An Alpha Class Mouse Glutathione S-Transferase with Exceptional Catalytic Efficiency in the Conjugation of Glutathione with 7β,8α-Dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene*

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The kinetics of the conjugation of glutathione (GSH) with anti-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (anti-BPDE) catalyzed by GSH S-transferase (GST) isoenzymes purified from the liver and forestomach of female A/J mouse has been investigated. The GST isoenzymes studied included an alpha class isoenzyme of forestomach (GST 9.5), alpha class hepatic isoenzymes mGSTA3-3 and mGSTA4-4, pi class hepatic isoenzyme mGSTP1-1, and mu class hepatic isoenzyme mGSTM1-1. When the concentration of (+)-anti-BPDE was varied (5–120 μM) at a fixed GSH concentration (2 mM), linear Lineweaver-Burk plots were observed for each isoenzyme. The $k_{cat}$ values for GST 9.5, mGSTA3-3, mGSTP1-1, mGSTM1-1, and mGSTA4-4 were 2.0, 0.02, 0.40, 0.05, and 0.01 s⁻¹, respectively, with corresponding $K_m$ values of 16, 12, 29, 27, and 49 μM. The catalytic efficiency ($k_{cat}/K_m$) of GST 9.5 in the conjugation of GSH with (+)-anti-BPDE, which is believed to be the ultimate carcinogenic metabolite of benzo(a)pyrene, was about 9–625-fold higher as compared with other mouse GST isoenzymes. These results indicate that GST 9.5 of forestomach is different among mammalian alpha class GSTs because (+)-anti-BPDE has been shown to be a poor substrate for alpha class rat or human GST isoenzymes. The catalytic efficiency of GST 9.5 was approximately 4.5-fold higher than that of pi class human isozyme (hGSTP1-1), which among human GSTs is reported to be most efficient in the detoxification of (+)-anti-BPDE. Unlike rat GST isoenzymes, linear Lineweaver-Burk plots were observed for mouse GSTs when GSH was used as a variable substrate. The catalytic efficiencies of the mouse GSTs toward (+)-anti-BPDE were about 2–20-fold higher as compared with the (-)-enantiomer of anti-BPDE. The results of the present study suggest that GST 9.5 may play an important role in the detoxification of (+)-anti-BPDE.

Benzo(a)pyrene (BP)¹ is the prototype of a class of wide-spread environmental pollutants, known as polycyclic aromatic hydrocarbons (PAH), which are thought to be etiological factors in human chemical carcinogenesis (1, 2). It is well known that PAHs require metabolic activation to generate electrophilic intermediates that react with nucleophilic centers in DNA to initiate carcinogenesis (3–5). For example, activation of BP through mediation of cytochrome P450-dependent monoxygenases leads to the formation of 7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE), which is believed to be the ultimate carcinogenic metabolite of BP (3, 4, 6–8). BPDE exists as a pair of diastereomers (syn- and anti-), and each diastereomer of BPDE can be resolved into a pair of optical enantiomers (7). Of the four isomers, (+)-anti-BPDE has been shown to be the most active mutagen in vitro, as well as the most potent carcinogen in vivo (6–8). Several pathways of biotransformation of BPDE compete with its reaction with nucleophilic centers in DNA, including (i) hydrolysis to tetrods and keto diols (3, 9); (ii) nonenzymatic and cytochrome P450-dependent metabolism to triols and triol epoxides (10, 11); (iii) hydration by microsomal epoxide hydrolase (12, 13); and (iv) conjugation with cellular nucleophiles such as glutathione (GSH) (14–17). Since BPDE is a poor substrate for epoxide hydrolase (12, 13, 18), the most important mechanism of BPDE inactivation seems to be its conjugation with GSH, a reaction catalyzed by glutathione S-transferases (GSTs) (19) (14–16). In the presence of GSH, both rat liver cytosol and purified cytosolic rat liver GST isoenzymes reduce the binding of anti-BPDE to nuclear DNA (19), which suggest that GSTs play a major role in the detoxification of anti-BPDE.

GSTs belong to a superfamily of multifunctional isoenzymes that contribute to the detoxification processes through several different mechanisms, including (a) catalytic inactivation of a wide variety of xenobiotics through conjugation with GSH; (b) chemical removal of certain xenobiotics through non-catalytic binding; and (c) reduction of lipid- and DNA hydroperoxides through expression of GSH peroxidase II activity (20–23). The cytosolic GST activity in mammalian tissues is expressed by multiple isoenzymes, which arise from binary combinations of identical or non-identical subunits (20, 23). A species-independent classification of the cytosolic GST isoenzymes into four major classes, alpha, mu, pi, and theta, has been suggested on the basis of their structural, immunological, and functional properties (24, 25). Tissue-specific expression of GST isoenzymes/subunits is another interesting feature of this enzyme system (20, 21, 23). Furthermore, GST isoenzymes belonging to different classes have been shown to exhibit overlapping yet distinct substrate specificities (20, 23).

Specificities of human and rat GST isoenzymes in the conjugation of GSH with (+)-anti-BPDE has been studied previously (14–16). These studies have shown that while (+)-anti-
BPDE is a poor substrate for alpha class rat and human GSTs, isoenzymes belonging to the pi class are highly efficient in the conjugation of GSH with (+)-anti-BPDE (14–17). In this communication, we report that an alpha class GST isoenzyme of the forestomach of female A/J mouse (designated as GST 9.5) is exceptionally efficient in the conjugation of GSH with (+)-anti-BPDE. In fact, catalytic efficiency of GST 9.5 in the conjugation of GSH with (+)-anti-BPDE is severalfold higher as compared with the pi class human GST isoenzyme. The significance of these findings in relation to cancer prevention is discussed.

EXPERIMENTAL PROCEDURES

Materials—Female A/J mice (8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME). The use of mice for the studies presented in this paper was approved by the Mercy Hospital Animal Care and Use Committee. Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), and epoxy-activated Sepharose 6B were purchased from Sigma. Reagents for chromatofocusing were obtained from Pharmacia Biotech Inc. The (+)- and (-)-enantiomers of anti-BPDE were procured from the National Cancer Institute Chemical Carcinogen Reference Standard Repository (Chemysn Science Laboratories, Lenexa, KS). All other reagents were the same as described previously.

Purification of GST Isoenzymes—GST isoenzymes of the liver and forestomach of female A/J mouse were purified by a protocol involving GSH-linked to epoxy-activated Sepharose 6B affinity chromatography, followed by chromatofocusing. GSH affinity chromatography was performed by the method of Simons and Vander Jagt (27). Details of GSH affinity chromatography and chromatofocusing have been described by us previously. During purification of the isoenzymes, the GST activity toward CDNB was monitored according to the method of Habig et al. (28). Protein content was determined by the method of Bradford (29).

The homogeneity and classification of GST isoenzymes used for kinetic studies were ascertained by reverse-phase HPLC and Western blot analysis, respectively, as described by us previously.

Determination of GST Activity toward (+)- and (-)-Anti-BPDE—A reverse-phase HPLC method was employed to study GST-catalyzed conjugation of GSH with anti-BPDE. The quantitation of conjugate was performed by generating standard curves of GSH conjugates of (+)- and (-)-anti-BPDE. Briefly, GSH conjugates of (+)- and (-)-anti-BPDE of known specific radioactivity were prepared by reacting 33 nmol (±)- or (±)-anti-BPDE with 240 nmol of [3H]GSH (specific activity, 49.6 μCi/μmol) for 12 h at room temperature in tubes covered with aluminum foil. The unreacted anti-BPDE was removed by extracting three times with ethyl acetate saturated with 50 mM Tris/HCl buffer (pH 7.5) containing 2.5 mM KC1 and 0.5 mM EDTA (TKE buffer). The unreacted GSH from the conjugate was separated by a solid-phase extraction procedure using an Extract-Clean C18 cartridge (Alltech, Deerfield, IL).

Thin layer chromatography (Whatman LK6F silica gel plates 250 μm) followed by visualization of ninhydrin-positive spots were utilized to ascertain separation of anti-BPDE-GSH conjugates from unreacted GSH. The mobile phase for thin layer chromatography consisted of isopropyl alcohol/isobutanol/acetic acid:water (4:3:1:2). The Rf values for GSH and GSH conjugate of anti-BPDE were about 0.026 and 0.34, respectively. To generate standard curves, known amounts of (+)- and (-)-anti-BPDE-[3H]GSH conjugates (3.3–203 pmol and 9.9–119 pmol, respectively) were subjected to reverse-phase HPLC and monitored at 247 nm. Fractions corresponding to the anti-BPDE-GSH conjugates were pooled for scintillation counting. Correlation coefficients for standard curves of GSH conjugates of (+)- and (-)-anti-BPDE were 0.999 and 0.997, respectively.

A Waters C18 reverse-phase column was used for the separation of GSH conjugates of (+) or (−)-anti-BPDE. The column was pre-equilibrated with 78% solvent A (5% acetonitrile, 0.1% trifluoroacetic acid) and 22% solvent B (90% acetonitrile, 0.1% trifluoroacetic acid). The GSH conjugates of anti-BPDE were eluted with 22% of solvent B in 0–3 min followed by a 22–24.5% gradient of solvent B in 3–8 min at a flow rate of 1 ml/min. Under these conditions, the GSH conjugates of (+)- and (−)-anti-BPDE eluted at retention times of about 5.5 and 6.3 min, respectively. Representative HPLC profiles of GSH conjugates of (+)- and (−)-anti-BPDE are illustrated in Fig. 1.

The purified GST isoenzymes were dialyzed against TKE buffer and stored at −20°C until used. GST activity toward CDNB was determined immediately before enzyme activity determination toward anti-BPDE. The reaction mixture in a final volume of 0.1 ml contained TKE buffer, 2 mM GSH, desired concentration of the (+)- or (−)-anti-BPDE, and appropriate amount of the GST isoenzyme protein. GST-catalyzed conjugation of GSH with anti-BPDE was determined as a function of varying enzyme protein concentration for each isoenzyme to optimize incubation conditions. For (+)-anti-BPDE, the GST isoenzymes were used at the following concentrations: alpha class hepatic mGSTA3-3, 200 μg/ml; pi class hepatic and forestomach mGSTP1-1, 14 μg/ml; mu class hepatic mGSTM1-1, 62 μg/ml; alpha class hepatic mGSTA4-4, 130 μg/ml, and alpha class forestomach GST 9.5, 14 μg/ml. We did not assign a name to forestomach GST 9.5, according to the nomenclature recently recommended by Hayes and Pulford (23), due to lack of information on the structures of its subunits. For (−)-anti-BPDE, the concentrations of GST isoenzymes were the same as described above except for mGSTP1-1 which was used at a concentration of 280 μg/ml. The reaction was initiated by adding anti-BPDE, and the reaction mixture was incubated for 30 s at 37°C. The reaction was terminated by rapid mixing with 0.1 ml of cold acetone, and the reaction mixture was extracted with ethyl acetate. The GST conjugates of anti-BPDE in the aqueous phase were quantitated by reverse-phase HPLC. A control without the enzyme protein was also included to account for non-enzymatic conjugation of GSH with anti-BPDE.

RESULTS AND DISCUSSION

Previous studies from our laboratory have shown that the GSH affinity purified GST preparations from the liver of female A/J mouse can be resolved into seven isoenzymes, which arise from different homo- or heterodimeric combinations of at least seven structurally distinct subunits. In liver, approximately 94% of the total GST activity is accounted for by four isoenzymes with pI values of 9.3 (alpha class mGSTA3-3), 8.8 (pi class mGSTP1-1), 8.6 (mu class mGSTM1-1), and 5.9 (alpha class mGSTA4-4). While constitutive expression of mGSTA3-3 is very low in the forestomach of female A/J mouse, mGSTP1-1, mGSTM1-1, and mGSTA4-4 of the liver and forestomach appear to be identical by the criteria of immunoreactivities with isoenzyme-specific antibodies, specific activities toward (+)- and (−)-anti-BPDE, N-terminal region amino acid sequence, and/or elution profile on reverse-phase HPLC. An additional

Fig. 1. HPLC separation of GSH conjugates of (+) and (−) anti-BPDE. The chromatographic conditions and other details are described under “Experimental Procedures.” A, 55 pmol of GSH conjugate of (+) anti-BPDE, which eluted at a retention time of about 5.5 min. B, 60 pmol of GSH conjugate of (−) anti-BPDE, which eluted at a retention time of about 6.3 min.
Kinetic parameters for mouse GST isoenzymes with (+)-anti-BPDE as the variable substrate

The purified mouse GST isoenzymes were incubated with 5–120 μM (+)-anti-BPDE and 2 mM GSH in 50 mM Tris/HCl (pH 7.5) containing 2.5 mM KCl and 0.5 mM EDTA for 30 s at 37°C. The GST isoenzymes were used at the following concentrations: forestomach GST 9.5, 14 μg/ml; hepatic mGSTA3–3, 200 μg/ml; hepatic mGSTP1–1, 14 μg/ml; hepatic mGSTM1–1, 62 μg/ml; and hepatic mGSTA4–4, 125 μg/ml. The BPDE-GSH conjugate formed was quantitated by HPLC as described under “Experimental Procedures.” Km and Vmax values were estimated by linear regression analysis. For calculation of Km and Vmax values for the respective GST isoenzymes were taken from Hayes and Pulford (23). A molecular weight of 54,000 was used for calculation of kcat value for GST 9.5. The molecular weight of GST 9.5 was estimated from the relative mobility of its subunits during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Values represent mean ± S.D. from three independent experiments.

| Isoenzyme           | Vmax (nmol/mg−1min−1) | kcat (μM) | Km (μM) | Catalytic efficiency (kcat/Km) |
|---------------------|------------------------|-----------|---------|-------------------------------|
| Forestomach GST 9.5 | 2232 ± 162             | 2.0       | 16 ± 4  | 125                           |
| Hepatic mGSTA3–3    | 17 ± 1                 | 0.02      | 12 ± 2  | 1.7                           |
| Hepatic mGSTP1–1    | 515 ± 79               | 0.40      | 29 ± 9  | 13.8                          |
| Hepatic mGSTM1–1    | 56 ± 10                | 0.05      | 27 ± 13 | 1.9                           |
| Hepatic mGSTA4–4    | 14 ± 3                 | 0.01      | 49 ± 20 | 0.2                           |

alpha class heterodimeric GST isoenzyme with pI of 9.5 (designated as GST 9.5) is expressed in the forestomach, which was not detected in the liver. Similar to liver enzymes, GST 9.5, mGSTP1–1, mGSTM1–1, and mGSTA4–4 account for more than 95% of total GST activity in the forestomach of female A/J mouse. We, therefore, selected hepatic mGSTA3–3, mGSTP1–1, mGSTM1–1, and mGSTA4–4 and forestomach GST 9.5 to investigate the kinetics of the GST-catalyzed conjugation of GSH with anti-BPDE. The kinetic constants for murine GST isoenzymes in catalyzing the conjugation of GSH with (+)-anti-BPDE are summarized in Table I. When concentration of (+)-anti-BPDE was varied (5–120 μM) at a fixed GSH concentration (2 mM), linear Lineweaver-Burk plots were obtained for each isoenzyme (plots not shown; the correlation coefficients were >0.97). Fig. 2 exemplifies the reverse-phase HPLC analysis of water-soluble products resulting from the reaction of 2 mM GSH with 120 μM (+)-anti-BPDE in the absence and presence of 14 μg/ml of forestomach GST 9.5. As can be seen, the nonenzymatic conjugation of GSH with (+)-anti-BPDE was negligible, but this reaction was accelerated severalfold in the presence of GST 9.5 (Fig. 2). Fig. 3 exemplifies the relationship between the rate of GSH (+)-anti-BPDE conjugation and the concentration of (+)-anti-BPDE in the presence of forestomach GST 9.5 and hepatic mGSTP1–1. As shown in Table I, the Vmax values for murine GST isoenzymes were in the order of GST 9.5 > mGSTP1–1 > mGSTM1–1 > mGSTA3–3 > mGSTA4–4. The Vmax value for forestomach GST 9.5 was approximately 131-, 4-, 40-, and 159-fold higher than the values for mGSTA3–3, mGSTP1–1, mGSTM1–1, and mGSTA4–4, respectively. The kcat value determined for GST 9.5 was approximately 5–200-fold higher as compared with other murine GST isoenzymes examined in the present study (Table I). The Km values for GST 9.5, mGSTA3–3, mGSTP1–1, and mGSTM1–1, and mGSTA4–4, respectively, were 16–, 12–, 29–, 27–, and 49 μM. Calculation of the catalytic efficiency (kcat/Km) revealed that while forestomach GST 9.5 was most competent in the conjugation of (+)-anti-BPDE with GSH, (+)-anti-BPDE appeared to be a poor substrate for two other alpha class mouse GST isoenzymes (mGSTA3–3 and mGSTA4–4). The catalytic efficiency of GST 9.5 in the conjugation of GSH with (+)-anti-BPDE, as compared with mGSTA3–3, mGSTP1–1, mGSTM1–1, and mGSTA4–4, was higher by about 74–, 9–, 66–, and 625-fold, respectively (Table I). Exceptionally high catalytic efficiency of GST 9.5 toward (+)-enantiomer of BPDE indicates that this isoenzyme is different among the alpha class mammalian GSTs because (+)-anti-BPDE has been shown to be a poor substrate for rat and human alpha class GST isoenzymes (15, 16). The kinetic parameters for mG-
Kinetics of the GST-catalyzed Conjugation of GSH with Anti-BPDE

The purified GSTs were incubated with 5–120 μM (-)-anti-BPDE and 2 mM GSH as described in Table I, except for mGSTP1–1 which was used at a concentration of 280 μg/ml. Details of incubation and chromatographic conditions are described under “Experimental Procedures.” Values are mean ± S.D. of three separate experiments.

### Table II

| Isoenzyme          | V_{max} | K_{m} | nmol·mg⁻¹·min⁻¹ | μM |
|---------------------|---------|-------|-----------------|----|
| Forestomach GST 9.5 | 1588 ± 104 | 57 ± 17 | 1588 ± 104 | 57 ± 17 |
| Hepatic mGSTA3–3   | 140 ± 4  | 176 ± 31 | 140 ± 4  | 176 ± 31 |
| Hepatic mGSTP1–1   | 234 ± 29 | 43 ± 6  | 234 ± 29 | 43 ± 6  |
| Hepatic mGSTM1–1   | 12 ± 1  | 167 ± 44 | 12 ± 1  | 167 ± 44 |
| Hepatic mGSTA4–4   | 18 ± 1  | 197 ± 14 | 18 ± 1  | 197 ± 14 |

Kinetic constants for mouse GST isoenzymes with GSH as the variable substrate

GST isoenzymes were incubated with 50–1600 μM GSH and 120 μM (-)-anti-BPDE in 50 mM Tris/HCl (pH 7.5), containing 2.5 mM KCl and 0.5 mM EDTA for 30 s at 37 °C. The concentrations of the GST isoenzymes were the same as described in Table I. Details of incubation and chromatographic conditions are described under “Experimental Procedures.” K_{m} and V_{max} values are mean ± S.D. of three separate experiments.

### Table III

| Isoenzyme          | V_{max} | k_{cat} | K_{m} | Catalytic efficiency |
|---------------------|---------|---------|-------|---------------------|
| Forestomach GST 9.5 | 603 ± 100 | 0.5 | 57 ± 22 | 8.8 |
| Hepatic mGSTA3–3   | 4 ± 1  | 0.003 | 9 ± 3 | 0.3 |
| Hepatic mGSTP1–1   | 18 ± 3  | 0.02 | 27 ± 14 | 0.7 |
| Hepatic mGSTM1–1   | 12 ± 1  | 0.01 | 97 ± 48 | 0.1 |

Kinetic parameters for mouse GST isoenzymes with (-)-anti-BPDE as the variable substrate

STP1-1 of theorestomach were also estimated. V_{max}, k_{cat}, and K_{m} values and catalytic efficiencies of hepatic and forestomach mGSTP1-1 isoenzymes were comparable (data not shown).

Kinetic constants were also estimated with GSH as the variable substrate at a fixed concentration of anti-BPDE (120 μM). These experiments were performed by using racemic anti-BPDE, and the results are summarized in Table II. The double-reciprocal plots were also linear for each GST isoenzyme when the concentration of GSH was varied (plots not shown). This is in contrast to the results obtained with purified rat liver GST isoenzymes where a nonlinear relationship was observed (14, 15). The V_{max} value for forestomach GST 9.5 was about 7–159-fold higher as compared with other GST isoenzymes. The K_{m} value toward GSH for forestomach GST 9.5 was about 33% higher as compared with that for hepatic mGSTP1-1 (Table II). The K_{m} values for mGSTA3-3, mGSTM1-1, and mGSTA4-4 were 176, 167, and 197 μM, respectively. With the exception of mGSTA4-4, the V_{max} values for other murine GST isoenzymes were lower by about 29% (for GST 9.5) to 79% (for mGSTM1-1) when racemic anti-BPDE was used as a substrate as compared with the values obtained with (+)-anti-BPDE (Table I). This may be attributed to the inhibition of GSH conjugation of (+)-anti-BPDE by the (-)-enantiomer, a phenomenon reported for hGSTP1-1 (16).

Linear Lineweaver-Burk plots were also observed for each isoenzyme when the concentration of (-)-anti-BPDE was varied while GSH concentration was kept constant (2 mM) (Table III). Fig. 3 exemplifies the rate of (-)-anti-BPDE-GSH conjugation at different concentrations of the (-)-anti-BPDE in the presence of forestomach GST 9.5 and hepatic mGSTP1-1. The (-)-anti-BPDE appeared to be a relatively poor substrate for each GST isoenzyme as compared to the (+)-anti-BPDE. The catalytic efficiency of forestomach GST 9.5 in the conjugation of GSH with (-)-anti-BPDE (8.8 nmol·mg⁻¹·min⁻¹) was in the range observed for other compounds that are considered as good substrates of GSTs (30). In general, the murine GST isoenzymes were about 2–20-fold more efficient in catalyzing the conjugation of GSH with (+)-anti-BPDE as compared with the (-)-anti-BPDE. The enantioselectivity was relatively more pronounced for mGSTP1-1 where the catalytic efficiencies toward (+)- and (-)-enantiomers differed by about 20-fold. It is noteworthy that the murine pi class mGSTP1-1 investigated in the present study was able to catalyze the conjugation of (-)-anti-BPDE with GSH. On the contrary, the corresponding human isoenzyme (hGSTP1-1) is unable to catalyze the conjugation of GSH with (-)-anti-BPDE (16).

The results of the present study indicate that an alpha class heterodimeric GST isoenzyme (GST 9.5) of the forestomach of female A/J mouse is exceptionally efficient in catalyzing the conjugation of GSH with (+)-anti-BPDE. These results are noteworthy because (+)-anti-BPDE has been reported to be a poor substrate for alpha class human and rat GST isoenzymes (14–16). In the present study, the (+)-anti-BPDE appeared to be a rather poor substrate for the other two homodimeric alpha class mouse GST isoenzymes of the female A/J mouse tissues (mGSTA3-3 and mGSTA4-4). Further studies are needed to determine whether or not murine GST 9.5 exhibits a similar substrate specificity in catalyzing the conjugation of bay-region dihydrodiol epoxides of other PAHs, such as chrysene, dibenz(a,h)anthracene, etc. Similar to anti-BPDE, the anti-dihydrodiol epoxides of these PAHs possess the highest mutagenic and carcinogetic potency (31,32) and are configurated analogously to the (+)-anti-BPDE (7R,8S,9S,10R absolute configuration) (33). Therefore, it is more than likely that the GST 9.5 of forestomach will be efficient in the detoxification of anti-dihydrodiol epoxides of other PAHs as well.

Previous studies from our laboratory have shown that GST 9.5 of forestomach is composed of two distinct alpha class GST subunits.2 The antibodies raised against alpha class mGSTA4-4 do not recognize mGSTA3-3 (34), whereas both subunits of the forestomach GST 9.5 cross-react with these antibodies.2 While mGSTA3-3 of female A/J mouse liver is recognized by the antibodies raised against a mixture of human liver alpha class GST isoenzymes (GST alpha-epsilon), mGSTA4-4 does not cross-react with these antibodies.2 Interestingly, both subunits of the forestomach GST 9.5 are also recognized by the antibodies raised against alpha class human liver GST isoenzymes.2 These results indicate that the subunits of GST 9.5 are structurally different from those of mGSTA3-3 and mGSTA4-4 and suggest that an isoenzyme immunologically related to mouse GST 9.5 may be present in human liver. However, further studies are needed to identify the human orthologue of mouse GST 9.5 and to determine if the human isoenzyme is as efficient as murine GST 9.5 in the detoxification of anti-BPDE.

The pi class human GST isoenzyme (hGSTP1-1) has been shown to be highly efficient in the detoxification of (+)-anti-BPDE (16). The results of the present study indicate that the mouse pi class GST isoenzyme (mGSTP1-1) is comparatively less efficient in catalyzing the conjugation of GSH with (+)-anti-BPDE as compared with the corresponding human isoenzyme. The catalytic efficiency of hGSTP1-1 toward (+)-anti-BPDE is about 2-fold higher as compared with the mGSTP1-1. Even though catalytic efficiency of mGSTP1-1 is relatively lower than that of hGSTP1-1, this isoenzyme is likely to play an important role in the detoxification of (+)-anti-BPDE in mouse tissues. The pi class mGSTP1-1 accounts for approximately 21 and 23%, respectively, of total cytosolic GST protein in the liver and forestomach of female A/J mouse. On the other hand, the constitutive expression of GST 9.5 amounts to only...
about 5% of total cytosolic GST protein in the forestomach of A/J mouse. Therefore, it seems reasonable to postulate that high levels of mGSTP1-1 expression may overcome the relatively lower catalytic efficiency of this isoenzyme in the detoxification of (+)-anti-BPDE. The relative contributions of the GST 9.5 and mGSTP1-1 to the overall detoxification of (+)-anti-BPDE, however, remains to be determined.

The results of the present study indicate that the kinetic properties of the pi class GST isoenzymes of rat (rGSTP1-1, Ref. 15), human (hGSTP1-1, Ref. 16), and mouse (present study) in the conjugation of GSH with anti-BPDE are different. For example, the $K_m$ value for hGSTP1-1 toward (+)-anti-BPDE is approximately 2.9-fold higher than that for mGSTP1-1. On the other hand, $K_m$ for mGSTP1-1 is about 2-fold higher as compared with that for rGSTP1-1. Second, the (-)-enantiomer of anti-BPDE is not a substrate for hGSTP1-1 (16), whereas mGSTP1-1 can catalyze the conjugation of (-)-anti-BPDE with GSH. The activity of rGSTP1-1 toward (-)-enantiomer of anti-BPDE has not been investigated. Finally, the rGSTP1-1, but not hGSTP1-1 or mGSTP1-1, shows a biphasic kinetics with GSH as the variable substrate (15).

BP-induced cancer of the forestomach and lung in female A/J mouse has been used extensively as an experimental model in studies to identify naturally occurring inhibitors of PAH-induced neoplasia (35). Even though the mechanism by which chemoprotectors attenuate the carcinogenic effects of BP is not fully understood, increased detoxification of the carcinogenic metabolites of BP through induction of GST activity appears to be an important mechanism of their chemopreventive activity (23, 26, 36). It seems reasonable to postulate that the effectiveness of a chemoprotective agent against BP-induced carcinogenesis may, at least in part, be dependent upon its ability to increase the expression of GST isoenzyme(s) which is/are most efficient in the detoxification of (+)-anti-BPDE. The results of the present study suggest that an agent that selectively induces GST 9.5 and/or mGSTP1-1 is likely to be a relatively more effective inhibitor of BP-induced carcinogenesis than those increasing expression of mGSTA3-3, mGSTA4-4, or mGSTM1-1, which have weak activity toward (+)-anti-BPDE. However, further studies are needed to test the validity of this contention.

In conclusion, the results of the present study indicate that an alpha class mouse GST isoenzyme is highly efficient in the detoxification of both (+) and (-)-enantiomers of anti-BPDE. The relatively high activity of GST 9.5 toward the (+)-enantiomer is noteworthy because (+)-anti-BPDE is the most active mutagen in vitro and the most potent carcinogen in vivo (6–8). The catalytic efficiency of mouse GST 9.5 is about 4.5-fold higher than that of human pi class GST isoenzyme (hGSTP1-1), which among human GSTs is most efficient in the detoxification of (+)-anti-BPDE (16). Whether or not an orthologue of mouse GST 9.5 is expressed in human tissues remains to be seen. Also, the molecular basis for exceptionally high catalytic efficiency of the GST 9.5 in the conjugation of GSH with (+)-anti-BPDE remains to be understood.

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