Protection of HLE B-3 Cells against Hydrogen Peroxide– and Naphthalene-Induced Lipid Peroxidation and Apoptosis by Transfection with hGST*A1* and hGST*A2*

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Purpose. To investigate the physiological role of two major α-class glutathione *S*-transferases (GSTs), hGSTA1-1 and hGSTA2-2 in protection against oxidative stress and lipid peroxidation (LPO) in human lens epithelial (HLE B-3) cells.

Methods. Total GSTs were purified from HLE B-3 cells by glutathione (GSH)-affinity chromatography and characterized by Western blot analysis, isoelectric focusing, and kinetic studies. The relative contributions of the α-class GSTs and the Se-dependent glutathione peroxidase (GPx)-1 in GSH-dependent reduction of phospholipid hydroperoxide (PL-OOH) were quantitated through immunoprecipitation studies using separately the specific polyclonal antibodies against human α-class GSTs and GPx-1. HLE B-3 cell membranes were prepared, peroxidized, and used to examine whether hGSTA1-1 and hGSTA2-2 catalyzes the reduction of membrane PL-OOH in situ using the microiodometric and spectrophotometric assays. The protective effects of the α-class GSTs against H_2O_2- and naphthalene-induced LPO and apoptosis were examined by transfecting HLE B-3 cells with cDNAs of hGST*A1* and hGST*A2*.

Results. HLE B-3 cells expressed only the α and π class GSTs. The Michaelis-Menten constant (k_m) and turnover number (k_{cat}) of purified total GSTs toward phosphatidylcholine hydroperoxide (PC-OOH) were found to be 30 ± 4 μM and 1.95 ± 0.26 seconds, respectively. The α-class GSTs accounted for approximately 65% of the total GPx activity of HLE B-3 cells toward PC-OOH. Our results demonstrate for the first time that hGSTA1-1 and hGSTA2-2 effectively catalyzed GSH-dependent reduction of membrane PL-OOH in situ in HLE B-3 cells. Transfection with hGST*A1* or hGST*A2* protected these cells from H_2O_2- and naphthalene-induced LPO and attenuated H_2O_2- and naphthalene-induced apoptosis through inhibiting caspase 3 activation.

Conclusions. These results demonstrate that the α-class GSTs hGSTA1-1 and hGSTA2-2 play a major role as antioxidant enzymes and are the main determinants of the levels of LPO caused by oxidative stress in human lens epithelial cells. (*Invest Ophthalmol Vis Sci.* 2002;43:434–445)

The reactive oxygen intermediates (ROIs) generated during the mitochondrial electron transport chain, the biotransformation of xenobiotics by the cytochrome P-450 system, and exposure to environmental agents such as UV light and ionizing radiation can cause oxidative stress within cells by reacting with macromolecules and causing damage, such as mutations in DNA, destruction of protein structure and function, and peroxidation of lipids.[1] Among these effects of ROIs, lipid peroxidation (LPO) is perhaps the most damaging to cells, because it is an autocatalytic chain process initiated by the abstraction of electrons from unsaturated fatty acids, and a single ROI species can lead to the formation of large amounts of phospholipid hydroperoxides (PL-OOH) and breakdown toxic products such as 4-hydroxy-2-nonenal (4-HNE).[2] LPO has been implicated in the pathogenesis of a number of diseases, including cataract,[3–6] atherosclerosis,[7] Alzheimer disease,[8] cancer,[9] degenerative retinal disease,[10] and Parkinson disease.[11] In isolated systems both PL-OOH[12] and 4-HNE[5,6] have been shown to cause cataract.

In mammalian cells, the primary defenses against LPO consist of enzymes, including the superoxide dismutases (SODs), catalase (CAT), and selenium-dependent glutathione peroxidases (GPxs) and antioxidants such as glutathione (GSH), ascorbate, and urate, which can scavenge ROIs before the initiation of LPO.[13] Secondary defenses against LPO include enzymes such as the glutathione *S*-transferases (GSTs) and Se-dependent GPxs, which can catalyze GSH-dependent reduction of PL-OOH and fatty acid hydroperoxides (FA-OOH) and terminate the autocatalytic chain reaction of LPO.[13] Four selenium-dependent GPx isozymes—cellular glutathione peroxidase (GPx-1),[14] gastrointestinal GPx (GPx-2),[15] plasma GPx (GPx-3),[16,17] and phospholipid glutathione peroxidase (GPx-4),[18,19] have been cloned and characterized in mammalian cells. GPx-1, GPx-2, and GPx-3 have similar substrate specificities and can effectively catalyze the reduction of H_2O_2 and FA-OOH but poorly metabolize PL-OOH,[15,20] whereas GPx-4 reduces PL-OOH much more effectively.[21]

In addition to the conjugation of toxic electrophilic xenobiotics to GSH, GSTs also catalyze GSH-dependent reduction of PL-OOH and FA-OOH through their Se-independent GPx activity.[22] In human tissues including lens, GST isoenzymes belonging to the α-, μ-, and π-class constitute the major portion of cytosolic GST activity. However, the GPx activity of GSTs is only displayed by the cationic α-class GST isoenzymes.[22,23] hGSTA1-1 and hGSTA2-2, which constitute the bulk (>90%) of the cationic α-class GSTs have relatively high GPx activities toward PL-OOH and FA-OOH, but cannot use H_2O_2 as the substrate.[24,25] Our recent studies have shown that the overexpression of hGSTA2-2 in K562 cells can significantly decrease LPO levels during oxidative stress and block H_2O_2-induced apoptosis through inhibition of stress-activated protein kinase/c-Jun N-terminal protein kinase (SAPK/JNK) and caspase 3

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activation, suggesting that these GST isozymes play an important role in regulation of the intracellular concentrations of LPO products that may be involved in the signaling mechanisms of apoptosis.[25]

In vitro studies have demonstrated that H_2O_2-induced opacification of rat lenses is preceded by apoptosis of lens epithelial cells.[26] Consistent with these observations, the apoptosis of rat lens epithelial cells has been observed during cataractogenesis by naphthalene[27] and galactose[28] in vivo. The α-class GSTs have been shown to provide protection to cells against oxidative stress and apoptosis through their Se-independent GPx activity in cultured cells.[25] The protective role of the α-class GSTs in lens epithelial cells is suggested by studies showing induction of GSTs in rat lens epithelial cells during 4-HNE–induced cataractogenesis.[5,6] A study showing overexpression of the α-class murine GSTs, mGSTA1-1 and mGSTA2-2 (designations based on the currently accepted nomenclature of mammalian GSTs used in this communication),[29] in H_2O_2-resistant murine lens epithelial cells[30] is also consistent with the idea of a protective role of the α-class GST against oxidative stress.

The mechanisms through which GSTs provide protection to lens epithelial cells against oxidative stress are not known and should be investigated. In the present studies, we examined the physiological role of the α-class GSTs hGSTA1-1 and hGSTA2-2 in cultured human lens epithelial cells (HLE B-3). We characterized the kinetic properties of GSTs toward PL-OOH and quantitated the contributions of the α-class GSTs and the Se-dependent GPx-1 in the GSH-dependent reduction of PL-OOH in these cells. We also investigated whether the α-class GSTs or Se-dependent GPx can catalyze the reduction of membrane PL-OOH in situ. Finally, we investigated whether the cells transfected with hGST*A1* or hGST*A2* cDNA acquire resistance to H_2O_2- and naphthalene-induced apoptosis. Results of experiments presented in this communication demonstrate for the first time that the α-class GSTs contribute to a major portion of GPx activity of HLE cells toward PL-OOH and that these enzymes catalyze the GSH-dependent reduction of PL-OOH of lens epithelial cell membranes in situ. Transfection of cells with hGST*A1* or hGST*A2* provides strong protection against H_2O_2- and naphthalene-induced LPO and apoptosis by inhibiting the activation of caspase 3. This study shows that GSTs provide protection from oxidative stress to lens epithelial cells through attenuation of LPO.

Materials and Methods

Materials

Epoxy-activated Sepharose 6B, GSH, 1-chloro-2,4-dintrobenzene (CDNB), protein A immobilized on Sepharose 6MB, β-reduced nicotinamide adenine dinucleotide phosphate (NADPH), GPx-1, and naphthalene were obtained from Sigma (St. Louis, MO). Dilinoleoyl phosphatidylcholine (PC) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL), and phosphatidylcholine hydroperoxide (PC-OOH) was synthesized as described previously.[22] PC-OOH was stored at −70°C in a nitrogen atmosphere. H_2O_2 was purchased from Fisher Chemicals (Fairlawn, NJ). All reagents for SDS-PAGE and Western blot analysis were purchased from Bio-Rad (Hercules, CA).

Antibodies

The polyclonal antibodies raised against the human α-, μ-, and π-class GSTs were the same as those used in our previous studies.[22] For immunoprecipitation studies, the IgG fraction of the anti-α-class GSTs was purified by affinity chromatography on columns of protein A bound to Sepharose beads. For raising polyclonal antibodies against GPx-1, the peptide H_2N-CLRRYSRRFQTIDIEPDIEA-COOH, which corresponds to C-terminal residues 174-192of human GPx-1, was synthesized in the Protein Chemistry Laboratory of the University of Texas Medical Branch (Galveston, TX). A cysteine residue was included on the NH_2 terminus for coupling purposes. The GPx-1 peptide was conjugated to maleimide-activated keyhole limpet hemocyanin (Pierce, Rockford, IL) and purified by gel filtration. The conjugate was used to immunize the rabbits. National Institutes of Health guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research were strictly adhered to for the welfare of the rabbits and the protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Medical Branch. Polyclonal antibodies against GPx-1 were obtained 12 weeks after the initial immunization. The Western blot analysis showed that these antibodies specifically recognized GPx-1, but not other GPx isoenzymes. A protein A–purified IgG fraction obtained from these antibodies as described earlier was used in these studies. Caspase 3 (CPP32) antibodies were obtained from PharMingen (San Diego, CA). Poly(ADP-ribose) polymerase (PARP) antibodies were purchased from Biomol (Plymouth Meeting, PA).

Cell Culture

A human lens epithelial cell line with extended life span (HLE B-3) was established by infecting the infant HLE cells with adenovirus 12-SV40 hybrid virus, as described previously by Andley et al.[31] These cells ceased to produce infectious virus after a few passages. The cells were cultured in minimal essential medium (MEM) with 20% fetal bovine serum at 37°C in a 5% CO_2 humidified atmosphere. The cells used for present studies were between 17 and 20 passages.

Purification of GSTs

Heterologous expression and purification of recombinant hGSTA1-1 and hGSTA2-2 has been described by us previously.[25] For purification of total GSTs from HLE B-3 cells, 1×10^9 HLE B-3 cells were pelleted by centrifugation at 500*g* for 5 minutes and washed twice with phosphate-buffered saline (PBS). The cell pellets were resuspended in 10 mM potassium phosphate buffer (pH 7.0), containing 1.4 mM β-mercaptoethanol (buffer A) and homogenized by sonication on ice (three times for 5 seconds at 40 W) followed by centrifugation for 45 minutes at 28,000*g* at 4°C. The supernatants were collected and subjected to affinity chromatography using GSH-linked to epoxy-activated Sepharose 6B.[25] After overnight binding, the unbound proteins were thoroughly washed off the resin with 22 mM potassium phosphate buffer (pH 7.0) containing 1.4 mM β-mercaptoethanol (buffer B) until absorbance of the wash at 280 nm was undetectable. Total GSTs were eluted from the GSH-affinity resin in 50 mM Tris-HCl (pH 9.6), containing 10 mM GSH and 1.4 mM β-mercaptoethanol. The purified total GSTs were dialyzed overnight against buffer A and subjected to kinetic and immunologic characterization.

Preparation of HLE B-3 Membrane Fraction and Induction of LPO

The cell membrane fraction was prepared according to the method described by Hipfner et al.[32] and Gao et al.[33] Briefly, the cells were harvested from the culture media by centrifugation and lysed by incubation in hypotonic buffer containing 0.5 mM sodium phosphate (pH 7.0), 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) for 1.5 hour, followed by homogenization. After centrifugation of the homogenate at 12,000*g* for 10 minutes, the postnuclear supernatant was further centrifuged at 100,000*g* for 40 minutes at 4°C. The resultant crude membrane pellet was suspended in the reconstitution buffer (250 mM sucrose, 10 mM Tris-HCl [pH 7.4]) and homogenized using a glass homogenizer (Dounce; Bellco Glass Co., Vineland, NJ) and layered over 38% sucrose in 5 mM HEPES-KOH (pH 7.4). After centrifugation at 280,000*g* for 2 hour at 4°C, the interphases were collected and washed by centrifugation in the reconstitution buffer (100,000*g*). The membrane pellet was resuspended in 10 mM Tris-HCl (pH 7.4) 1.4 mM β-mercaptoethanol and 0.1 mM EDTA and stored under nitrogen at −70°C to minimize auto-oxidation. Induction of

membrane LPO and microiodometric determination of PL-OOH have been described by us previously.[25]

Enzyme Assays and Kinetic Studies

GST activity toward CDNB was determined spectrophotometrically at 340 nm by the method of Habig et al.[34] One unit of GST activity was defined as the amount of enzyme catalyzing the conjugation of 1 μmol CDNB with GSH per minute at 25°C. GPx activity toward PC-OOH and H_2O_2 was determined using the glutathione reductase (GR)–coupled assay as described by us previously.[25] Briefly, the reaction mixture contained 3.2 mM GSH, 0.32 mM NADPH, 1 U glutathione reductase, and 0.82 mM EDTA in 0.16 M Tris-HCl (pH 7.0). When H_2O_2 was used as the substrate, 1 mM sodium azide was added to the reaction mixture to inhibit endogenous CAT activity. The reaction mixture was preincubated with an appropriate amount of GST isozyme or the Se-dependent GPx-1 at 37°C for 5 minutes. The reaction was started by addition of PC-OOH (prepared in methanol) or H_2O_2 with the final concentration of 100 μM. The consumption of NADPH was monitored at 340 nm for 4 minutes at 37°C. One unit of GPx activity was defined as the amount of enzyme necessary to consume 1 μmol NADPH per minute in the coupled assay. A nonsubstrate blank and a nonenzyme additional blank in which the enzyme was replaced with equal volume of buffer A were used to correct for non–GR-dependent NADPH oxidation and nonenzymatic peroxidase activity. For determination of the Michaelis-Menten constant (k_m) and turnover number (k_{cat}) of the GSTs for CDNB and PC-OOH, fixed concentration of GSH and increasing concentrations of CDNB (0.2–1 mM) and PC-OOH (20–100 μM) were used. The kinetic constants were determined using double-reciprocal plots of activity versus increasing concentrations of substrates.

Immunoprecipitation of GPx Activity in Cell Extracts

HLE B-3 cells were washed with PBS and pelleted by centrifugation at 500*g*. The cell pellets were resuspended in buffer A and homogenized by sonication in ice. The homogenates were centrifuged at 28,000*g* for 45 minutes at 4°C and the supernatants, after dialysis against 200 × volumes of buffer A with three changes, were used for immunoprecipitation studies. Fixed aliquots (100 μL) of 28,000*g* supernatants containing 50 μg protein were incubated with purified GST-α antibodies or GPx-1 antibodies (2.5 μg IgG) at 4°C. Equal amounts of purified preimmune serum were used in control experiments and additional controls containing only buffer were also used. After 2 hours of incubation, 20 μL protein A Sepharose beads was added to the reaction mixtures and incubated overnight at 4°C. The reaction mixtures were centrifuged at 10,000*g* for 30 minutes, and the GPx activities toward PC-OOH and H_2O_2 were determined in the supernatants. To confirm the complete immunoprecipitation of the α-class GSTs in these experiments, the pellet and supernatant fractions were subjected to Western blot analysis using biotin-labeled antibodies against the human α-class GSTs, followed by detection with streptavidin-horseradish peroxidase (HRP), according to the manufacturer's suggested protocol to exclude the detection of IgG (Amersham Pharmacia Biotech, Piscataway, NJ).

Transfection with hGST*A1* and hGST*A2*

Based on the cDNA sequences of hGST*A1* and hGST*A2*, PCR primers were designed to amplify the complete coding sequence of hGST*A1* and hGST*A2* from pET-30a(+)/hGST*A1* or pET-30a(+)/hGST*A2* vector, respectively.[24] The amplified cDNA was ligated into the pTarget-T mammalian expression vector (Promega, Madison, WI). The HLE B-3 cells were transiently transfected with pTarget-T/hGST*A1,* pTarget-T/hGST*A2* vector, or with the vector alone, using a lipofection reagent (Lipofectamine Plus; Invitrogen, San Diego, CA). The transfection efficiency was monitored by Western blot analysis, using GST-α antibodies and determination of GST activity toward CDNB, GPx activity toward PC-OOH in cell extracts.

Determination of Intracellular Malondialdehyde and 4-HNE Levels

Malondialdehyde (MDA) and 4-HNE levels in HLE B-3 cells were determined with a kit (Biotech LPO-586; Oxis International, Portland, OR) according to the manufacturer's protocol. For each determination, 10^7 cells were collected by centrifugation at 500*g* for 10 minutes and washed twice with PBS. The pellet was resuspended in 0.2 mL of buffer A containing 5 mM butylated hydroxytoluene (BHT) and frozen at −70°C until assayed. To each sample, 650 μL *N*-methyl-2-phenylindole and 150 μL of either 12 N HCl (for MDA determination) or 15.4 M methanesulfonic acid (for 4-HNE plus MDA determination) was added. The reaction mixture was mixed by vortexing and incubating at 45°C for 60 minutes. After centrifugation at 15,000*g* for 10 minutes, the absorbances of the supernatant were determined at 586 nm. Standards of MDA or 4-HNE were prepared from the hydrolysis of 1,1,3,3-tetramethoxypropane in HCl or 4-HNE diethylacetal in methanesulfonic acid, respectively. Extinction coefficient for MDA and 4-HNE (1.1×10^5 M/cm) determined from the standard curves was used and the data expressed as picomoles of MDA or 4-HNE per milligram protein.

Detection of Apoptosis by DNA Laddering

For the detection of DNA laddering, the cells were treated with indicated concentrations of H_2O_2 and naphthalene (stock solution was dissolved in methanol and final concentration of methanol in the media was 0.5%) in complete medium. After the indicated periods, cells were harvested, washed with PBS and resuspended in 200 μL of PBS. The genomic DNA was isolated with a kit (QIAamp DNA Mini Kit; Qiagen, Valencia, CA) and stored in Tris-EDTA buffer (10 mM Tris-HCl [pH 7.4] and 1 mM EDTA [pH 8.0]). The concentrations of DNA were determined spectrophotometrically at absorbance of 260 nm. For electrophoresis, DNA samples (1 μg) were loaded on 2% agarose gels containing ethidium bromide. After electrophoresis for 2 hours at 50 V, gels were photographed with an imaging system (Model T 2000; Alpha Innotech, San Leandro, CA) under UV illumination.

Western Blot Analysis

Cells were centrifuged for 5 minutes at 500*g*, washed twice with PBS, and resuspended in hypotonic lysis buffer (buffer A). After sonication for 15 seconds at 28,000*g*, supernatants of cell lysates were separated with 12% polyacrylamide gels (for detection of caspase 3, 15% of gels were used). For detection of PARP, pellets from 10^6 cells were first resuspended in 50 μL of denaturing lysis buffer containing 62.5 mM Tris-HCl (pH 6.8), 6.0 M urea, 2% SDS, 10% glycerol, 1.4 mM β-mercaptoethanol, 0.00125% bromophenol blue, 0.5% Triton X-100, and 1 mM PMSF and then sonicated three times for 5 seconds each on ice to disrupt protein-DNA interaction. Cell lysates (20 μL) were resolved by 10% polyacrylamide gels. The proteins in gels were electrophoretically transferred to nitrocellulose membrane. The nitrocellulose membranes were incubated with primary antibodies, as indicated in the figure legends, followed by HRP-conjugated secondary antibodies (Sigma). The antigens were detected by chemiluminescent substrate (SuperSignal; Pierce) or by HRP color-developing reagent (Bio-Rad).

Statistical Analysis

The results are expressed as mean ± SD. Significant differences were evaluated with the unpaired Student's *t*-test or one-way analysis of variance. All statistical tests were performed at the 5% level of significance.

Results

Immunologic and Kinetic Characterization of GSTs Purified from HLE B-3 Cells

To investigate the role of GSTs in protective mechanisms against oxidative stress and LPO in HLE B-3 cells, the profile of

TABLE 1. Purification of Total GSTs from HLE B-3 Cells

Fraction	GST Activity (U/mL)	GST Activity (Total Units)	Total Protein (mg)	Specific Activity (U/mg)	Yield (%)	Purification (fold)
28,000*g* supernatant	0.26 ± 0.02	1.31 ± 0.10	3.44 ± 0.25	0.38	100	
GSH-affinity chromatography	0.17 ± 0.02	0.75 ± 0.06	0.033 ± 0.003	22.73	57.3	59.8

HLE B-3 (1×10^9) cells were harvested and subjected to purification using GSH-affinity chromatography. Total GSTs refers to the mixture of GST isozymes purified by GSH-affinity chromatopgraphy. GST activity during purification was monitored using CDNB as the substrate. Data are the mean ± SD of three determinations.

GST isozymes in these cells was studied. Total GSTs from HLE B-3 cells were purified using GSH-affinity chromatography. Results of purification (Table 1) showed that purified total GSTs accounted for approximately 1% of the total soluble proteins in HLE B-3 cell extracts. In SDS-PAGE, the purified total GSTs showed a major band at 23 kDa, with the expected value of the π-class GSTs and a minor band at 25 kDa corresponding to the molecular mass (M_r) of α-class GSTs (Fig. 1A, lane 3). Results of Western blot analysis using polyclonal antibodies specific for the α-, μ-, and π-class GSTs confirmed that HLE B-3 cells expressed only the α- and π-class GSTs (Figs. 1B, 1C) and the μ-class GSTs were not present in these cells (Fig. 1D). Purified total GSTs of epithelial cells when subjected to column isoelectric focusing, showed two peaks corresponding to isoelectric point (pI) values of 4.8 and 9.6. In Western blot analysis, a peak corresponding to pI 4.8 was identified as the π-class isozyme, hGSTP1–1, whereas the peak at pI 9.6 consisted of a mixture of immunologically similar cationic α-class GSTs. The kinetic constants for the GPx activity of purified total GSTs from the cells toward dilinoleoyl phosphatidylcholine (PC-OOH) were also determined. The k_m and k_{cat} of the purified total GSTs toward PC-OOH were found to be 30 ± 4 μM and 1.95 ± 0.26 seconds, respectively. The densitometric scan of the bands (Figs. 1A–C) on the imager (Alpha Innotech) indicated that the π- and α-class GSTs comprised approximately 85% and 15% of the total GSTs, respectively. Because it has been shown that the π-class GSTs do not display GPx activity,[22] the GPx activity of total GSTs purified from HLE B-3 cells must be contributed by the α-class GSTs. The estimated k_{cat} value of the α-class GSTs for PC-OOH should be approximately 13 seconds. These results strongly suggested that the α-class GSTs can effectively catalyze GSH-dependent reduction of PL-OOH.

Immunoprecipitation of GPx Activity in HLE B-3 Cells Using Antibodies against GST-α and GPx-1

Several Se-dependent[35–37] and Se-independent[38–40] GPx activities have been identified in mammalian tissues, including the eye. To quantitate the relative contributions of the cationic α-class GSTs and the major selenoenzyme, GPx-1 in GSH dependent reduction of PC-OOH in HLE B-3 cells, immunoprecipitation experiments were designed. In these experiments, polyclonal antibodies specific to the selenoenzyme GPx-1 and those against the cationic α-class GSTs were separately used to quantitatively immunoprecipitate the GPx activity of the cell extracts toward PC-OOH. Quantitative immunoprecipitation of GPx-1 and the cationic α-class GSTs by their respective anti-

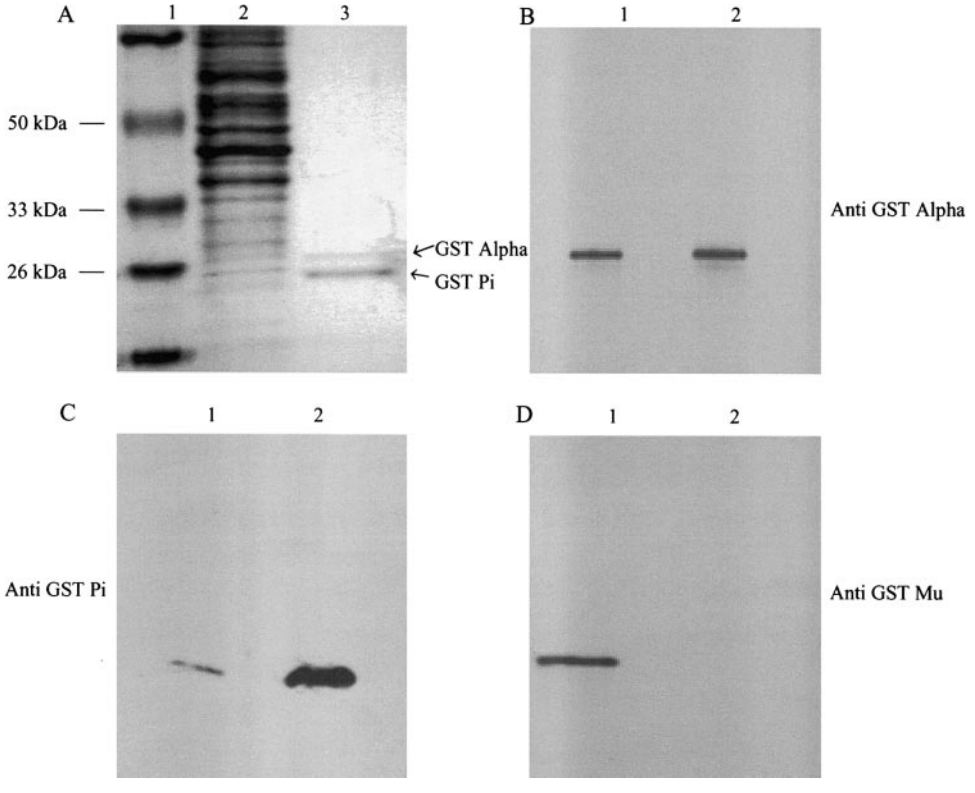

FIGURE 1. Immunological characterization of the GSTs expressed in HLE B-3 cells. (**A**) SDS-β-mercaptoethanol polyacrylamide gel electrophoresis of GSH-affinity purified total GSTs from HLE B-3 cells. *Lane 1*: prestained broad-range molecular weight markers; *lane 2*: 28,000*g* supernatant (15 μg) of the cell lysate; *lane 3*: purified total GSTs (1 μg) from HLE B-3 cells. (**B**, **C**, **D**) Western blots of purified total GSTs from HLE B-3 cells, using the polyclonal antibodies specific to the cationic α- (**B**), π- (**C**), and μ- (**D**) class GSTs. In all panels: *lane 1*: respective positive controls containing 0.1 μg protein; *lane 2*: purified total GSTs (1 μg) of HLE B-3 cells.

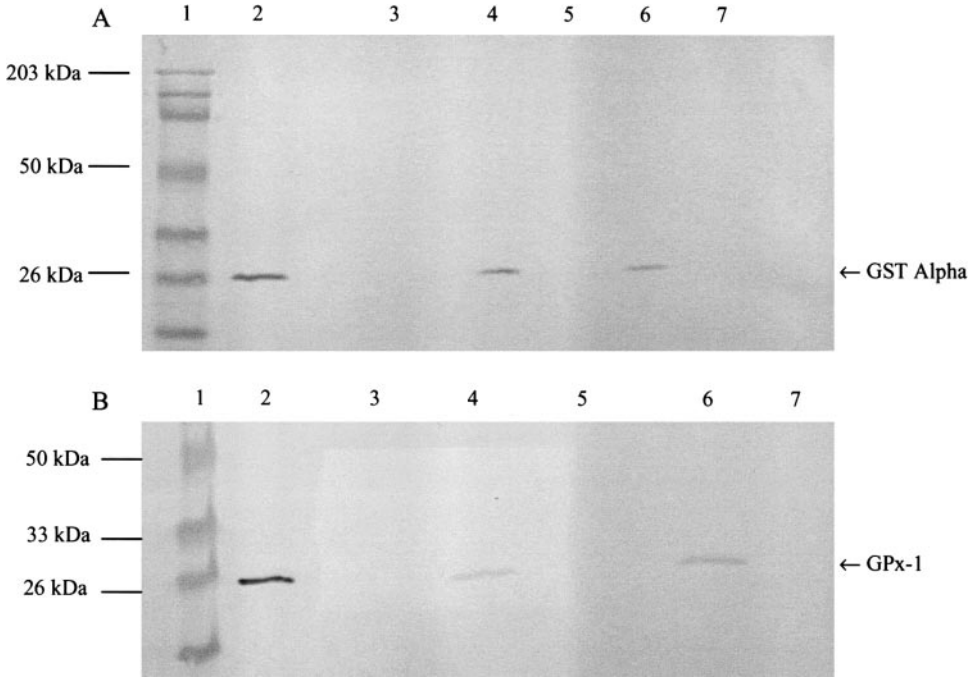

FIGURE 2. Immunoprecipitation of the cationic α-class GSTs (**A**) and the selenoenzyme GPx-1 (**B**) from HLE B-3 cell extracts. HLE B-3 cell extracts (100 μl, containing 50 μg protein) were immunoprecipitated with 2.5 μg purified anti-human cationic α-class GST IgG, or anti-human GPx-1 IgG. The proteins in the supernatant and the pellet were subjected to Western blot analysis, using the biotinylated polyclonal antibodies specific to human cationic α-class GSTs (**A**) or GPx-1 (**B**). *Lane 1*: prestained broad-range molecular weight markers; *lane 2*: cationic α-class GSTs (0.2 μg) purified from human liver (**A**) or GPx-1 (**B**) as positive controls; *lanes 3* and *4*: proteins from the immunoprecipitated pellet and supernatant fractions, respectively, using 2.5 μg IgG from the preimmune serum as the control; *lanes 6* and *7*: proteins from the immunoprecipitated pellet and supernatant fractions using the α-class GST antibodies (**A**) or GPx-1 antibodies (**B**).

bodies was confirmed by Western blot analyses. When HLE B-3 cellular extracts (100 μL) containing 50 μg protein were immunoprecipitated with anti-GST-α IgG (2.5 μg), GSTs were exclusively detected in the pellet fraction (Fig. 2A, lane 6), and not in the supernatant fraction (Fig. 2A, lane 7). These results indicate that under these conditions, the cationic α-class GSTs were quantitatively immunoprecipitated by these antibodies. Similarly, the results presented in Figure 2B show that the antibodies against GPx-1 also completely immunoprecipitated GPx-1 antigen, because it was detected only in the pellet (Fig. 2B, lane 6), not in the supernatant (Fig. 2B, lane 7). After standardizing the conditions for quantitative immunoprecipitation, the supernatant fractions of the immunoprecipitation reaction mixture were assayed for GPx activity using PC-OOH and H_2O_2 as substrates. The results of a prototypical experiment from several such experiments are presented in Figure 3. These results show that the α-class GSTs antibodies precipitated approximately 65% of GPx activity toward PC-OOH. On the contrary, only an insignificant fraction of GPx activity toward PC-OOH was immunoprecipitated by GPx-1 antibodies. However, when H_2O_2 was used as the substrate, approximately 80% of GPx activity was immunoprecipitated by the GPx-1 antibodies, whereas the α-class GSTs antibodies did not immunoprecipitate any GPx activity toward H_2O_2. These results are consistent with those of our previous studies[25] and confirm that the α-class GSTs do not use H_2O_2 as the substrate. More important, our results demonstrated for the first time that the α-class GSTs account for the major portion of the GPx activity of HLE B-3 cells toward PL-OOH.

In Situ Reduction of Membrane PL-OOH in HLE B-3 Cells by Cationic α-Class GSTs

GSTs are presumably cytosolic enzymes, and the question arises whether these enzymes can reduce membrane PL-OOH in situ. Therefore, to determine whether the α-class GSTs catalyze the GSH-dependent reduction of the membrane PL-OOH of HLE B-3 cells in situ, we prepared purified recombinant hGSTA1-1 and hGSTA2-2, two major cationic α-class GSTs, and measured their GPx activity toward PL-OOH present in plasma membranes. The membrane fractions prepared from HLE B-3 cells were peroxidized by incubation with 1 mM H_2O_2 and 1 mM $FeSO_4$ at 37°C for 1 hour, as described by us recently.[25] The amount of PL-OOH generated in the membranes under these conditions was determined to be approximately 450 ± 36 nmol/mg membrane protein as determined by the previously described microiodometric assay.[25] The peroxidized membrane preparations containing 15 nmol PL-OOH were then used as the substrate and incubated at 37°C with an excess of GSH (4 mM) in the presence or absence of hGSTA1-1 or GPx-1. The results show that incubation with GSH alone caused the reduction of PL-OOH content from 15.02 to 8.18 nmol (~46% reduction) in 4 minutes (Table 2). Addition of 2 μg hGSTA1-1 in the presence of GSH led to an accelerated reduction of PL-OOH—a 77% reduction in 4 minutes. However, addition of 2 μg Se-dependent GPx-1 did not cause any significant increase in the reduction of PL-OOH over that observed in the presence of GSH only. The specific activity of hGSTA1-1 calculated from the data presented in Table 2 was found to be 0.59 ± 0.06 μmol/min · mg protein. Similar results were obtained when hGSTA2-2 was added to the reaction mixture (data not presented). These results indicate that the cationic α-class GSTs hGSTA1-1 and hGSTA2-2 can catalyze the reduction of membrane PL-OOH of HLE B-3 cells in situ.

These findings were further confirmed using a GR-coupled spectrophotometric assay. In these experiments, the HLE B-3 cell membranes containing 4 nmol PL-OOH were incubated with either the reaction mixture alone (3.2 mM GSH, 0.32 mM NADPH, 0.82 mM EDTA, and 1 U GR in 0.16 M Tris-HCl [pH 7.0]) or with the reaction mixture containing increasing amounts of hGSTA1-1 (0.2–1 μg) at 37°C for 4 minutes. In this assay, the reduction of PL-OOH was linked to NADPH consumption, which was monitored by absorbance change at 340 nm. The data presented in Figure 4 show the nonenzymatic reduction of PL-OOH by GSH as the linear rate of NADPH consumption was observed. When hGSTA1-1 was added to the reaction mixture, an accelerated, dose-dependent rate of PL-OOH reduction was observed. However, addition of heat-inactivated hGSTA1-1 did not cause any significant change in the

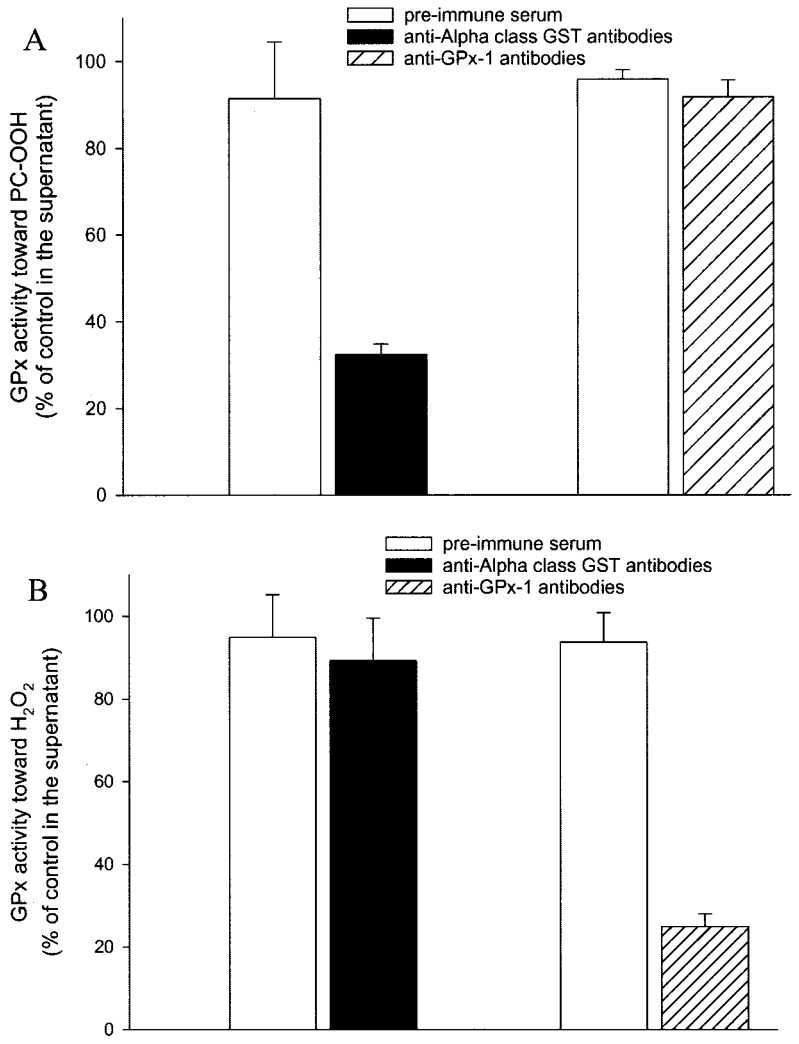

FIGURE 3. Quantitative immunoprecipitation of the GPx activity of HLE B-3 cells toward PC-OOH (**A**) and H_2O_2 (**B**), using polyclonal antibodies specific to the cationic α-class GSTs or GPx-1. Immunoprecipitation was performed as described in the legend of Fig. 2. In control experiments, serum was replaced by 50 μL of buffer. The proteins recovered in the supernatant fraction were used for determining GPx activity toward PC-OOH (**A**) and H_2O_2 (**B**). The activities were normalized to the controls. Results are the mean ± SD of three determinations. Representative results from one of the three independent experiments are presented.

rate of PL-OOH reduction observed in the presence of GSH only. The GPx activity of hGSTA1-1 toward membrane PL-OOH calculated from the curves was around 0.65 μmol/min · mg protein, regardless of the different amounts of enzymes used. These similar results obtained from two different assay methods demonstrated that hGSTA1-1 and hGSTA2-2 catalyzed the reduction of PL-OOH of HLE cell membranes in situ.

TABLE 2. GSH-Dependent Reduction of Membrane PL-OOH by GSTA1-1 and GPx

Incubation Conditions	Residual PL-OOH Content (nmol)
No incubation	15.02 ± 1.20
Peroxidized membranes + buffer only	15.73 ± 1.38
Peroxidized membranes + GSH	8.18 ± 0.63
Peroxidized membranes + GSH + hGSTA1-1	3.45 ± 0.03
Peroxidized membranes + GSH + GPx-1	7.71 ± 0.42

Equal amounts of peroxidized membrane preparations containing 33 μg protein and 15.02 ± 1.20 nmol PL-OOH, as determined by microiodometric assay, were incubated with 4 mM GSH in 0.16 mM Tris-HCl (pH 7.0), with or without 2 μg recombinant hGSTA1-1 or GPx-1 for 4 minutes at 37°C (in a total volume of 1 mL). After the incubation, 2 mL methanol-chloroform (1:2, vol/vol) was added to stop the reaction, and the reaction mixture was centrifuged at 4000*g* for 5 minutes at 4°C to extract the lipid. Residual PL-OOH was determined as described by us previously.[25] The mean ± SD of three separate experiments is shown.

Effect of Overexpression of hGSTA1-1 and hGSTA2-2 on H_2O_2- and Naphthalene-Induced LPO

The physiological significance of the GPx activity of the cationic α-class GSTs toward PL-OOH and their capability to reduce the membrane PL-OOH in situ was assessed through transfection studies. HLE B-3 cells were transiently transfected separately with pTarget-T/hGST*A1* or pTarget-T/hGST*A2* vector. Expression of protein in the transfected cells was examined through Western blot analyses. The transfected cells overexpressed hGSTA1-1 (Fig. 5A lanes 4 and 5) and hGSTA2-2 (lanes 6 and 7) compared with the wild-type cells (lane 3) for at least 48 hours, even though a noticeable decline in its expression was observed after 24 hours (lanes 5 and 7). To examine whether the hGSTA1-1 and hGSTA2-2 expressed in these cells were functional, GPx activities in the wild-type and transfected cells toward PC-OOH were compared. These results show that GPx activity toward PC-OOH of the hGST*A1*- and hGST*A2*-transfected cells was increased by 3.6-fold and 3.4-fold, respectively, compared with that in the wild-type cells at the 24-hour time point and declined at the 48-hour time point (Fig. 5B). GST activity toward CDNB in transfected cells also increased significantly (Fig. 5C), confirming the expres-

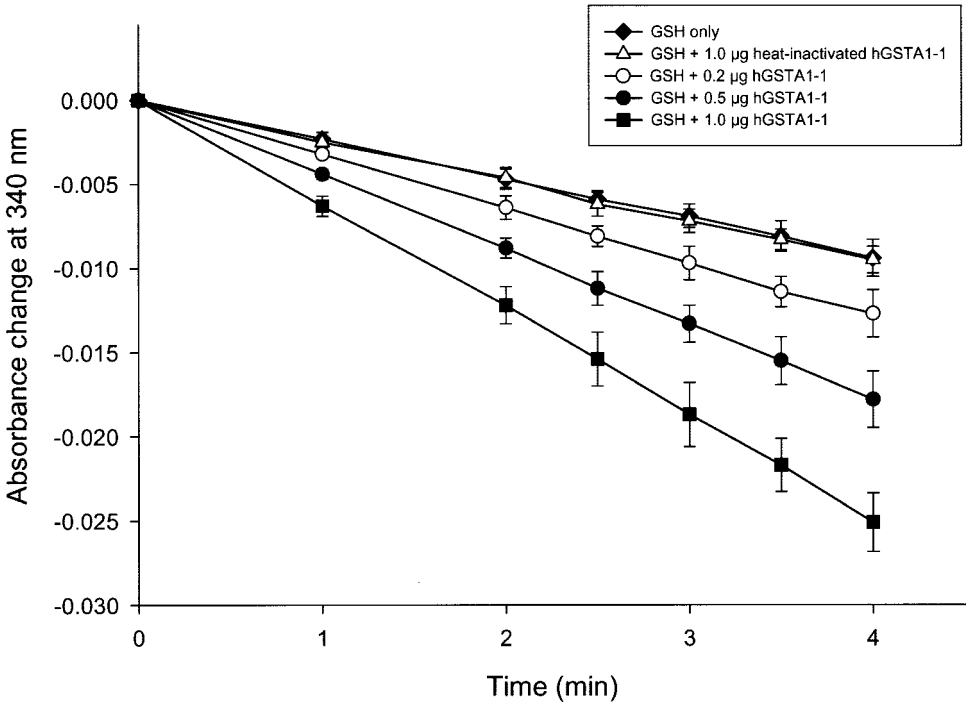

FIGURE 4. In situ reduction of PL-OOH by hGSTA1-1 in HLE B-3 cell membranes. Cell membranes were prepared and peroxidized by Fenton reaction, and the peroxidized membrane was used as the substrate to determine GPx activity of hGSTA1-1. Varying amounts of recombinant hGSTA1-1 (0.2–1.0 μg) were preincubated with GPx assay buffer containing 3.2 mM GSH, 0.32 mM NADPH, 1 U GSH reductase and 0.82 mM EDTA in 0.16 mM Tris-HCl (pH 7.0) at 37°C for 5 minutes. The reaction was started by addition of peroxidized membranes containing 4.0 nmol of PL-OOH, as determined by the microiodometric assay with a final volume of 1 mL and was monitored spectrophotometrically by measuring absorbance at 340 nm for 4 minutes. Two separate control samples, one containing no hGSTA1-1, which was replaced with equal volume of buffer A, and the other containing heat-inactivated (90°C, 5 minutes) hGSTA1-1, were used. The mean ± SD of four determinations is shown.

sion of functional hGSTA1-1 or hGSTA2-2 protein in the transfected cells.

H_2O_2-induced LPO, as measured by MDA content, has been shown in a variety of cell lines.[25,41,42] In the present studies, the hGST*A1*-transfected cells and the control HLE B-3 cells at the 24-hour time point after transfection were incubated with 100 μM H_2O_2 at 37°C for 3 hours to initiate LPO. The cells were pelleted and washed, and the extent of LPO was determined by the spectrophotometric assay of intracellular MDA and 4-HNE concentrations, the end products of LPO. A marked attenuation of MDA and 4-HNE levels was clearly shown in hGST*A1*-transfected cells (Fig. 6A), demonstrating the role of hGSTA1-1 in mechanisms protecting against LPO. Similar results were obtained when the cells were transfected with hGST*A2* (data not presented). Because neither hGSTA1-1 nor hGSTA2-2 can use H_2O_2 as a substrate, their protective effect against LPO can be attributed entirely to their ability to reduce lipid hydroperoxides through their GPx activity.

Naphthalene is toxic to the eye and cataractogenic in rodents.[43–45] In vivo studies have demonstrated the generation of reactive free radical intermediates and enhanced LPO during its phase I metabolism by cytochrome P-450. Oxidative stress due to the redox cycling of its major metabolite, 1,2-naphthoquinone has been implicated in the mechanisms of naphthalene-induced cataractogenesis.[43,46] Therefore, we treated the wild-type, vector-transfected, and hGST*A1*-transfected HLE B-3 cells with 200 μM naphthalene for 24 hours and compared the MDA and 4-HNE levels in these cells (Fig. 6B). The results clearly indicate that naphthalene caused LPO in these cells and that hGST*A1*-transfected cells were relatively resistant to naphthalene-induced LPO. It should be noted, however that naphthalene is weakly soluble in aqueous media and precipitates to form a turbid suspension. Thus, the exact concentration of naphthalene or its metabolites formed through mediation of the cytochrome P-450 system, which cause these effects cannot be ascertained from these experiments.

Resistance to H_2O_2- and Naphthalene-Induced Apoptosis of hGST*A1*- or hGST*A2*-Transfected HLE B-3 Cells through Blocking of Caspase 3 Activation

Previous studies have suggested that apoptosis of HLE cells due to a deficient defense system against factors such as oxidative stress and UV light may be a general mechanism and an early event in cataractogenesis.[26] We therefore studied the effect of overexpression of hGSTA1-1 and hGSTA2-2 on the apoptosis of HLE B-3 cells induced by H_2O_2 or naphthalene. Conditions under which H_2O_2 and naphthalene induced apoptosis in the wild-type cells were first determined, and it was established that inclusion of 100 μM H_2O_2 (6 hours) or 200 μM naphthalene (48 hours) in complete MEM induced apoptosis in these cells. Results show that under these conditions, the vector-transfected cells underwent apoptosis, as indicated by characteristic DNA laddering (Figs. 7A, 7B, lane 2). On the contrary, cells transfected with hGST*A1* showed no detectable apoptosis by these agents, under identical conditions (Figs. 7A, 7B, lane 3). A similar protective effect was observed in hGST*A2*-transfected cells (data not presented) indicating that overexpression of hGSTA1-1 or hGSTA2-2 protected these cells from H_2O_2- and naphthalene-induced apoptosis. More important, these results strengthen the assumption that lipid hydroperoxides or their downstream products such as 4-HNE are obligate intermediates in H_2O_2-induced apoptosis in HLE B-3 cells, because neither hGSTA1-1 nor hGSTA2-2 uses H_2O_2 directly as a substrate.

The activation of caspases, a family of specific cysteine proteases, is critical in the execution of apoptosis. Among the more than 10 identified caspases,[47] caspase 3, a key protease in the effector phase of apoptosis, is activated by a variety of stimuli that causing apoptosis.[48,49] Procaspase 3, a 32-kDa inactive proenzyme, is processed into a 17-kDa active subunit. Our study showed that treatment of wild-type and vector-transfected cells with H_2O_2 resulted in the appearance of the characteristic 17-kDa band (Fig. 8A, lanes 1 and 2), indicating activation of caspase 3. In hGST*A1*-transfected cells, a minimal activation of caspase 3 was observed, as indicated by a faint

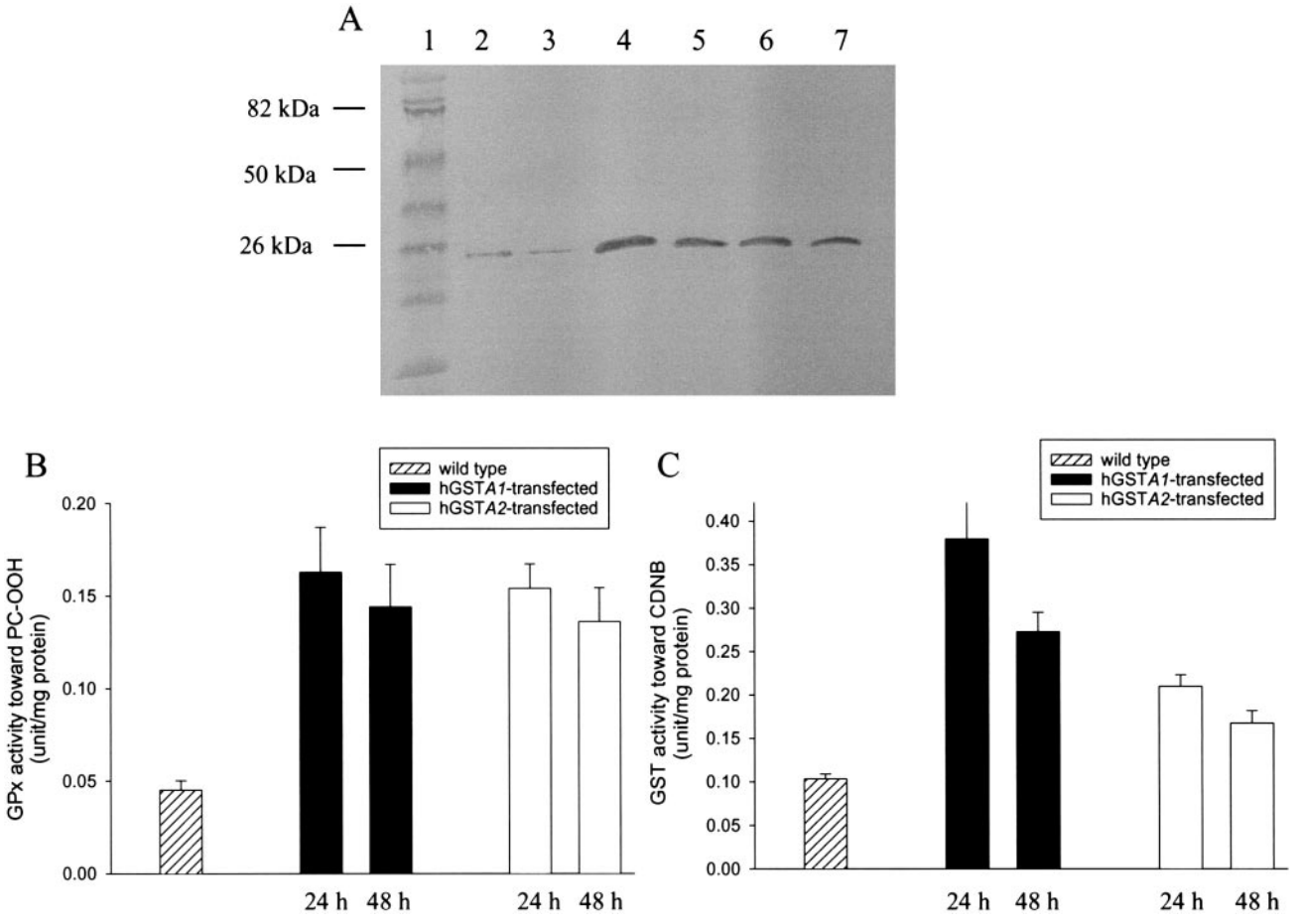

FIGURE 5. (**A**) Western blot analysis of expression of hGSTA1-1 and hGSTA2-2 in transfected cells. Aliquots of 28,000*g* supernatant fractions of lysates of the control and transfected HLE B-3 cells containing 50 μg protein were subjected to Western blot analysis, using polyclonal antibodies against the cationic α-class GSTs, and the blots were developed using HRP color-developing reagent. *Lane 1*: prestained broad-range molecular weight markers; *lane 2*: 0.1 μg recombinant hGSTA1-1 as the positive control; *lane 3*: extracts from wild-type cells; *lanes 4* and *5*: extract from hGST*A1*-transfected cells harvested at 24 and 48 hours after transfection, respectively; *lanes 6* and *7*: extracts from hGST*A2*-transfected cells harvested at 24 and 48 hours after transfection, respectively. (**B**, **C**) GPx activity toward PC-OOH (**B**) and GST activity toward CDNB (**C**) in the extracts from wild-type, hGST*A1*-transfected, and hGST*A2*-transfected HLE B-3 cells determined at 24 and 48 hours after transfection. The mean ± SD of three determinations is shown.

band at 17 kDa (Fig. 8A, lane 3). Similarly, activation of caspase 3 by naphthalene was observed only in the wild-type and vector-transfected cells (Fig. 8B, lanes 1 and 2) and not in hGST*A1*-transfected cells (Fig. 8B, lane 3). The activation of caspase 3 only in the wild-type and vector-transfected cells after H_2O_2 and naphthalene exposure was further confirmed by the Western blot analyses of cell extracts, using antibodies against PARP, a substrate of caspase 3 (Figs. 8C, 8D). The results showed that the characteristic proteolytic cleavage of the 116-kDa native PARP into the 89-kDa fragment was observed only in the wild-type or vector-transfected cells that underwent apoptosis and not in the hGST*A1*-transfected cells that were resistant to apoptosis. hGST*A2*-transfection also inhibited caspase 3 activation and PARP cleavage induced by H_2O_2 and naphthalene (data not presented). These results show that overexpression of hGSTA1-1 or hGSTA2-2 protected HLE B-3 cells from H_2O_2- and naphthalene-induced apoptosis by inhibiting caspase 3 activation.

DISCUSSION

It is well established that oxidative stress and LPO are involved in the pathogenesis of noncongenital cataractogenesis,[3–6,50] and it has been shown that the antioxidants such as ascorbate, vitamin E, and β-carotene,[51,52] and antioxidant enzymes, including CAT and Se-dependent GPx,[37,53] can prevent ROI-mediated damage to lens DNA, proteins, and membrane. Our results provide strong evidence that GSTs play a major role in protection against oxidative stress in HLE cells by attenuation of LPO. This conclusion is supported by the following findings presented in this article: GSTs in human lens epithelial cells (HLE B-3) effectively reduced PC-OOH; hGSTA1-1 and hGSTA2-2 catalyzed the in situ reduction of PL-OOH in HLE B-3 membranes; hGSTA1-1 and hGSTA2-2 contributed a major fraction of GPx activity (approximately 65%) toward PC-OOH in HLE B-3 cells; overexpression of these isozymes in HLE B-3 cells significantly decreased H_2O_2- and naphthalene-induced LPO; and overexpression of these isozymes attenuated H_2O_2- and naphthalene-induced apoptosis by inhibiting caspase 3 activation.

Previous studies have suggested that the epithelial cell is the initial target of oxidative stress-induced cataract.[54] Therefore, HLE cells in culture were used to study the role of GSTs against oxidative stress. Our results show that HLE B-3 cells expressed only the π-class and the cationic α-class GSTs, and in this regard HLE cells are different from bovine lens epithelial cells,

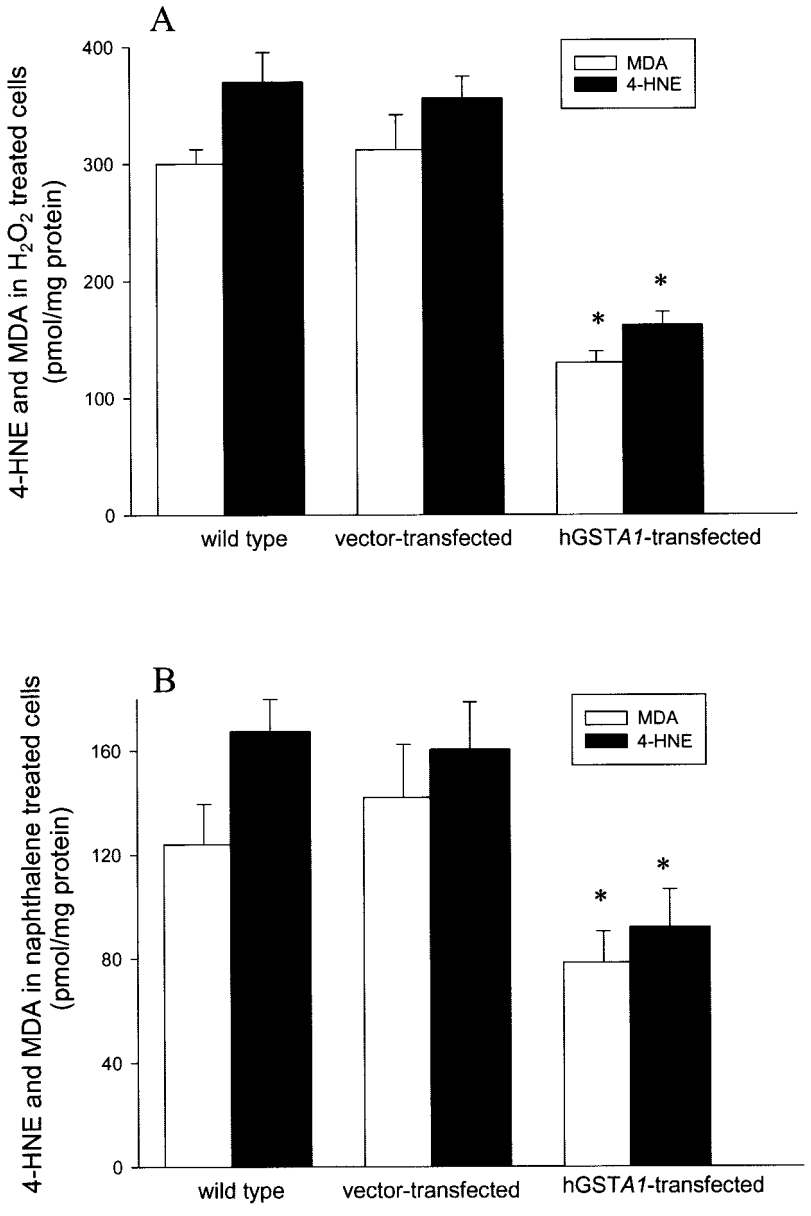

FIGURE 6. Effect of hGSTA1-1 overexpression on H_2O_2- and naphthalene-induced LPO in HLE B-3 cells. Wild-type, vector-transfected, and hGST*A1*-transfected HLE B-3 cells (1×10^7) were incubated with complete MEM containing 100 μM H_2O_2 for 3 hours (**A**) or 200 μM naphthalene for 24 hours (**B**). The cells were harvested, washed, and homogenized in 200 μL of 10 mM potassium phosphate buffer (pH 7.0) containing 5 mM BHT. LPO was determined in the whole homogenates by colorimetric assays for 4-HNE and MDA. The mean $\pm$ SD ($n = 3$) are presented in the bar graph. *Significant difference from the control results ($P < 0.01$). MDA and 4-HNE concentrations in the untreated wild-type cells were found to be 58 $\pm$ 6 and 70 $\pm$ 4 pmol/mg protein, respectively.

where the expression of μ-class GSTs alone has been demonstrated.[55] Because the cationic α-class GSTs displayed Se-independent GPx activity, we postulated that these GST isozymes may play an important role in protection against oxidative stress and LPO in HLE cells, and our results substantiate this postulation. The k_m of GSTs purified from HLE B-3 cells toward PC-OOH is 30 μM, suggesting that in these cells, GSTs can efficiently catalyze the reduction of lipid hydroperoxides under physiological conditions, in that the levels of these hydroperoxides in cells during oxidative stress has been estimated to be approximately 50 μM.[22] This is substantiated by our immunoprecipitation studies, which show that approximately 65% of GPx activity toward PC-OOH was contributed by the α-class GSTs. Our results for the first time demonstrate that the major Se-dependent GPx-1 does not provide any significant protection against PL-OOH, which are the major components of the autocatalytic chain of LPO. We hypothesize that the remaining 35% of GPx activity of HLE B-3 cells toward PC-OOH is perhaps contributed by the selenoenzyme, GPx-4, which is known to catalyze the GSH-dependent reduction of PL-OOH.[21] Currently, we are trying to raise the antibodies against recombinant GPx-4 to test this hypothesis.

The importance of the role of the α-class GSTs, hGSTA1-1 and hGSTA2-2, in protection against LPO in HLE B-3 cells is underscored by our results, which show for the first time that these isozymes reduce membrane PL-OOH of HLE B-3 cells in situ. It may be argued that the activity of GSTs are in fact due to the reduction of FA-OOH, either present as contaminants in membrane preparations or released from PL-OOH through the action of phospholipase A2 (PLA2) associated with membrane preparations. This possibility is ruled out, however, by our results in studies with the selenoenzyme GPx-1. FA-OOH is a substrate of GPx-1[15,20] and should therefore be reduced by this enzyme. Our data (Table 2) clearly show that GPx-1 caused only a minimal, or insignificant, reduction of membrane hydroperoxides, indicating that our preparations were relatively free of FA-OOH and that the GPx activity of the α-class GSTs was directed toward intact membrane PL-OOH rather than FA-OOH released from PL-OOH. Therefore, the cleavage of Syn-2 FA-OOH of membrane PL-OOH by PLA2 may be not

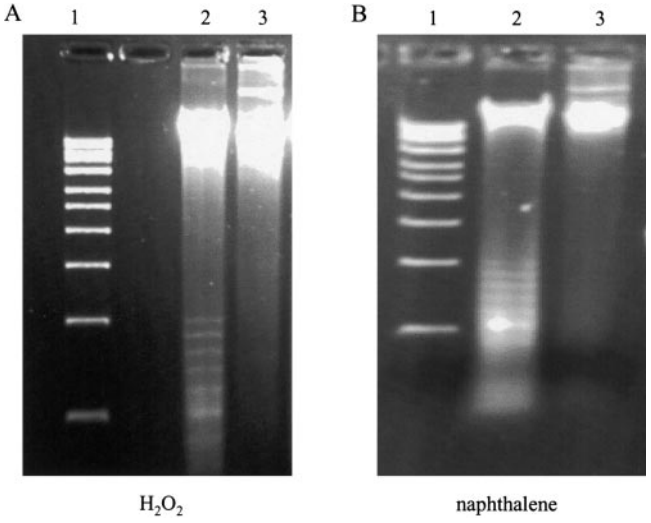

FIGURE 7. The effect of overexpression of hGSTA1-1 in HLE B-3 cells on H_2O_2- and naphthalene-induced apoptosis. The vector-transfected, and hGST*A1*-transfected HLE B-3 cells were treated with 100 μM H_2O_2 for 6 hours (**A**) or 200 μM naphthalene for 48 hours (**B**) in complete MEM. After incubations, genomic DNA was extracted from pelleted cells and electrophoresed on 2% agarose gel. In both panels, *lane 1*: DNA markers; *lane 2*: genomic DNA from vector-transfected cells; *lane 3*: genomic DNA from hGST*A1*-transfected cells. Apoptosis was indicated by the appearance of characteristic DNA laddering.

necessary for the reduction of membrane hydroperoxides by GSTs. The exact mechanisms by which the presumably cytosolic cationic α-class GSTs interact with membranes are unknown and should be studied. Results of our unpublished immunohistochemical studies using immunofluorescence and immunogold electron microscopy suggest a strong interaction of the α-class GSTs with plasma and nuclear membranes. This may explain why GSTs reduce membrane PL-OOH in situ.

In aerobic organisms, H_2O_2 is continually generated in the mitochondria, cytosol, and peroxisomes during physiological processes as a product of intracellular oxidases and SOD. It has been reported in normal human lenses and aqueous humor that H_2O_2 concentrations are in the range of 20 to 30 μM,[3] whereas in the lenses taken from cataractous eyes, H_2O_2 concentrations are two to seven times higher than the normal range.[56] Cataract develops in animal lenses in organ cultures exposed to H_2O_2 in the range observed in human cataract, showing patterns similar to that observed in human cataract.[57] It has been suggested that the mechanism of cytotoxicity of H_2O_2 involves LPO, particularly mediated by its metabolic product $OH^{\cdot}$ generated through the Fenton reaction.[58,59] Our results demonstrate for the first time that overexpression of hGSTA1-1 and hGSTA2-2 can significantly decrease intracellular concentration of the LPO end products MDA and 4-HNE, generated by H_2O_2 in HLE B-3 cells. It should be noted that 4-HNE is not a preferred substrate of hGSTA1-1 or hGSTA2-2.[24,60] These isozymes must decrease the intracellular 4-HNE or MDA levels through the reduction of PL-OOH, which gives rise to these end products of LPO autocatalytically propagated by PL-OOH. Thus, GSTs may play a crucial role in the protection mechanisms against cataractogenesis caused by factors that induce LPO through oxidative stress. This suggests that nontoxic compounds that may induce GSTs have the potential of being effective agents for retarding cataractogenesis. This idea is consistent with our previous studies showing a positive correlation of the protective effect of curcumin against 4-HNE–induced cataract with the induction of GSTs in rat lens epithelial cells by curcumin.[6]

In animal models of naphthalene and galactose cataract, apoptosis of lens epithelial cells is observed.[27,28] Involvement of apoptosis of lens epithelial cells has also been suggested in selenite cataract.[61] Similarly, apoptosis of lens epithelial cells in the mechanisms of radiation-induced cataract has been implicated.[62] However, there are conflicting reports on the role of apoptosis of lens epithelial cells in age-related cataract in humans.[26,63] Our results show that a 6-hour exposure of HLE B-3 cells to 100 μM H_2O_2, which falls in the concentration range found in the cataractous lens,[56] caused apoptosis. Similarly, exposure to naphthalene (200 μM for 48 hours) also caused apoptosis in these cells. Overexpression of hGSTA1-1 and

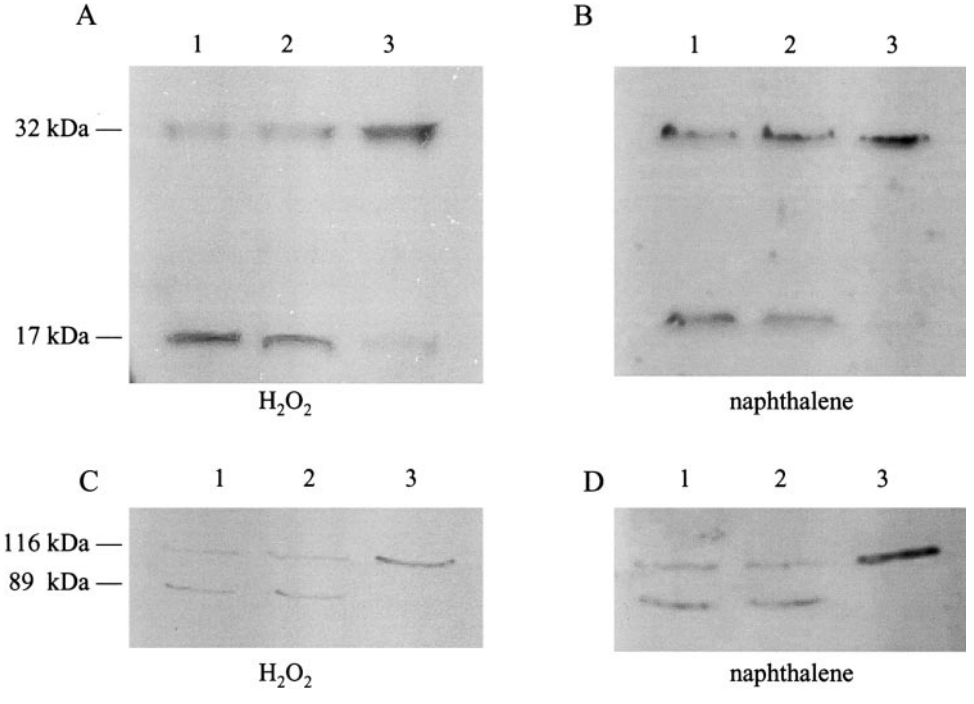

FIGURE 8. Effect of hGSTA1-1 overexpression on H_2O_2- and naphthalene-induced caspase 3 activation (**A**, **B**) and PARP cleavage (**C**, **D**). Cells (1×10^6) were incubated with 100 μM H_2O_2 in medium for 6 hours (**A**, **C**) or with 200 μM naphthalene in medium for 48 hours (**B**, **D**). For detection of caspase 3, cell lysates containing 20 μg protein were loaded in each lane and subjected to Western blot analysis, using caspase 3 polyclonal antibodies that recognize 32-kDa procaspase 3(CPP32) as well as its active 17-kDa subunit. For detection of PARP, cell lysates prepared from 4×10^5 treated HLE B-3 cells were subjected to Western blot analysis, using the polyclonal antibodies against PARP that recognize the full-length PARP (116 kDa) as well as its 89-kDa fragment. In all panels, *lane 1*: lysates from the wild type; *lane 2*: lysates from the vector-transfected cells; and *lane 3*: lysates from hGSTA1-transfected cells.

hGSTA2-2 attenuated the apoptosis caused by both these reagents. Because hGSTA1-1 and hGSTA2-2 do not decompose H_2O_2, attenuation of H_2O_2-induced apoptosis of HLE B-3 cells by transfection with these enzymes should be due to their GPx activity toward PL-OOH. Similarly, attenuation of naphthalene-induced apoptosis by hGSTA1-1 and hGSTA2-2 overexpression may be attributed to their GPx activity. However, it is also possible that GSTs provide protection against naphthalene toxicity by conjugating its metabolite 1,2-naphthoquinone to GSH. Nonetheless, conjugation of 1,2-naphthoquinone should also attenuate naphthalene-induced LPO, because the quinone metabolite exerts oxidative stress through redox cycling, and its removal should alleviate naphthalene-induced oxidative stress. Overexpression of these isozymes inhibited activation of caspase 3 and apoptosis induced by H_2O_2 and naphthalene and lowered the levels of LPO products caused by these agents. These results strongly suggest that the LPO products may be the common mediators for H_2O_2- and naphthalene-induced apoptosis and caspase 3 activation, and that hGSTA1-1 or hGSTA2-2 modulate LPO through GSH-dependent reduction of lipid hydroperoxides.

In conclusion, our results demonstrate that GSTs should be regarded as important antioxidant enzymes in mechanisms of protection against oxidative stress–mediated cataractogenesis. Thus, the possibilities of generally regarded as safe (GRAS) compounds that induce GSTs as potential cataract-preventing agents should be explored.

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