Novel Human Ocular Glutathione S-Transferases With High Activity Toward 4-Hydroxynonenal

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Purpose. To study the distribution and expression of glutathione S-transferase isozymes involved in detoxification of endogenously generated toxic products of lipid peroxidation, namely, 4-hydroxynonenal (4-HNE) in human lens, retina, cornea, iris, ciliary body and to study their kinetic and structural properties.

Methods. The authors have previously cloned and sequenced cDNA of mouse mGSTA4-4, which shows high activity towards 4-HNE. They have expressed it in Escherichia coli and have raised antibodies against the recombinant mGSTA4-4. In the present study, these antibodies were used in Western blot analysis and immunoaffinity chromatography to study the expression and to purify the human ortholog(s) of mGSTA4-4 from ocular tissues.

Results. Western blot analyses of human ocular tissues indicated that a glutathione S-transferases (GST) isozyme immunologically similar to mGSTA4-4 was expressed in cornea, retina, and iris and ciliary body, but not in lens. This isozyme designated as hGST 5.8 was purified to homogeneity from human retina, cornea, and iris and ciliary body by immunabsorption on immobilized antibodies against mGSTA4-4. The human ortholog of mGSTA4-4, designated as hGST 5.8 purified from all these tissues and pi value of 5.8, subunit Mr value of 25 k and blocked N-terminal. Amino acid sequences of CNBr fragments of hGST 5.8 isozymes of human ocular tissues showed a high degree of primary structure homologies with the corresponding regions of mGSTA4-4. There were noticeable differences in the amino acid sequences of hGST 5.8 of cornea, retina, and iris and ciliary body, suggesting the presence of several closely related hGST 5.8 subunits in the ocular tissues. This heterogeneity was due to tissue-specific expression rather than simple allelic polymorphism. The hGST 5.8 had about sixfold to eightfold higher activity toward 4-hydroxynonenal than 1-chloro-2,4-dinitrobenzene, or CDNB. The catalytic efficiency (Kcat/Km) of ocular hGST 5.8 for 4-HNE was about 100-fold higher than those for the α, β, or γ classes of GST. In addition, hGST 5.8 expressed glutathione peroxidase activity toward phospholipid hydroperoxides and GSH-conjugating activity toward 9,10-epoxy stearic acid.

Conclusions. The results indicate that hGST 5.8 isozyme(s) distinct from the α, β, or γ classes of GSTs, are differentially expressed in human ocular tissues and may play an important role in protective mechanisms against endogenous toxicants generated during lipid peroxidation.

zymes of bovine retina, cornea, sclera, and iris and ciliary body differ slightly in their primary structures and kinetic properties, but all these isozymes are related to mGSTA4-4 and show remarkably high activity toward 4-hydroxyhexenal (4-HNE). GST isozymes orthologous to bGST 5.8 and mGSTA4-4 have also been reported in human tissues.\(^5,6\) These isozymes, designated as hGST 5.8, appear to be expressed in a tissue-specific manner because they are present in tissues such as liver, brain, pancreas, bladder, and heart, but are absent in other tissues, such as lung, skeletal muscle, colon, spleen, and erythrocytes. The human ocular tissues have so far not been investigated for hGST 5.8 isozymes.

Because major differences exist in the constitutive GSTs of the same organs of different species, the results of our recent studies with bovine ocular tissues\(^3\) showing the presence of bGST 5.8 isozymes and their potential role in the detoxification of 4-HNE in bovine ocular tissues cannot be extrapolated to humans. For example, the expression, relative abundance, and kinetic characteristics of the \(\alpha\), \(\mu\), and \(\pi\) class GSTs of human and bovine ocular tissues differ remarkably.\(^7,8\) With this in view, the present study was designed to examine the expression of hGST 5.8 isozymes in human ocular tissues to evaluate their potential physiological role in the defense against 4-HNE and other toxicants generated during lipid peroxidation. Using antibodies against the recombinant mouse enzyme mGSTA4-4, we studied the expression of human (h)GST 5.8 isozymes in retina, cornea, lens, and iris and ciliary body by Western blot analyses. The hGST 5.8 isozymes of human retina, cornea, and iris and ciliary body were then purified to homogeneity, and their structural and functional relatedness to the GST isozymes of other human tissues and mGSTA4-4 and bGST 5.8 was examined, with particular emphasis on the kinetic properties of these isozymes toward 4-HNE, hydroperoxides, and fatty epoxides. Results of those studies reported here show that several hGST 5.8 isozymes closely related to bGST 5.8 and mGSTA4-4 are expressed in human ocular tissues and that these isozymes appear to be involved in the detoxification of the toxicants formed during lipid peroxidation.

MATERIALS AND METHODS

Materials

Human eye globes were obtained after donor death from the Lion’s Eye Bank (Houston, TX). Eye globes were stored at 4°C for not more than 24 hours. Human ocular tissues (cornea, retina, iris and ciliary body, and lens) were dissected or separated from eyes under the supervision of a trained ophthalmologist using a binocular microscope and microsurgery instruments. The lens, cornea, retina, and iris and ciliary body were carefully removed and freed of pigmented epithelium, washed with phosphate-buffered saline, pH 7.0, and stored at \(-20^\circ C\) until used. Pooled tissue from several donors was used as starting material for subsequent enzyme purification.

4-HNE was kindly provided by Professor Dennis R. Petersen, University of Colorado Health Sciences Center (Denver, CO). Sources for most of the chemicals and the polyclonal antibodies raised against the mixture of cationic GSTs (\(\alpha\)-class), the neutral GSTs of human liver (\(\mu\)-class), and the anionic GST of human placenta (\(\pi\)-class) used in this study were the same as those used in previous studies.\(^5,6\) Class specificities of these antibodies have been established by us previously.\(^8\) The polyclonal antibodies against the recombinant mGSTA4-4 obtained by its heterologous expression in Escherichia coli (rec-mGSTA4-4) were raised in rabbit and were used as the same as those used by us previously.\(^5,6\) These antibodies did not recognize the \(\alpha\), \(\mu\), and \(\pi\)-classes of GSTs.

Methods

Details of the protocols for the determinations of GST and GSH-peroxidase activities using various substrates, SDS-PAGE, and Western blot analysis have either been described or referenced in our earlier studies.\(^10\) One unit of enzyme used 1 \(\mu\)mol substrate/minute at 25°C for GST and at 37°C for GSH-peroxidase. Protein was determined according to the method of Bradford,\(^15\) using bovine serum albumin as standard.

PURIFICATION OF THE GST ISOZYMES

Total GSTs From Human Lens, Cornea, Retina, and Iris and Ciliary Body

All purification steps were performed at 4°C. GST activity during the purification was monitored with 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate.\(^16\) Human lenses, corneas, retinas, and iris and ciliary bodies were homogenized separately in 10 mM potassium phosphate, pH 7.0, containing 1.4 mM \(\beta\)-mercaptoethanol (buffer A) using a Tekmar tissue homogenizer to make a 25% (wt/vol) homogenate. The homogenates were centrifuged at 28,000g for 45 minutes, and the supernatants were dialyzed overnight against buffer A (50 volumes, two changes). The dialyzed enzymes were subjected to affinity chromatography over a column of GSH linked to epoxy-activated Sepharose 6B\(^17\) to obtain purified GSTs. Consistent with the results of previous studies,\(^7,9\) these preparations consisted of a mixture of GST isozyme and were free of non-GST proteins.
Immunoaffinity Separation of hGST 5.8 Isozymes From Other Isozymes

For these experiments, antibodies raised against the recombinant mGSTA4-4 (rec-mGSTA4-4) were bound to CNBr-activated Sepharose 4B as described by us previously.5 GSH-affinity purified mixtures of GST isozymes of human lens, cornea, retina, and iris and ciliary body were dialyzed separately against buffer A and then incubated with the anti-rec-mGSTA4-4 antibodies bound to CNBr-activated Sepharose 4B beads (calculated twofold excess of antibodies required to titrate their antigen were used in these experiments) with gentle shaking for 12 hours at 4°C. The GST fraction not absorbed by the immunoaffinity matrix consisted of the a-, ß-, and Ï¾-class GSTs and was collected in the supernatant after centrifugation at 3000g. The enzyme absorbed on the immunoaffinity matrix was extracted with 4 M potassium thiocyanate (KCNS) and immediately subjected to dialysis against buffer A to remove KCNS. The dialyzed enzyme was then subjected to second GSH-affinity column chromatography step to exclude the possibility of any IgG contamination originating from the immunoaffinity purification. The enzymes from all the four tissues were prepared in identical parallel protocols using fresh immunoaffinity matrices for each tissue to avoid cross-contamination.

For kinetic studies, the purified enzymes obtained after the second GSH-affinity chromatography step were dialyzed against buffer A. On the other hand, the enzyme preparations used for structural studies were dialyzed against distilled water containing 0.1% acetic acid. To determine the pI values, a portion of the enzyme preparations obtained after immunoaffinity step was subjected to isoelectric focusing in a column (LKB model #8100) with Ampholines in the pH range of 3.5 to 10 using a 0% to 50% sucrose density gradient. After isoelectric focusing at 1600 V for 24 hours, 0.8-ml fractions were collected and monitored for pH and GST activity using CDNB as the substrate.4.5

Kinetic Studies

GST activity toward 4-HNE was determined spectrophotometrically with slight modification described by us previously.4 In brief, the reaction mixture (final volume, 1 ml) contained 780 µl of 100 mM potassium phosphate buffer (pH 6.5), 100 µl of 5 mM GSH, and an appropriate amount of the enzyme in 20-µl solution. The reaction was started by adding 100 µl of 1 mM 4-HNE, and the use of 4-HNE was followed at 224 nm in a Shimadzu UVPC-2101-PC spectrophotometer against a blank that contained all the above reactants except the enzyme. For determinations of the Km and Kcat values, the enzyme activity was measured in triplicate at varying concentrations of 4-HNE (20 µM to 100 µM) at a fixed concentration (1 mM) of GSH. Double reciprocal plots of 1/v versus 1/[s] were used to determine Km values. The catalytic efficiency was defined as Kcat/Km.

Peptide Mapping and Automated Amino Acid Sequence Analysis

For digestion with staphylococcal V-8 protease, approximately 5 µg of the GST protein was incubated with 50 µl of SV-8 protease in 0.1 M ammonium bicarbonate, pH 7.8, for 6 hours at 37°C at a protease substrate ratio of 1:25 (wt/wt).7 Proteolysis was terminated by lyophilization. The resulting peptides were dissolved in an appropriate volume of electrophoresis sample buffer and analyzed by SDS-PAGE in 17.5% gels using the buffer system of Laemmli.11 CNBr-fragments were generated as described by us previously.14 The samples for N-terminal amino acid sequence analysis were prepared by transblotting the peptide bands on Immobilon-P PVDF membranes.19 Details of protocols were the same as reported previously.14 The stained bands were cut with a razor blade and used directly for microsequence analysis on an Applied Biosystems Model 475A protein-peptide sequencer equipped with an on-line model 120A microbore HPLC phenylthiobodytioanalyzer and a model 900 data processor.

RESULTS

Expression of hGST 5.8 in Ocular Tissues

The results of Western blot analyses of the purified GST isozymes of human ocular tissues using primary
Glutathione S-Transferases of Human Ocular Tissues

TABLE 1. Purification of the hGST 5.8 From Cornea and Lens

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Enzyme Activity* (U)</th>
<th>Total Protein (mg)</th>
<th>Specific Activity Toward CDNB (U/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28,000g supernatant GSH-affinity column chromatography (total GSTs)</td>
<td>8.2</td>
<td>7.2</td>
<td>0.013</td>
</tr>
<tr>
<td>Enzyme not absorbed by the anti-rec-mGSTA4-4 antibodies</td>
<td>6.2</td>
<td>5.4</td>
<td>0.013</td>
</tr>
<tr>
<td>Enzyme absorbed by anti-rec-mGSTA4-4 and eluted by KCNS</td>
<td>5.6</td>
<td>4.0</td>
<td>0.013</td>
</tr>
<tr>
<td>Second GSH-affinity column chromatography§</td>
<td>ND</td>
<td>0.11</td>
<td>ND</td>
</tr>
</tbody>
</table>

*5 and 6 g of wet weight of humans lens and cornea were used as starting materials for purification.
† One unit enzyme uses 1 μmol CDNB substrate per minute at 25°C.
‡ All the α, μ, and π class GSTs were recovered in this fraction.
§ Fraction containing hGST 5.8 was not detected (ND) in lens.

* Purification of Ocular hGST 5.8

The results of immunoaffinity purification of hGST 5.8 of retina, cornea, and iris and ciliary body (Fig. 1) indicated that GST isozyme(s) immunologically similar to mGSTA4-4 were present in retina, cornea, and iris and ciliary body (Fig. 1, lanes 3, 4, 5, respectively) but absent in lens (Fig. 1, lane 2). The isozyme(s) were immunologically distinct from the α, μ, and π classes of GSTs that constitute the bulk of GST isozymes of human ocular tissues and could be separated from the α, μ, and π-class GSTs by immunoaffinity using antibodies against mGSTA4-4.

**Purification of Ocular hGST 5.8**

The results of immunoaffinity purification of hGST 5.8 of retina, cornea, and iris and ciliary body were similar, and only a representative purification protocol of hGST 5.8 from cornea is presented in Table 1. Results from purification of GSTs from lens demonstrating the absence of hGST 5.8 in this tissue are also presented in Table 1 for comparison. When the "total GST" fraction of cornea obtained by GSH-affinity column chromatography was incubated with immobilized anti-rec-mGSTA4-4 antibodies, approximately 95% was not absorbed by the anti-rec-mGSTA4-4 antibodies and recovered in the unabsorbed fraction (Table 1). Similar results were obtained with retina and iris and ciliary body enzymes (data not presented). No human lens GST was, however, retained on the immunoaffinity matrix. These results were consistent with those of Western blot analyses, which indicated the absence of mGSTA4-4 like enzyme in lens (Fig. 1, lane 2). A small but significant amount of the cornea enzyme absorbed on the immunoaffinity matrix could be eluted with 4 M KCNS (Table 1). Similar results were observed with the GSTs of retina and iris and ciliary body (data not presented). The enzyme absorbed on the mGSTA4-4 immunoaffinity matrix and eluted with KCNS, as expected, showed positive cross-reactivity with the anti-rec-mGSTA4-4 antibodies (data not presented) and did not cross-react the antibodies raised against the α, μ, and π classes of GSTs. The π values of the cornea (π 5.75) and retina (π 5.8) enzymes were found to be close to 5.8 and similar to the π of bovine ocular GSTs. The human isozymes were temporarily designated as hGST 5.8 of human ocular tissues. Their final designation, in accordance with the recently revised nomenclature, will follow only after their complete primary structure is determined. hGST 5.8 isozyme(s) accounted for about 1.3%, 1.1%, and 1.4% of total GST protein of human cornea, retina, and iris and ciliary body, respectively.

![SDS-PAGE](image-url)
Structural Properties of Ocular hGST 5.8

In reduced denaturing gels, hGST 5.8 from cornea, retina, and iris and ciliary body (Fig. 2, lanes 2, 3, 4, respectively) showed identical sharp single bands corresponding to Mr values of 25 kDa. Attempted N-terminal sequence determination revealed that the enzyme had a blocked N-terminus, which was consistent with the previously reported blocked N-termini of mGSTA4-4,21 and bGST 5.8 of bovine ocular tissues.3 The peptide maps of the CNBr digested hGST 5.8 from cornea, retina, and iris and ciliary body were similar and showed in SDS-gels two major bands (data not presented) similar to those observed with mGSTA4-421 and with the bovine ocular tissue isozymes,3 and hGST 5.8 of bovine ocular tissues.3 The peptide maps of the CNBr digested hGST 5.8 and with the bovine ocular tissue isozymes (hGST 5.8) characterized recently in our laboratory. Similarly, the SV-8 protease peptide maps of ocular hGST 5.8 were similar to each other (Fig. 5) and to mGSTA4-4 and bGST 5.8 of bovine ocular tissues (data not presented). These results indicated that a close structural relationship existed among mGSTA4-4, bGST 5.8 of bovine ocular tissues, and hGST 5.8 of human ocular tissues.

N-terminal amino acid sequence analyses was performed on the smaller (~6 kd) of the two predominant CNBr fragments and resulted in at least two superimposed sequences. This indicated that the ~6-kd peptide was a mixture of at least two fragments of hGST 5.8 with similar Mr values. When aligned with the sequence of mGSTA4-4,4 one of these fragments corresponded to the region between methionine residues 105 and 166 of mGSTA4-4, whereas the other fragment corresponded to the region between the methionine residue at position 166 and the C-terminus of mGSTA4-4. Approximately 10 pmol of the peptide fragments of the enzyme from each tissue was used for sequence analyses. The residue yields in the first 10 cycles for the sequence, beginning after the methionine residue at position 105, were in the range of 3 to 1.5 pmol, whereas for the sequence beginning after methionine at position 166, they were in the range of 1.7 to 1.0 pmol. The residue yields in both sequences for the last cycle were about 0.3 pmol. The sequences of CNBr peptide fragments of hGST 5.8 of human cornea, retina, and iris and ciliary body were highly homologous with each other and also with bovine bGST 5.8 and mouse mGSTA4-4.4 As shown in Figure 4, hGST 5.8 of cornea, retina, and iris and ciliary body showed 85%, 90%, and 75% identities with mGSTA4-4 in the region corresponding to residues 106 to 125. Interestingly, there were noticeable differences in the sequences of retina, cornea, and iris and ciliary body hGST 5.8 isozymes. This was consistent with the observed heterogeneity of bGST 5.8 in bovine ocular tissues reported by us previously.7 The leucine residue present at position 120 (Fig. 4) in iris and ciliary body and cornea hGST 5.8 was substituted by a serine residue in the retina hGST 5.8, indicating that it was distinct from the corneal and iris and ciliary body enzymes. The serine residue of the retina hGST 5.8 at 120 was, however, similar to that reported for mouse mGSTA4-4. This finding is important because it points out a continuing divergence among this subgroup of GSTs during the evolution and also generates additional confidence in the results of N-terminal sequence analysis of these peptide fragments.

Except for the residues at position 120, cornea and retina hGST 5.8 showed identical sequences in the region after the methionine residue at position 105. The sequences of cornea and retina hGST 5.8, however, differed at two positions when compared with the sequence of iris and ciliary body GST 5.8. The lysine and glutamic acid residues present at positions 117 and 119, both in the cornea and retina hGST 5.8 were substituted by aspartic acid and alanine, respectively, in hGST 5.8 of iris and ciliary body enzymes (Fig. 4). Interestingly, ocular hGST 5.8 also showed significant differences with the sequences of hGST 5.8 of human liver reported previously.5 The residue at 115 (lys) in liver hGST 5.8 was substituted by proline in cornea, retina, and iris and ciliary body enzyme. The residues at position 118 of the cornea, retina, and iris and ciliary body hGST 5.8 isoforms were similar (glu) but differed from that of hGST 5.8 of liver (ala). These results further corroborate the notion of heterogeneity among the GST isoforms of human tissues orthologous to mGSTA4-4 as proposed by us previously.5,6

Comparatively less homology was observed between the ocular hGST 5.8, and mGSTA4-4 in the region corresponding to residues 167 to 186. Sequences of hGST 5.8 isozyme from cornea, retina, and iris and ciliary body also showed homologies with the corresponding regions of rat GST 8-8 and chicken GSTCL3 indicating an evolutionary relatedness among these enzymes as proposed previously. On the
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of the hGST 5.8 of cornea and retina toward 4-HNE were approximately eightfold higher than that toward CDNB, which is one of the most preferred substrates of the hGST 5.8 of cornea and retina en-...

**Kinetic Properties**

Substrate specificity profiles of hGST 5.8 from cornea, retina, and iris and ciliary body were determined using various electrophilic substrates of GSTs (Table 2). Specific activities of hGST 5.8 of cornea, retina, and iris and ciliary body were generally similar toward these substrates. As shown in Table 2, the specific activities of the hGST 5.8 of cornea and retina toward 4-HNE were approximately eightfold higher than that toward CDNB, which is one of the most preferred substrates of GST isozymes except GST θ. Specifc activity of the iris and ciliary body hGST 5.8 toward 4-HNE was noticeably less than those of cornea and retina enzymes. These isozymes also showed GSH-peroxidase activity toward the intact phospholipid hydroperoxides, as well as the hydroperoxides of linoleic acid, suggesting their role in the protection mechanisms against the endogenous toxicants formed during lipid...

**TABLE 2. Substrate Specificities of the Human Cornea, Retina, and Iris and Ciliary Body hGST 5.8**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Cornea</th>
<th>Retina</th>
<th>Iris and Ciliary Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-chloro-2,4-dinitrobenzene†</td>
<td>5.9</td>
<td>5.7</td>
<td>4.8</td>
</tr>
<tr>
<td>1,4-dihydroxy-2-nonenal†</td>
<td>44.6</td>
<td>49.8</td>
<td>32.5</td>
</tr>
<tr>
<td>dihydroxydiol phosphatidylcholine hydroperoxide§</td>
<td>0.32</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td>dihydroxydiol phosphatidylethanolamine</td>
<td>0.30</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>hydroperoxide§</td>
<td>0.36</td>
<td>0.27</td>
<td>0.29</td>
</tr>
<tr>
<td>dihydroxydiol phosphatidylglycerol hydroperoxide§</td>
<td>0.18</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>9-hydroperoxy linoleic acid§</td>
<td>0.16</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>15-hydroperoxy linoleic acid§</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Means of two closely similar (within 5%) values.
† GST activity determined according to reference 16.
‡ GST activity determined according to reference 1.
§ GSH-peroxidase activities determined according to reference 14.
TABLE 3. Specific Activities and Kinetic Constants of Human Ocular GSTs for 4-HNE

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific Activity (μmol/min per mg protein)</th>
<th>Km (μM)</th>
<th>kcat (mol/mol per sec)</th>
<th>kcat/Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea hGST 5.8</td>
<td>44.6 ± 2.7</td>
<td>80 ± 4.0</td>
<td>68.8 ± 5.0</td>
<td>0.86</td>
</tr>
<tr>
<td>Mixture of other GSTs of cornea*</td>
<td>1.04 ± 0.03</td>
<td>111 ± 7.0</td>
<td>1.35 ± 0.04</td>
<td>0.012</td>
</tr>
<tr>
<td>Retina hGST 5.8</td>
<td>49.8 ± 3.1</td>
<td>71 ± 4.0</td>
<td>75.8 ± 3.0</td>
<td>1.07</td>
</tr>
<tr>
<td>Mixture of other GSTs of retina*</td>
<td>1.11 ± 0.04</td>
<td>118 ± 5.0</td>
<td>1.43 ± 0.07</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation of three determinations. Km and kcat values were determined as described previously. * These fractions consisted of the isozymes not absorbed by the anti-rec-mGSTA4-4 antibodies during the purification and were completely free from hGST 5.8.

Furthermore, the Kcat/Km ratios (catalytic efficiency) of hGST 5.8 were approximately two orders of magnitude higher than those for the other GSTs that comprised the three major classes (α, μ, and π) of GSTs (Table 3).

DISCUSSION

The results of present study demonstrate for the first time that several closely related GST subunits (hGST 5.8) with structural and immunologic similarities to mouse mGSTA4 subunit are differentially expressed in human ocular tissues. These results are consistent with the presence of a similar group of GST subunits in bovine ocular tissues reported by us recendy. 3 The presence of several closely related subunits among human hGSTs 5.8 is also consistent with our previous studies that have shown that the mouse ortholog, mGSTA4-4, is heterogenous and coded by several related but distinct genes.13 Differences in the primary structures of the cornea, retina, iris and ciliary body, and liver hGST 5.8, observed during the present study, provide further evidence for the presence of at least four distinct but closely related hGST 5.8 subunits in humans which appear to be selectively expressed in different tissues. It is important to note that pooled tissues from several donors were used for the enzyme purifications. No mixed sequences were obtained from these samples. This would indicate that each individual expressed the same hGST 5.8 isoform in any given tissue, even though that isoform might have differed from one present in another ocular tissue. It appears likely, therefore, that expression of the isoforms is tissue specific rather than due to allelic polymorphism between individuals.

Because the hGST 5.8 subunits belong to a group of GSTs with a high degree of structural homology, the subunits present in each tissue could associate with each other to give active GST dimers.23 For example, four distinct hGST 5.8 subunits could give rise to a maximum of 10 different active dimeric GST isozymes. Several active dimers of hGST 5.8 could, then, be present in ocular tissues. The physiological significance of
the heterogeneity among this subgroup of GST isozymes remains to be established. hGST 5.8 isozymes display very high specific activities toward 4-HNE, which suggests that these isozymes potentially represent the major cellular defense mechanism against highly electrophilic, \( \alpha \)-unsaturated aldehydes (alkenals) generated during peroxidation of polyunsaturated fatty acids. The catalytic efficiencies (Kcat/Km) of hGST 5.8 isozymes toward 4-HNE were found to be about 100-fold higher than other GSTs of ocular tissues. This underscores the physiological importance of hGST 5.8.

It has been postulated that antioxidants such as vitamin E function by scavenging free radicals at the site of their formation in the membrane, whereas enzymes such as GSH-peroxidase and GST may be involved in the detoxification of hydroperoxides and other products of oxidative processes once they are formed. Inflammation is one of the more frequent situations in which oxidative stress may initiate lipid peroxidation in ocular and other tissues. In addition, the photochemical effects of light, and especially of ultraviolet light — which is becoming an increasingly serious environmental problem, can also lead to oxidative stress and to enhanced lipid peroxidation, resulting in the formation of toxic compounds, such as 4-HNE, hydroperoxides, and epoxides. The GSH-conjugating activity of hGST 5.8 isozymes toward 4-HNE and epoxides, and their GSH-peroxidase activity toward phospholipid hydroperoxides, may play a key role in the defense against putative damaging effect of these compounds in retina, cornea, and iris and ciliary body, particularly during the conditions conducive to enhance lipid peroxidation. Retinal damage has been associated with hyperoxia and free-radical generation.

The oxidant-defense mechanism mediated by GSH and hGST 5.8 may be of particular importance to retina because of its high unsaturated fatty acid content.

The comparison of hGST 5.8 isofrom sequences with those of bovine, mouse, rat, and chicken orthologs suggests a continuum of divergence during evolution because some of the residues of hGST 5.8 isofroms not shared with each other are common with mGSTA4-4, bGST 5.8, or rGST 8-8. Construction of a phylogenetic interrelationship will only be possible after complete primary structures of all these isozymes are determined. As yet, complete primary structures have been determined only for three members of this group (rat GST 8-8, mouse GSTA4-4, and chicken liver GST CL3), and determination of complete primary structures of hGST 5.8 and bGST 5.8 isozymes through cloning and sequencing of their cDNA is currently under way in our laboratory.

**Key Words**

glutathione S-transferases, 4-hydroxynonenal, human ocular tissues, lipid peroxidation

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**References**