Purification, molecular cloning and heterologous expression of a glutathione S-transferase from the Australian sheep blowfly (*Lucilia cuprina*)

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Three glutathione S-transferases from *Lucilia cuprina* (Australian sheep blowfly) pupae were purified by affinity chromatography and anion-exchange chromatography. One isoenzyme was composed of M_r -24800 subunits, and two isoenzymes had subunits of M_r 23900. The M_r -23900 subunits showed immunological identity and were immunologically distinct from the M_r -24800 subunits. All three enzymes were active with the substrate 1-chloro-2,4-dinitrobenzene and had low activity with 1,2-dichloro-4-nitrobenzene. A cDNA clone encoding a M_r -23900 subunit (LuGST1) was isolated and sequenced. The sequence has close

similarities (> 81 %) to that of GSTs from the fruitfly *Drosophila melanogaster* and *Musca domestica* (housefly). The deduced amino acid sequence of the Lu GST1 subunit showed no significant similarity to that of the mammalian GSTs to the Alpha, Mu and Pi classes, but shows some similarity (33 %) over the first 100 residues with the rat subunit 12 Theta-class GST. Southern blots of genomic DNA hybridized with the LuGST1 cDNA identified many hybridizing fragments. Taken together, these data indicated that the *L. cuprina* genome contains multiple glutathione S-transferase genes.

INTRODUCTION

The glutathione transferases (GST) are a family of enzymes that catalyse the conjugation of GSH to a variety of electrophilic compounds. The mammalian species studied so far express a number of isoenzymes with some overlapping substrate specificities (Mannervik and Danielson, 1988; Board et al., 1990). These isoenzymes have been implicated in the detoxication and biotransformation of many xenobiotics, including several carcinogens and a number of therapeutic drugs [for a review, see Tsuchida and Sato (1992)]. The mammalian cytosolic GSTs are dimers and can be classified into a number of classes based on their structure and substrate specificities (Mannervik and Danielson, 1988).

GSTs appear to be found in multiple forms in most eukaryotes, and their presence in insect species has been reviewed by Clark (1989). Several studies have correlated resistance to insecticides with increased levels of GST activity and the production of different isoforms. These studies have involved organochlorines (Tanaka et al., 1976; Clark and Shamaan, 1984), organophosphates (Motoyama and Dauterman, 1974; Oppenoorth et al., 1977, 1979), and other xenobiotics (Ottea and Plapp, 1981).

The sheep blowfly, *Lucilia cuprina*, is a major economic problem for the Australian wool industry. The long-term use of organophosphorus insecticides to control fly strike has resulted in the development of resistance to this class of insecticide (Arnold and Whitten, 1976; Hughes and Raftos, 1985; Raftos, 1986; Russell et al., 1990). Since GSTs have been implicated in the expression of insecticide resistance in the housefly (*Musca domestica*) (Oppenoorth et al., 1977, 1979; Motoyama and Dauterman 1980), it was of interest to study the GSTs of *L. cuprina*.

We have identified three GSTs in *L. cuprina* pupae that fall into two immunologically distinct groups. A cDNA encoding an isoenzyme with high activity for 1-chloro-2,4-dinitrobenzene

(CDNB) has been cloned and its sequence shows strong similarity to that of GST 1-1 from *Drosophila melanogaster* (Toung et al., 1990) and GST-1 from *Musca domestica* (Wang et al., 1991).

EXPERIMENTAL

Materials

All chemicals used were of analytical grade and readily available from commercial sources. A λ zap *L. cuprina* pupal cDNA library was generously provided by Dr. Peter Atkinson, CSIRO Division of Entomology, Canberra, Australia. All DNA-modifying enzymes used are commercially available.

The *L. cuprina* used in this study were from the LS-2 strain (Parker et al., 1991). Larvae were raised on sheep liver, and at the third-instar stage were allowed to pupate in vermiculite. The pupae were stored at -70° until used for protein purification.

Enzyme assays

Enzyme assays were carried out at 30°C . The methods of Habig and Jacoby (1981) were used for the measurement of GST activity with CDNB, 1,2-dichloro-4-nitrobenzene (DCNB) and ethacrynic acid. GST activity with *trans*-alk-2-enals and *trans*-, *trans*-alk-2,4-dienals were determined by the procedures described by Brophy et al. (1989). Glutathione peroxidase was determined with *t*-butyl hydroperoxide by the method described by Beutler (1975).

Enzyme purification

The *L. cuprina* GSTs were isolated by affinity chromatography on GSH-agarose (Simons and Vander Jagt, 1977). Typically 5 g of pupae were homogenized in 50 mM Tris/HCl buffer, pH 7.2, containing 10 mM β -mercaptoethanol. The homogenate was centrifuged for 20 min at 10000 *g* and the supernatant was

Abbreviations used: GST, glutathione S-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; IPTG, isopropyl thio- β -D-galactoside.

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The sequence of LuGST1 has been submitted to the GenBank Nucleotide Sequence Database under the accession number L23126.

immediately applied to a 20 ml column of GSH-agarose equilibrated in 50 mM Tris/HCl, pH 7.2. The column was washed with several bed volumes of 50 mM Tris/HCl, pH 7.2, until the eluate was free of protein as determined by A_{280} measurement. The GST isoenzymes were then eluted by addition of 5 mM GSH in 50 mM Tris/HCl, pH 9.6. Preliminary studies showed that addition of a pH 9.6 washing step, as advocated by Simons and Vander Jagt (1977), resulted in the elution of at least one larger isoenzyme.

The affinity-purified fraction was concentrated by filtration on an Amicon UM10 filter and then subjected to ion-exchange chromatography on an f.p.l.c. mono Q column (Pharmacia). The column was equilibrated in 20 mM Tris/HCl, pH 7.2, and the protein was eluted with a gradient from 0 to 250 mM NaCl in the Tris buffer. The elution was monitored by continuous A_{280} measurement, and GST activity was determined with CDNB. GST activity was found to be associated with the major peaks of protein.

Electrophoresis and Immunoblotting

SDS/PAGE was carried out using the buffer system of Laemmli (1970) in 12% gels. Electrophoresis of fractionated proteins on to nitrocellulose was carried out by the method of Towbin et al. (1979), and specific proteins were identified by the method of Board (1984) using antiserum raised in rabbits against *L. cuprina* GST and a second goat anti-rabbit IgG-alkaline phosphatase conjugate.

cDNA library screening

A cDNA library prepared from LS-2 strain *L. cuprina* pupae in the λ Zap vector (Stratagene) was screened with affinity purified antibodies to *L. cuprina* GST. The methods used for the affinity purification of specific antibodies and the detection procedure have been previously described in detail by Board and Webb (1987). Positive plaques were picked and rescreened until pure cultures were obtained.

DNA sequencing

All cDNA fragments were subcloned into M13 phage mp18 or mp19 to generate single-stranded DNA templates for sequencing. Sequencing was carried out by the dideoxy-chain-termination procedure of Sanger et al. (1980). The cDNA sequence was obtained from both strands, and all restriction sites used in subcloning were contained in overlapping sequences.

Expression of recombinant GST

A ribosome-binding site was added upstream of the ATG translation initiation codon and a *SalI* site was added downstream of the TAA termination codon by PCR (Saiki et al., 1985). The 5' oligonucleotide primer had the sequence 5'-AGGAGAC-TGCTATCATGGATTCTACTAC, and the 3' oligonucleotide primer had the sequence 5'-AAGTCGACTTTAGTTGAAG-AATTC. The amplified DNA was cloned between the *SmaI* and *SalI* sites downstream of a *tac* promoter in the plasmid pKKPS (Board et al., 1990). The resulting plasmid pLUC1-EX was transformed into *E. coli* TG1 and was grown in the presence of isopropyl thio- β -D-galactopyranoside (IPTG) to induce expression of recombinant *Luc* GST1-1, as previously described by Board and Pierce (1987). The amplified cDNA fragment cloned in pLUC1-EX was sequenced to confirm that no mutations had

been introduced into the sequence by the PCR procedure. The recombinant GST was purified by affinity chromatography on GSH-agarose as described above.

Southern blots

Genomic DNA was isolated from *L. cuprina* eggs by the procedure of Davis et al. (1986), digested with restriction enzymes and size-fractionated by electrophoresis through agarose gels. The DNA was blotted on to nylon membranes (Gene Screen Plus; Du Pont) and probed with a 32 P-labelled cDNA probe. The methods used are standard and have been described in detail by Sambrook et al. (1989).

Native size determination

Purified recombinant GSTs were passed through an f.p.l.c. Superose-12 column (Pharmacia). Elution volumes were determined in a buffer consisting of 150 mM NaCl/10 mM sodium phosphate, pH 7.2, in comparison with protein standards of known size (BSA, M_r 66000; ovalbumin, M_r 45000; trypsinogen, M_r 24000).

Isoelectric focusing

Isoelectric focusing was carried out in solution in a Rotofor apparatus (Bio-Rad). Pupae were homogenized in water and, after removal of particulate matter by centrifugation, the extract was combined with a pH 3–10 or pH 4–6.5 ampholyte solution and focused at 12 W for 4 h. The pH and GST activity were determined in the 20 fractions collected at the end of the experiment. Alternatively, purified enzymes were applied to isoelectric-focusing gels on a PHAST electrophoresis apparatus (Pharmacia).

RESULTS

GST purification

Affinity purification of pupal extracts on GSH-agarose produced a fraction with high GST activity for CDNB. As shown in Figure 1, a significant proportion of the material with CDNB activity passed through the affinity column. As this unbound material was not retained by passage through an additional affinity column, the lack of binding was not apparently due to overloading of the original affinity matrix. The failure of a significant fraction of GST to bind to GSH-agarose was observed repeatedly in several experiments. The possibility of purifying the unbound fraction on other affinity matrices has yet to be tested. The affinity-purified fraction contained at least five proteins detectable by SDS/PAGE (lane 2, Figure 2). The most abundant of these proteins had M_r values of 24800 and 23900 and were in the size range expected for GST subunits. The affinity-purified fraction was concentrated and subjected to ion-exchange chromatography on an f.p.l.c. mono Q column. Three major protein peaks were resolved, and each peak contained GST activity for CDNB (Figure 3). SDS/PAGE of the three fractions (Figure 2) indicated that peak 1 contained the larger M_r -24800 protein, while the proteins in peaks 2 and 3 appeared to have an M_r identical with that of the smallest protein in the affinity-purified fraction. Thus, on the basis of size and charge, three distinct GSTs were identified in the affinity-purified fraction. A summary of the purification of these isoenzymes is presented in Table 1.

In preliminary studies the affinity matrix was washed with 50 mM Tris, pH 9.6, prior to elution with 5 mM GSH. The high-

Figure 1 Affinity chromatography on GSH-agarose

GSH-agarose (20 ml) was equilibrated with 50 mM Tris/HCl buffer, pH 7.2, containing 10 mM β -mercaptoethanol. A pupal cytosol preparation was applied to the column and eluted with the equilibration buffer. Fractions of approx. 4 ml were collected and the A_{280} ($\square$) and activity towards CDNB ($\blacklozenge$) were monitored. The eluting buffer was changed to 5 mM GSH in 50 mM Tris/HCl, pH 9.6, at the position of the vertical arrow.

Figure 2 SDS/PAGE analysis of *L. cuprina* GSTs

Electrophoresis was performed in a 12% gel. Protein size markers are shown in lane 1. Lane 2 contained the affinity-purified fraction. Lane 3 contains affinity-purified recombinant LuGST1-1. Lanes 4, 5 and 6 contain peaks 1, 2 and 3 respectively from mono Q anion-exchange chromatography (see Figure 3).

pH washing step eluted the M_r -24800 protein. The M_r -23900 protein was subsequently eluted with GSH, and this fraction was used initially to generate antibodies.

Isoelectric point

Isoelectric focusing of pupal extracts over a wide range (pH 3–10) in a Rotofor apparatus indicated that the GST isoenzymes with activity for CDNB are generally acidic. Narrow-range

Figure 3 Purification of *L. cuprina* pupal GSTs by anion-exchange f.p.i.c.

The material purified by affinity chromatography was applied to an f.p.i.c. mono Q column equilibrated with 20 mM Tris/HCl, pH 7.2, and the protein was eluted with a 0–0.25 M NaCl gradient in the equilibration buffer. A_{280} was monitored continuously and fractions of 2 ml were collected. Activity with CDNB was detected in peaks 1, 2 and 3.

Table 1 Purification of GSTs from *Lucilia cuprina* pupae

Stage	Total protein (ml)	Total protein (mg)	Specific activity ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	Yield (%)
Homogenate	46	300	0.3	100
GSH-agarose	3.8	2.3	12.3	31.5
Mono-Q				
Peak 1	3	0.47	2.3	1.2
Peak 2	3	0.22	9.6	2.3
Peak 3	3	0.3	5.7	1.9

isoelectric focusing (pH 4–6.5) suggested two peaks of GST activity, with pI values in the range 4.3–4.7 and 4.9–5.2. Isoelectric focusing of the purified recombinant Lu GST1-1 isoenzyme on polyacrylamide gels indicated a pI of pH 5.2, which is in agreement with the values obtained from the crude unpurified isoenzymes (results not shown).

cDNA cloning

Screening of a *L. cuprina* cDNA library with an antibody to the M_r -23900 protein fraction identified five clones expressing cross-reacting proteins. One of these clones was sequenced completely and was found to contain a 790 bp cDNA that encoded a complete GST subunit that has been termed 'LuGST1' (Figure 4). The other four clones have not yet been studied in detail. The coding sequence was flanked by 11 bp of 5' and 152 bp of 3' non-coding sequence. The deduced amino acid sequence of 208 residues has a calculated M_r of 23702. The amino acid sequence of LuGST1 is 81 % similar to that of a GST from *D. melanogaster* (Toung et al., 1990), and 91 % identical with that of a GST from the housefly (*Musca domestica*) (Wang et al., 1991). Although the

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      M D F Y Y L P G S T P Y H S V L      16
TAAATAAACATGGATTCTACTTGCCTCCCTCCCTACCTCAGTCTTGA      60
      M T A K A L G I E L N K K L N L Q A G      36
TGACTGCTAAAGCTCTTGGCATTGAGCTTAACAAGAAATTTGGAAGCTGCAAGCTGGCG      120
      E H L K P E F L K I N P Q H T I P T L V      56
AACACTTGAAACCTGAATCTTGAAGATCAATCCCCAGCACACCATTCCTCCTTTGGTGC      180
      D G D F A L W E S R A I M V Y L V E K Y      76
ATGGTGACTTCGCTTGTGGGAATCTCGTGCCATTATGGTTTACTTGGTTGAGAAGTACG      240
      G K N D S L F P K C P K K R A V I N Q R      96
GTAAGAACGACTCCTGTTCCTCAAGTGCCTCAAGAGCGCGCTCATCAACCAACGTT      300
      L Y F D M G T L Y K S F A D Y Y Y P Q I      116
TGACTTCGATATGGTACCTCTACAAGAGCTTCGCTGACTACTACCCACAAATCT      360
      F A K A P A D P E L Y K K M E A A F D F      136
TCGCAAGGCTCCCGTGATCTGAGTTGTACAAGAAATGGAAGCTGCCTTCGATTCT      420
      L N T F L E G H Q Y V A G D S L T V A D      156
TGAACACCTTCTTGAAGGTACCAATACCTGCTGGTGATTCTTGCCTTCCGCTT      480
      L A L L A S V S T F E V A G F D F S K Y      176
TGCTTTGTTGGCAGTGTACAGCCTTTGAAGTTGCCGTTTCGATTTCAGCAAATATG      540
      A N V A K W Y A N A K T V A P G F D E N      196
CCAATGTGCTAAGTGTACGCTAATAGCTAGACTGTTGCTCCCGTTTCGATGAAACT      600
      W E G C L E F K K F F N      208
GGGAAGTTGCTTGAATTAAGAAATCTTCACTAAATATAAGTTATTATTTTGT      660

      TTTTTCATTATTTTAAATTTTAAATTTATATTTTAAATTTAGAGTTAAAA      720
      CAATGCACAATATTATTTCTAATTTCTTAGAAAAATAATAAAAAAATATTTTCT      780
      AAAAAAATT      790

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Figure 4 cDNA nucleotide sequence and deduced amino acid sequence of LuGST1

A poly(A) tail has been deleted from the 3' end of the nucleotide sequence.

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Lu 1  MDFFYYLPGSTPYHSLMTAKALGIELNKKLLNLQAGEHLKPEFLKINPQH      50
      | | | | | | | | | | | | | | | | | | | | | | | | | |
Ra 12 MELYLDLLSQPSRAVYIFAKKNGIPFQLRTVDLLKGQHLSEQFSQVNCCLK

Lu 1  TIPTLVGDGFALWESRAIMVYLVEKYGNDSLFPKCPKKRAVINQRLYFD      100
      | | | | | | | | | | | | | | | | | | | | | | | | | |
Ra 12 KVPVLKDGSLVLTSTAILIYLSKQYQVADHWYPADLQARAQVHEYLGW

Lu 1  MGTLYKSFADYYYPQIFAKAPADPELYKKMEAFDFLNTFLEGHQYVAGD      150
      | | | | | | | | | | | | | | | | | | | | | | | | | |
Ra 12 ADNIRGTGVLWLTGVLGPLIGVQVPEEKVERNRRNSMVLALQRLEDKFLR

Lu 1  SLTVADLALLASVSTFEVAGDFDSKYANVAKWYANAKTVAPGFDENWEGC      200
      | | | | | | | | | | | | | | | | | | | | | | | | | |
Ra 12 DRAFIAGQQVTLDLMSLEELIQPVALGCNLFEGRPQLTAWRERVEAFLG

Lu 1  LEFKKFFN      208
      |
Ra 12 AELCQEAHNPIMSVLGQAAKKTLVPVPEAHASMMRLRIARIP

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Figure 5 Alignment of the amino acid sequences of LuGST1 (Lu1) and rat subunit 12 (Ra 12)

Identical residues are indicated by a vertical line.

LuGST1 amino acid sequence has little similarity to the Alpha-, Mu- and Pi-class GSTs from mammals, there is a notable (33%) similarity with the first 100 residues of the rat Theta-class subunit 12 (Figure 5) (Ogura et al., 1991).

Expression of recombinant LuGST1-1

The LuGST1 cDNA sequence was cloned downstream of a *tac* promoter, and the resulting plasmid, pLuGST1EX, was used to generate recombinant LuGST1-1 protein. Preliminary studies indicated that the recombinant enzyme was active with CDNB as

Figure 6 SDS/PAGE and Western-blot analysis of *Lucilia cuprina* GSTs

The gel loading is identical with that in Figure 2. Size markers are shown in Lane 1. Lane 2 contains the affinity-purified fraction. Lane 3 contains affinity purified recombinant LuGST1-1. Lanes 4, 5 and 6 contain peaks 1, 2 and 3 respectively from the Mono Q anion-exchange chromatography step (see Figure 3). The blot was developed with antiserum affinity purified on immobilized recombinant LuGST 1-1 (lane 3).

a substrate. The recombinant LuGST1-1 was purified by affinity chromatography for further characterization. The purified recombinant enzyme was homogeneous after SDS/PAGE and had a subunit electrophoretic mobility equivalent to, or slightly greater than that of the GSTs in peaks 2 and 3 from mono Q ion-exchange chromatography (Figure 2).

The recombinant GST was subjected to gel filtration on an f.p.l.c. Superose-12 column in comparison with standard proteins of known size (results not shown). The estimated M_r of the *L. cuprina* GST was 38000, which is clearly considerably larger than the M_r -23702 monomer and in the range expected for a dimer.

Immunological relationships

Western blots of SDS/PAGE gels were probed with antiserum raised against the M_r -23900 subunit complex and purified by affinity chromatography on immobilized recombinant LuGST1-1. As shown in Figure 6, the antiserum cross-reacted with the recombinant GST and the subunits found in peaks 2 and 3. There was no evidence for cross-reactivity with the higher- M_r (24800) protein from pupal extracts or peak 1 (lanes 2 and 4). In order to study these differences further, the protein from peak 1 was used to immunize a rabbit on two occasions. Surprisingly, in each case the serum obtained failed to cross-react with any GST. These data suggest that the M_r -23900 proteins fractionated in peaks 2 and 3 share some structural features, while the M_r -24800 protein in peak 1 appears to be structurally distinct.

Substrate specificities

The purified isoenzymes used a number of substrates, and their specific activities are compared in Table 2. The data indicate that, as is the case with many other GST isoenzymes, CDNB appears to be the substrate giving highest activity. It is evident that cloned LuGST1-1 has a higher specific activity with most substrates than the affinity-purified fraction or the isoenzymes in peaks 1, 2 and 3. The levels of activity in peaks 1, 2 and 3 were generally the lowest. This difference may result from the partial inactivation of the pupal isoenzymes during purification.

Although no substantial differences in specific activity were detected between the different isoenzymes in peaks 1, 2 and 3 with the most substrates, the peak 2 isoenzyme had noticeably lower activity with *t*-butyl hydroperoxide and *trans*-2-nonenal. The results of the immunological studies and the comparison of the subunit size show that the recombinant LuGST1-1 corre-

Table 2 Specific activities of purified natural and recombinant GSTs from *Lucilia cuprina*

Enzyme activity is expressed as $\mu\text{mol}/\text{min}$ per mg of protein at 30 °C. All values are the means of at least two determinations.

Substrate	Enzyme activity				Recombinant LuGST 1-1
	Total affinity-purified fraction	Peak 1	Peak 2	Peak 3	
CDNB	6.7	2.4	9.5	5.7	44.3
DCNB	0.62	0.13	0.15	0.25	0.8
Ethacrynic acid	0.3	0.78	0.75	1.15	1.17
t-butyl hydroperoxide	0.18	0.11	0.03	0.21	0.02
Decadienal	0.41	0.06	0.06	0.04	0.96
Octenal	0.53	0.32	0.52	0.47	1.12
Nonenal	0.6	0.17	0.06	0.49	1.09

Figure 7 Southern-blot analysis of *Lucilia cuprina* GST genes

DNA was digested with *Eco*RI (lane 1) and *Hind*III (lane 2) and, after fractionation in agarose gels, was blotted and hybridized with a ^{32}P -labelled LuGST1 cDNA fragment.

sponds with either peak 2 or peak 3. Analysis of the substrate specificities does not, however, clearly differentiate between these possibilities.

Southern blots

Southern blots of genomic DNA digested with *Eco*RI or *Hind*III were probed with the LuGST1 cDNA, and each revealed at least seven hybridizing fragments in the *L. cuprina* genome (Figure 7). These fragments exhibited large differences in the strength of their hybridization signals.

DISCUSSION

L. cuprina pupae contain several GSTs that use CDBN as a substrate. It is also evident that a significant proportion of the CDBN activity is not retained by a GSH affinity matrix, and the

isoenzyme(s) responsible for that activity have yet to be characterized. In a study of the GSTs of the housefly (*M. domestica*), Clark et al. (1990) also found that a significant fraction of material with CDBN activity was not retained by a GSH affinity matrix. In the present studies, ion-exchange chromatography of the GSH-binding GSTs identified one isoenzyme with subunits of M_r 24800 and two isoenzymes with subunits of M_r 23900. The two isoenzymes with M_r -23900 subunits (peaks 2 and 3) differ in charge, but appear to be immunologically related. Comparison of their activities with different substrates suggests that there are some differences in specificity, especially for t-butyl hydroperoxide and *trans*-2-nonenal. Since the enzyme preparations were prepared from multiple pupae, it is possible that peaks 2 and 3 may represent genetic heterogeneity and may be allelic variants of the same isoenzyme subunit. Kotze and Rose (1989) previously reported the presence of two GST isoenzymes in *L. cuprina* larvae. These isoenzymes may correspond to the M_r -24800 and M_r -23900 proteins detected in the affinity-purified fraction from *L. cuprina* pupae in the present study as our preliminary studies (results not shown) showed a similar distribution of enzymes in late stage larvae and pupae. Kotze and Rose (1989) also noted a significant difference in the activities of the two isoenzymes with CDBN and DCNB as substrates. None of the isoenzymes purified in the present study showed particularly strong activity with DCNB. However, the ratio of DCNB to CDBN activity was higher in the peak 1 isoenzyme. In unpublished studies we have not detected significant differences in the total GST activity with CDBN or DCNB as substrates between control and organophosphate-resistant fly strains. Although correlations between organophosphate resistance and GST activity have been noted in the housefly (Motoyama and Dauterman, 1974; Oppenoorth et al., 1977, 1979), further studies comparing the activities and expression of individual isoenzymes in control and resistant strains of *L. cuprina* will need to be completed before it will be clear if organophosphate resistance is related to GST expression.

The sequence of the *L. cuprina* GST has strong similarity (> 81 %) with other insect GST sequences. (Toung et al., 1990; Wang et al., 1991). There is little similarity between the insect GST sequences and the mammalian Alpha, Mu- and Pi-class GSTs. However, as shown in Figure 6, there is some similarity (33 %) between the first 100 residues of the *L. cuprina* GST and the rat subunit 12 Theta-class GST (Ogura et al., 1991). It is noteworthy that the LuGST 1-1 cloned and characterized here is retained by a GSH affinity matrix and utilized CDBN as a substrate. In contrast the rat Theta-class 12-12 isoenzyme has neither of these properties. As the structures of either the *L. cuprina* gene or the rat Theta-class genes are not yet known, it is not clear if the loss of similarity in the C-terminal halves of the subunit sequences is aligned with the intron-exon boundaries. However, this loss of similarity suggests that there may have been a major rearrangement in one of these GST genes during evolution.

The existence of GST subunits of various sizes and the failure of some GST activity to bind to the affinity matrix indicates that *L. cuprina* has several GST genes. The multiple hybridizing fragments detected in the Southern blots also suggests that several GST genes are encoded in the *L. cuprina* genome. However, these data do not indicate whether some hybridizing fragments represent different regions of the same gene. Toung et al. (1991) have shown that the *D. melanogaster* GST (DmGST 1-1) is encoded by an intronless gene. If a similar situation exists in the *L. cuprina*, hybridizing fragments may provide an upper estimate of the number of GST genes in this species. There is a large range in the hybridization signals obtained for different

fragments on the Southern blots. This may reflect the length of the hybridizing sequence in each fragment, or may indicate large variations in sequence identity between the probe and the target genes.

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