (M_r 25,000), whereas the GST isoenzyme with a pI value of 8.0 was a heterodimer with subunit M_r values of 25,000 and 27,000. The GST isoenzymes of *E. loftini* with pI values of 9.7 and 7.7 were homodimers of subunits with M_r values of 25,000 and 26,000, respectively. However, the GST isoenzyme with a pI value of 5.3 was a heterodimer of subunits having M_r values of 26,000 and 27,000. Peptide fingerprint analysis by SV-8 protease digestion revealed primary structural differences in these isoenzymes. The N-termini of all GST isoenzymes from both the species of larvae were blocked. Substrate specificities and kinetic properties of the purified GSTs from both the species have been described. The low K_m and high V_{max} values toward 1-chloro-2,4-dinitrobenzene of GST enzymes isolated from both *D. saccharalis* and *E. loftini* indicate that these enzymes have a high affinity for electrophilic substrates compared with most human GSTs.

KEY WORDS Insecta, glutathione *S*-transferase, *Diatraea saccharalis*, *Eoreuma loftini*

RESISTANCE TO PESTICIDES is often encountered in pests invading crops such as sugarcane (interspecific hybrids of *Saccharum*), which is a major source of sugar consumed in the world. Two pests of sugarcane in the Lower Rio Grande Valley of Texas are the Mexican rice borer, *Eoreuma loftini* (Dyar), and the sugarcane borer, *Diatraea saccharalis* (F.). *D. saccharalis* was a threat to the Lower Rio Grande Valley sugarcane industry until the release of the parasite *Cotesia flavipes* (Cameron) in 1977 (Fuchs et al. 1979). *E. loftini*, a recent immigrant from Mexico, was first detected in the Lower Rio Grande Valley in 1980 (Johnson & van Leerdam 1981) and has essentially replaced *D. saccharalis* as the primary pest.

Because the enzymes involved in the metabolism of insecticides are important in the development of resistance, the detoxification mechanisms of these insects must be defined to develop protocols for their effective and economic control. Glutathione S-transferases (GST, EC:2.5.1.18) are involved in the metabolism of many insecticides, including organophosphorus and chlorinated hydrocarbon insecticides (Motoyama & Dauterman 1980, Tanaka et al. 1981, Clark & Shamaan 1984). GSTs represent a family of multifunctional enzymes that catalyze the conjugation of glutathione (GSH) to a variety of xenobiotics, including insecticides (Lamoureux & Rusness 1989). These enzymes are ubiquitous, and their presence has been demonstrated in most of the organisms investigated so far, including mammals (Jakoby 1978, Awasthi & Singh 1985, Mannervik & Danielson 1988), plants (Lamoureux & Rusness 1989), bacteria (Lau et al. 1980), and insects (Yu 1989).

The structural, functional, and immunological characteristics of mammalian GSTs are fairly well understood. On the basis of structural homologies and substrate specificities, they have been divided into three classes designated as α, μ, and π (Mannervik et al. 1985). Even though GSTs have been characterized in many insects, including houseflies (*Musca domestica* L.) (Motoyama & Dauterman 1977, Clark et al. 1984), cockroaches (*Periplaneta americana* L.) (Usui et al. 1977), grass grubs (*Costelytra zelandica* (White)) (Clark et al. 1985), fruit flies (*Ceratitis capitata* (Wiedemann)) (Yawatz & Koren 1984), and caterpillars from five phytophagous lepidopterous species (Yu 1989), functional and structural interrelationships among these isoenzymes is less well understood compared with those among the mammalian GSTs. Because GSTs have been suggested to play a role in the development of resistance to insecticides (Dauterman

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1983), structural and functional interrelationships among various GST isoenzymes of insect pest species must be understood. In the studies described here, we purified GST isoenzymes from *E. loftini* and *D. saccharalis* larvae by GSH-affinity chromatography. We studied the structural and immunological properties of these isoenzymes and determined their substrate specificities toward various electrophilic xenobiotics. Finally, we defined the kinetics of inhibition by various inhibitors of GST.

Materials and Methods

Materials. Sources of most of the chemicals used in this study were the same as described previously by Singhal et al. (1990). Epoxy-activated Sepharose 6B, γ-hexachlorocyclohexane (lindane), chenodeoxycholate, 2,4-dichlorophenoxy acetate (2,4-D), and 2,4,5-trichlorophenoxy acetate (2,4,5-T) were purchased from Sigma Chemical Company, St. Louis, Mo. The antibodies against the human α, μ, and π class GST isoenzymes were raised in rabbits and were the same as those used in our previous studies (Ahmad et al. 1988). Colonies of *E. loftini* and *D. saccharalis* were originally collected from sugarcane in the Lower Rio Grande Valley and have been reared at the Animal and Plant Health Inspection Service (APHIS), USDA, Mission, Tex., for >2 yr without exposure to insecticides. Larvae were reared in large trays containing artificial diet (Martinez et al. 1986). The trays were transferred to the Texas Agricultural Experiment Station, Weslaco, where they were held at 27°C and a photoperiod of 14:10 (L:D). Late instars (fifth or sixth) were removed, frozen (−20°C) immediately, and shipped frozen to Galveston, where biochemical studies were performed.

Enzyme Assays. GST activity with different substrates, including 1-chloro-2,4-dinitrobenzene (CDNB), was determined with the method described by Habig et al. (1974). GST activity toward γ-hexachlorocyclohexane (lindane) was determined as described by Portig et al. (1979) with slight modifications. The incubation mixture (0.2 ml) contained appropriate amounts of GST protein; 100 m*M* potassium phosphate buffer (pH 7.4), (^{3}H) glutathione (0.15 μCi/μmol), and 3.5 m*M* lindane. Reaction mixtures were incubated at 37°C for 4 h. The reaction was stopped by adding 100 μl of cold methanol. The reaction product, 2,4-dichlorophenyl glutathione (DCPG, Rf. 0.45), was separated by thin-layer chromatography (TLC). TLC plates were developed for 4 h in *n*-butanol/acetic acid/water (4:2:2, vol/vol) and visualized by 0.2% ninhydrin reagent. Formation of DCPG was quantitated by scraping and measuring radioactivity of the ninhydrin-positive spot. Glutathione peroxidase activity with cumene hydroperoxide and hydrogen peroxide as substrates was determined as described previously (Singhal et al. 1990). One unit of enzyme used 1 μmol substrate/min at 25°C for GST and at 37°C for GSH peroxidase. Protein was determined as described by Bradford (1976).

Inhibition Studies. The effects of chenodeoxycholate, 2,4-dichlorophenoxy acetate (2,4-D), and 2,4,5-trichlorophenoxy acetate (2,4,5-T) on GST activity toward CDNB were studied by comparing the reaction rate in the absence and presence of six different inhibitor concentrations. The enzyme (20 μl containing 100–150 ng protein) was preincubated with 2,4-D and 2,4,5-T at 25°C in 100 m*M* potassium phosphate buffer (pH 6.5) for 5 min. Preincubation of the enzyme with chenodeoxycholate in 100 m*M* potassium phosphate buffer was done at pH 7.5 at 37°C for 5 min before addition of the substrates. After the preincubation, 1 m*M* CDNB and 1 m*M* GSH were added as substrates in the assay system and an increase in OD at 340 nm was recorded. Because of the limited solubility of the inhibitors, the inhibition of GST isoenzymes by chenodeoxycholate was studied in the presence of 5% methanol (Singh et al. 1988b). Inhibition with 2,4,5-T was performed in the presence of 5% ethanol. The controls contained equivalent concentrations of respective solvents. The I_{50} values (the concentration of the inhibitor giving 50% inhibition of enzyme activity) were determined from the plots of percentage control activity versus inhibitor concentration. The nature of inhibition was determined by double reciprocal plots; the inhibitor constants (K_i) were determined by the replots of the double reciprocal plots.

Purification of GST Isoenzymes. Unless otherwise specified, all purification steps were performed at 4°C. GST isoenzymes of *D. saccharalis* and *E. loftini* were purified according to procedures previously reported for human GST isoenzymes (Singhal et al. 1990). Briefly, 4–5 g *D. saccharalis* and *E. loftini* larvae stored at −20°C were washed with phosphate buffered saline (10 m*M* potassium phosphate buffer [pH 7.0] containing 150 m*M* NaCl) and homogenized separately in four volumes of 10 m*M* potassium phosphate buffer (pH 7.0) containing 1.4 m*M* β-mercaptoethanol (buffer A). The homogenates were centrifuged at 28,000 × *g* for 45 min at 4°C, and the supernatants were dialyzed for 24 h against buffer A (120 vol, three changes). After the dialysis, the samples were centrifuged at 28,000 × *g* for 30 min, and the supernatants were subjected to affinity chromatography (Simons & VanderJagt 1977) over a column of GSH linked to epoxy-activated Sepharose 6B.

The affinity column (1.0 by 10 cm) was equilibrated with 22 m*M* potassium phosphate buffer (pH 7.0) containing 1.4 m*M* β-mercaptoethanol (buffer B) at 10 ml/h, and this flow rate was maintained throughout affinity chromatography. After sample application, the column was thoroughly washed with buffer B until the absorbance of the eluate at 280 nm had fallen to a value close to zero. The enzyme bound to the GSH affinity column was eluted with 10 m*M* GSH in 50 m*M* Tris·HCl buffer (pH 9.6) containing 1.4 m*M* β-mercaptoethanol.

Table 1. Substrate specificities of *D. saccharalis* and *E. loftini* GSTs toward different substrates

Substrates	Specific activities, μmol/min per mg protein[a]			
	D. saccharalis		*E. loftini*	
	28,000 × *g* supernatant	GST-purified by GSH affinity	28,000 × *g* supernatant	GST-purified by GSH affinity
CDNB	0.74 ± 0.064 (6)	31.25 ± 0.96 (6)	1.06 ± 0.088 (6)	51.25 ± 1.47 (6)
DCNB	0.08 ± 0.006 (4)	1.96 ± 0.36 (4)	0.03 ± 0.002 (4)	1.13 ± 0.044 (4)
EA	0.034 ± 0.007 (4)	3.76 ± 0.78 (4)	0.062 ± 0.014 (3)	1.64 ± 0.081 (4)
p-NBC	0.096 ± 0.021 (3)	5.18 ± 1.04 (4)	0.18 ± 0.014 (4)	8.92 ± 0.896 (4)
NPNO	0.025 ± 0.0084 (4)	4.48 ± 0.88 (4)	0.036 ± 0.007 (5)	1.36 ± 0.368 (3)
BSP	0.007 ± 0.003 (3)	ND	ND	ND
TPBO	0.004 ± 0.001 (3)	0.18 ± 0.042 (4)	0.006 ± 0.0024 (4)	0.088 ± 0.0088 (4)
Cumene hydroperoxide[b]	0.038 ± 0.004 (4)	0.86 ± 0.031 (5)	0.026 ± 0.0017 (4)	0.82 ± 0.0073 (4)
H_2O_2[b]	0.044 ± 0.0062 (4)	1.25 ± 0.074 (5)	0.042 ± 0.0062 (4)	0.98 ± 0.042 (4)
Lindane[c]	0.008 (2)	0.014 (2)	0.0014 (2)	0.012 (2)

[a] Values are means ± SD. Numbers of determinations are given in parentheses. ND, not detected.
[b] GSH-peroxidase activities determined at 37°C.
[c] GST activity determined at 37°C.

The eluted enzyme was dialyzed against buffer A and subjected to isoelectric focusing in a column (LKB Model No. 8100) with Ampholines in the pH range 3.5–10 using a 0–50% sucrose density gradient. After isoelectric focusing at 1,600 V for 20 h, 0.8-ml fractions were collected and monitored for pH and GST activity using CDNB as the substrate (Singh et al. 1988a). For kinetic studies, the purified isoenzymes were dialyzed against buffer A. Isoenzymes used in structural studies were dialyzed against distilled water containing 0.1% acetic acid.

Electrophoresis and Western Blotting. SDS–β-mercaptoethanol–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using the buffer system described by Laemmli (1970). The stacking and resolving gels contained 7.1% (wt/vol) and 12.5% (wt/vol) acrylamide, respectively. The concentrations of the cross linker, N,N¹-methylenebisacrylamide, in the stacking and resolving gels were 0.15% (wt/vol) and 0.35% (wt/vol), respectively. Western blots were done according to the method of Towbin et al. (1979) with slight modifications as described previously by Singh et al. (1988a).

SV-8 Protease Digestion. For digestion with staphylococcal V-8 protease, each dialyzed GST isoenzyme was incubated with 20 μl of SV-8 protease in 0.1 *M* ammonium bicarbonate (pH 7.8) at an enzyme/substrate ratio of 1:25 (wt/wt) for 2 h at 37°C. Proteolysis was terminated by lyophilization. Resulting peptides were dissolved in an appropriate volume of electrophoresis sample buffer and analyzed by SDS–PAGE (BioRad mini gel system) using the buffer system described by Laemmli (1970). The acrylamide concentration of the stacking gel was 7.1%. The top and bottom halves of the resolving gel contained 12.5 and 15% of acrylamide, respectively.

Automated Amino Acid Sequence Analysis. The N-terminal sequences of the purified GST isoenzymes were determined by microsequence analysis with protein–peptide sequencer (Applied Biosystems model 470A) according to the procedure described previously (Ahmad et al. 1990).

Statistical Analysis. Enzyme assay data were subjected to analysis of variance and Student's *t* test analysis (Steel & Torrie 1960).

Results

Larvae of both species had significant amounts of GST activity which was expressed toward a wide variety of electrophilic compounds including CDNB, 3,4-dichloronitrobenzene (DCNB), ethacrynic acid (EA), para-nitrobenzylchloride (p-NBC), 4-nitropyridine *n*-oxide (NPNO), bromosulfophthalein (BSP), and *trans*-4-phenyl-3-buten-2-one (TPBO) examined in this study (Table 1). The total GST activity as measured with the commonly used substrate CDNB was 31.12 ± 0.68 units/g wet weight for *E. loftini* and 24.8 ± 1.04 units/g wet weight for *D. saccharalis* ($\bar{x}$ ± SEM of three experiments). This indicated that the total GST activity of *E. loftini* was significantly higher ($t = 4.5$, df = 4, $P < 0.05$) than that of *D. saccharalis*. The specific activities of the crude homogenates of *E. loftini* were higher for CDNB, EA, p-NBC, NPNO, and TPBO. *D. saccharalis* had higher specific activities toward DCNB and BSP (Table 1). Both larvae had comparable activity toward γ-hexachlorocyclohexane, which is used as an insecticide for sugarcane in some parts of the world.

GST from both larvae were purified with affinity chromatography over a column of GSH linked to epoxy-activated Sepharose 6B resin according to the method described by Simons & VanderJagt (1977). Purification of GST from *D. saccharalis* larvae was achieved by GSH-affinity chromatography in an overall yield of ≈40%. When the enzyme purified from *D. saccharalis* was subjected to isoelectric focusing, two distinct peaks corresponding to the pI values of 9.3 and 8.0 were obtained (Fig. 1). The specific activity of the more

Table 2. Purification of GST isoenzymes of *D. saccharalis*

Fraction	GST activity[a]		Total protein, mg	Specific activity, units/mg protein	% Yield	Purification, fold
	Units/ml	Total units				
28,000 × *g* supernatant	6.0	120	162	0.74		—
Affinity chromatography	1.25	52.5	1.68	31.25	100	42
Isoelectric focusing[b]					43.75	
GST 9.3	1.014	10.14	0.217	46.73		—
GST 8.0	0.487	5.26	0.174	30.25	27.0	—

[a] One unit of enzyme used one μmol substrate/min at 25°C.
[b] Total units applied on the isoelectric focusing column were 25.

basic isoenzyme (pI 9.3) was higher than that of the less basic isoenzyme (pI 8.0). About 48-fold purification of the enzyme was obtained when the crude supernatant from *E. loftini* homogenates was subjected to affinity chromatography. When purified GST from *E. loftini* was subjected to isoelectric focusing, three distinct peaks of activity were observed (Fig. 2), corresponding to pI values of 9.7, 7.7, and 5.3. In increasing order, the specific activities of these isoenzymes toward CDNB were GST 9.7 > GST 7.7 > GST 5.3. The combined specific activity of the *E. loftini* total GSTs purified by GSH affinity was 51.0 units/mg protein compared with 31.2 units/mg protein for GSTs of *D. saccharalis*. Specific activities of the individual GST isoenzymes of *E. loftini* were also higher compared with the corresponding GST isoenzymes of *D. saccharalis* (Tables 2 and 3). Purified enzymes from both species expressed GSH-peroxidase activity toward cumene hydroperoxide. Some GST isoenzymes of insects are known to have GSH-peroxidase activity toward cumene hydroperoxide (Weinhold et al. 1990). Purified GSTs from both species also had GSH-peroxidase activity toward hydrogen peroxide.

Structural Properties. In gel filtration studies, the molecular weights of GST isoenzymes purified by GSH-affinity chromatography from both species were ≈50,000 (data not presented). In reduced denaturing gels, total purified GST isoenzymes of *D. saccharalis* obtained by GSH-affinity chromatography showed the presence of two polypeptide bands corresponding to 25,000 and 27,000 (data not presented). Individually, the more basic isoenzyme of *D. saccharalis* (pI 9.3) had a single band corresponding to an M_r value of 25,000 (Fig. 3, lane 2), whereas the isoenzyme with a pI value of 8.0 showed two distinct bands corresponding to M_r values of 25,000 and 27,000 (Fig. 3, lane 3). These results indicated that the more basic GST isoenzyme of *D. saccharalis* (pI 9.3) was a dimer of two identical-sized subunits, whereas the enzyme peak corresponding to the pI value of 8.0 was either a heterodimer of 25,000 and 27,000 subunits or represented a mixture of two homodimeric enzymes with subunit M_r values of 25,000 and 27,000 (Fig. 3). In SDS–PAGE, total GST isoenzymes of *E. loftini* obtained by GSH-affinity chromatography showed the presence of three polypeptide bands corresponding to M_r values of 25,000, 26,000, and 27,000 (data not presented). The GST isoenzyme of *E. loftini* having a pI value of 9.7 showed a single band corresponding to 25,000 M_r value (Fig. 3, lane 5). The GST isoenzyme of *E. loftini* (pI 7.7) also showed a single peptide band, but its M_r value corresponded to 26,000 (Fig. 3, lane 6). The *E. loftini* isoenzyme with a pI value of 5.3 showed two bands corresponding with M_r values of 26,000 and 27,000 (Fig. 3, lane 7). These results indicated significant differences in the subunit composition of the GST isoenzymes of the two insects. In Western blots, none of the GST isoenzymes of either species recognized the antibodies raised against any of the three classes (α, μ, and π) of human GSTs.

The SV-8 protease digests of the two isoenzymes of *D. saccharalis* were analyzed by SDS polyacryl-

Table 3. Purification of GST isoenzymes of *E. loftini*

Fraction	GST activity[a]		Total protein, mg	Specific activity, units/mg protein	% Yield	Purification, fold
	Units/ml	Total units				
28,000 × *g* supernatant	7.5	120	112.96	1.06	100	—
Affinity chromatography	1.64	68.88	1.35	51.0	57.4	48
Isoelectric focusing[b]						
GST 9.7	0.874	12.68	0.177	71.64	—	—
GST 7.7	0.4027	5.84	0.171	34.13	35.74	—
GST 5.3	0.3166	1.90	0.117	16.24	—	—

[a] One unit of enzyme used 1 μmol substrate/min at 25°C.
[b] Total units applied on the isoelectric focusing column were 32.8.

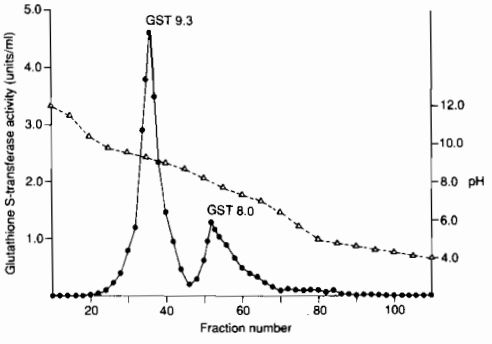

Fig. 1. Isoelectric focusing profile of GSH affinity-purified GST from *D. saccharalis*. The pH range for Ampholine used in isoelectric focusing was 3.5–10. △, pH gradient; ●, GST activity with CDNB as the substrate.

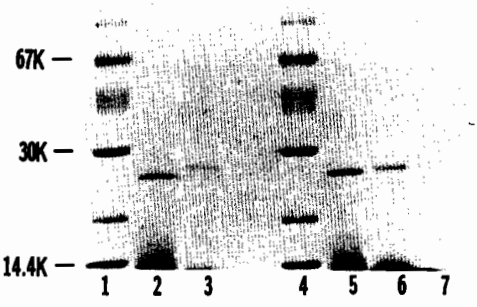

Fig. 3. SDS–β-mercaptoethanol–polyacrylamide slab gel electrophoresis of GST isoenzymes from *D. saccharalts* and *E. loftini*. Lanes 1 and 4, protein standards; lanes 2 and 3 contain GST 9.3 and 8.0, respectively, from *D. saccharalis;* lanes 5, 6, and 7 contain GST, pI 9.7, 7.7, and 5.3 of *E. lofttni*, respectively. K, thousand.

amide gel electrophoresis (Fig. 4). Although a number of protease-generated peptides were common for both the isoenzymes, some differences were seen in the peptide fingerprints. The GST 9.3 isoenzyme generated a peptide corresponding to an M_r value of 12,000, which was not observed in the peptide maps of GST 8.0 isoenzyme. These results indicated that the two isoenzymes were distinct proteins. The peptide maps of SV-8 cleaved *E. loftini* GSTs; pI 9.7, 7.7, and 5.3 are presented in Fig. 5. Despite the fact that all the three isoenzymes showed several common peptides, some marked differences among the SV-8-cleaved peptides of these isoenzymes were apparent. For example, the peptides corresponding to approximate M_r values of 22,000 and 16,000 were observed only in the maps of GST 9.7 (Fig. 5, lane 3). These results indicated primary structural differences in these isoenzymes, and differences in their pI values were not due to posttranslational modifications.

Kinetic Characteristics. K_m and V_{max} values for both CDNB and GSH for the purified total enzyme preparation obtained after GSH-affinity chromatography from both species are presented in Fig. 6. The K_m values of the purified GST of *E. loftini* for GSH and CDNB were found to be 0.26 m*M* and 0.19 m*M*, respectively. The K_m values of the purified enzyme from *D. saccharalis* were 0.29 m*M* and 0.16 m*M* for GSH and CDNB, respectively. These results indicate that the K_m of the purified enzymes for CDNB from both species was lower compared with the K_m values for CDNB of the human GST isoenzymes (Awasthi et al. 1980). However, the K_m values of both enzymes for GSH were in the same range as that reported for human enzymes (Awasthi et al. 1980). The V_{max} value of 40 μmol/min per mg of *D. saccharalis* enzymes for CDNB was also significantly higher compared with those reported for human enzymes. Likewise, the V_{max} value of *E. loftini* enzyme for CDNB (65

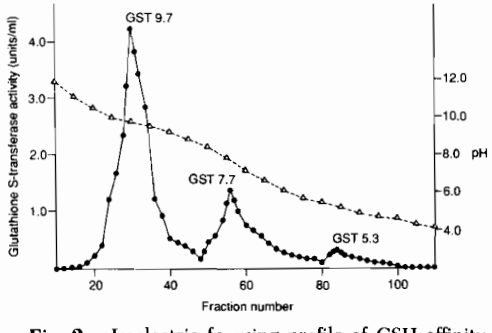

Fig. 2. Isoelectric focusing profile of GSH affinity-purified GST from *E. loftini*. Ampholine in the pH range 3.5–10 was used. △, pH gradient; ●, GST activity determined with CDNB.

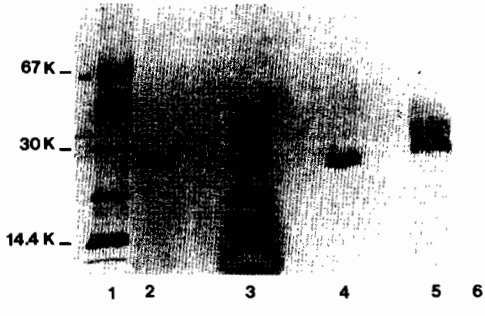

Fig. 4. SDS polyacrylamide gel electrophoresis of the SV-8 protease digests of *D. saccharalis* GST isoenzymes. Experimental details are indicated in the text. Lane 1, mixture of standard proteins; lanes 2 and 4, *D. saccharalis* GSTs Peak I and Peak II; lanes 3 and 5, SV-8 digests of GSTs of *D. saccharalis* Peak I and Peak II isoenzymes, respectively; lane 6, SV-8 protease only. K, thousand.

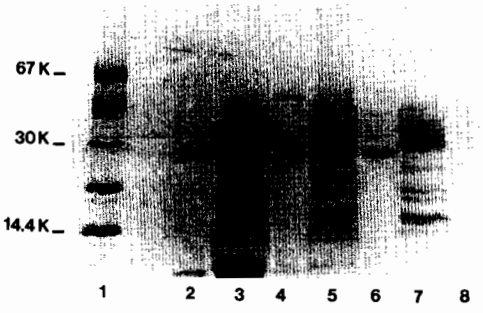

Fig. 5. SDS polyacrylamide gel electrophoresis of the SV-8 protease digests of *E. loftini* GST isoenzymes. Experimental details are given in the text. Lane 1, protein standards; lanes 2, 4, 6, *E. lofttni* GSTs Peak I, Peak II, and Peak III, respectively; lanes 3, 5, 7, SV-8 digests of GSTs of *E. loftini* Peak I, II and III isoenzymes, respectively; lane 8, SV-8 protease only. K, thousand.

μmol/min per mg) was also significantly higher than those of human GSTs (Awasthi et al. 1980).

The inhibitory characteristics of both species' GSTs purified by GSH-affinity were also studied with some of the inhibitors known to inhibit GST. 2,4-dichlorophenoxy acetate (2,4-D), and 2,4,5-trichlorophenoxy acetate (2,4,5-T) are known to be inhibitors of GST from higher organisms (Singh & Awasthi 1985). GST isoenzymes of *D. saccharalis* as well of *E. loftini* were inhibited by both these herbicides (Table 4). The I_{50} values and K_i for the most basic isoenzymes from both species were comparable for both 2,4-D and 2,4,5-T, indicating that the basic isoenzymes from both species had similar kinetic characteristics. This is further reflected by inhibition of these isoenzymes by the bile acid chenodeoxycholate. The inhibitory effect of 2,4,5-T and 2,4-D on the isoenzymes, pI 8.0 of *D. saccharalis* and pI 7.7 of *E. loftini*, were also found to be similar (Table 4).

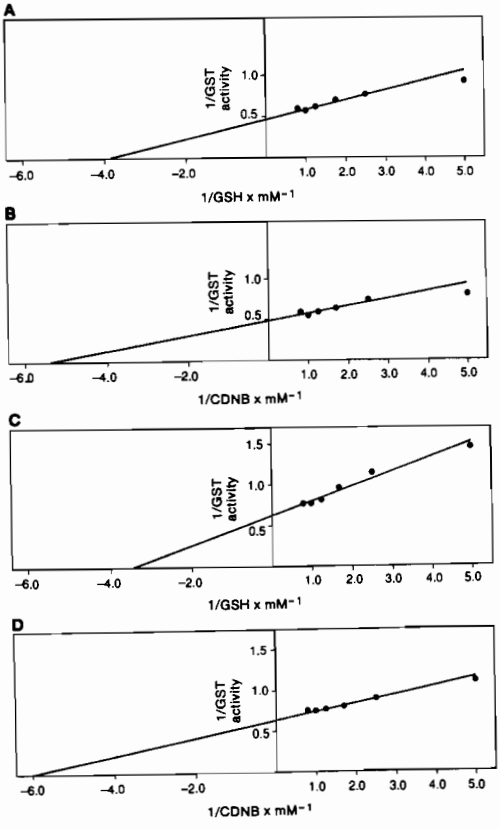

Fig. 6. Lineweaver–Burk plot of GSH-affinity purified total GST from *D. saccharalis* and *E. lofttni*. GST activity, product formed (μmol/min per ml); CDNB and GSH, substrate concentration (m*M*). Each point represents the mean of three determinations. (A, B) *E. lofttni*. (C, D) *D. saccharalis*.

Discussion

Both *E. loftini* and *D. saccharalis* larvae had significantly high levels of GST activity to a number of electrophilic substrates. Data presented in Table 5 show the comparison of GST activities toward CDNB from these two insect species and from higher organisms. Enzyme activity (expressed

Table 4. Kinetics of inhibition of GST isoenzymes of *D. saccharalis* and *E. loftini*

Isoenzyme	Inhibitors								
	2,4-D			2,4,5-T			Chenodeoxycholate		
	K_i, m*M*	I_{50}, m*M*	Type[a]	K_i, m*M*	I_{50}, m*M*	Type[a]	K_i, m*M*	I_{50}, m*M*	Type[a]
D. saccharalis									
pi 9.3	0.44	0.76	Uncompetitive	0.09	0.14	Noncompetitive	0.30	0.41	Noncompetitive
pi 8.0	0.53	0.66	Noncompetitive	0.27	0.36	Competitive	0.42	0.54	Noncompetitive
E. loftini									
pi 9.7	0.60	1.36	Uncompetitive	0.26	0.52	Competitive	0.24	0.30	Noncompetitive
pi 7.7	0.50	1.12	Uncompetitive	0.28	0.64	Competitive	0.54	0.60	Noncompetitive
pi 5.3	0.70	0.78	Noncompetitive	0.25	0.40	Competitive	0.38	0.51	Noncompetitive

[a] Type of inhibition with respect to CDNB. 2,4-D, 2,4-dichlorophenoxy acetate 2,4,5-T, 2,4,5-trichlorophenoxy acetate.

Table 5. Comparison of GST activities of *D. saccharalis* and *E. loftini* larvae with those of rat and human tissue

Tissue	μmol/min per g wet wt	Reference
D. saccharalis	24.8	Current study
E. loftini	31.12	Current study
Human muscle	6.5	Singh et al. (1988a)
Human liver	14.0	Singhal et al. (1990)
Human kidney	13.1	Singh et al. (1987)
Human lung	2.2	Gupta et al. (1990)
Human brain	3.8	Theodore et al. (1985)
Rat pancreas	2.4	S. Gupta & Y.C.A., unpublished data
Rat liver	26.7	Awasthi et al. (1983)
Rat kidney	3.8	Partridge et al. (1983)
Rat lung	1.0	Partridge et al. (1983)

in units per gram wet weight of tissue) of these insect species is about twice as high as that in human liver, which is the major detoxification organ. The spectrum for substrate specificities of GSTs from both the species in this study appear to be somewhat similar. GST activities of lepidopterous insects are known to vary considerably among themselves, and the values reported in this study for *D. saccharalis* and *E. loftini* fall within the range of values reported by Yu (1989) for five lepidopterous insects. Total GST activities for both *E. loftini* and *D. saccharalis* were higher than those from velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, tobacco budworm, *Heliothis virescens* (F.), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), but lower than those of corn earworm, *Helicoverpa zea* (Boddie), and cabbage looper, *Trichoplusia ni* (Hübner) (Yu 1989). The low K_m values of *D. saccharalis* as well as *E. loftini* GSTs for CDNB indicate that the enzymes have a high affinity for electrophilic substrates compared with most of the rat and human enzymes. The K_m reported for CDNB is in the range of 0.4–0.8 m*M* for most of the rat and human enzymes (Habig et al. 1976, Awasthi et al. 1980). These values are higher than the K_m values of *E. loftini* and *D. saccharalis* determined in this study. The higher V_{max} values for CDNB for *D. saccharalis* as well as *E. loftini* and the lower K_m values indicate that the insect enzyme has a higher catalytic efficiency compared with rat and human GSTs.

In *D. saccharalis*, GST activity is expressed by two basic isoenzymes, whereas an acidic form of the isoenzymes in *E. loftini* is also present. This is consistent with the results of subunit studies, which indicate that two different kinds of GST subunits are present in GSTs of *D. saccharalis* rather than the three distinct types of GST subunits in *E. loftini*. Results of SV-8 peptide fingerprint analyses indicate that these isoenzymes are distinct proteins rather than being posttranslational modification products. Results on the N-terminal amino acid analysis of *E. loftini* as well as *D. saccharalis* GST subunits indicated that their N-termini were blocked. Among human, rat, and mice GSTs, the N-termini of α and ζ class GSTs are blocked (Mannervik & Danielson 1988, Singh et al. 1988a, Singhal et al. 1990). The α class GSTs of humans and rodents express GSH-peroxidase activity toward lipid hydroperoxides (Mannervik & Danielson 1988, Singhal et al. 1990). The expression of GSH-peroxidase activity toward cumene hydroperoxide as well as H_2O_2 by these insect enzymes may indicate their functional relatedness to the α class GSTs of higher organisms. Because the antibodies raised against the α, μ, and π classes of human GSTs failed to recognize any of the subunits of *D. saccharalis* or *E. loftini*, the primary structures of these insect GSTs appear to be considerably different from human GSTs.

Results of these studies strongly suggest the important physiological role of GST in the detoxification of various electrophilic chemicals by these insects. Despite significant differences in GST isoenzyme profiles of *D. saccharalis* and *E. loftini*, the kinetic characteristics of both these enzymes are remarkably similar. Both species not only have high concentrations of enzyme, but the isoenzymes present in both these species are catalytically efficient compared with that observed for plant enzymes (S.S.S. & Y.C.A., unpublished data) and the enzymes in higher organisms (Awasthi et al. 1980). *E. loftini* has significantly higher GST activity than *D. saccharalis*. The kinetic characteristics of GSTs of *D. saccharalis* and *E. loftini* also compared well with those of corn earworm and tobacco budworm reported by Yu (1989). Similar to corn earworm and tobacco budworm GSTs, both *E. loftini* and *D. saccharalis* had lower K_m values and higher V_{max} values for CDNB compared with some other Lepidoptera such as velvetbean caterpillar and fall armyworm (Yu 1989), which indicate higher catalytic efficiency of these enzymes. Noncompetitive inhibition of most of the *D. saccharalis* and *E. loftini* GST isoenzymes by 2,4-D and 2,4,5-T indicated that these herbicides could hamper the capabilities of both insects for detoxifying various electrophilic toxicants. Although the use of 2,4,5-T has been discontinued, 2,4-D is still used in some of the cash crops, including corn. Possibly, 2,4-D may augment the effect of various insecticides because of its inhibitory effect on GSTs.

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