Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1480–1484, February 1994
Biochemistry

Detoxication of base propenals and other α,β-unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases

KIFLU BERHANE*, MIKAEL WIDERSTEN*, ÅKE ENGSTRÖM†, JOHN W. KOZARICH‡, AND BENGT MANNERVIK*§

*Department of Biochemistry, P.O. Box 576, and †Department of Immunology, P.O. Box 582, Uppsala University, Biomedical Center, S-751 23 Uppsala, Sweden; and ‡Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742

Communicated by Bengt Samuelsson, September 20, 1993

ABSTRACT **Radiation and chemical reactions that give rise to free radicals cause the formation of highly cytotoxic base propenals, degradation products of DNA. Human glutathione transferases (GSTs; RX:glutathione R-transferase, EC 2.5.1.18) of classes Alpha, Mu, and Pi were shown to promote the conjugation of glutathione with base propenals and related alkenes. GST P1-1 was particularly active in catalyzing the reactions with the propenal derivatives, and adenine propenal was the substrate giving the highest activity. The catalytic efficiency of GST P1-1 with adenine propenal (k_{cat}/K_m = 7.7 × 10^5 $M^{-1}·s^{-1}$) is the highest so far reported with any substrate for this enzyme. In general, GST A1-1 and GST M1-1, in contrast to GST P1-1, were more active with 4-hydroxyalkenals (products of lipid peroxidation) than with base propenals. The adduct resulting from the Michael addition of glutathione to the alkene function of one of the base propenals (adenine propenal) was identified by mass spectrometry. At the cellular level, GST P1-1 was shown to provide protection against α,β-unsaturated aldehydes. GST P1-1 added to the culture medium of HeLa cells augmented the protective effect of glutathione against the toxicity of adenine propenal and thymine propenal. No protective effect of the enzyme was observed in the presence of the competitive inhibitor *S*-hexylglutathione. GST P1-1 introduced into Hep G2 cells by electroporation was similarly found to increase their resistance to acrolein. The results show that glutathione transferases may play an important role in cellular detoxication of electrophilic α,β-unsaturated carbonyl compounds produced by radical reactions, lipid peroxidation, ionizing radiation, and drug metabolism.**

The human population is under persistent exposure to a wide variety of toxic and carcinogenic α,β-unsaturated aldehydes derived from the metabolism of natural cellular constituents and foreign compounds as well as from the environment. Endogenously, genotoxic α,β-unsaturated aldehydes, notably 4-hydroxyalkenals, are produced as a result of free-radical-initiated lipid peroxidation (1). 4-Hydroxyalkenals are reactive α,β-unsaturated aldehydes, which are able to react with cellular constituents including DNA (1–3). Acrolein and crotonaldehyde also are among the hazardous α,β-unsaturated aldehydes, which occur in the environment, for example as pollutants from industrial waste, tobacco smoke, gasoline, and diesel exhaust (4). They may also be produced intracellularly—e.g., acrolein is generated as a result of metabolic activation of the cytostatic drug cyclophosphamide (5, 6).

Another group of α,β-unsaturated aldehydes known to be highly cytotoxic are base propenals, major low-molecular-mass products of DNA degradation generated by activated bleomycin (7–9), a drug used in cancer chemotherapy (10). The formation of base propenals is initiated by the hydroxyl radical generated in the presence of molecular oxygen, a process also elicited by γ-irradiation (11).

Cytosolic glutathione transferases (GSTs; RX:glutathione R-transferase, EC 2.5.1.18) are a group of enzymes catalyzing the conjugation of reduced glutathione (GSH) with a wide variety of electrophilic compounds (12, 13). In mammalian species, GSTs have been divided into four classes based on their primary structure (14). The classes are named Alpha, Mu, Pi, and Theta (15, 16).

The GSTs are expressed in a tissue-specific manner (17, 18). Furthermore, the expression of GSTs in tumor cells is usually both qualitatively and quantitatively different from that in normal cells. In many human tumors, the class Pi GST P1-1 is present at high concentrations (19–21).

GST-mediated detoxication of a broad spectrum of electrophilic groups is important for the survival of cells, since many of the electrophilic compounds acted upon by GSTs are acutely cytotoxic as well as genotoxic. The present study shows the capacity of human GSTs to inactivate toxic α,β-unsaturated aldehydes of relevance to oxygen-linked radical reactions, lipid peroxidation, and anti-cancer drug metabolism.

MATERIALS AND METHODS

Chemicals. Adenine propenal [9-(3-oxoprop-1-enyl)adenine], cytosine propenal [1-(3-oxoprop-1-enyl)cytosine], thymine propenal [1-(3-oxoprop-1-enyl)thymine], and uracil propenal [1-(3-oxoprop-1-enyl)uracil] were synthesized by published procedures (22). All other chemicals were standard commercial products.

Expression and Purification of Recombinant GSTs. Recombinant human GST A1-1, GST P1-1, and GST M1-1 (allelic variant M1a-1a) were purified as described (23–25).

Assay of Enzyme Activities. Enzyme activities with base propenals were based on the reaction between 0.1 mM of the appropriate propenal and 1 mM GSH in 1 ml of 0.1 M sodium phosphate (pH 6.5). The conjugation of the base propenals with GSH was monitored spectrophotometrically. The base propenal concentration was limited to 0.1 mM because of the high absorbance of the compounds at wavelengths useful for spectrophotometric activity measurements: 264 nm for adenine propenal ($\Delta\varepsilon$ 7 $mM^{-1}·cm^{-1}$), 312 nm for cytosine propenal ($\Delta\varepsilon$ 10.4 $mM^{-1}·cm^{-1}$), 302 nm for thymine propenal ($\Delta\varepsilon$ 14.2 $mM^{-1}·cm^{-1}$), 296 nm for uracil propenal ($\Delta\varepsilon$ 10.6 $mM^{-1}·cm^{-1}$), 248 nm for 4-vinylpyridine ($\Delta\varepsilon$ 7.4 $mM^{-1}·cm^{-1}$), and 230 nm for crotonaldehyde ($\Delta\varepsilon$ 10.7 $mM^{-1}·cm^{-1}$). The $\Delta\varepsilon$ values (molar absorption coefficient) were determined by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GST, glutathione transferase; GSH, reduced glutathione.

§To whom reprint requests should be addressed.

Table 1. Specific activities of human GSTs with base propenals and related compounds as electrophilic substrates

Substrate	Specific activity, μmol·min^{-1}·mg^{-1}		
	GST A1-1	GST M1-1	GST P1-1
Adenine propenal	0.7 ± 0.1	3.7 ± 0.2	77 ± 1
Cytosine propenal	<0.1	<0.1	1.0 ± 0.1
Thymine propenal	<0.1	1.0 ± 0.1	8.7 ± 0.8
Uracil propenal	<0.1	1.2 ± 0.1	15 ± 0.1
4-Vinylpyridine	<0.1	<0.1	17 ± 1
Crotonaldehyde	<0.1	<0.1	1.6 ± 0.2
Acrolein	0.86	7.05	26.3

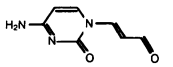
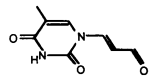
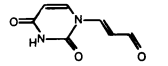
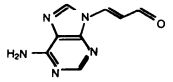
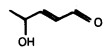
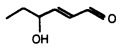
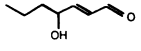
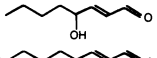
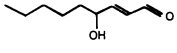
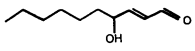
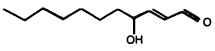
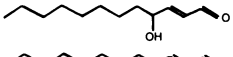
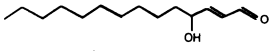
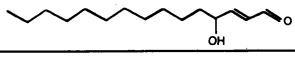
Values are given as means ± SD ($n \geq 5$). Values for acrolein are from ref. 29.

allowing a standardized solution of GSH to react to completion with an excess of alkenal.

Cell Culture and Cytotoxicity Assay. The cytotoxicity assay was performed essentially as described by Wilson (26). The human cell lines, Hep G2 and HeLa, were kindly provided by Dr. S. Braesch-Andersen (Department of Immunology, Stockholm University). Cells were grown in minimal essential medium, supplemented with 10% fetal calf serum, 2 mM glutamine, 200 μg/l gentamicin at 37°C in 5% CO_2 in air. For assaying acrolein toxicity, the cells (Hep G2) were exposed to different concentrations of acrolein for 5 min. The toxicity of base propenals to HeLa cells was assayed after addition of 0.5 mM GSH, 5 μM recombinant GST P1-1 or GST A1-1, and 200 μM *S*-hexylglutathione (GST inhibitor) in different combinations to the microtiter wells together with the propenal. The propenals were diluted with PBS and exposure times were 30 min (thymine propenal) or 60 min (adenine propenal).

Electroporation of Hep G2 Cells with GST P1-1. Hep G2 cells were cultured as described above and harvested during exponential growth. After detachment and two washings in electroporation buffer, phosphate-buffered sucrose (272 mM sucrose/7 mM potassium phosphate/1 mM $MgCl_2$, pH 7.4), 1.5×10^8 cells were divided into three equal portions and washed once more in phosphate-buffered sucrose. The cells were resuspended in 0.8 ml of phosphate-buffered sucrose containing 0, 0.2, or 2.0 mg of GST P1-1 per ml and were transferred to sterile electroporation cuvettes with an electrode gap of 0.4 cm and cooled on ice for 10 min. Electroporation was performed in a GenePulser (Bio-Rad) with the voltage set at 300 V and the capacitor set at 25 μF. After electroporation and 5-min cooling on ice, the cells were washed twice in growth medium [minimal essential medium supplemented with 10% (vol/vol) fetal calf serum] and were spread for cytotoxicity assay as described (26). Simultaneously, cells were spread onto microscope glass slides to

Table 2. k_{cat}/K_m values for human GSTs when using α,β-unsaturated aldehydes substrates

Structure	Compound	k_{cat}/K_m, mM^{-1}·s^{-1}		
		GST A1-1	GST M1-1	GST P1-1
	Acrolein	10	60	350
	Crotonaldehyde	<0.1	<0.1	29 ± 0.7
	Cytosine propenal	<0.1	<0.1	7 ± 0.8
	Thymine propenal	<0.1	<0.1	104 ± 10
	Uracil propenal	<0.1	<0.1	222 ± 9
	Adenine propenal	6 ± 0.6	46 ± 2	769 ± 54
	4-Vinylpyridine	<0.1	<0.1	154 ± 5
	4-Hydroxyalkenals			
	C-5	0.4 ± 0.9	49 ± 14	34 ± 15
	C-6	6 ± 5	120 ± 6	19 ± 3
	C-7	26 ± 3	401 ± 50	10 ± 3
	C-8	76 ± 11	740 ± 13	26 ± 2
	C-9	111 ± 21	708 ± 58	39 ± 4
	C-10	166 ± 21	839 ± 100	54 ± 7
	C-11	227 ± 15	915 ± 125	93 ± 5
	C-12	258 ± 20	901 ± 61	93 ± 24
	C-14	295 ± 19	786 ± 110	87 ± 8
	C-15	197 ± 19	430 ± 79	48 ± 11

k_{cat}/K_m values were determined by measurements ($n \geq 5$) at low concentration of electrophilic substrates (5 and 10 μM) and saturating concentration of glutathione (1 mM). Values for acrolein were from ref. 29, and values for 4-hydroxyalkenals were from ref. 30.

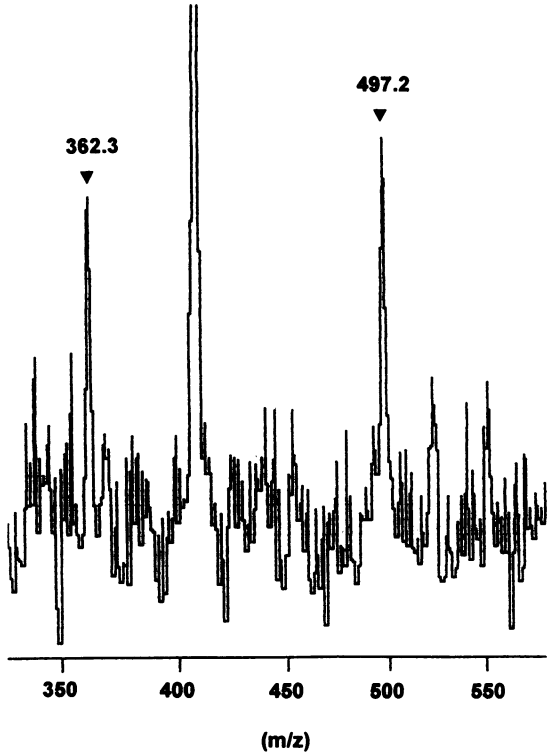


FIG. 1. Plasma desorption mass spectrum of the reaction mixture of the GST P1-1-catalyzed conjugation of GSH with adenine propenal. Arrows mark peaks corresponding to *S*-(3-oxoprop-1-enyl)-glutathione and the adduct of adenine propenal and GSH.

attach overnight for immunostaining of GST P1-1 (27) with affinity-purified polyclonal rabbit anti-human GST P1-1 antibodies. The immunocomplexes were visualized by addition of goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad) at a dilution of 1:2000 and stained with diaminobenzidine-cobalt (27).

MS Analysis. Plasma-desorption MS (28) was performed with a BioIon 20 mass spectrometer (Applied Biosystems).

RESULTS

Base Propenals as Substrates for Human GSTs. Table 1 shows specific activities of human GSTs with adenine propenal, cytosine propenal, thymine propenal, and uracil propenal as substrates. GST P1-1 displayed the highest activity, while GST A1-1 showed the lowest activity with all four base propenals. The activities with adenine propenal were higher for all three GSTs than those of the other propenals. Cytosine propenal was the poorest substrate and did not afford detectable activity with GST A1-1 and GST M1-1. The parent compound acrolein (i.e., propenal) of the base propenals, as well as crotonaldehyde and 4-vinylpyridine were also tested as structurally related activated alkene substrates (Table 1).

Catalytic efficiencies (k_{cat}/K_m) for the human GSTs are shown in Table 2. In comparison with GST M1-1 and GST A1-1, GST P1-1 shows 1 order and 2 orders of magnitude higher k_{cat}/K_m values with adenine propenal as substrate. GST P1-1 is also more efficient than GSTs A1-1 and M1-1 with thymine and uracil propenal.

Analysis of Reaction Products by MS. Fig. 1 shows a mass spectrum obtained from the enzyme-catalyzed reaction of adenine propenal with GSH. Characteristic m/z peaks were obtained at 362 and 497, corresponding to the calculated m/z for glutathionylpropenal [*S*-(3-oxoprop-1-enyl)glutathione] and 497 for the adduct of adenine propenal and GSH. The spectra of samples were compared with the signals obtained with empty sample foils and of spectra of the reaction mixture without GSH. The large peak at approximately m/z 410 is normally present in all spectra recorded with the analytical system used and does not derive from the sample. To further eliminate the possibility of matrix-derived signals, several unrelated mass spectra obtained from nitrocellulose foils were examined for the presence of m/z peaks at 362 and 497. No such m/z values were detected in any of the unrelated spectra examined, demonstrating that these signals originated from products of the reaction studied.

Cytotoxicity of Base Propenals and Protection by GSH and Extracellular GST P1-1. Toxic effects of adenine and thymine propenals on HeLa cells were examined. Cell cytotoxicity was estimated from the degree of radioactive leucine incorporation into proteins as compared with controls in which base propenals were omitted from the culture medium (Fig. 2 *Left*). HeLa cells were found to be very sensitive (IC_{50} = 10 μM) when adenine propenal or adenine propenal and GST P1-1 were added to the medium. Addition of GSH to the incubation medium that contained adenine propenal shifted the IC_{50} value to 125 μM. The value increased to 230 μM when 5 μM GST P1-1 was added in addition. In contrast, 5 μM GST A1-1 did not give any detectable protective effect (data not shown). Addition of the competitive inhibitor *S*-hexylglutathione (200 μM) to the medium containing en-

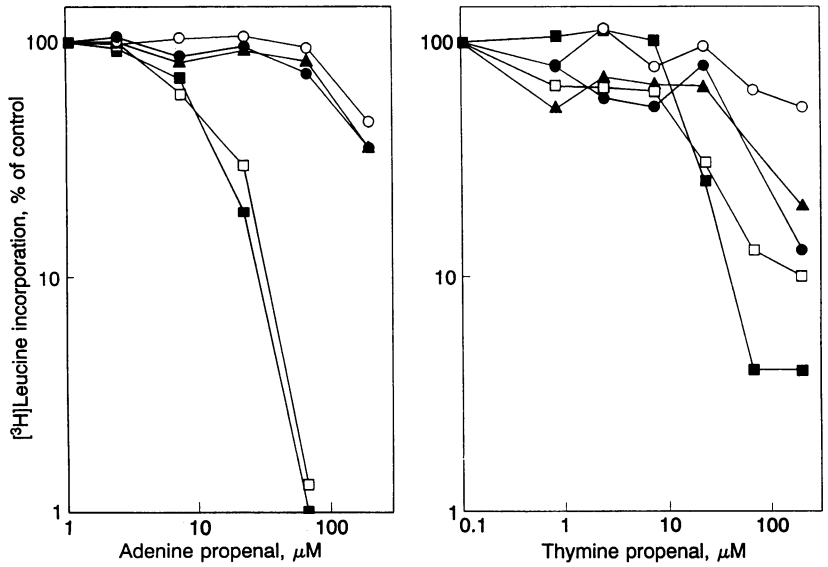


FIG. 2. Dose–response curves of HeLa cells exposed to adenine propenal for 60 min (*Left*) or thymine propenal for 30 min (*Right*). The propenals were added to the cell culture medium together with the following supplements: propenal alone (■), propenal + 5 μM GST P1-1 (□), propenal + 0.5 mM GSH (●), propenal + 0.5 mM GSH + 5 μM GST P1-1 (○), and propenal + 0.5 mM GSH + 5 μM GST P1-1 + 200 μM *S*-hexylglutathione (▲).

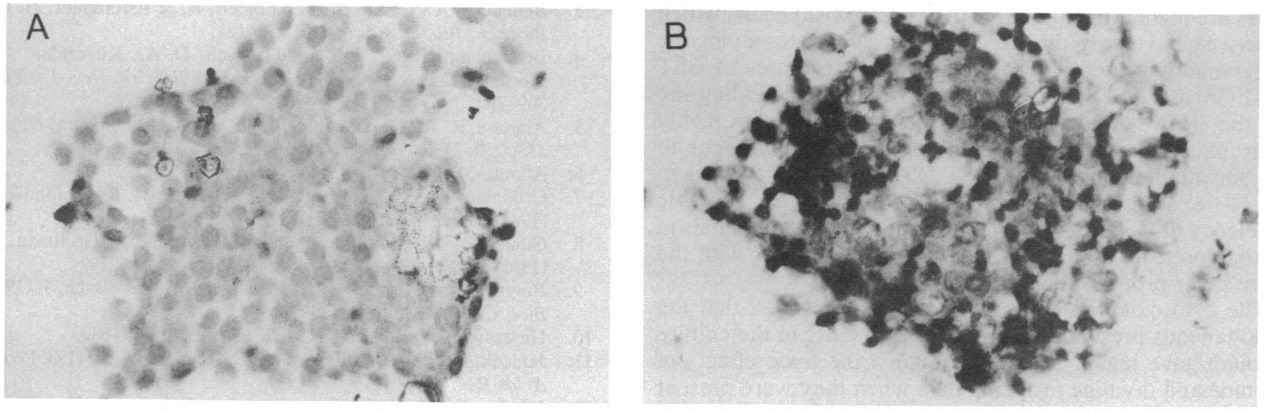


FIG. 3. Immunostaining for the presence of GST P1-1 after electroporation into Hep G2 cells, which were electroporated in the presence of buffer containing 0 (*A*) or 2 mg (*B*) of GST P1-1 per ml. Immunocomplexes were detected by addition of goat anti-rabbit IgG conjugated with horseradish peroxidase and development with diaminobenzidine cobalt. (×200.)

zyme and GSH gave a protection comparable to that of GSH alone. A similar trend was observed when HeLa cells were treated with thymine propenal (Fig. 2 *Right*). However, at higher thymine propenal concentrations, GST P1-1 appears to provide some protection even in the absence of GSH.

Protective Effect of GST P1-1 Introduced by Electroporation into Hep G2 Cells. To further study the protective effect of GST P1-1 towards toxic propenals, the intracellular level of the enzyme was increased by electroporation prior to exposure of the cells to acrolein. The Hep G2 cell line was selected, since it does not express detectable amounts of GST P1-1 under the growth conditions used (21). Hep G2 cells are also far more sensitive to acrolein than are HeLa cells (M.W. and B.M., unpublished results).

Electroporated cells were stained for GST P1-1 content using anti-human GST P1-1 antibodies, and a clear difference in staining intensity was seen between the control cells and cells treated with 2 mg of GST P1-1 per ml, demonstrating the incorporation of the enzyme (Fig. 3). When the electroporated cells were exposed to acrolein for 5 min, the sensitivity towards the propenal decreased with GST P1-1 concentration in a dose-dependent manner (Fig. 4).

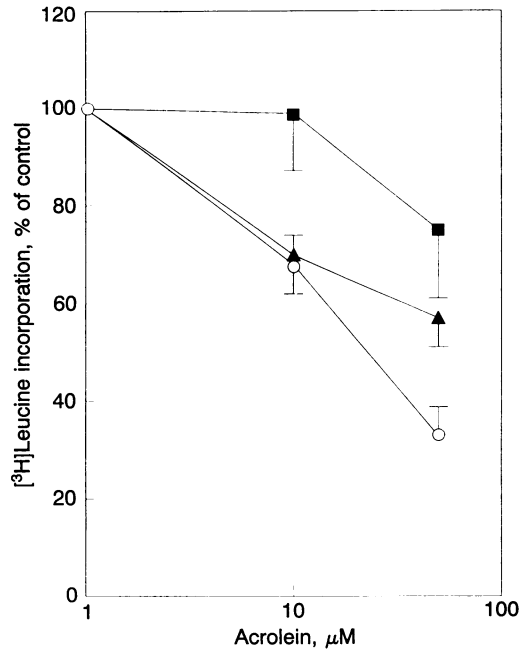


FIG. 4. Dose–response curves for Hep G2 cells electroporated in the presence of 0, 0.2, and 2 mg of GST P1-1 per ml after exposure to 0, 10, and 50 μM acrolein for 5 min. ○, Hep G2 cells electroporated in phosphate-buffered sucrose (control); ▲, Hep G2 cells electroporated in the presence of 0.2 mg of GST P1-1 per ml; ■, HepG2 cells electroporated in the presence of 2 mg of GST P1-1 per ml. Bars indicate standard deviations (n = 4).

DISCUSSION

In this study the role of GSTs in cellular protection against activated alkenes was approached at two levels. First, base propenals were evaluated as possible substrates for GSTs; second, the protective effect was evaluated on cells *in vitro*. The base propenals were found to be good substrates, and GST P1-1 showed the highest specific activity with the four base propenals tested, followed by GST M1-1 and GST A1-1 (Table 1). Catalytic efficiency, as represented by k_{cat}/K_m, demonstrated that GST P1-1 was by far the best catalyst, and GST M1-1 was better than GST A1-1, in inactivating the base propenals tested (Table 2). For GST P1-1, the k_{cat}/K_m value obtained with adenine propenal (769 $mM^{-1}\cdot s^{-1}$) was significantly higher than that obtained with 1-chloro-2,4-dinitrobenzene (130 $mM^{-1}\cdot s^{-1}$; ref. 29).

Previous studies by Grollman *et al.* (7) showed that GSH reacts with thymine propenal under physiological conditions, generating thymine and GS—CH=CH—CHO. By MS analysis of the GST P1-1-catalyzed reaction between adenine propenal and GSH, two peaks with m/z values of 362 and 497 were detected (Fig. 1). These values are in good agreement with the molecular mass of glutathionylpropenal and of a GSH conjugate formed by a Michael addition—i.e., a nucleophilic attack of GSH at the double bond of the aldehydic moiety of adenine propenal, respectively. It appears possible that the GSH adduct of adenine propenal is formed as a result of specific GST catalysis, whereas the glutathionylpropenal derives from the uncatalyzed reaction. For comparison, in the reaction of GSH with the cytostatic drug chlorambucil, several GSH conjugates have been identified (31), whereas only one of the products (the monoglutathionyl adduct) has been identified as the product of the GST-mediated reaction (32).

The finding that GSTs are active in the detoxication of activated alkenes suggests that they play a major role in the cellular protection against oxygen toxicity. It has previously been shown that GSTs efficiently catalyze the GSH conjugation of 4-hydroxyalkenals (30, 33), with rat GST 8-8 showing a particularly high catalytic efficiency (34, 35). Table 2 shows catalytic efficiencies of human GSTs with 4-hydroxyalkenals for comparison with values for the propenal derivatives as electrophilic substrates for the same enzymes. It is noteworthy that the activities of the base propenals in many

cases are higher than those for the 4-hydroxyalkenals, which otherwise have been ranked among the substrates giving the highest activity with GSTs (30). Crystal structures of GST A1-1 (36) and GST P1-1 (37) have shown that the binding site for the electrophilic second substrate (the H-site) is highly hydrophobic in GST A1-1 but more polar and more open to the surrounding solution in GST P1-1. These differences in the H-site structure are consistent with the higher catalytic efficiency of GST A1-1 with long-chain 4-hydroxyalkenals in distinction from that of GST P1-1, which is higher with the more polar propenal derivatives.

The cytotoxicity studies on HeLa cells showed that the simultaneous presence of GST P1-1 and GSH in the culture medium gave higher protection against the toxic effects of adenine and thymine propenal than when they were present separately (Fig. 2). The protection against adenine propenal obtained with GST P1-1 plus GSH as compared with that with GSH alone may be even higher than the 2-fold effect observed, since constituents in the medium were found to inhibit the enzyme irreversibly (data not shown). The degree of protection was >3-fold against thymine propenal (Fig. 2).

We have also investigated the protective effect of GST P1-1 introduced by electroporation into Hep G2 cells, which normally do not express detectable levels of GST P1-1 (21). Our results show that a substantial protection against acrolein cytotoxicity was achieved (Fig. 4) and that the protective effect is afforded also intracellularly. The effect observed also implies that the GSH adduct formed, which still retains the electrophilic aldehyde functionality, is less toxic than acrolein.

In terms of the physiological function, it appears relevant that class Pi GSTs are often expressed in response to chemical insults, as exemplified by the rat hepatocyte nodule model (38). GST P1-1 is also produced at high levels in many tumor cells (19–21, 39). Therefore, the high activity observed with the propenal derivatives supports the notion that GST P1-1 serves an important function in the cellular response to oxidative stress. The differences in substrate selectivities of the different GSTs suggest that GST P1-1 is associated with oxidative damage to nucleic acids, whereas GST A1-1 and GST M1-1 are linked to lipid peroxidation, even if the substrate specificities are largely overlapping, implicating that different GSTs to some degree can substitute for one another.

It should be noted that in addition to the GST-catalyzed reactions examined in the present investigation, other GSH-linked enzymatic reactions contribute to the cellular resistance phenotype. In particular, the GSH peroxidase activity (40) is an important contributor to the protection against organic hydroperoxides and H_2O_2 formed by oxidative processes and radical reactions.

In conclusion, the results of the present investigation show that human GSTs, especially GST P1-1, are capable of inactivating base propenals and acrolein as well as related activated alkenes. This GST activity therefore contributes to the cellular resistance phenotype that is of fundamental importance for the protection of the organism against toxic effects of drug metabolism, lipid peroxidation, and ionizing radiation.

This work was supported by the Swedish Cancer Society and the Swedish Natural Science Research Council.

1. Esterbauer, H. (1982) in *Free Radicals, Lipid Peroxidation, and Cancer*, eds. McBrien, D. C. H. & Slater, T. F. (Academic, London), pp. 101–122.
2. Benedetti, A., Comporti, M. & Esterbauer, H. (1980) *Biochim. Biophys. Acta* **620**, 281–296.
3. Benedetti, A., Comporti, M., Fulceri, R. & Esterbauer, H. (1984) *Biochim. Biophys. Acta* **792**, 172–181.
4. Beauchamp, R. O., Jr., Andjelkovich, D. A., Kligerman, A. D., Morgan, K. T. & Heck, H. d'A. (1985) *CRC Crit. Rev. Toxicol.* **14**, 309–380.
5. Alarcon, R. A. & Meienhofer, J. (1971) *Nature (London) New Biol.* **233**, 250–252.
6. Alarcon, R. A. (1976) *Cancer Treat. Rep.* **60**, 327–335.
7. Grollman, A. P., Takeshita, M., Pillai, K. M. R. & Johnson, F. (1985) *Cancer Res.* **45**, 1127–1131.
8. Giloni, L., Takeshita, M., Johnson, F., Iden, C. & Grollman, A. P. (1981) *J. Biol. Chem.* **256**, 8608–8615.
9. Burger, R. M., Projan, S. J., Horwitz, S. B. & Peisach, J. (1986) *J. Biol. Chem.* **261**, 15955–15959.
10. Umezawa, H. (1971) *Pure Appl. Chem.* **28**, 665–680.
11. Janicek, M. F., Haseltine, W. A. & Henner, W. D. (1985) *Nucleic Acids Res.* **13**, 9011–9029.
12. Jakoby, W. B. (1978) *Adv. Enzymol. Rel. Areas Mol. Biol.* **46**, 383–414.
13. Chasseaud, L. F. (1979) *Adv. Cancer Res.* **29**, 175–274.
14. Mannervik, B., Awasthi, Y. C., Board, P. G., Hayes, J. D., Di Ilio, C., Ketterer, B., Listowsky, I., Morgenstern, R., Muramatsu, M., Pearson, W. R., Pickett, C. B., Sato, K., Widersten, M. & Wolf, C. R. (1992) *Biochem. J.* **282**, 305–306.
15. Mannervik, B., Ålin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. & Jörnvall, H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7202–7206.
16. Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M. & Ketterer, B. (1991) *Biochem. J.* **274**, 409–414.
17. Mannervik, B., Guthenberg, C., Jensson, H., Warholm, M. & Ålin, P. (1983) in *Functions of Glutathione: Biochemical, Physiological, Toxicological, and Clinical Aspects*, eds. Larsson, A., Orrenius, S., Holmgren, A. & Mannervik, B. (Raven, New York), pp. 75–88.
18. Tu, C.-P. D., Weiss, M. J., Li, N.-Q. & Reddy, C. C. (1983) *J. Biol. Chem.* **258**, 4659–4662.
19. Shea, T. C., Kelley, S. L. & Henner, W. D. (1988) *Cancer Res.* **48**, 527–533.
20. Mannervik, B., Castro, V. M., Danielson, U. H., Tahir, M. K., Hansson, J. & Ringborg, U. (1987) *Carcinogenesis* **8**, 1929–1932.
21. Castro, V. M., Söderström, M., Carlberg, I., Widersten, M., Platz, A. & Mannervik, B. (1990) *Carcinogenesis* **11**, 1569–1576.
22. Johnson, F., Pillai, K. M. R., Grollman, A. P., Tseng, L. & Takeshita, M. (1984) *J. Med. Chem.* **27**, 954–958.
23. Board, P. G. & Pierce, K. (1987) *Biochem. J.* **248**, 937–941.
24. Kolm, R. H., Sroga, G. E. & Mannervik, B. (1992) *Biochem. J.* **285**, 537–540.
25. Widersten, M., Pearson, W. R., Engström, Å. & Mannervik, B. (1991) *Biochem. J.* **276**, 519–524.
26. Wilson, A. P. (1986) in *Animal Cell Culture—A Practical Approach*, ed. Freshney, R. I. (IRL, Oxford), pp. 206–207.
27. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 359–420.
28. Sundqvist, B. & Macfarlane, R. D. (1985) *Mass Spectrom. Rev.* **4**, 421–460.
29. Berhane, K. & Mannervik, B. (1990) *Mol. Pharmacol.* **37**, 251–254.
30. Danielson, U. H., Esterbauer, H. & Mannervik, B. (1987) *Biochem. J.* **247**, 707–713.
31. Dulik, D. M. & Fenselau, C. (1987) *Drug Metab. Disp.* **15**, 195–199.
32. Meyer, D. J., Gilmore, K. S., Harris, J. M., Hartley, J. A. & Ketterer, B. (1992) *Br. J. Cancer* **66**, 433–438.
33. Ålin, P., Danielson, U. H. & Mannervik, B. (1985) *FEBS Lett.* **179**, 267–270.
34. Jensson, H., Guthenberg, C., Ålin, P. & Mannervik, B. (1986) *FEBS Lett.* **203**, 207–209.
35. Stenberg, G., Ridderström, M., Engström, Å., Pemble, S. E. & Mannervik, B. (1992) *Biochem. J.* **284**, 313–319.
36. Sinning, I., Kleywegt, G. J., Cowan, S. W., Reinemer, P., Dirr, H. W., Huber, R., Gilliland, G. L., Armstrong, R. N., Ji, X., Board, P. G., Olin, B., Mannervik, B. & Jones, T. A. (1993) *J. Mol. Biol.* **232**, 192–212.
37. Reinemer, P., Dirr, H. W., Ladenstein, R., Huber, R., Lo Bello, M., Federici, G. & Parker, M. W. (1992) *J. Mol. Biol.* **227**, 214–226.
38. Eriksson, L. C., Blanck, A., Bock, K. W. & Mannervik, B. (1987) *Toxicol. Pathol.* **15**, 27–42.
39. Sato, K. (1989) *Adv. Cancer Res.* **52**, 205–255.
40. Flohé, L. (1989) in *Glutathione, Chemical, Biochemical and Medical Aspects, Coenzymes and Cofactors*, eds. Dolphin, D., Poulsen, R. & Avramović, O. (Wiley, New York), Vol. 3A, pp. 643–731.