# Quantitative profiling of tissue- and gender-related expression of glutathione S-transferase isoenzymes in the mouse

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Cytosolic glutathione S-transferase (GST) isoenzymes from brain, heart, lung, liver, kidney and gonads of male and female CD-1 mice were identified and quantified with a combination of affinity purification, electrospray ionization MS, Edman micro-sequencing, Western blot analysis and reverse-phase HPLC. The three principal hepatic GST subunits, mGSTA3 (25271 Da), mGSTP1 (23478 Da), and mGSTM1 (25839 Da), were isolated from liver, lung, kidney, testes and female heart, whereas brain, ovaries and male heart contained mGSTM1 and mGSTP1. Additional isoenzymes were detected in tissues, including mu class subunits mGSTM2 (25580 Da) and mGSTM3 (25570 Da), an N-terminally blocked Alpha subunit (25480 Da) assigned as mGSTA4, and proteins of molecular masses 25490, 22540, 24493, 24378 and 25383 Da. Distinct gender differences in expression of GST subunits were observed for liver, heart, kidney and gonads, whereas GST expression was similar in brain and lung for both genders. In contrast with patterns of expression in liver (high ratio of mGSTA3 to mGSTP1 in females relative to males), mGSTP1 was the most abundant subunit in female gonads, whereas mGSTA3 was not present in detectable quantities. The profile of GST expression in kidney was similar to that in liver; however, male kidneys expressed 30% more soluble GST than female kidneys. A striking gender-related difference in GST expression was found in cardiac tissue, where female animals expressed 50% more soluble GST than male tissues, and the GST isoenzyme with the greatest documented activity towards lipid hydroperoxides, mGSTA3, was present in female tissue yet absent from male tissue. These results point to complex gender- and tissue-dependent expression of individual mouse GST isoenzymes.

## INTRODUCTION

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of multifunctional enzymes that contribute to detoxification of a number of potentially harmful chemicals [1–3]. Substrates for GSTs include many environmental pollutants, pesticides, antibiotics, antineoplastics and carcinogenic products of phase I metabolism. Biochemical functions of GST enzymes include catalysing the addition of glutathione (GSH) to electrophilic xenobiotics, facilitating the transfer of reducing equivalents to toxic products generated during oxidative stress, and chemical sequestration [4,5]. Cytosolic GST isoenzymes are composed of two subunits that exist as homo- or hetero-dimers and are encoded by five distantly related gene families designated as class Alpha, Mu, Pi, Sigma and Theta [6–8]. Microsomal forms of GSTs exist as trimers and are encoded by a separate gene. Individual isoenzymes display distinctive catalytic properties and marked substrate specificities.

Biological mechanisms responsible for controlling the expression and regulation of GST isoenzymes are complex and not well characterized. Individual isoenzymes are induced by an extensive range of xenobiotics, regulated by hormones and expressed in a manner specific to gender, species, tissue, age and tumour status [9–13]. The induction of GST isoenzymes is thought to have evolved as an adaptive response to chemical stresses within the cell. The structural diversity of compounds that enhance GST expression (polycyclic aromatic hydrocarbons, Michael acceptors, reactive oxygen species, barbiturates and phenolic antioxidants) suggests several distinct mechanisms of GST induction. In addition, many drugs and xenobiotics enhance the expression of GSTs in an isoenzyme-specific manner. Differences in expression of specific GST isoenzymes within tissues might reflect various mechanisms of biological regulation and have been proposed as one underlying cause of organ-selective toxicity.

Profiles of GST isoenzymes constitutively expressed within tissues and changes subsequent to chemical exposure point to mechanisms regarding cellular responses to changes in environment. Profiling metabolic enzymes within tissues permits better predictions of sites of toxicity, metabolic intermediates and responses to exposures to specific environmental pollutants. Distinct differences in GST isoenzyme profiles have been found in comparisons of human tumour cell lines and breast tumour tissue [11,14], and changes in expression of individual GSTs have been proposed as early biomarkers for human disease. Polymorphic expression of GST isoenzymes has been described in several species and populations [15]. Consequently, distinguishing distribution patterns of GST isoenzymes within populations will be beneficial for identifying populations at greater risk to effects of chemical exposures. Gender-, strain- and tissue-selective expression of GST isoenzymes has been reported in several toxicologically important animal models, including the mouse [12,13,16–18]. Although gender-related expression of GSTs has been recognized in mouse liver, systematic studies identifying and comparing isoenzymes in extrahepatic tissues between genders in this rodent model are still lacking [18].

Differences in physical properties of purified GST isoenzymes have been reported by several groups [12]; however, it is not clear whether such discrepancies have a biological basis or can be attributed to differences in experimental techniques. Despite

Abbreviations used: CDNB, 1-chloro-2,4-dinitrobenzene; ESI, electrospray ionization; GSH, glutathione; GST, glutathione S-transferase.

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demonstrations that multiple GST isoenzymes are expressed in all vertebrate species studied, many investigations still rely on a single method [rate of conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) by an aggregate of isoenzymes] to measure GST induction. Such an approach can yield inaccurate and misleading results when some isoenzymes are induced while the expression of others is suppressed. A reliable evaluation of factors that regulate GST expression (e.g. tissue, gender, age or environment) demands an approach that (1) resolves all individual isoenzymes, (2) affords unambiguous identification of each isoenzyme and (3) exhibits quantitative accuracy and precision over a wide dynamic range. Enzyme assays and immunochemical methods such as Western blotting can differentiate families but not individual isoenzymes within a family [19]. Alternative separation methods such as SDS/PAGE and isoelectric focusing yield partial information for isoenzyme identification but usually fail to resolve all isoenzymes [16,20]. Furthermore, experimental pI values show variations of approx. $\pm 0.5$, impeding unambiguous assignments to isoenzymes [13]. Enzyme activities are even less reliable indicators owing to marked differences in specific activities reported for individual isoenzymes purified from different tissues [18].

The existence of multiple forms of GST with similar primary structure necessitates the use of high-resolution analytical techniques to allow the identification of individual GST subunits. Electrophoretic methods can distinguish many GST isoenzymes; however, molecular masses and pI values reported for similar isoenzymes exhibit substantial variability; misclassification of GSTs can result from a variability in composition of the resolving gel [20]. In addition, these techniques are incapable of resolving GST subunits that have undergone chemical modifications, differ in only a few amino acid residues, or have undergone post-translational modifications such as C-terminal truncations [21]. Western and Northern blotting techniques have found a routine use in distinguishing and quantifying GST isoenzymes. However, these techniques are not reliable quantitative tools owing to the high intraclass similarity of GST isoenzymes, the existence of multiple GSTs of the same class, and differences in mRNA stabilization. HPLC has emerged as the preferred technique for resolving and quantifying individual GST subunits. This approach has been applied in studies of chemical induction [22], strain- and sex-related differences in expression [12,16], measurement of isoenzyme profiles in human liver [23] and assessment of the methylation of rat liver isoenzymes [24]. Because several reports document the apparent co-elution of GSTs, improved HPLC separations are needed to provide better accuracy and precision in quantifying individual GST isoenzymes [16]. Rigorous evaluation of methods for GST identification in tissues are scarce and highlight a need for improved standardization, particularly because GST isoenzymes identified in some studies have not been observed in others [17,22].

Although HPLC is capable of resolving individual subunits, retention times provide the only information about isoenzyme identities, and isoenzymes might go unidentified unless authentic standards are available. In recent years electrospray ionization (ESI) MS has emerged as a powerful tool capable of measuring protein molecular masses with an accuracy ($\pm 3$ Da) sufficient to distinguish individual subunits. Mass spectra have been used to characterize microsomal GSTs [25] and GST isoenzymes isolated from rat and mouse [26,27]. This information has been used to identify post-translational modifications and has made it possible to identify isoenzymes on the basis of their molecular masses.

In the current study we describe a methodology for quantifying individual isoenzymes by HPLC with diode-array spectrophotometric detection combined with ESI MS for isoenzyme identification. Isoenzyme levels in liver, lung, heart, gonads and kidney have been measured and compared in both male and female CD-1 mice.

## MATERIALS AND METHODS

### Chemicals

Acetonitrile (Optima grade) was purchased from Fisher Scientific Corp. (Fair Lawn, NJ, U.S.A.). GSH–agarose [attached through sulphur to epoxide-activated 4% (w/v) cross-linked beaded agarose] was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dye reagent for protein assays was purchased from Bio-Rad (Richmond, CA, U.S.A.). All other chemicals used were of analytical grade.

### Animals

Male and female CD-1 mice (25–30 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). Animals were divided into groups by gender, housed on autoclaved pine shavings in approved animal care facilities and fed with Purina rodent chow *ad libitum* for 1 week. Mice were killed by cervical dislocation; tissues were removed, minced in ice-cold PBS [10 mM potassium phosphate buffer (pH 7.0)/150 mM NaCl] and immediately frozen under liquid nitrogen. Tissues for each gender were combined separately and stored at $-80$ °C until GSTs were purified.

### Enzyme assays

GST activity was measured at 25 °C with CDNB, bromosulphophthalein and ethacrynic acid and GSH as substrates following the methods of Habig et al. [28]. Rates of product formation were monitored by measuring the changes in absorbance at 340, 330 and 270 nm respectively with a Shimadzu PC-2101 UV/visible spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) and were corrected for non-enzymic reaction. GSH peroxidase activity was measured with methods established by Pagalia et al. [29]. Specific activities are based on protein concentrations as determined with the method of Bradford [30].

### Purification of GST isoenzymes

Frozen tissues were thawed on ice and homogenized in 10 mM Tris/HCl buffer, pH 7.4, containing 250 mM sucrose, 1 mM dithiothreitol and 1 mM EDTA, with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY, U.S.A.) set at 70% maximum speed for 1 min (1 g of tissue/2 ml of buffer). Centrifugation and all subsequent purification steps were carried out at 4 °C. Homogenate was centrifuged at 10000 ***g*** for 30 min to pellet cellular debris; the supernatant fraction was collected and centrifuged at 100000 ***g*** for 80 min. The resulting supernatant was diluted 1:10 (v/v) with 25 mM phosphate buffer (pH 7.0) containing 1 mM dithiothreitol and 1 mM EDTA (buffer A) and loaded on a GSH–agarose affinity column (5.5 cm × 1.0 cm) equilibrated with the same buffer. The affinity column was washed with buffer A containing 500 mM NaCl, until no protein could be detected in the effluent by monitoring absorbance at 280 nm with an Isco-UA5 detector. GSTs were eluted with approx. 20 ml of 25 mM phosphate buffer, pH 9.5, containing 75 mM GSH, 500 mM NaCl, 1 mM dithiothreitol and 1 mM EDTA. Affinity column eluents were analysed within 2 h by HPLC.

### HPLC determination of GST subunits

HPLC separations provide improved resolution of GST isoenzymes [16] relative to electrophoretic methods, and also facilitate the removal of salts and buffers from proteins, making them amenable to ESI MS. Individual GST subunits isolated from affinity columns were quantified with reverse-phase HPLC [31] on a Vydac 214TP54 (25 cm × 4.6 mm internal diam., 30 nm pore size) $C_{18}$ column. Chromatographic analyses were performed with a Varian 9010 solvent delivery system and a Hewlett Packard series 1050 diode-array detector monitoring absorbance at 214 and 280 nm. Proteins were separated by using a flow rate of 1.0 ml/min with an analysis time of 35 min per sample. The initial mobile-phase composition was held at acetonitrile/water (40:60, v/v) containing 0.1 % (v/v) trifluoroacetic acid for 5 min followed by a 30 min gradient to acetonitrile/water (57:43, v/v) containing 0.1 % (v/v) trifluoroacetic acid. Injections of 20 $\mu$l were made with a Rheodyne injector. A memory effect observed with liver samples was attributed to large quantities of protein present in affinity-column eluents. As a precaution, blank samples of purified water were analysed between samples of hepatic extracts.

### MS

Fractions corresponding to HPLC peaks were collected and concentrated under vacuum. Proteins were reconstituted in acetonitrile/water (50:50, v/v) containing 0.05 % (v/v) formic acid; molecular masses for individual GST subunits were determined with ESI MS on a Quattro-BQ triple quadrupole mass spectrometer (VG Biotech, Altrincham, Greater Manchester, U.K.). The mobile phase was composed of acetonitrile/water (50:50, v/v) containing 0.05 % (v/v) formic acid, delivered at 5 $\mu$l/min by an Isco $\mu$LC-500 syringe pump. Proteins were analysed by direct-flow injection of 20 $\mu$l samples. Spectra were obtained in positive-ion mode with a capillary voltage of +3.5 kV, with a source temperature of 75 °C and a cone voltage of +50 V. Spectra were scanned over $m/z$ 600–1400 at 20 s per scan and summed. Mass calibration was performed with horse heart myoglobin as reference. Mathematical transformations of electrospray spectra to true mass scale were attained with the MaxEnt® algorithm (Fisons Masslynx software), with 1 Da per point. Matrix-assisted laser desorption ionization mass spectra were obtained on a Hewlett Packard G2025A time-of-flight mass spectrometer with sinapinic acid as matrix and recombinant human insulin as internal mass calibrant.

### Amino acid sequencing

N-terminal amino acid sequences of purified GST isoenzymes were determined by Edman microsequence analysis at the UC Davis Protein Structure Laboratory with a Hewlett Packard Series G1000A protein sequencer system.

### Electrophoresis and immunoblotting

HPLC-purified proteins were concentrated under vacuum and reconstituted to give a solution of 1 $\mu$g of protein/$\mu$l of 0.1 M Tris-buffered saline, pH 7.4. Individual GST isoenzymes were separated (5 $\mu$g per lane) on gradient (4–20 %, w/v) polyacrylamide gels (Mini-PROTEAN II; Bio-Rad Laboratories) in the presence of SDS. Proteins were transferred to Immobilon-P transfer membranes (Millipore Corporation, Bedford, MA, U.S.A.) and immunoblotted with rabbit anti-human polyclonal antibodies against Alpha, Pi and Mu GST isoenzymes (Novocastra Laboratories, Newcastle upon Tyne, Tyne and Wear, U.K.). Binding of the primary antibody was revealed by using biotinylated goat anti-(rabbit peroxidase–antiperoxidase) as described previously [32].

## RESULTS AND DISCUSSION

### Isolation and recovery of GST

Reproducibility of the GST isolation protocol was assessed in triplicate with pooled hepatic tissues from both male and female animals. Homogenized tissue subsamples were centrifuged and GSTs were isolated from the supernatant fraction by affinity chromatography. Catalytic activity of GST towards CDNB was measured in triplicate for supernatant, affinity-column flow-through fractions and affinity-column eluate. Fractional recoveries are reported relative to the 100000 ***g*** supernatant and are based on enzyme activities towards CDNB. Affinity-column eluate accounted for 72 ± 5 % (mean ± S.D.) of original supernatant activity for male tissues and 81 ± 4 % for female tissues. For male liver tissues, 8 ± 1 % of GST activity was not retained on the affinity column, whereas 13 ± 3 % of GST activity was not retained for female tissues. Total recovered GST activity accounted for 80 ± 6 % of supernatant activity for males and 94 ± 7 % for females. Some GST isoenzymes show no activity towards CDNB, and others such as the Theta class isoenzymes are not retained on GSH–agarose columns and are not included in the current study. Activity present in non-retained fractions was attributed to proteins other than the principal cytosolic GST isoenzymes and was not an indication of inadequate affinity-column capacity. These results demonstrate efficient recoveries of GST isoenzymes from the affinity column, with the precision of measurements limited by uncertainties in measuring enzyme activities.

### Quantitative HPLC profiling of GST subunits

Concentrations of individual GST subunits in affinity column eluate were quantified from integrated HPLC peak areas for absorbance at 214 nm, with external standardization. Pure standards were not available for several mouse GST isoenzymes at the start of this study. As a result, instrument response was calibrated with purified mGSTA3, mGSTP1 and mGSTM1 standards that had been isolated from CD-1 mouse liver during an earlier study [27] and shown to be at least 98 % pure by HPLC. External calibration curves were linear ($r^2 > 0.997$) over the range of 0–10 $\mu$g of protein injected, and isoenzyme concentrations in affinity-column eluates were calculated from calibration curve regression equations. Quantitative determinations of mGSTA3 and mGSTP1 subunits were based on calibration curves determined for the two respective standards of these proteins. For all other GST subunits, protein concentrations were determined from a calibration curve generated with standard mGSTM1 because this standard displayed the smallest variance and because more Mu class subunits have been reported in mouse tissues than GST subunits from other classes. Molar absorbances at 214 nm for all three GST subunit standards agreed to within 18 % of one another.

HPLC determinations of subunit concentrations (based on standard curves) are reported as means of triplicate injections of affinity eluates from each purification. The S.D. determined for measuring the concentration of GST isoenzymes in affinity eluates by HPLC analysis was less than 6 %. The limit of quantification, based on the smallest peak detected at chosen integration threshold values, was 8 pmol (approx. 200 ng) of GST injected. Isoenzyme quantities in affinity eluates were determined from external calibration curves and normalized on the basis of wet weight of tissue.

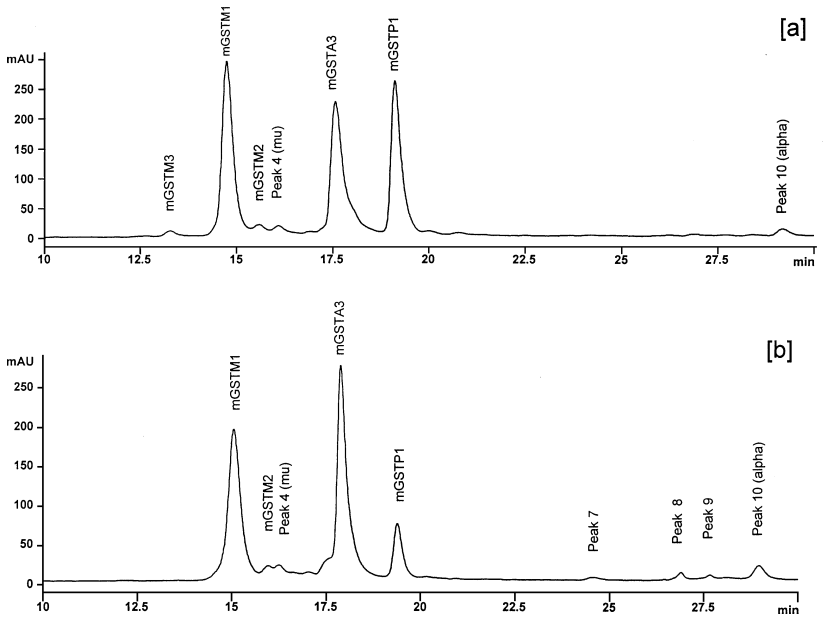


**Figure 1 Reverse-phase HPLC chromatographic separation of male hepatic GST subunits (a) and female hepatic GST subunits (b)**

Abbreviation: mAU, milliabsorbance units.

**Table 1 A comparison of HPLC-purified GST isoenzyme molecular masses obtained by ESI MS with reported molecular masses calculated from cDNA sequences**

Abbreviation: n.a., not available.

| HPLC retention time (min) | ESI/MS molecular mass (Da) | Calculated molecular mass (Da) | Isoenzyme identification | Reference |
|---|---|---|---|---|
| 13.3 | 25570 ± 5 | 25570 | mGSTM3 | [10] |
| 14.7 | 25839 ± 2 | 25839 | mGSTM1 | [10] |
| 15.7 | 25580 ± 5 | 25585 | mGSTM2 | [34] |
| 16.1 | 25490 | 25488* | Peak 4 (des-Pro[1]-mGSTM2) | n.a. |
| 17.7 | 25271 ± 2 | 25271† | mGSTA3 | [35] |
| 19.2 | 23478 ± 2 | 23478 | mGSTP1 | [36] |
| 24.5 | 22540<br>24493 | n.a. | Peak 7 | n.a. |
| 26.9 | 24378 ± 5 | 24380 | Peak 8 | n.a. |
| 27.7 | 25383 ± 5 | n.a. | Peak 9 | n.a. |
| 29.0 | 25480 ± 5 | 25487† | Peak 10 (mGSTA4)* | [33] |

* Tentative assignment.
† N-terminal acetylation after removal of N-terminal methionine.

## Mouse GST isoenzyme identification

Ten chromatographic peaks accounted for all GSTs observed in affinity-column eluates throughout this study. Each peak was characterized by its HPLC retention time and molecular mass determined by ESI MS. The assignment of each GST to an isoenzyme class was accomplished with the help of Edman microsequencing and Western blot analysis in conjunction with comparisons with reported amino acid sequences of mouse GST subunits. Several GST isoenzymes isolated in this study were expressed in minimal amounts (less than 3 % of total) and were detected only when concentrated extracts (e.g. from hepatic tissue) were analysed. All extrahepatic tissues were found to express at least three of the GST isoenzymes isolated from liver. Representative HPLC chromatograms of GST isoenzymes isolated from male and female hepatic tissue are shown in Figures 1(a) and 1(b) respectively.

By using ESI MS we were able to assign molecular masses (to within 0.02 %) to HPLC-purified GST isoenzymes (Table 1). The molecular mass (25570 ± 5 Da) of the earliest eluting GST isoenzyme (retention time $t_r$ 13.3 min) matched that calculated from the cDNA sequence for Mu class subunit mGSTM3. This isoenzyme also reacted strongly with anti-(Mu class GST) antibodies during Western blot analysis (Figure 2, lane 2). A weak band observed with anti-(Pi class) antibodies is attributed to weak non-specific binding of antibody to Mu class GST. ESI mass spectra showed a major peak at 25570 Da, and no peak for either mGSTP1 or mGSTP2 was observed above background. The second HPLC peak ($t_r$ 14.7 min; 25839 ± 2 Da) was identified as mGSTM1 in our earlier study [27]. ESI mass spectra of peak

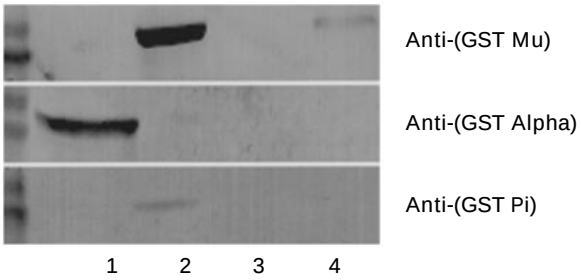


**Figure 2 Western blot analysis of HPLC-purified GST isoenzymes**

Lane 1, peak 10 (mGSTA4) protein with a molecular mass of 25480 Da; lane 2, mGSTM3; lane 3, peak 9 protein with a molecular mass of 25383 Da; lane 4, peak 4 protein with a molecular mass of 25490 Da.

**Table 2 A comparison of N-terminal amino acid sequences of mouse GSTs isolated in this study and in earlier studies**

| GST isoenzyme | Amino acid sequence | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peak 3 | P | M | T | L | G | Y | W | D | I | R | | | | | | |
| mGSTM2* | P | M | T | L | G | Y | W | D | I | R | G | L | A | H | I | A |
| mGSTA4† | M | A | A | K | P | K | L | Y | Y | F | N | G | R | G | R | M |
| Peak 9 | ‡ | M | Y | L | I | X | Y | P | I | R | G | L | A | H | A | I |
| Peak 10 | BLOCKED | | | | | | | | | | | | | | | |

* Sequence reported by Townsend et al. [34].
† Sequence reported by Zimniak et al. [33].
‡ Multiple amino acids.

3 ($t_r$ 15.7 min) revealed its molecular mass to be 25580 ± 5 Da, which agreed with calculated molecular masses of an Alpha class isoenzyme mGSTA4 (25576 Da) [33] and a Mu class isoenzyme mGSTM2 (25585 Da) [34] within experimental error. N-terminal amino acid sequencing (Table 2) yielded agreement with the N-terminal sequence of the mGSTM2 subunit. This and knowledge that mGSTA4 has a blocked N-terminus allowed mGSTA4 to be ruled out. A minor component of 25627 Da was also observed in ESI mass spectra of peak 3. This molecular mass does not correspond to any known mouse GST sequences, although N-acetylation of mGSTM2 would yield a protein of molecular mass 25627 Da, in agreement with this result. ESI mass spectra for Mu class GST isoenzymes mGSTM2 and mGSTM3 appear in Figures 3(a) and 3(b) respectively.

The molecular mass of the GST that was eluted next ($t_r$ 16.1 min; peak 4) was determined as 25490 Da. ESI mass spectra of fractions collected for this peak showed evidence of heterogeneity and suggest the presence of a minor co-eluting isoenzyme with molecular mass of 25577 Da. Western blot analysis of peak 4 indicated that it reacted weakly with anti-(Mu class GST) antibodies but gave no recognition with anti-(Alpha class GST) antibodies (Figure 2, lane 1). Although these results indicate the major isoenzyme belongs to the Mu class GSTs, there remains a possibility that insufficient quantities of the minor isoenzyme were present for detection by Western blot analysis. The molecular mass of the major isoenzyme present in this fraction corresponds to the deletion of a proline residue from the N-terminus of mGSTM2. Quantities of protein were insufficient to confirm this by Edman sequencing, but the molecular mass, Western blot results and HPLC elution near other Mu class isoenzymes suggest the deletion of N-terminal proline from mGSTM2 (des-Pro$^1$-mGSTM2) to yield a protein with a calculated molecular mass of 25488 Da (in comparison with 25490 Da measured). This could also be explained by post-translational removal of the N-terminal methionine of mGSTA4 followed by acetylation of the new N-terminus (post-translational processing identical with that of mGSTA3, calculated to be 25487 Da), but this isoenzyme is better explained by peak 10 (below). The ESI mass spectrum obtained for peak 4 is shown in Figure 4(a).

GST isoenzymes with chromatographic retention times of 17.7 min (25271 ± 2 Da) and 19.2 min (23478 ± 2 Da) were identified as Alpha class mGSTA3 and Pi class mGSTP1 isoenzymes in this and in previous studies [27,35,36].

The ESI mass spectrum of peak 7 (24.5 min) indicates that this fraction consists of a mixture of two substances of molecular masses 22540 and 24493 Da. Neither value matched those

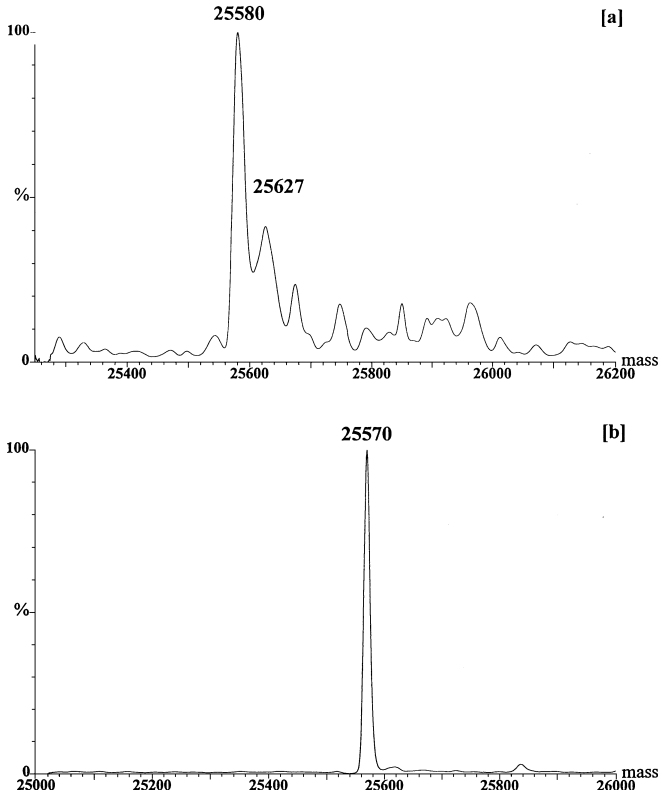


**Figure 3 Transformed ESI mass spectra of Mu class GST mGSTM3 (a) and Mu class GST mGSTM2 (b) isolated from mouse liver**

calculated from sequences of known GST isoenzymes, and both are lower than nearly all reported cytosolic GST molecular masses. These proteins are attributed either to products of post-translational truncation or to other proteins bound by the GSH–agarose column. The ESI mass spectrum obtained for peak 7 appears in Figure 4(b).

GST isoenzymes with HPLC retention times of 26.9 min (peak 8; 24378 ± 5 Da) and 27.7 min (peak 9; 25383 ± 5 Da) also did not match sequences reported for known GST isoenzymes. Edman sequencing and Western blot analysis of peak 8 were unsuccessful owing to low yields of isolated protein (low pmol level). Again, the low molecular mass suggests post-translational truncation. N-terminal sequence information obtained for peak 9 indicated substantial sequence similarity to Mu class GSTs. This isoenzyme was not recognized by any GST antibodies

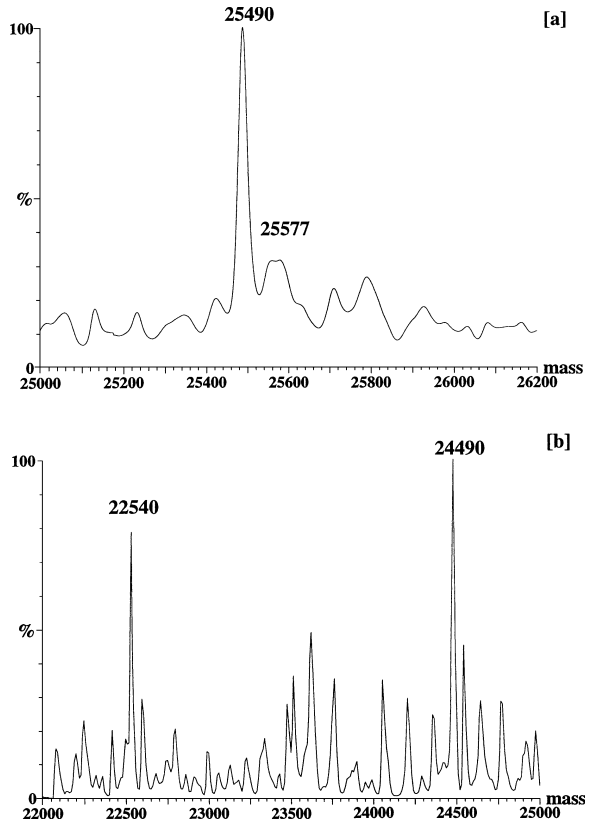


**Figure 4 Transformed ESI mass spectra of GST peak 4 (a) and GST peak 7 (b) isolated from mouse liver**

Peak 7 transform was conducted as a survey scan with 10 Da per point to highlight the major components.

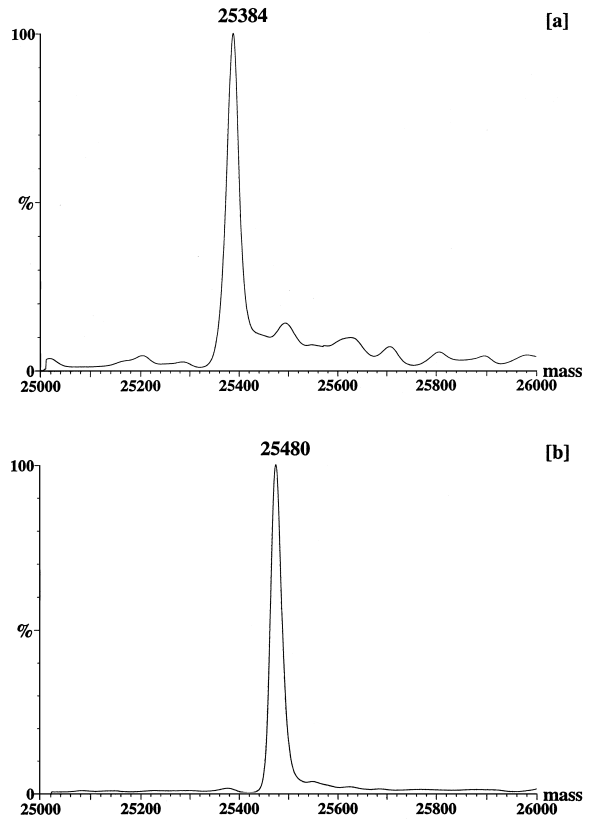


**Figure 5 Transformed ESI mass spectra of GST peak 9 (a) and GST peak 10 (b) isolated from mouse liver**

during Western blot analysis (Figure 2, lane 3), but this was attributed to insufficient yield of protein during HPLC separations. ESI mass spectra of peak 9 are presented in Figure 5(a).

The GST isoenzyme that was eluted last (peak 10; $t_r$ 29.0 min) was determined as having a molecular mass of $25480 \pm 5$ Da. This isoenzyme was recognized by anti-(Alpha class GST) antibodies during Western blot analysis (Figure 2, lane 4). The molecular mass of this isoenzyme is similar to that calculated for an Alpha class GST isoenzyme (25477 Da) sequenced earlier by Daniel et al. [36a], but a blocked N-terminus prevented Edman N-terminal sequencing. The calculated mass above does not take into account the mass of the N-terminal blocking group. However, post-translational removal of the N-terminal methionine residue of the acidic isoenzyme mGSTA4 followed by acetylation yields a theoretical molecular mass of 25487 Da, which is close to the experimental value. Digestion of the protein with CNBr followed by analysis of the product mixture by matrix-assisted laser desorption ionization/time-of-flight MS yielded peaks that combined to account for the entire protein. The ESI mass spectrum for peak 10 is shown in Figure 5(b).

Molecular mass determinations of all hepatic GST isoenzymes are based on averages of three separate ESI mass spectral determinations, except for peaks 4 and 7. These samples showed extensive heterogeneity and were obtained in low yield; reported molecular masses were therefore based on single ESI mass spectra.

Chromatographic retention times and molecular masses obtained for GSTs did not differ between isoenzymes isolated from male and female tissues, indicating comparable primary structures of GSTs in both genders.

## Liver GSTs

Hepatic GSTs of male and female mice were isolated by isocratic elution from GSH-linked agarose. Results of typical purifications for male and female mice are summarized in Table 3. More than 85% of total GST activity applied to the affinity column was recovered. Hepatic tissues yielded similar amounts of soluble GST protein from male (1136 $\mu$g of GST/g of tissue) and female (1006 $\mu$g of GST/g of tissue) animals as determined by combining levels measured for all isoenzymes by HPLC. The specific activity of affinity-purified hepatic GSTs was measured towards a number of isoenzyme-specific substrates; the results are shown in Table 4. The specific activity towards CDNB was found to be $92.5 \pm 6.0$ $\mu$mol/min per mg of protein in male livers, compared with $68.6 \pm 2.1$ $\mu$mol/min per mg of protein in female livers. The specific activity of GSTs extracted from male livers was 35% higher than that of GSTs from female livers. These results are consistent with findings obtained by other investigators for this strain of mouse [12,13] and can be explained by comparing the relative expression of specific GST isoenzymes. For instance, GST activities towards ethacrynic acid and CDNB (Pi class-specific substrates) were greater in male livers than in female livers. Measures of enzymic activity suggest that male hepatic tissues express more mGSTP1 isoenzyme than do female tissues. However, precise quantitative measurements of protein ex-

**Table 3 Summary of recoveries and activities for mouse liver GSTs at different stages of isolation**

Total protein was determined by the method of Bradford [30] with BSA as reference. Enzymic activity was measured by the catalysis of formation of *S*-(2,4-dinitrophenyl)glutathione from CDNB and GSH.

| | Total protein (mg) | | Total activity ($\mu$mol/min) | | Specific activity ($\mu$mol/min per mg) | | Activity remaining (% of original) | |
|---|---|---|---|---|---|---|---|---|
| Purification step | Male | Female | Male | Female | Male | Female | Male | Female |
| Liver supernatant | | | 1081 | 451 | 4.0 | 2.0 | | |
| 100 000 ***g*** centrifugation | 149 | 112 | 679 | 289 | 4.6 | 2.1 | 100 | 100 |
| GSH–agarose eluate | 5.3 | 3.3 | 520 | 225 | 98 | 68 | 77 | 78 |
| Non-retained material | 173 | 124 | 53 | 31 | 0.3 | 0.3 | 8 | 11 |
| Total recoveries | | | | 85 | 89 | | | |

**Table 4 Specific activities towards several substrates of affinity-isolated hepatic GSTs isolated from male and female mice**

Affinity effluent was used for activity determination. Activity was measured at 27 °C for all assays except for the cumene hydroperoxide assay, which was measured at 37 °C. All values are based on an average of two (*) or three separate isolations.

| | Specific activity ($\mu$mol/min per mg of protein) | | Tissue activity ($\mu$mol/min per g of tissue) | |
|---|---|---|---|---|
| Substrate | Female | Male | Female | Male |
| CDNB | 68.6 ± 2.1 | 92.5 ± 6.0 | 98.4 ± 3.3 | 133.4 ± 0.4* |
| Bromosulphophthalein | 0.26 ± 0.04 | 0.27 ± 0.08 | 0.38 ± 0.06 | 0.43 ± 0.15 |
| Ethacrynic acid | 4.6 ± 0.6 | 5.6 ± 0.1 | 6.6 ± 1.0 | 9.4 ± 0.2 |
| Cumene hydroperoxide | 3.6 ± 0.7* | 2.9 ± 0.2* | 5.0 ± 0.8* | 4.8 ± 0.1* |

pression cannot be inferred from these measurements. Specific activities towards bromosulphophthalein (Mu class-specific substrate) and cumene hydroperoxide (Alpha class-specific substrate) were also measured in both male and female tissues: activities were found to be equivalent in both genders. Quantitative HPLC analyses of hepatic extracts indicate that livers from male and female animals contain different amounts of mGSTA3 (male, 387 $\mu$g/g of tissue; female, 474 $\mu$g/g) and mGSTP1 (male, 295 $\mu$g/g; female, 91 $\mu$g/g) isoenzymes and equivalent amounts of subunit mGSTM1 (male, 358 $\mu$g/g; female, 325 $\mu$g/g). The mGSTP1 isoenzyme accounts for a greater proportion of cytosolic GST in male liver (26 %) than in female liver (9 %), whereas subunit mGSTA3 is expressed in greater proportion in livers of females (47 %) than in males (34 %). A comparison of the relative proportions of major GST isoenzymes, reported as a percentage of total GST recovered from various tissues, is provided Table 5.

In addition, HPLC analyses of hepatic extracts show that mGSTM3 was present only in extracts of male tissues, whereas peak 7 isoenzyme(s) were isolated only from female livers. The expression of minor GST isoenzymes was similar for both genders. Absolute quantities ($\mu$g of GST/g wet weight of tissue) of major GST isoenzymes present in liver are presented in Table 6, and indicate that males express twice as much mGSTP1 isoenzyme as females, whereas females express 1.7-fold as much mGSTA3 isoenzyme as males.

**Table 5 Percentage composition of mouse GST isoenzymes isolated from tissues from male and female animals**

Abbreviation: n.d., GST isoenzyme not present in detectable quantities. Dashes indicate entries where isoenzymes were detected but were below the level of quantification.

| | | | Proportion of GST isoenzyme (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HPLC retention time (min) | ESI MS molecular mass (Da) | GST subunit identity | Liver | | Brain | | Heart | | Lung | | Kidney | | Gonads | |
| | | | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 13.3 | 25 570 ± 5 | mGSTM3 | 1 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 6 | n.d. | n.d. | n.d. |
| 14.7 | 25 839 ± 2 | mGSTM1 | 32 | 34 | 33 | 34 | 30 | 33 | 43 | 45 | 60 | 26 | 34 | 15 |
| 15.7 | 25 580 ± 5 | mGSTM2 | 3 | 3 | n.d. | n.d. | n.d. | n.d. | 14 | 17 | 10 | 26 | 21 | 22 |
| 16.1 | 25 490 | Peak 4 (des-Pro[1]-mGSTM2) | 2 | 3 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 17.7 | 25 271 ± 2 | mGSTA3 | 34 | 47 | n.d. | n.d. | n.d. | 18 | 15 | 14 | 7 | 8 | 7 | n.d. |
| 19.2 | 23 478 ± 2 | mGSTP1 | 26 | 9 | 34 | 38 | 24 | 22 | 19 | 21 | 14 | 15 | 6 | 34 |
| 24.5 | 22 540<br>24 493 | Peak 7 | n.d. | – | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 16 | 13 | n.d. |
| 26.9 | 24 378 ± 5 | Peak 8 | – | – | 13 | 12 | n.d. | 5 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 27.7 | 25 383 ± 5 | Peak 9 (mu) | – | 1 | 7 | 5 | n.d. | 7 | n.d. | n.d. | 6 | – | 7 | n.d. |
| 29.0 | 25 480 ± 5 | Peak 10 (mGSTA4) | 2 | 3 | 12 | 11 | 46 | 15 | 9 | 3 | 4 | 9 | 12 | 29 |

**Table 6 Quantities of GST isoenzymes isolated from male and female mouse tissues by the affinity-column technique and quantified by HPLC**

Abbreviation: n.d., GST isoenzyme not present in detectable quantities. Dashes indicate entries where isoenzymes were detected but were below the level of quantification.

| HPLC retention time (min) | ESI MS molecular mass (Da) | GST subunit identity | Quantity of GST subunit ($\mu$g/g wet weight of tissue) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Liver | | Brain | | Heart | | Lung | | Kidney | | Gonads | |
| | | | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 13.3 | 25570 ± 5 | mGSTM3 | 16 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 7 | n.d. | n.d. | n.d. |
| 14.7 | 25839 ± 2 | mGSTM1 | 358 | 325 | 15 | 19 | 12 | 27 | 46 | 51 | 77 | 25 | 85 | 27 |
| 15.7 | 25580 ± 5 | mGSTM2 | 30 | 28 | n.d. | n.d. | n.d. | n.d. | 17 | 17 | 13 | 24 | n.d. | n.d. |
| 16.1 | 25490 | Peak 4 (des-Pro[1]-mGSTM2) | 29 | 28 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 53 | 39 |
| 17.7 | 25271 ± 2 | mGSTA3 | 387 | 474 | n.d. | n.d. | n.d. | 15 | 14 | 18 | 9 | 8 | 17 | n.d. |
| 19.2 | 23478 ± 2 | mGSTP1 | 295 | 91 | 15 | 21 | 10 | 17 | 21 | 22 | 19 | 14 | 16 | 59 |
| 24.5 | 22540<br>24493 | Peak 7 | n.d. | – | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 15 | 34 | n.d. |
| 26.9 | 24378 ± 5 | Peak 8 | – | – | 6 | 6 | n.d. | 4 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 27.7 | 25383 ± 5 | Peak 9 (mu) | – | 13 | 3 | 3 | n.d. | 6 | n.d. | n.d. | 6 | – | 19 | n.d. |
| 29.0 | 25480 ± 5 | Peak 10 (mGSTA4) | 21 | 33 | 6 | 6 | 18 | 12 | 4 | 11 | 4 | 9 | 30 | 50 |
| | | Total | 1136 | 1006 | 45 | 55 | 40 | 81 | 102 | 119 | 135 | 95 | 254 | 175 |

## Brain GSTs

GSTs of the brain were isolated from separate pools of tissue ($n = 20$ for each gender) from male and female mice. The viscosity and physical characteristics of the neuronal homogenate made it necessary to incorporate an additional step in the affinity purification protocol as follows: (1) supernatant from the initial 10000 ***g*** spin was collected; pelleted material was resuspended in 10 ml of buffer A; (2) samples were centrifuged at 10000 ***g*** for an additional 20 min; and (3) the resulting supernatant was added to supernatant retained from the first centrifugation step.

Recoveries of CDNB activity in affinity eluates were 80 % in male tissues and 77 % in female tissues. Less than 3 % of GST activity applied to the affinity matrix passed unretained in these studies. Neuronal tissue isolated from female animals yielded greater quantities of soluble GST (55 $\mu$g/g wet weight of tissue) than male animals (45 $\mu$g/g wet weight of tissue). GSTs isolated from the brains of females displayed higher specific activity towards CDNB than GSTs from the brains of males (87.3 and 75.2 $\mu$mol/min per mg respectively).

Reverse-phase HPLC of affinity effluents indicate that brain (both genders) contains five GST isoenzymes with HPLC retention times of 14.4, 18.5, 26.4, 27.0 and 28.3 min. Molecular masses determined for each of these fractions indicate that these proteins were identical with proteins previously identified in liver and are as follows: mGSTM1, mGSTP1, peak 8, peak 9 and mGSTA4 respectively. The two principal GSTs expressed in brain are the mGSTM1 and mGSTP1 isoenzymes.

The profiles of GST expression in neuronal tissues were similar in male and female animals (Table 5), although tissues from female animals displayed greater quantities of mGSTP1 (19 $\mu$g/g of tissue) and mGSTM1 (21 $\mu$g/g of tissue) than tissues from male animals (15 $\mu$g/g for each isoenzyme). The three minor GST isoenzymes isolated from this tissue were expressed in similar amounts in both male and female animals. A summary of these results is given in Table 6.

## Heart GSTs

GSTs of the heart were isolated from separately pooled tissues ($n = 20$ for each gender) of male and female mice. Recoveries of GST activity in affinity eluates were 92 % for male tissues and 90 % for female tissues. Of total enzyme activity applied to the affinity matrix, 13 % passed unretained in isolations of GSTs from male tissues and 11 % passed unretained in isolations from female tissues. Female cardiac tissue displayed twice the amount of soluble GST (81 $\mu$g/g wet weight of tissue) compared with male cardiac tissue (40 $\mu$g/g wet weight of tissue). In addition, the specific activity measured towards CDNB in female tissues (59.1 $\mu$mol/min per mg) was approximately twice that in male tissues (32.8 $\mu$mol/min per mg).

HPLC chromatograms of affinity effluents revealed six GST isoenzymes (mGSTM1, mGSTA3, mGSTP1, peak 8, peak 9 and mGSTA4) isolated from hearts of females, whereas male heart yielded only three GST isoenzymes (mGSTM1, mGSTP1 and mGSTA4). Peak 8 and peak 9 were present in quantities near the limit of detection in female tissue. These isoenzymes might have been absent from eluates from male tissues owing to the elution of GSTs from male tissues in a 40 % greater volume than those of isolated from female tissues. Consequently these minor isoenzymes might have been present in male extracts but at quantities below the limit of detection.

A striking difference in the distribution of mGSTA3 was seen between male and female cardiac tissue. This isoenzyme comprised 18 % of the soluble GST from female tissues yet was absent from tissues isolated from male animals (Table 5). This finding suggests an important gender-dependent role for mGSTA3 in cardiotoxicity in mice because the mGSTA3 isoenzyme demonstrates the greatest activity towards organic hydroperoxides. In addition, the absence of mGSTA3 from cardiac tissue isolated from male mice might render them more susceptible to the cardiotoxic side-effects of antineoplastic drugs such as adriamycin and doxorubicin that stimulate production of reactive oxygen species.

A gender-related difference in the distribution of the mGSTA4 isoenzyme was also seen in cardiac tissue. This Alpha class GST was the principal soluble GST isoenzyme in male heart (18 $\mu$g/g) but only 12 $\mu$g/g was isolated from female heart. It has been recognized that the regulation of GST expression in humans is different from that in rodents [37,38]. Because the enzymic properties and substrate specifities of this, or analogous, isoenzymes have not yet been characterized, the implications for gender-related heart disease in humans remain unclear.

## Lung GSTs

Lung GSTs were isolated from separately pooled tissues ($n = 20$)

from male and female mice. The isolation of GSTs from GSH–agarose resulted in a minimal loss of enzymic activity (only 4 % passed unretained by the affinity matrix for both genders). Of total GST activity applied to the affinity matrix, 96 % was recovered from male tissues, whereas 79 % was recovered from female tissues. The specific activity towards CDNB was comparable in male (67.9 $\mu$mol/min per mg) and female tissues (70.7 $\mu$mol/min per mg). Tissues from both genders yielded similar amounts of soluble GST protein (102 $\mu$g/g wet weight of tissue from male tissue, and 120 $\mu$g/g from female). Reverse-phase HPLC of affinity effluents indicated that pulmonary tissue (from both genders) expressed five GST isoenzymes with chromatographic retention times of 14.0, 15.7, 17.0, 18.0 and 28.0 min. ESI mass spectra indicated that these isoenzymes were identical with GSTs described previously in liver, heart and brain tissue and are as follows: mGSTM1, mGSTM2, mGSTA3, mGSTP1 and mGSTA4 respectively.

The overall patterns of isoenzyme distribution in male and female pulmonary tissue were similar and isoenzymes were expressed in equivalent amounts (Table 6). The mGSTM1 isoenzyme was the major GST expressed in lung, comprising approx. 44 % of soluble GSTs. The mGSTP1 isoenzyme made up approx. 20 % of soluble pulmonary GSTs, the mGSTM2 isoenzyme approx. 15 % and the mGSTA3 isoenzyme approx. 14 %. Minor differences were observed in the expression of the mGSTA4 isoenzyme between male and female animals. This isoenzyme constituted 9 % of soluble GSTs in female lung and only 3 % in male lung (Table 5).

## GSTs of kidney

GSTs of the kidney were isolated from pooled tissues (3.5 g) of male and female mice. Of total GST activity applied to the affinity matrix, from male tissues 82 %, and from female tissues 79 %, was recovered. Less than 5 % of total enzyme activity applied to the affinity column passed unretained in samples from either gender. Extracts from male kidneys yielded greater quantities of soluble GSTs (135 $\mu$g/g wet weight of tissue) than female kidneys (93 $\mu$g/g). The specific activities of GSTs towards CDNB were greater in those from male kidneys (86.3 $\mu$mol/min per mg) than in those from female kidneys (55.5 $\mu$mol/min per mg). Reverse-phase HPLC and ESI mass spectra indicate that male kidneys yielded the following seven GST isoenzymes: mGSTM1, mGSTM2, mGSTM3, mGSTA3, mGSTP1, peak 9 and mGSTA4. Female kidney tissue also contained seven GST isoenzymes; however, female tissues lack the mGSTM3 isoenzyme but expressed the peak 7 isoenzyme(s). The peak 9 protein was present in extracts from both male and female kidneys but below the limit of quantification in females.

The mGSTM1 isoenzyme was the major GST present in male kidney (77 $\mu$g/g), whereas this isoenzyme was only present at 25 $\mu$g/g in female kidney. The mGSTM2 isoenzyme accounted for 26 $\mu$g/g of soluble GSTs from female kidney but only 13 $\mu$g/g from male kidney. The mGSTA3 and mGSTP1 isoenzymes were isolated in similar amounts from both genders. Minor differences were also seen in the expression of the mGSTA4 isoenzyme: this was present at 9 $\mu$g/g in female tissues but only 4 $\mu$g/g in male kidney.

## Gonadal GSTs

Amounts of GST isolated from gonads were higher per gram of tissue than from all other tissues except liver. GSTs of the ovary were isolated from 1.03 g of pooled tissue. Affinity purification resulted in 95 % recovery of total GST activity applied to the affinity matrix (9 % passed unretained). Ovarian tissue yielded 175 $\mu$g of soluble GST/g wet weight of tissue and the specific activity towards CDNB was 103 $\mu$mol/min per mg. Ovaries contained four GST isoenzymes. ESI mass spectra of fractions collected during HPLC analysis indicated that GSTs of ovaries correspond to the mGSTM1, mGSTM2, mGSTP1 and mGSTA4 isoenzymes.

The mGSTP1 isoenzyme was the major GST expressed in the ovary, comprising 34 % of soluble GSTs of this tissue (59 $\mu$g/g). The mGSTA4 isoenzyme (Alpha class) accounted for 50 $\mu$g/g of tissue; this isoenzyme was found in greater abundance in the ovary than in any other tissue (Table 6). Isoenzymes mGSTM1 and peak 4 (des-Pro$^1$-mGSTM2) accounted for 27 and 39 $\mu$g/g respectively.

GSTs of the testis were isolated from 2.88 g of pooled tissue. Isolation of GSTs from affinity agarose resulted in 89 % recovery of total GST activity, with only a 7 % loss in activity applied to the affinity column. Testis yielded 254 $\mu$g of soluble GST/g wet weight of tissue, and the specific activity towards CDNB was 69.0 $\mu$mol/min per mg. Reverse-phase HPLC of affinity effluents indicated that testis contained seven GST isoenzymes. The molecular masses and retention times of these isoenzymes indicated that they were identical with the GST isoenzymes identified from liver. The isoenzymes present in testes are as follows: mGSTM1, mGSTM2, mGSTA3, mGSTP1, peak 4, peak 9 and mGSTA4.

The mGSTM1 isoenzyme was found to be the major GST expressed in testes (85 $\mu$g/g; Table 5), and constituted 21 % of the soluble GSTs of the testes, with comparable levels of expression of the remaining minor isoenzymes.

## Conclusions

Affinity purification of GST isoenzymes yielded efficient and reproducible (to within $\pm 10$ %) recoveries of total GST activity as measured towards CDNB for all tissues examined in this study. The quantitative HPLC method developed for this study demonstrated a precision better than $\pm 6$ % and was successful in separating all major GST isoenzymes retained by the affinity column. HPLC analyses yielded information about differences in tissue concentrations of GST isoenzymes that would not be apparent from analyses based on comparisons of catalytic activities towards 'isoenzyme-specific' substrates or from Western blot analyses.

Eleven proteins were identified in affinity eluates. ESI MS and reverse-phase HPLC analyses of affinity eluates yielded evidence of three minor isoenzymes expressed in concentrations too low for unambiguous identification. Isoenzymes representing the three major classes of GST isoenzyme, mGSTA3 (25571 Da), mGSTP1 (23478 Da) and mGSTM1 (25839 Da), were identified in male and female hepatic tissues. Additional isoenzymes were isolated from liver and also from extrahepatic tissues, including mGSTM2 (25580 Da), mGSTM3 (25570 Da), peak 4 (25490 Da; tentatively assigned as des-Pro$^1$-mGSTM2), peak 8 (24378 Da), peak 9 (25383 Da) and peak 10 (25480 Da; assigned as acidic isoenzyme mGSTA4). ESI mass spectra of peak 7 revealed a mixture of two proteins with molecular masses of 22540 and 24493 Da. Molecular masses obtained for three of the minor GST isoenzymes were so low as to suggest N- or C-terminal truncations of major GST isoenzymes. The HPLC and mass-spectrometric analyses showed no evidence of isoenzymes reported in other studies, including mGSTP2 or Theta-class isoenzymes, which were not expected to be retained by the affinity column.

Distinct gender-related differences in the expression of individual GST subunits were observed in liver, heart, kidney and gonads, whereas GST expression was similar in brain and lung of both male and female mice. Marked gender-dependent differences were observed in the expression of mGSTM3 and mGSTA3 in mouse hepatic tissue. In contrast with patterns of expression in liver (higher ratio of mGSTA3 to mGSTP1 in females than in males), mGSTP1 was the most abundant subunit in female gonads, whereas mGSTA3 was not present in detectable quantities. Ovarian tissue was found to express the greatest amount of the Alpha class mGSTA4 isoenzyme compared with other tissues examined in this study.

The pattern of GST subunits in kidneys was similar to that in liver, but male kidneys expressed more soluble GST than did female kidneys. A gender-related difference in the pattern of expression of mGSTM2 was found in this tissue, with more of this isoenzyme being expressed by male animals than by female animals.

Cardiac tissues exhibited striking differences in levels of GST between male and female animals. Female animals expressed twice as much soluble GST per gram of tissue as male animals. Furthermore the GST isoenzyme with greatest activity towards lipid hydroperoxides, mGSTA3, was present in female tissues yet absent from male cardiac tissue. The absence of mGSTA3 from male cardiac tissue might have an important role in gender-dependent cardiotoxicity. To our knowledge this is the first report of a gender-related difference in the pattern of expression of mGSTA3 in cardiac tissue.

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