Identification, Characterization, and Crystal Structure of the Omega Class Glutathione Transferases*

Received for publication, March 2, 2000, and in revised form, April 10, 2000
Published, JBC Papers in Press, April 26, 2000, DOI 10.1074/jbc.M001706200

Philip G. Board‡‡, Marjorie Coggan‡, Gareth Chevlandayagam¶, Simon Eastal‡, Lars S. Jermiin**, Gayle K. Schulte***, Dennis E. Danley‡‡, Lise R. Hoth‡‡, Matthew C. Griffon‡‡, Ajith V. Kamath‡‡, Michele H. Rosner‡‡, Boris A. Chrunya‡‡, David E. Perregaux‡‡, Christopher A. Gabel††, Kieran F. Geoghegan‡‡, and Jayvardhan Pandit‡‡‡

From the ‡Molecular Genetics Group and ¶Human Genetics Group, John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory 2601, Australia, **Australian Genomic Information Centre, University of Sydney, New South Wales 2006, Australia, and ‡‡‡Pfizer Global Research and Development, Groton, Connecticut 06340

A new class of glutathione transferases has been discovered by analysis of the expressed sequence tag data base and sequence alignment. Glutathione S-transferases (GSTs) of the new class, named Omega, exist in several mammalian species and Caenorhabditis elegans. In humans, GSTO 1-1 is expressed in most tissues and exhibits glutathione-dependent thiol transferase and dehydroascorbate reductase activities characteristic of the glutaredoxins. The structure of GSTO 1-1 has been determined at 2.0-Å resolution and has a characteristic GST fold (Protein Data Bank entry code 1eem). The Omega class GSTs exhibit an unusual N-terminal extension that abuts the C terminus to form a novel structural unit. Unlike other mammalian GSTs, GSTO 1-1 appears to have an active site cysteine that can form a disulfide bond with glutathione.

The glutathione transferases (GSTs)1 are a family of phase II enzymes that utilize glutathione in reactions contributing to the biotransformation and disposition of a wide range of exogenous and endogenous compounds. These include chemical carcinogens, therapeutic drugs, and products of oxidative stress (1). GST expression levels and the factors modulating them can have significant biological and clinical implications. Overexpression of some GSTs is implicated in resistance to several anti-cancer drugs (1). In contrast, genetically determined GST deficiencies are risk factors for cataract and several forms of cancer (1, 2), and homozygosity for the GSTM 1 null allele is a positive prognostic indicator for successful chemotherapy and long term survival in childhood acute lymphoblastic leukemia (3). Genetic polymorphisms influencing the substrate specificity of GSTP 1–1 (4) are risk factors for Parkinson's disease in subjects exposed to pesticides (5).

Based on their sequences, the mammalian cytosolic GSTs form six evolutionarily distinct classes termed Alpha, Mu, Pi, Sigma, Theta, and Zeta (1, 6). There may also be additional classes in plants, insects, and other species (6). Although some members of the different GST classes exhibit overlapping substrate specificities, others are highly specific. Despite the diversity of substrates metabolized by this large family, many GSTs were originally identified through their activity with the xenobiotic 1-chloro-2,4-dinitrobenzene and their ability to bind to glutathione affinity matrices. To identify new members of the glutathione transferase gene family on the basis of sequence similarity rather than substrate specificity, we have searched a database containing sequences of more than 106 human expressed sequence tags.

This analysis has led to identification of the class Omega glutathione transferases, a group of GSTs with novel structural and functional characteristics. We have identified a human cDNA, expressed and characterized the recombinant protein, and determined its crystal structure to 2.0-Å resolution. Recombinant human Omega class GST (GSTO 1-1) exhibits a glutathione-dependent thiol transferase activity and catalyzes glutathione-dependent reduction of dehydroascorbate. These activities are not associated with other human GSTs but are characteristic of the glutaredoxins (7). The Omega class GSTs have a unique N-terminal extension, and the crystal structure reveals an active site cysteine residue distinct from the tyrosine and serine residues characteristic of other eukaryotic GSTs (8–10).

EXPERIMENTAL PROCEDURES

**EST Identification and DNA Sequencing**—The human EST data base was searched using the TBLASTn version of the BLAST program (11) with human Theta and Zeta class amino acid sequences as query sequences. An EST (GenBank accession number W38456) encoding an unknown protein with low similarity to both Theta and Zeta class GSTs was identified, and the cDNA clone was obtained from the I.M.A.G.E. consortium. The complete cDNA sequence was obtained on each strand by subcloning restriction fragments and by the use of a Thermo sequenase kit (Amersham Pharmacia Biotech). The cDNA sequence has been submitted to GenBank as accession number AF212303.

Alignment and Phylogenetic Analysis of Representative GST and GST-like Amino Acid Sequences—Although a large number of GSTs and related protein sequences are available, to simplify computations and to promote legibility, representative GST and GST-like amino acid sequences were selected from previously described GST classes and related proteins. The sequences were obtained from GenBank and aligned using CLUSTAL W (12) and GDE (13). The revised alignment,
which agrees with predictions based on molecular modeling and crystal structure, is available from Dr. P. Board at the John Curtin School of Medical Research web site.

A phylogenetic tree was obtained by maximum likelihood analysis of all the sites in the above-mentioned alignment. The data was analyzed using the F81+F model and local bootstrap probabilities were estimated for the internal branches using the PROMTL program (15).

The phylogenetic analysis involved two examinations of the sequence alignment with different input order of the sequences. Each analysis involved two steps: stepwise addition and nearest neighbor interchanges. The most likely tree was compared with the remaining trees using the test of Kishino and Hasegawa (16). The phylogenetic tree constructed from the sequence alignment of 2.1 Å resolution maps calculated after density modification by histogram matching and solvent flipping in SOLOMON (27) were found in anomalous difference Patterson maps calculated at the peak anomalous wavelength using the program PATSOL (25), and another peak anomalous wavelength using the program SHARP (26). Examination of log-likelihood was employed, and data were collected in two 75° sweeps in two dimensions. Relevant statistics are given in Table II.

Northern and Western Blotting—Northern blots containing mRNA from multiple human tissues obtained from CLONTECH (Palo Alto, CA) were hybridized with 28P-labeled GSTO 1-1 cDNA in ExpressHyb (CLONTECH, Palo Alto, CA) at 68 °C and washed according to the manufacturer’s instructions. The filters were subsequently hybridized with a labeled 28-actin cDNA probe to evaluate track loading. Western blots were performed with a 1:400 dilution of rabbit antiserum raised against His-tagged recombinant GSTO 1-1, and goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma) (22). The primary antiserum does not cross-react with native GSTs from the other classes; however, we have noted some cross-reactivity with recombinant proteins expressed with the poly-His tag.

Data Collection—Multilength anomalous dispersion data were collected from crystals of SeMet-substituted apo-GSTO 1-1 on Beam Line X12-C of the National Synchrotron Light Source at the Brookhaven National Laboratory, Brookhaven, New York. Before data collection at 100 K, crystals were transferred to a cryoprotectant solution of 2.0 M Li2SO4 and 5% glycerol. The Se-absorption edge was collected with a 1.400 dilution of rabbit antiserum raised against His-tagged recombinant GSTO 1-1, and goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma) (22). The primary antiserum does not cross-react with native GSTs from the other classes; however, we have noted some cross-reactivity with recombinant proteins expressed with the poly-His tag.

TABLE I

| Substrate                  | Activity (µmol/min/mg) |
|----------------------------|------------------------|
| 1-Chloro-2,4-dinitrobenzene| 0.18 ± 0.006           |
| 1,2-Dichloronitrobenzene   | ND                     |
| 7-Chloro-4-nitrobenzo-2-oxa,1,3-diazole| 0.05 ± 0.004 |
| 1,2-Epoxy-3-(4-nitrophenyl)propane| ND                 |
| 4-Phenybut-3-en-2-one      | ND                     |
| Ethacrynic acid            | ND                     |
| p-Nitrobenzyl chloride     | ND                     |
| p-Nitrophenyl acetate      | 0.06 ± 0.012           |
| Methacrylic acid sulfate   | ND                     |
| Dichloromethane            | ND                     |
| Dichloroacetic acid        | ND                     |
| t-Butyl hydroperoxide      | ND                     |
| Cumene hydroperoxide       | ND                     |
| trans-2-Nona-2,4-dienal    | ND                     |
| trans-Deca-2,4-dienal      | ND                     |
| trans-Hexenal              | ND                     |
| trans-Octanal              | 0.03 ± 0.006           |
| trans-Nonenol              | 0.03 ± 0.003           |
| 1,2-Dichloronitrobenzene   | 0.16 ± 0.005           |
| Thiolsulfate               | 2.92 ± 0.120           |

Further details are available from Dr. Philip Board at the John Curtin School of Medical Research web site.
for all of the protein backbone and side chains except for the first seven amino acids. The first three (Gly-Ser-Thr) were a legacy of the recombinant construct. The protein model was refined against 2.0-Å data collected from native (non SeMet) crystals of the GSTO 1-1 complex with GSH. Refinement was carried out using conjugate-gradient least squares and simulated-annealing protocols in X-PLOR 3.851 (29) and monitored with the $R_{\text{free}}$ value using a 5% randomly selected test set. A model for GSH covalently bound to Cys-32 as well as chemically reasonable water molecules and sulfate ions was built into $F_o - F_c$ maps (30). The final model contained 110 water molecules, 2 sulfate ions, disulfide-linked GSH, and the entire polypeptide minus the first seven residues. Final model statistics are shown in Table III.

### RESULTS

**DNA and Protein Sequence**—BLAST searches of the human EST data base with residues 1–100 of human GSTZ1 (6) revealed clones with around 40% sequence identity with the residues between positions 19 and 77 of GSTZ1. Preliminary alignments suggested these were distinct from previously described human GSTs, and a representative EST clone
The poly(A) addition signal is identified GSTO 1-1 as a human EST, searches of additional GSTO 1-1 and the previously defined classes. Although we first lights the similarities and significant differences between total atoms (non-hydrogen) 2045 (protein 1 Root mean square deviation R pre‑viously described GST classes and GST-like proteins high‑duced amino acid sequence with representative sequences from others with accession number U90313. Alignment of the de‑molecular mass of 27,566 Da. A similar cDNA sequence encod‑ing the same peptide has been submitted to GenBank™ by ing the same peptide has been submitted to GenBank™ by

The sequence alignment was used to generate a phylogenetic tree using the maximum‑likelihood approach. The tree in Fig. 3 is the most likely tree; another five locally optimal trees were found, none of which differ significantly from the most likely tree. The differences between the six trees were confined to the branches, indicated by dashed lines. The sequences are (species name; GenBank™ accession number): nematode Omega (C. elegans; L23651), mouse Omega (Mus musculus; U80819), rat Omega (Rattus rattus; AB008807), human Omega (Homo sapiens; AF212303), soybean heat‑shock protein (HsPr) (Glycine max; M20363), potato GST (Solanum tuberosum; J03952), human Zeta (H. sapiens, NM_001513), carnation Zeta (Dianthus caryophyllus, M64268), Escherichia stringent starvation protein (SisPPr) (E. coli, X05088), Proteus GST (Proteus mirabilis, U38482), Escherichia GST (E. coli, D38497), Ochrobactrum GST (Ochrobactrum anthropi, Y17279), Arabidopsis Phi (A. thaliana, D17672), Petunia Phi (Petunia hybrida, Y07721), blowfly Delta (L. cuprina, L23126), house fly Delta (Mus domestica, X61302), fruit fly Delta (Drosophila melanogaster, X14233), mouse Theta (M. musculus, U38482), human Theta (H. sapiens, NM_000844), mouse Pi (P. morum, NM_008939), human Pi (M. musculus, NM_000848), chicken Pi (Gallus gallus, X59248), rat Pi (Rattus norvegicus, L29427), human Omega (H. sapiens, NM_000852), rat Sigma (R. norvegicus, M36937), human Sigma (H. sapiens, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Omega (H. sapiens, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. musculus, AF212303), human Omega (M. musculus, NM_001513), human Mu (M. muscul,
bean heat-shock protein are part of the Omega class.

A notable feature of the human, mouse, pig, and nematode Omega GSTs is a 19–20-residue N-terminal extension not found in any previously described GST. This was not noted in the homologous rat sequence reported as a glutathione-dependent dehydroascorbate reductase (32), but translation with a reading frame adjustment of the rat sequence upstream of the reported translation-initiating ATG codon reveals an N-terminal sequence similar to that in the other species. Additional rat cDNAs need to be sequenced to determine if there is a species difference or if there is a cloning artifact in the clone reported by Ishikawa et al. (32).

**GSTO 1 Expression**—Northern blots of RNA from a range of human tissues showed a single hybridizing species of approximately 0.8 kilobases (Fig. 4). The level of hybridization indicates that GSTO 1 mRNA is expressed in all tissues tested, with the greatest expression in liver, skeletal muscle, and heart and relatively low levels in brain, placenta, and lung. The level of expression in skeletal muscle and heart is difficult to judge accurately because the loading control hybridization with a β-actin probe shows strong hybridization to an extra 1.8-kilobase band that tends to obscure the 2-kilobase β-actin band found in all tissues. A survey of the EST data base has confirmed that GSTO 1 is expressed in a wide range of human tissues including aorta, bone, brain, breast, central nervous system, colon, esophagus, foreskin, germ cell, heart, kidney, lung, lymph, muscle, ovary, pancreas, parathyroid, placenta, prostate, spleen, stomach, testis, uterus, and whole embryo. The broad range of expression of GSTO 1 contrasts with many other GSTs that show distinct tissue-specific expression and suggests that it plays a fundamental role in cellular metabolism.

**Characterization of Recombinant GSTO 1-1**—A Western blot of cytosolic extracts from human liver and Jurkat cells revealed a single cross-reacting protein with an apparent mass of 31 kDa (not shown), co-migrating with purified recombinant GSTO 1. As the deduced amino acid sequence predicts a mass of 27.5 kDa, GSTO 1 migrates anomalously in reducing SDS-PAGE. Size exclusion chromatography of recombinant GSTO 1 on a calibrated Superdex 75 HR 10/30 column estimated its mass as 56 kDa, indicating that GSTO 1 forms a dimer (GSTO 1-1) under native conditions. Sedimentation equilibrium experiments were conducted at protein concentrations of 0.11, 0.17, and 0.34 mg/ml. For the two higher concentrations, NONLIN analysis yielded good fits to molecular masses of 54,545 Da (0.34 mg/ml) and 54,145 Da (0.17 mg/ml), each indicating a dimer. The data collected at 0.11 mg/ml displayed evidence of some non-ideality and fit better to a model taking account of some non-equilibrium behavior (90% dimer and 10% incompetent monomer). More extensive studies would be needed to confirm this observation and extend it into a lower concentration range.

To test recombinant GSTO 1-1 for GST-like activities, assays were performed with compounds that are substrates for GSTs of other classes. Also, as the homologous rat sequence was reported to encode a glutathione-dependent dehydroascorbate reductase (32), activity and glutathione-dependent thiol transferase activity were determined. GSTO 1-1 has little activity with most GST substrates (Table I). There was low but detectable activity with 1-chloro-2,4-dinitrobenzene, which is generally a good substrate for members of the Alpha, Mu, and Pi classes. Similarly, there was low but measurable activity with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-CI) trans-cyclooctene and trans-nonenal, substrates utilized preferentially by Alpha class GSTs (22).

Glutathione-dependent dehydroascorbate reductase activity was detected in preparations of recombinant human GSTO 1-1. This has been reported in a number of tissues from several species (33) and, in most cases, attributed to glutaredoxin (also termed thiol transferase). Consequently, the thiol transferase activity of the recombinant enzyme was determined and found to be the highest of all the activities investigated (Table I). Similar results were obtained with GSTO 1-1 prepared from a GST fusion or with an N-terminal His tag. Thus the Omega class GSTs have activities similar to those of the glutaredoxins.

Similarities in the protein fold of the glutaredoxins and the N-terminal domain of the cytosolic GSTs have been noted (34), and low thiol transferase activity (74.3 nmol/mg/min) has been attributed to a Mu class GST from bovine lens (35). In other unpublished studies we examined the thiol transferase activity of a range of GSTs from the Alpha, Mu, Pi, Theta, and Zeta classes (GSTA 1-1, GST 2-2, GSTA 4-4, GSTM 1-1, GSTM 2-2, GSTM 3-3, GSTM 4-4, GSTP 1-1, GSTT 2-2, GSTT 1-1, GST Z 1-1). With the exception of GSTM2-2, which had low activity, all were essentially inactive. It is therefore clear that the glutaredoxin-like activities of GSTO 1-1 are not a common feature of other cytosolic GSTs despite similarities in their structure.

**Crystal Structure of GSTO 1-1**—The structure of a covalent complex between GSTO 1-1 and GSH was determined to 2.0-Å resolution by multiwavelength anomalous dispersion. Multiwavelength anomalous dispersion data to 2.1 Å resolution were collected from crystals of SeMet-substituted apo-GSTO 1-1 (Table II). The crystals are trigonal, belong to space group P3_2_1, and have a single polypeptide in the asymmetric unit. Two monomers related by the crystallographic 2-fold axis form a dimer, as is characteristic of GSTs (Fig. 5A).

GSTO 1-1 has modest sequence identity with the GSTs for which crystal structures are known but clearly adopts the canonical GST fold (Fig. 5B). There are two domains, an N-terminal thioredoxin-like domain, and a C-terminal domain that is all α-helical. The N-terminal domain consists of a central four-stranded β-sheet flanked on one side by two α-helices
(a1, residues 32–45; a3, residues 85–97) and on the other side by a 3_10 helix (a2, residues 60–66). The N-terminal extension identified by sequence alignment\(^2\) forms a distinct structural unit that other structures lack (Fig. 6). Residues 5–22 form an extended structure on the surface of the protein that leads into the first \(\beta\)-strand (b1, 23–28) (Figs. 5A and 6).

Of the seven \(\alpha\)-helices in the C-terminal domain, five (a4, a5, a6, a7, a8) are common to most members of the GST folding superfamily (Fig. 6), but the last two (a9, 219–230 and a10, 235–239) fold back over the top of the N-terminal domain and set the present structure apart. This C-terminal extension makes several H-bonds with the N-terminal domain and forms a continuous surface with the N-terminal extension (Fig. 7).

**Fig. 5. Structure of human GSTO 1-1.** A, the GSTO 1-1 dimer, as seen looking down the 2-fold axis. The N- and C-terminal extensions (see text under “Crystal Structure of GSTO 1-1”) are highlighted in red. The GSH molecules and bound sulfate ions are indicated in ball and stick representation. B, a ribbon representation of the monomer. Residues were assigned to secondary structural elements according to criteria defined by Kabsch and Sander (51). The secondary structure nomenclature has been made consistent with other published GST structures. Helices a2 and a10 are 3_10 helices, and other helices are \(\alpha\)-helices. These images were generated using the program RIBBONS (52).

**Fig. 6. The common chain fold of the GST superfamily.** The coordinates of all nine structures were superimposed and then pulled apart for clarity. Helices are represented as cylinders, and \(\beta\)-strands are represented as arrows. Assignment of secondary structure was done according to criteria defined by Kabsch and Sander (51). Protein data bank codes and references for the structures used are: alpha, 1gse (53); beta, 2pmt (36); delta, (40); theta, 1ljr (54); mu, 1hna (55); pi, 1glp (56); sigma, 1gsq (57); phi, 1gnw (39); omega, 1eem (this work). Images in this figure were drawn using the programs MOLSCRIPT (58) and RASTER3D (59).

**Fig. 7. Surface representation of the GST-Omega dimer, looking down the 2-fold axis.** The N- and C-terminal extensions (see text under “Crystal Structure of GSTO 1-1”) are colored green and purple, respectively. GSH molecules are shown in ball and stick representation. This figure was drawn using the program GRASP (60).
G-site and the Recognition of GSH—Two features of GSH binding distinguish GSTO 1-1 from typical GSTs. The first is that Cys-32 makes a mixed disulfide with GSH, as seen also in the bacterial enzyme PmGST B1-1 (36). As noted in that case, the presence of this disulfide prompts caution in considering possible catalytic functions of GSTO 1-1. It is unclear what catalytic residues would stabilize the thiolate form of glutathione for the standard GST function of transferring it to an electrophile. In the human Mu class enzyme GSTM2-2 it has been suggested that movement of a positively charged Arg residue into the catalytic pocket promotes the ionization of the sulphydryl group of GSH (37). However, examination of the GSTO 1-1 structure has not identified any atoms within 5 Å of the Cys-32 thiol that could be involved in stabilizing a thiolate ion.

Cys-32 is located at the N terminus of α1, with its thiol precisely over the helix axis. This placement is exactly as seen for the thiol of the N-terminal cysteine of the Cys-Xaa-Xaa-Cys motif of thioredoxin, glutaredoxin, and DsbA (Fig. 8 shows structural overlap of GSTO 1-1 and glutaredoxin). This structural feature appears likely to have major implications for the function of GSTO 1-1. As shown by Kortemme and Creighton (38), the proximity of the Cys side chain to the positive end of a helix dipole substantially lowers the thiol $pK_a$ and creates the potent nucleophilicity of the exposed cysteine in thioredoxin and its relatives. The proline following this cysteine, as Pro-33 follows Cys-32 of GSTO 1-1, promotes optimal positioning of the Cys-32 thiol for stabilization of the thiolate ion.

The second novel feature is the absence of interactions between GSH bound to one polypeptide and groups of the other polypeptide. In the other known GST structures, except for the Theta-like GSTs from plant (Arabidopsis thaliana (39)) and insect (Lucilia cuprina (40)) species, there is a salt bridge between the N-terminal nitrogen of GSH and an acidic residue on helix α4 of the second subunit (Asp-101 in GST-Alpha, Glu-104 in GST-Beta, Asp-105 in GST-Mu, Asp-98 in GST-Pi, Asp-101 in GST-Sigma, and Asp-104 in mammalian GST-Theta). The corresponding residue in GSTO 1-1 is Lys-122. The GSTO 1-1 dimer has an atypically open, V-shaped configuration. Contacts between the subunits are confined to side chains from β4, α3, and α4. Most of the interactions at the interface are non-polar in character, with no H-bonds and only two salt bridges (the two instances of Lys-114–Glu-91). The relative orientation of the monomers at the subunit interface is consistent with the orientation seen in the other GST crystal structures (10), but the interface is more open than in any of the other GST dimers. The buried area at the interface is 1960 Å², compared with 2700–3400 Å² in most other GST dimers. The plant Theta-like GSTs (39), which also lack an inter-subunit interaction involving GSH, have a relatively open dimer inter-
face with a buried surface area of 2370 Å².

In all other respects, GSH binding is analogous to what has been observed in other GSTs. All the interactions of GSH with the protein are made with the N-terminal domain, and residues that contribute to binding GSH are either conserved or conservatively replaced in all the GSTs (Fig. 9). Glu-85, which forms a H-bond with the amino group of GSH, has strained backbone stereochemistry and is in the generously allowed region of the Ramachandran plot. The equivalent residue in other GST structures also adopts a strained conformation (10).

The H-site—Many GSTs catalyze conjugation of the thiol group of GSH to an electrophile, often a component of a toxic xenobiotic with some hydrophobic character. The canonical GST structure has a binding site known as the H-site, which accommodates a hydrophobic moiety and is adjacent to the glutathione binding G-site. The H-site is constructed from elements of both the N- and C-terminal domains, and variations of these between different GST classes reflect the varying substrate specificities of GSTs.

GSTO 1-1 possesses a well defined cavity adjacent to the GSH binding site in the same relative location as the H-site in other GSTs, and this is presumed to be the binding site for a substrate or other molecular target (Fig. 10). One side of this wide and deep pocket is formed by Phe-31 and Pro-33, which flank the putative catalytic or otherwise reactive Cys-32. Residues from the C-terminal helix α8, a distinctive feature of GSTO 1-1, form the top and back of this pocket. Particularly interesting is Trp-222, with its indole nitrogen pointing into the pocket. The indole nitrogen contributes one additional H-bond to the pocket, making the pocket that much less hydrophobic. Another polar atom is contributed by the side chain of Arg-183, the Ne atom of which forms the bottom of the pocket. The open subunit interface and the relatively polar nature of the H-site suggest that the substrate or other binding partner for GSTO 1-1 could be a large and not entirely hydrophobic molecule. The cleft between the subunits is large enough that binding to another protein appears possible.

**DISCUSSION**

Our sequence-based searches of the human EST data base identified what appeared to be a new member of the GST structural family. Independently, a recent study evaluating a new data base searching tool suggested the existence of an additional GST family in the mouse (41). The unknown enzyme was termed GSTX. Sequence comparisons indicate that GSTX is the orthologue of the human GSTO 1-1 characterized in this study. Because the term GSTX does not conveniently lend itself to the established nomenclature (31), we have named the new gene family Omega. Sequence alignment suggested that GST Omega was a new member of the GST structural family, and phylogenetic analysis confirmed that the Omega class GSTs form a cluster distinct from the previously described classes. Further detailed phylogenetic studies are required to gain a greater understanding of the evolutionary history of the GSTs and the clear demarcation of the different classes. Our analysis of the GSTO 1-1 structure has also confirmed its membership of the GST superfamily. GSTO 1-1 is a dimer of subunits that adopt a characteristic GST fold with an N-terminal α/β GSH binding domain and a C-terminal domain composed entirely of α-helices.

Several features set GSTO 1-1 apart from the other members of the GST superfamily. Its N-terminal region has a unique extension of approximately 19 residues when compared with other cytosolic GSTs. This contains a proline-rich segment that in conjunction with the C terminus forms a distinct structural unit with an unknown function. An active site Cys is another notable feature of GSTO 1. Previously characterized eukaryotic GSTs have a tyrosine or serine residue within hydrogen-bonding distance of the sulfur atom of the bound glutathione (8, 10). It is generally considered that a H-bond between the GSH sulfur and the OH of the tyrosine or serine stabilizes the thiolate anion of GSH (42). Mutation of the active site tyrosine and serine residues found in other GSTs generally results in a substantial, if not complete, inactivation (8, 9). Consequently the absence of an equivalent residue in the Omega class GSTs suggests that they may not catalyze the glutathione conjugation reactions typical of many GSTs. The Theta class isoenzyme GSTT 2-2 provides an interesting example of a novel reaction that does not require the presence of a H-bond from a nearby hydroxyl to stabilize the GSH thiolate. GSTT 2-2 acts as a sulfatase with menaphthyl sulfate and generates menaphthyl glutathione and free sulfate. Mutagenesis has suggested that this reaction is not dependent on the presence of a serine and may be a product of the environment generated by a number of residues (43, 44). This notable difference demonstrates that some reactions catalyzed by GSTs can take place in the absence of a tyrosine or serine residue, and an equivalent mechanism may be utilized by the Omega class GSTs.

Studies of the enzymic properties of GSTO 1-1 also indicated that it probably is not a typical GST. Known substrates for other members of the GST superfamily were not turned over by GSTO 1-1. Interestingly, GSTZ 1-1, the human GST with the closest sequence similarity to GSTO 1-1, catalyzes glutathione-dependent conversion of dichloroacetic acid to glyoxylic acid (45), but GSTO 1-1 has no significant activity against dichloroacetic acid. Thus, the enzymatic capabilities of GSTO 1-1 appear distinct from those of other GSTs. The only significant activity observed for GSTO 1-1 was the ability to act as a glutathione-dependent S-thioltransferase. This type of activity is consistent with expectations that Cys-32 serves as a nucleophilic center, based on structural homology to thioredoxin and glutaredoxin and preliminary unpublished studies indicating that the enzymatic activities of GSTO 1-1 are sensitive to alklylation by N-ethylmaleimide. Potential disulfide-containing molecules that may serve as natural substrates for this type of activity include S-thiolated polypeptides. For example, following oxidative stress a number of cellular proteins form S-thiol adducts with glutathione and cysteine (46, 47), and formation of these adducts can inactivate enzymatic functions of the affected polypeptides (48, 49). A conceivable function for GSTO 1-1 is to reduce this type of S-thiol adduct and restore enzymatic function. Interestingly, the crystal structure of GSTO 1-1 indicates that its H site is open to the surface and large enough to accommodate a polypeptide chain.

Mouse and rat orthologues of GSTO 1-1 have been identified and characterized in recent reports. Comparison of the cDNAs prepared from radiation-sensitive and radiation-resistant populations of mouse lymphoma cells identified a 28-kDa polypeptide (p28) as more highly expressed in the resistant cells (50). The p28 protein (GenBank™ number accession U80819) is predicted to be expressed as a 240-residue polypeptide. This is one amino acid shorter than human GSTO 1-1, with the extra residue in the human sequence (Thr-162) occurring in a surface loop, p28 has 72% sequence identity with human GSTO 1-1, with many conservative replacements where the sequences differ.

In apparent contrast to our findings with GSTO 1-1, p28 is reported to bind to agarose-coupled glutathione. However, our studies were conducted using Glutathione-Sepharose (Amer sham Pharmacia Biotech), in which glutathione is linked to the support through its sulfydryl. The earlier study was conducted using Glutathione-agarose (Sigma), in which glutathione is immobilized through its N terminus and retains the free thiol.
Like GST 1-1, p28 lacks demonstrable GST activity, and the recombinant mouse protein is devoid of glutathione peroxidase activity (50). Moreover, message encoding GST 1-1 and p28 is expressed by many tissues, with liver and heart containing the highest levels. Interestingly, p28 is reported to change its subcellular location in response to heat (50). On this basis p28 may represent a component of the stress response mechanism of the cells.

A rat orthologue of GSTO 1-1 (GenBankTM number accession AB008807) has been purified from rat liver on the basis of its p28 may represent a component of the stress response mechanism of the cells.

A rat orthologue of GSTO 1-1 (GenBankTM number accession AB008807) has been purified from rat liver on the basis of its p28 may represent a component of the stress response mechanism of the cells.

A rat orthologue of GSTO 1-1 (GenBankTM number accession AB008807) has been purified from rat liver on the basis of its p28 may represent a component of the stress response mechanism of the cells.

A rat orthologue of GSTO 1-1 (GenBankTM number accession AB008807) has been purified from rat liver on the basis of its p28 may represent a component of the stress response mechanism of the cells.

A rat orthologue of GSTO 1-1 (GenBankTM number accession AB008807) has been purified from rat liver on the basis of its p28 may represent a component of the stress response mechanism of the cells.