A Distinct Human Testis and Brain \( \mu \)-Class Glutathione \( S \)-Transferase

MOLECULAR CLONING AND CHARACTERIZATION OF A FORM PRESENT EVEN IN INDIVIDUALS LACKING HEPATIC TYPE \( \mu \) ISOENZYMES*

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\( \mu \)-Class glutathione \( S \)-transferases (GSTs) were identified in all 13 human testes and 28 brains examined; even subjects whose livers were devoid of \( \mu \)-GSTs expressed extrahepatic GSTs of this class. Testes and brains from individuals with \( \mu \)-class GSTs in their livers had additional forms that also reflected the liver phenotypes. An isoenzyme with an isoelectric point of 5.2, which was a major GST in testis and present as well in cerebral cortex but not detected in any livers, was identified and purified. Sequence analysis of peptides derived by cleavage of the testicular \( \mu \)-class GST by Achromobacter protease I revealed distinct aspects of primary structure not found previously in any mammalian \( \mu \)-class GSTs. These unique features included a blocked and extended amino terminus and 3 additional residues (Pro-Val-Cys) at the carboxyl terminus. This structure was confirmed by molecular cloning and sequencing of cDNAs derived from human testis and brain libraries. In the coding region the mRNA of the brain-testis \( \mu \)-class GST was 75% homologous with that of the liver form, and its 3' untranslated sequence was mostly divergent, indicating that it is the product of a separate gene. Distinct catalytic and structural properties of the testis-brain \( \mu \)-class GSTs suggest that these GSTs may be uniquely involved in blood-barrier functions common to both organs.

Multiple forms of mammalian glutathione \( S \)-transferases (GSTs)\(^*\) are encoded by supergene families (1-4). Based on sequence homologies, substrate specificities, and other common properties, mammalian cytosolic GSTs may be subdivided into three different classes designated as \( \alpha \), \( \mu \), and \( \pi \) (5). The proteins assemble in dimeric combinations of subunits within each class. Three distinct genetic loci (GST1 or \( \mu \)-class, GST2 or \( \alpha \)-class, and GST3 or \( \pi \)-class) have been identified in humans (6-9) with additional genetic polymorphisms. There are three alleles of the GST1 locus (GST1 type 1 and GST1 type 2 and a null allele (GST1-0)) to give rise, in liver, to four phenotypes (1; 1, 2, and 0). The prevalent null allele has been attributed to a gene deletion (10, 11), and about half of the human population does not express \( \mu \)-class GSTs (GST1) in their livers or peripheral mononuclear leukocytes (11-15). It has been suggested that this genotype may be correlated to the increased susceptibility of individuals to cancer of the lung and certain other tissues (16, 17) since GSTs are intracellular binding proteins that detoxify electrophilic compounds by catalyzing the formation of GSH conjugates (18-21).

This investigation was undertaken to determine if the GST1 (\( \mu \)) phenotype of liver is reflected in the GST composition of other tissues and to explore possible consequences of the occurrence of \( \mu \)-class GST null alleles in tissues other than liver. Human testis and brain were selected for study in view of earlier observations that \( \mu \)-class GSTs are major forms in rat brain and testis, which selectively express a specific \( \gamma \) subunit (22, 23). In the course of this investigation a novel human \( \mu \)-class GST was identified, characterized, and cDNA clones for this form were sequenced.

EXPERIMENTAL PROCEDURES

Tissue Sources and Purification of GSTs—Testis, cerebral cortex, and liver were obtained 5-12 h postmortem from 11 apparently normal male subjects ranging in age from 2 to 67 years who died of motor vehicle accidents or gunshot wounds with no evidence of any preexisting disease. Additional samples of brain and liver were obtained from 8 female and 20 male subjects. The tissue was snap-frozen in liquid \( N _ { 2 } \) after excision and stored at \(-70^\circ C\).

Approximately 10 g of frozen tissue was used for each GST purification. The tissue was homogenized in 0.1 M sodium phosphate buffer, pH 7.4, and 20,000 x g supernatant fractions were subjected to gel permeation chromatography (Sephadex G-100) and affinity chromatography according to described methods (24). GSTs were eluted from immobilized GSH (25) or epoxy-GSH-agarose affinity matrices using 10 mM Tris buffer, pH 8.9, containing 5 mM S-methyl-GSH or 5 mM GSH, respectively; in that way the entire complement of cytosolic GSTs from each tissue was obtained. Testicular GSTs were resolved further by anion exchange chromatography on DEAR-Sepharose (25).

Antisera and Immunoblotting—A synthetic oligomeric peptide of 24 residues which corresponds to a common sequence of rat \( \gamma \) type subunits (residues 43-66, sequence Ser-Gln-Val-Leu-Asn-Glu-Lys-Phe-Lys-Leu-Glu-Gly-Leu-Asp-Asp-Pro-Asn-Leu-Pro-Tyr-Leu-Ile-Asp-Asp-Lys-Ser) was used to raise antibodies to \( \mu \)-class GSTs in rabbits (26). This peptide is also identical to the corresponding mammalian liver \( \mu \)-GST sequence (with the exception of alanine instead of serine at the carboxyl terminus (11, 27)). Antisera to \( \alpha \)-class GSTs were raised in rabbits using human placental GST as antigen (28). Rabbit antihuman \( \alpha \)-class GST antisera were described previously (29). There was no cross-reactivity among antibodies of the \( \alpha \), \( \pi \), or \( \mu \)-classes of GSTs. Immunoblotting was performed by transfer of protein from SDS-PAGE gels to nitrocellulose filters (30) and incubation with antisera. 

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\( \ddagger \) The abbreviations used are: GST(s) glutathione \( S \)-transferase(s); tPBO, trans-4-phenyl-3-buten-2-one; SDS, sodium dodecyl sulfate; HPLC, high pressure liquid chromatography; kb, kilobase(s).
polycrylamide or isoelectric focusing gels to nitrocellulose membranes (Bio-Rad Trans-Blot) according to methods described previously (30). The membranes were blocked with 5% nonfat milk in 50 mM Tris-HCl buffer, pH 7.9, for 30 min and then incubated for 1 h with the antibodies in the same buffer. Two methods were used to detect bands. Antibodies were visualized by a silver-enhanced procedure with 0.1% Tween 20, the membranes were incubated with [125I]-protein A in the Tris buffer containing 5% nonfat milk, washed, and prepared for autoradiography (30). Alternatively, after interaction with specific anti-α, π-, or γ-GST antisera and washing, the blots were incubated for 1 h with a goat anti-rabbit IgG horseradish peroxidase conjugate in the Tris-HCl buffer containing 0.15 M NaCl and 5% nonfat milk. Blots were washed with the same buffer and color developed with 0.06% (w/v) 4-chloro-1-naphthol containing 0.01% H2O2 in the buffer.

**Enzymatic Assays**—Conditions were those described previously (31) with 1 mM 1-chloro-2,4-dinitrobenzene or other substrates and 1 mM GSH in 10 mM sodium phosphate buffer, pH 6.7. GST peroxidase activities were measured by an assay coupled to GS-SG reduction and changes in absorbance at 340 nm; 0.2 mM NADPH, 1.5 mM H2O2, or cumene hydroperoxide acid and 1 mM GSH were used.

**Proteolytic Cleavage with Achromobacter Protease I and Analysis of Peptides**—The GST was reduced using a 20-fold m excess of dithiothreitol in the presence of 6 M guanidinium chloride, 0.5 M Tris, pH 8.0, in the dark to remove the excess reagents. Digestion with Achromobacter protease I (kindly provided by Dr. Y. Burstein, Weizmann Institute, Rehovot, Israel) which selectively cleaves C-terminal peptide bonds was carried out at 37 °C for 5 h in 50 mM Tris-HCl buffer, pH 9.0, containing 3 M urea. With ratios of enzyme to GST of 1/250 (w/w), digestions were specific and essentially complete.

The peptides generated by this treatment were resolved and purified by reversed-phase HPLC on Aquapore RP-5000 (C8) columns (4.6 × 250 mm, 5 μm particle size) eluted with a linear gradient of acetonitrile (5-65%) in 0.1% trifluoroacetic acid. Peptides were analyzed using a Spectra-Physics modular chromatograph model SP 8800/8870/8500/8773/4290 equipped with a Barspec model 1937 Chrom-A-Scope fast scan detector. Sequence analysis of the HPLC peptides was performed with an automated Applied Biosystems model 470A microsequenator equipped with an on-line phenylthiohydantoin analyzer. Data obtained at each cycle were processed using a Spectra-Physics Chromstation-AT program.

**Molecular Cloning—**mRNA isolated from the testicle (not hormonally induced) of a 50-year-old healthy man was used to prepare a λgt11 human testis cDNA library (Clontech Laboratories, Inc.). Six plates with approximately 4 × 108 plaques were screened, and 38 positive clones with inserts ranging in size from 0.75 to 1.7 kb were identified. A λgt11 human brain cDNA library (Clonetech) was prepared from a surgical specimen of normal temporal cortex tissue excised from around a brain tumor of a 50-year-old male. Fifteen plates with approximately 3.8 × 108 plaques were screened from that library, and four positive plaques with inserts ranging in size from 0.5 to 0.8 kb were identified. A human cerebellum cDNA library (Strategene, Inc., LaJolla, CA) was prepared from a normal 2-year-old female and cloned in ZAP vectors. Thirteen plates with approximately 3.3 × 108 clones and two positive plaques with inserts of approximately 850 and 1200 bases were isolated from that library. A λgt11 human liver cDNA library was prepared by Dr. S. Woon, Baylor College of Medicine. Ten plates with approximately 2.5 × 108 plaques were screened, and one positive clone with an insert size of 1.2 kb was identified.

The λgt11 cDNA libraries were screened by DNA hybridization with a portion of a rat Ynα probe that cross-hybridized with all rat µ-class GSTs (30). The phage for screening was grown on an LEC92 strain of Escherichia coli to yield approximately 25,000 plaques on 150-mm-diameter Petri dishes. The Ynα probe was labeled with a Multivrimp DNA labeling system (Amersham) using [32P]dCTP to a specific activity of >1 × 109 dpm/μg of DNA. Hybridization was carried out at 42 °C in 50% formamide, 5 × Denhardt’s solution, 5 × SSPE, and 0.1% SDS. After hybridization, filters were washed in a solution containing 1× SSC and 0.1% SDS at 65 °C as described by Maniatis et al. (32). Positive plaques were isolated and then DNA purified. cDNA inserts were ligated into pUC19 plasmids (Bethesda Research Laboratories). Sequencing of plasmid DNA was performed by dideoxy chain termination techniques (33, 34) using a modified form of T7 DNA polymerase (Sequenase) and [α-32P]dATP (United States Biochemical Corp.).

**RESULTS**

**Identification of a Unique µ-GST in Testis and Brain**—In an extensive survey of µ-class GST composition of extraplastic tissue, GSTs were purified from the cerebral cortices of 28 individuals of both sexes. GSTs were also isolated from the livers of 18 of the male subjects, and from 13 testes of the same individuals. The proteins were analyzed by SDS-polyacrylamide gel electrophoresis and isoelectric focusing, and µ-, π-, and α-class GSTs were identified by immunoblotting methods using specific antisera. GSTs of the µ-type were detected in all brain and testis samples tested (Fig. 1B), even from individuals whose livers were devoid of the liver-type µ-class GSTs. Testis was particularly rich in a µ-class GST, with an isoelectric point of 5.2, and µ-class isoenzymes with the same isoelectric points were present in brain (Fig. 1A). The predominant GST of most brain specimens was κ (pI 4.7), whereas in testis, the π-enzyme is only a minor form, but α-class GSTs abound in testis.

In contrast to the pI 5.2 µ-class GSTs that were common to all testis and brain specimens, other µ-class isoenzymes showed significant variability among different subjects and were consistent with the isoenzyme compositions of their livers. Thus, each individual with type 1 (pI 5.6) or type 2 (pI 6.2) µ-class isoenzymes in liver (35, 36, 38) and heterozygotes (1, 2) displayed the same GST phenotypes in brain and testis (see examples of each in Fig. 1A). The heterozygotes also had a third isoenzyme with a pI of about 5.9 (Fig. 1A); this isoenzyme probably represents a heterodimeric form of the types 1 and 2 µ-subunits. The putative null alleles (GSTT1-0) had none of the liver µ-isoenzymes in their brain or testis.

**Peptide Maps and Primary Structure**—In view of differences in tissue distribution, pl, and other physical properties of the brain and testicular pI 5.2 GSTs when compared with the previously characterized µ-class GSTs of human liver and of rat (Ynα, Ymα, and Ymβ), studies of its primary structure were

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**FIG. 1. Electrophoretic properties of human testis and brain GSTs.** A, GSTs purified by use of affinity matrices were resolved by isoelectric focusing in 5% polyacrylamide gels with 0.5% amphotole solution, pH 3.5–10. Gels were stained with Coomassie Blue. The pl markers (lane M) were glucose oxidase (4.2), soybean trypsin inhibitor (4.6), β-lactoglobulin (5.15), carbonic anhydrase B (5.3, 6.6), and lactic dehydrogenase (8.55). The testis specimen of lane 4 and brain of lane 7 were from individuals containing a µ-class GST with an isoelectric point of 5.6 in their livers; brain specimens in lanes 3 and 5 were those from individuals with pl 6.1 in their livers, and lanes 2 of testis and lane 6 of brain were from putative heterozygotes with regard to liver µ-class GSTs. Testis of lanes 1, 3, and 5 and brains of lanes 2, 5, and 6 were from individuals lacking liver µ-class GSTs. B, immunoblot analysis of human brain GSTs. Soluble GSTs were isolated from the cerebral cortex of eight individuals using GSH-affinity matrices. Equivalent amounts of protein were resolved by SDS-polyacrylamide gel electrophoresis in 12% gels, and the gels were prepared for immunoblotting by methods described under "Experimental Procedures." M is a marker of rat liver Ynα, subunits; GSTs in lanes 1, 5, and 7 were isolated from subjects who had no detectable µ-class GSTs in their livers (null alleles).
undertaken. Since the pI 5.2 μ-class GST was a major component of testis (Fig. 1), this tissue was selected for purification and further characterization of that form. The protein was isolated from a single testis and purified to homogeneity. Its mobility on SDS-polyacrylamide gels was slightly slower than that of human liver μ-GST. The testicular protein did not undergo an Edman degradation reaction, suggesting that its amino terminus was blocked. The pI 5.2 GST was therefore subjected to proteolytic cleavage at lysine residues by Achromobacter protease I for analysis. Fifteen peptide fragments were identified from reversed-phase HPLC patterns (Fig. 2), and several were selected for sequence analysis. Fragment K12 did not react with phenylisothiocyanate and was therefore considered likely to be the blocked amino-terminal peptide. That fragment contained both tryptophan (based on its spectral properties in the 260-305 nm region) and cysteine (based on S-carboxymethylation with [14C]iodoacetate). The first lysine residue of rat and human liver μ-GSTs is at position 21. Although other μ-class GSTs have a single tryptophan near their amino-terminal regions, there are no cysteine residues in any other GST in this region.

Fragment K12, a second peptide lacking both tryptophan and cysteine, was shown to contain 21 residues with the expected lysine at its carboxyl terminus. Comparisons with human liver μ-GST sequences are shown in Fig. 3. Fourteen of the 21 residues of K12 are identical to corresponding sequences at positions 152-172 of the human liver enzyme. The latter sequence follows lysine 151. Residue 172 of the liver sequence however is an asparagine rather than the lysine that terminates K12.

A third peptide selected for sequence analysis because it contained both tryptophan and cysteine (fragment K3), was a decapeptide that lacked a carboxyl-terminal lysine but had an internal Lys-Pro sequence that is refractory to cleavage by Achromobacter protease I. This peptide terminated with a cysteine residue and was tentatively assigned as the carboxyl-terminal fragment of the testicular GST. Indeed, residues 211-217 at the carboxyl terminus of human liver μ-GST are identical to the first 7 residues of the K3 peptide with only a single exception (Fig. 3); but liver μ-GST terminates with a lysine at position 217, whereas the testis GST has the additional residues of proline, valine, and cysteine. To verify these unique differences and to search for other distinctive structural features and properties of the brain and testicular μ-class GSTs, enzymatic and molecular cloning studies were performed.

**Catalytic Properties**—Enzymatic activities of the purified pI 5.2 testicular GST with several commonly used substrates (Table I) show that its specific activity with 1-chloro-2,4-dinitrobenzene was much lower than that of the liver μ-isozyme, but activity of the testicular isoenzyme with ethacrynic acid was greater. A distinguishing property of human liver μ-class GSTs is their capacity to catalyze conjugation reactions with certain aromatic substrates such as trans-stilbene oxide, benz[a]pyrene oxides and other derivatives, and trans-4-phenyl-3-buten-2-one (tPB0) (12-14, 35). Indeed, cytosolic extracts of testes or brains from persons who also expressed liver μ-GSTs, but not those from null allele individuals, catalyzed the reaction with tPB0. The purified testicular μ-class GST (pI 5.2) was virtually inactive with tPB0 as a substrate. On the other hand, the testicular GST had much higher GSH peroxidase activity as compared with the liver isoenzyme (Table I).

**cDNA Cloning of Human Testis and Brain μ-GSTs**—To characterize the μ class GSTs specific for testis and brain, λgt11 phage expression vector libraries from testis, brain, and...
cerebellum were screened using a cDNA fragment obtained from a rat brain Yb9 (G-class) clone (22). That particular probe crossed-hybridized with mRNAs for all rat Yb or µ-class GSTs (30). A human liver cDNA library also screened with this probe yielded a µ-clone containing a 1.2-kb insert with a sequence that was virtually identical to that reported by DeJong et al. (27). Multiple positive plaques were isolated from the testis and brain cDNA libraries, and several with cDNA inserts of greater than 0.9 kb were selected for further characterization and sequence analysis.

Restriction endonuclease digests of the inserts suggested that the testis cDNA library was probably constructed from an individual lacking liver class µ-GSTs (null allele), since none of the inserts was identical to those of the liver clones. In the brain and testis cDNA libraries, at least two distinct groups of clones that hybridized with the µ-GST probe were identified. The restriction map for one type of insert (HBGT-18 from brain or HTGT-6 from testis) was unique and marked by EcoRV and BclI cleavage sites (notations in Fig. 4) not present in the cDNAs of human liver µ-class GSTs. On the other hand, the liver clones contained BglII, PstI, and two Ncol sites that were missing in the testis cDNA. Sequence analysis of the human testis clone (HTGT-6) yielded an open reading frame with an apparently complete coding region of 675 nucleotides which codes for a polypeptide with a chain length of 225 amino acids (including the methionine initiation codon). Its overall size (1.25 kb) and 570 nucleotides in the 3'-noncoding region were greater than the corresponding human liver µ-GST mRNA.

The nucleotide and deduced amino acid sequences of HTGT-6 (Fig. 4) show it to be a unique µ-class GST differing from the previously characterized human, mouse, and rat liver µ-class GSTs with chain lengths of 217 amino acids. Another novel feature of the testis and brain forms is the 9 additional nucleotides at the 3' end of the coding region which code for Pro-Val-Cys. The liver form terminates with lysine, which is the 4th residue from the carboxyl terminus in HTGT-6. These data confirm the structural analysis of the peptides (Figs. 2 and 3).

A TGA termination codon sequence that terminates the testis coding region is also found 5 nucleotides downstream of the TGA of the liver form. That TGA is 1 residue out of the reading frame and is also followed by a common 8-nucleotide motif (GCAGGAGG) for both forms (Fig. 4), but after that the sequences diverge in the 3'-noncoding region. A consensus sequence (CACC) for initiation of translation (37) immediately precedes the initiation codon.

The testis enzyme shows 72% amino acid and 75% nucleotide sequence homologies with the liver µ-GST in the coding region. The calculated pI of the testis isoenzyme based on its amino acid composition (PC-Gene, Intelligentic, Inc.) was 5.15, which is very close to the 5.2 value observed by isoelectric focusing. Disparities in amino acid composition of the liver and testis forms are highlighted by 11 asparagines as against 4 and 3 additional cysteines near the carboxyl end including the carboxyl-terminal residue in the testis GST. Even though the testis-brain isoenzymes and the liver forms probably originate from different genes, all are evidently µ-class GSTs. Thus, in spite of sequence differences at the amino- and carboxyl-terminal regions of the encoded polypeptides, there are long stretches of identical and homologous sequences. In particular, the peptide used to raise anti-µ-GST antibodies with a sequence that is common to all rat µ-GSTs (Yb9, Yb9a, and Yb) as well as to human liver µ-GST (residues 43–66), had 19 of its 24 residues identical to those of the corresponding sequence of the human testicular isoenzyme. Several divergent regions also exist: at residues 196–205 of the liver sequence near the carboxyl terminus, where only 3 of 13 residues are identical (Fig. 4), and at residues 106–119, where only 4 of 14 residues are identical. The 12 residues that are conserved in all mammalian forms of GST analyzed thus far (1, 4) are also conserved in the testis-brain sequence.

**DISCUSSION**

A human pl 5.2 GST that is prevalent in testis and found in brain but not in liver has been identified and characterized. This isoenzyme, which is more closely related to µ-class rather than α-class or γ-class GST categories, also has unique structural properties that distinguish it from other human or rat µ-class GSTs. Its primary structure features a blocked amino terminus with additional amino acid residues at both ends of the polypeptide chain. The testicular GST is 72% homologous with the human liver class µ-GST and has long stretches of amino acid sequence identity (particularly near the amino terminus). Nevertheless, discrete regions of sequence in the two forms of GST are substantially different. Moreover, the 3'-noncoding regions of their respective mRNAs are highly divergent. Earlier analyses of Southern blots of human genomic DNAs suggested the presence of multiple µ-GST genes or pseudogenes (11, 27). This prediction is borne out by the.
identification of the pl 5.2 testicular and brain GST which is evidently the product of a separate gene.

Even though about 50% of human livers are completely devoid of μ-class GSTs because of a frequently occurring gene deletion (11, 16), other μ-class GSTs persist in testis and brain of all individuals examined (Fig. 1B). In testis, the μ-form with an isoelectric point of 5.2 is a major GST isoenzyme. Individuals who did express liver-type μ-GSTs had an additional GST of the same liver phenotype (GST I type 1, GST I type 2, or the heterozygote) in testis and brain. It is thus possible to determine the liver phenotype of an individual by examining brain or testis GSTs. The isoelectric focusing data (Fig. 1A) disclose no evidence for the formation of natural heterodimers between subunits of liver and testis or brain μ-GSTs. It is not yet clear whether the absence of heterodimers is due to incompatibilities in assembly or because the subunits are synthesized in different cell types.

The pl 5.2 μ-class GSTs of testis and brain have extremely low enzymatic activities with tPBO as a substrate. This isoenzyme would probably escape detection in tissues if similar types of aromatic substrates are used as criteria for identification of μ-class GSTs (12, 14, 35). It follows that extrahepatic tissue lacking the liver-type μ-GST may not detoxify certain types of aromatic substrates efficiently.

There are some conflicting reports regarding the GST composition of human extrahepatic tissues. Isoelectric focusing studies by Polidoro et al. (39) suggested that human brain contains only a single acidic (π-type) isoenzyme. Theodore et al. (40) showed later that three GSTs with pl values of 8.3, 5.5, and 4.5 exist in human brain. Board and co-workers (38) detected an additional form designated as GST5, apparently specific for human brain. Testis was not examined, but GST5 was not present in all brains, and its electrophoretic mobility suggested that it was more basic (pl 5.9) than human liver GST. Those authors considered that GST5 may originate from possible post-translational modifications of GST1. Since the GST characterized here has a different isoelectric point, it is unlikely that GST5 corresponds to the pl 5.2 form of brain and testis described in the present study. In addition, human muscle GSTs with pl values of 5.0-5.2 were detected by Singh et al. (41) and an acidic form in heart (42), but their amino-terminal sequences were different from those of the testicular GST of the same isoelectric point described here. Those forms may correspond to the GST4 isoenzyme (pl 5.2) that was reported to be muscle specific and not found in brain (58).

Corrigall and Kirsch (43) studied GSTs in different tissues of nine male subjects by immunoquantitation and radial immunodiffusion and reported that the near-neutral forms (μ-GSTs) were missing in all tissues of five of the nine individuals. In view of the abundance of the pl 5.2 isoenzyme in all tissues, it is also not clear why μ-class GSTs were not detected in a study of seven normal human testis samples (44). Quantitation of GSTs by immunochemical methods may however yield varying results. For instance, it was noted that a human liver μ-class GST did not cross-react with rat liver Yba1 antibodies (27) in spite of their sequence homologies. Similarly, the pl 5.2 isoenzyme of testis and brain reacts poorly with some μ-class antibodies.

Similar patterns of tissue-specific expression of μ-class GST which are missing in livers but present in testis and brains have now been observed in both rats (22) and humans. Factors that determine this differential regulation of expression of the genes have not yet been defined, nor has the functional significance of this difference been established.

Both testis and brain, however, possess a blood barrier, and it has been proposed that GSTs govern uptake and detoxification of both endogenous compounds and xenobiotics at those barriers (45, 46). In addition, it has been suggested that GSTs may be important for fidelity of DNA replication by detoxifying pyrimidine hydroperoxides. Thus, a specific rat GST isozyme (6-6 or Yb3) that is abundant in spermatogenic tubules also exhibits high GSH peroxidase activity with pyrimidine hydroperoxides as substrates (47). It is therefore noteworthy that the testicular pl 5.2 GST characterized in this study has much higher GSH peroxidase activity as compared with liver μ-class GSTs (Table I). Functional studies of the human testis-brain GST isoenzyme may now be planned on the basis of its distribution and structural differences defined here.

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