Mouse hepatic glutathione transferase isoenzymes and their differential induction by anticarcinogens

Specificities of butylated hydroxyanisole and bisethylxanthogen as inducers of glutathione transferases in male and female CD-1 mice

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GSH transferase isoenzymes of class Mu (two forms), class Pi (one form) and class Alpha (two forms) were purified from liver cytosols of female CD-1 mice pretreated with an anticarcinogenic inducer, 2(3)-t-butyl-4-hydroxyanisole. GSH transferases GT-8.7, GT-8.8a and GT-8.8b, GT-9.0, GT-9.3, GT-10.3 and GT-10.6 contained a minimum of six types of subunits distinguishable by structural, catalytic and immunological characteristics. H.p.l.c. analysis of the subunit compositions of affinity-purified GSH transferases from liver cytosols of induced and non-induced male and female CD-1 mice showed that two anticarcinogenic compounds, 2(3)-t-butyl-4-hydroxyanisole and bisethylxanthogen, differed markedly in their specificities as inducers of GSH transferase.

INTRODUCTION

The cytosolic GSH transferases (EC 2.5.1.18) are a family of detoxification proteins that have many catalytic and binding functions [1–7]. Although differing in their specificities for substrates and non-substrate ligands, they collectively catalyse the conjugation of numerous toxic and carcinogen substrates with GSH and also inactivate reactive ligands by binding them covalently [8–12].

The thionosulphur compound BEX and the phenolic antioxidant BHA, although structurally dissimilar, have in common the ability to induce high activities of GSH transferases in livers and other organs of mice [13–15]. Administration of BHA protects against numerous carcinogenic and toxic chemicals [16,17]. BEX also inhibits chemical carcinogenesis [16,17]. A correlation has been noted between induction of GSH transferases and inhibition of chemical carcinogenesis [18,19]. Since GSH transferases participate in the inactivation of many reactive electrophiles, it has been postulated that induction of GSH transferases contributes to protection by BHA and BEX against carcinogenesis and toxicity [13–16,18,19].

We previously demonstrated that the multi-fold increases in GSH transferase activities in mouse liver cytosols in response to dietary BHA or BEX were accompanied by similar increases in the cytosolic concentrations of immunoreactive GSH transferases [15,20]. The concentration of the major hepatic GSH transferase mRNA and the rate of enzyme synthesis were

also greatly increased by the administration of BHA [20]. Pearson *et al.* [21] recently showed that the BHA-induced elevation of the concentration of GSH transferase mRNAs in female CD-1 mice was tissue-specific and resulted from increased transcription. Several GSH transferase isoenzymes have been purified from mouse liver cytosol [20,22–24]. We previously described the purification and some of the characteristics of two mouse class Mu [25] GSH transferases, GT-8.7 and GT-9.3 [20]. We now report the further characterization of these and other GSH transferases from livers of BHA-induced CD-1 mice, and the specificities of two anticarcinogens, BHA and BEX, as inducers of hepatic GSH transferases in male and female CD-1 mice.

MATERIALS AND METHODS

Materials

CD-1 mice, 4–6 weeks old, were from Charles River Breeding Laboratories. 1-Chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and *p*-nitrobenzyl chloride were bought from Eastman. BHA, cumene hydroperoxide, sulphobromophthalein, ethacrynic acid, GSH, *S*-hexyl-GSH, *S*-hexyl-GSH-Sepharose and yeast GSSG reductase were from Sigma Chemical Co. Androst-5-ene-3,17-dione was prepared as described by Kawahara [26]. Ampholytes, *trans*-4-phenylbut-3-en-2-one, CM-cellulose (CM 52), hydroxyapatite (HTP) and Polybuffer exchanger (PBE 118) were from LKB, Aldrich Chemical Co., Whatman, Bio-Rad Laboratories and Pharmacia

Abbreviations used: BHA, 2(3)-t-butyl-4-hydroxyanisole; BEX, bisethylxanthogen, (CH₃CH₂O-CS₂)₂.

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respectively. BEX was a gift from Fike Chemicals (Nitro, WV, U.S.A.) and was recrystallized five times from ethanol before use.

Measurement of enzyme activities and protein concentrations

Activities were measured at 25 °C by published procedures [27–29] except that 0.5 mM-1,2-dichloro-4-nitrobenzene was used and GSH was not added to steroid isomerase assays except as specified. One unit of enzyme produces 1 μ mol of product/min under the reaction conditions used. Protein was measured by the method of Lowry *et al.* [30], with bovine serum albumin as standard.

Purification of GSH transferases

Activities refer to the conjugation of 1-chloro-2,4-dinitrobenzene unless specified. Livers were obtained from female mice fed on a diet of 0.75% (w/w) BHA in powdered Purina Chow 5001 for the 14 days before they were killed by cervical dislocation. All purification procedures were performed at 0–4 °C. Adjustment of pH was at the temperatures at which solutions were to be used. Preparation of cytosol fractions. DEAE-cellulose chromatography and $(\text{NH}_4)_2\text{SO}_4$ precipitation were as described previously [20]. Activities were initially monitored with 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, androst-5-ene-3,17-dione and *p*-nitrobenzyl chloride. At least 95% of each activity was recovered from DEAE-cellulose. Gradient elution from CM-cellulose then separated some of the GSH transferase isoenzymes. In a typical experiment the dialysed $(\text{NH}_4)_2\text{SO}_4$ precipitate (42 ml; 2.1 g of protein; 46400 units) from 62 g of liver was applied to a 2.2 cm $\times$ 60 cm column of CM-cellulose in 10 mM-potassium phosphate buffer containing 1 mM-EDTA, 7 mM-2-mercaptoethanol and 0.15 mM-GSH (pH 6.1) [20]. A 1500 ml linear gradient of 0–200 mM-KCl in the pH 6.1 buffer eluted four peaks of activity, at KCl concentrations of 50–65 mM, 75–90 mM, 90–115 mM and 115–130 mM. Overall recovery at this stage was 75–97%, depending upon the substrate used. The pooled fractions, designated peak 1, peak 2, peak 3 and peak 4, were made 30% (v/v) in glycerol, frozen and stored at –80 °C.

GT-9.0 was obtained from peak 1. The pooled fractions (104 mg of protein; 10% of the cytosolic activity) were dialysed against 5 mM-potassium phosphate buffer containing 0.1 mM-EDTA, 2.9 mM-2-mercaptoethanol and 0.015 mM-GSH (pH 7.0) and electrofocused [31] at pH 8–11 in a 440 ml column containing a sucrose gradient. After the focusing, 40% of the activity was found in a peak centred at pH 9.0. After passage through Sephadex G-75, GT-9.0 was concentrated by vacuum dialysis against 10 mM-potassium phosphate buffer containing 0.1 mM-EDTA, 2 mM-GSH and 30% (v/v) glycerol (pH 7.0) (buffer A), frozen and stored at –80 °C.

GT-8.8a was purified from peak 2, which contained 155 mg of protein and 13% of the cytosolic activity. Dialysis, electrofocusing, gel filtration and concentration as described above yielded GT-8.8a, which represented 43% of the activity applied to the electrofocusing column.

GT-8.7, GT-8.8b and GT-10.3 were obtained from peak 3, which contained 63% of the cytosolic activity. The resolution of GT-8.7 and GT-9.3 by chromatography on CM-cellulose at pH 7.0 and their purification by

electrofocusing have been described previously [20]. These procedures also yielded GT-8.8b and GT-10.3. GT-8.8b was chromatographed on a hydroxyapatite column (11 cm $\times$ 175 mm) in buffer A. After application of 2 ml of the enzyme (25.2 mg of protein; 938 units) and 9 ml of buffer A, development of the column with a 200 ml linear gradient of 0–200 mM-KCl in Buffer A yielded GT-8.8b, which represented 82% of the activity applied to the hydroxyapatite column.

Peak 4 was the source of the most active preparation of GT-9.3. The pooled fractions (161 mg of protein; 11% of the cytosolic activity) were electrofocused at pH 9–11 as described above. GT-9.3 (7.8 mg; 22.2 units/mg) was then obtained by chromatofocusing on Poly-buffer exchanger PBE 118 with pH 9–11 ampholytes.

For the isolation of GT-10.6 a dialysed $(\text{NH}_4)_2\text{SO}_4$ precipitate (1946 mg of protein) was prepared from liver cytosol and chromatographed at pH 7.0 on CM-cellulose (2.5 cm $\times$ 28 cm) as described above. The major peak eluted by a 750 ml linear gradient of 0–0.1 M-KCl contained chiefly GT-8.7 and GT-10.3. After concentration and dialysis, 80% of this preparation was chromatofocused in three batches on PBE 118. The material that focused at pH 10.6 (23 mg of protein) was chromatographed on a 1.5 cm $\times$ 21 cm column of CM-cellulose with a 200 ml linear gradient of 0–0.1 M-KCl in buffer A. Rechromatography of the second of two activity peaks yielded 4.8 mg of GT-10.6 (14.5 units/mg).

Structural and immunological studies

M_r values were obtained by gel filtration [32] on Sephadex G-150 and by SDS/polyacrylamide-gel electrophoresis [33] on slab gels of 17.5% (w/v) acrylamide and 0.12% bisacrylamide. Enzymes, reduced and *S*-carboxymethylated [34] or oxidized with performic acid [35], as specified below, were hydrolysed in duplicate for 22 h either at 105 °C in 6 M-HCl or at 115 °C in methanesulphonic acid [36] and analysed on a Beckman model 120C amino acid analyser. *N*-Termini were examined by dansylation and Edman degradation [37,38]. Immunization of rabbits, preparation of γ -globulin fractions and immunoprecipitations by a double-antibody method were performed as described previously [20].

H.p.l.c. analysis of GSH transferase subunits

GSH transferases were first isolated from liver cytosol by affinity chromatography on *S*-hexyl-GSH-Sepharose 4B [39]. Portions (0.5–3.0 ml) of cytosol were dialysed against 2 litres of 20 mM-Tris/HCl buffer, pH 7.8, containing 200 mM-NaCl and 1 mM-2-mercaptoethanol. After application to a 1 cm $\times$ 18 cm *S*-hexyl-GSH-Sepharose 4B affinity column and extensive washing to remove non-specifically adsorbed protein, the GSH transferases were eluted in 6 mM-*S*-hexyl-GSH dissolved in the dialysis buffer.

The subunit compositions of the mouse liver GSH transferases eluted from the affinity column were determined by analytical reverse-phase h.p.l.c. on a Vydak 214TP matrix. A Waters system consisting of two M-45 pumps, a model U6K injector and a model 441 detector operating at 214 nm with a zinc lamp were used in conjunction with the Dynamic Solutions Baseline software package to generate gradients and collect and analyse data. The reverse-phase h.p.l.c. method developed for separation of mouse GSH transferase subunits

differs in several respects from that described by Ostlund Farrants *et al.* [40] for GSH transferase subunits from rat and human tissues. Samples of affinity column eluates containing 1–10 μg of GSH transferases were diluted into 0.5 ml of 0.075% (v/v) trifluoroacetic acid and injected on to the Vydak column, which had previously been equilibrated with 50% (v/v) solvent B. The compositions of the solvents were: solvent A, 0.075% trifluoroacetic acid in water; solvent B, 0.075% trifluoroacetic acid in 60% (v/v) acetonitrile. A linear gradient was run from 50% to 100% solvent B over 50 min. The amount of each specific GSH transferase subunit was calculated from its peak area by interpolation from previously established calibration curves of peak area versus micrograms of protein.

RESULTS AND DISCUSSION

Structural characteristics of the GSH transferases

The purification procedures described above yielded transferases designated GT-8.7, GT-8.8a, GT-8.8b, GT-9.0, GT-9.3, GT-10.3 and GT-10.6, according to their pI values. Gel filtration gave M_r values of 44000–48000. GSH transferases have been classified by their mobilities on SDS/polyacrylamide-gel electrophoresis as Ya, Yb etc. [24,41]. SDS/polyacrylamide-gel electrophoresis showed that these dimeric enzymes were composed of four different classes of subunits, based upon their M_r values. GT-8.7 consisted of Yb subunits, as did GT-8.8a, GT-8.8b and GT-9.3. GT-9.0 and GT-10.6 were composed of Yf and Ya subunits respectively. GT-10.3 yielded two bands of similar intensity, one with the M_r of the Ya subunit and the other with M_r approx. 1000 smaller than that of the Ya subunit. Dansylation and Edman degradation yielded no *N*-terminal residue for GT-10.6 or GT-10.3. Proline was found at the *N*-termini of GT-8.7 and GT-9.3, in agreement with the results of partial

amino acid sequencing [20]. GT-8.8a, GT-8.8b and GT-9.0 also yielded proline as the sole *N*-terminal residue.

Amino acid compositions of several GSH transferases, shown in Table 1, helped to clarify the relationships among some of the purified transferases and provided information relevant to studies on the molecular biology of these enzymes. GT-8.7, a Yb₁Yb₁ homodimer and a class Mu isoenzyme, is the major GSH transferase isoenzyme present in liver cytosols from BHA-induced female CD-1 mice [12,20]. Previous studies showed that the *N*-terminal amino acid sequence of GT-8.7 corresponded to the derived *N*-terminal sequence of the protein encoded by a cDNA clone, pGT875 [20]. The close agreement between the amino acid composition of GT-8.7 and the amino acid composition derived from the nucleotide sequence [21] of pGT875 provides further support for the conclusion by Pearson *et al.* [20,21] that GT-8.7 is the protein encoded by pGT875.

GT-8.8a and GT-8.8b, which were each about 15% as abundant as GT-8.7, were obtained from chromatographically distinct forms of GSH transferase that were resolved in the CM-cellulose gradient elution step of the purification procedure described in the Materials and methods section. Differences observed among the amino acid compositions of GT-8.7, GT-8.8a and GT-8.8b were generally within experimental error, and these proteins were not distinguishable by any of the other structural characteristics examined. They yielded essentially identical peptide profiles after cleavage with trypsin, as illustrated by the h.p.l.c. analyses of tryptic digests of GT-8.7 and GT-8.8b, shown in Fig. 1. Thus the chromatographic multiplicity may be due to conformational differences, bound ligands or post-translational modifications. Alternatively, separation of homodimeric and heterodimeric isoenzymes containing the same type of class Mu subunit may have occurred, followed by heterodimer dissociation and reassociation of the

Table 1. Amino acid compositions of GSH transferases

Amino acid compositions derived from the cDNA nucleotide sequences reported by Pearson *et al.* [21] are shown in parentheses for comparison with the compositions of isoenzymes GT-8.7 (pGT875), GT-9.3 (pGT55) and GT-10.6 (pGT41).

Amino acid	Amino acid composition (mol of residue/25 500 g of protein)					
	GT-8.7	GT-8.8a	GT-8.8b	GT-9.3	GT-9.0	GT-10.6
Lys	17.5 (18)	17.9	17.5	16.3 (16)	13.7	20.5 (22)
His	5.0 (5)	5.0	4.8	3.2 (3)	4.3	3.9 (3)
Arg	13.1 (14)	14.2	13.9	14.9 (15)	11.0	13.9 (13)
Asx	25.3 (25)	24.8	25.1	23.1 (22)	26.8	20.8 (20)
Thr	8.6 (9)	8.8	8.6	10.6 (11)	7.3	4.5 (8)
Ser	11.1 (11)	10.8	11.4	11.7 (12)	8.8	8.5 (7)
Glx	20.8 (20)	20.8	20.6	22.7 (23)	23.7	25.3 (26)
Pro	12.6 (12)	12.1	12.1	12.1 (12)	15.2	13.1 (12)
Gly	9.8 (9)	9.8	9.4	11.6 (11)	20.1	13.0 (11)
Ala	11.5 (11)	10.8	11.1	9.0 (9)	14.3	17.6 (16)
Cys	1.9 (2)	2.6	2.4	4.0 (4)	3.3	1.2 (2)
Val	4.6 (5)	5.2	4.9	7.5 (8)	12.1	15.5 (13)
Met	9.5 (10)	8.8	9.0	7.0 (7)	5.3	7.4 (7)
Ile	11.5 (13)	11.3	11.7	9.3 (10)	8.6	10.2 (10)
Leu	23.0 (23)	23.0	23.0	25.7 (25)	32.0	25.7 (32)
Tyr	13.1 (13)	12.5	12.5	10.8 (11)	11.4	9.7 (8)
Phe	12.8 (13)	13.2	13.4	13.5 (14)	7.6	10.4 (10)
Trp	3.7 (4)	3.7	3.7	3.8 (4)	2.1	1.1 (1)

Fig. 1. Separation of the tryptic peptides of GT-8.7 and GT-8.8b by reverse-phase h.p.l.c.

Tryptic peptides from *S*-carboxymethylated GT-8.7 (10 μ g) or GT-8.8b (20 μ g) were applied to a Vydak 214TP C_4 column equilibrated with 0.1% H_3PO_4 and eluted with a linear 60 min gradient of solvent B (0.1% H_3PO_4 in 60% acetonitrile) as described in the text.

subunits into homodimers during subsequent purification steps.

GT-9.3 is a class Mu homodimer that is structurally distinct from GT-8.7 [20]. The accordance between the amino acid composition of GT-9.3 and that derived from the nucleotide sequence of cDNA clone pGT55 reported by Pearson *et al.* [20,21] confirms the conclusion [21] that GT-9.3 is the GSH transferase isoenzyme encoded by pGT55. Conversely, a relatively high degree of disparity was observed between the amino acid compositions of the protein encoded by the class Alpha cDNA clone pGT41 [21] and GT-10.6.

Catalytic and immunological characteristics

With the exception of GT-10.6, which exhibited very high GSH peroxidase activity towards the organic hydroperoxide cumene hydroperoxide, each of the GSH

transferases was more active towards 1-chloro-2,4-dinitrobenzene than towards any of the other substrates examined (Table 2). Two catalytically distinct types of class Mu transferases were identified. 1,2-Dichloro-4-nitrobenzene and sulphobromophthalein, characteristic substrates of isoenzymes containing Yb_1 subunits [23,24], were most actively conjugated by GT-8.7, GT-8.8a and GT-8.8b. The close agreement between the specific activities of GT-8.8a and GT-8.8b is in accord with the suggestion (above) that these are the same protein. A basis for the appreciably lower specific activities of GT-8.7 towards several substrates was not evident, since there was no indication of structural differences between GT-8.7 and the two forms of GT-8.8. GT-9.3, a structurally distinct class Mu homodimer, was distinguished by very low activity towards 1,2-dichloro-4-nitrobenzene and sulphobromophthalein.

GT-9.0 had relatively high activity towards the characteristic class Pi substrate [24,25], ethacrynic acid. The isomerization of the double bond of the steroidal substrate androst-5-ene-3,17-dione was catalysed primarily by GT-9.0 and GT-10.3. It has been noted previously that the steroid isomerase activity of mouse liver cytosol and of the GSH transferase isoenzyme primarily responsible for this activity were inhibited by GSH [13,23,42]. The paradoxical effects of GSH on the catalysis of this reaction by GT-9.0 and GT-10.3 are shown in Fig. 2. The activity of GT-9.0 towards androst-5-ene-3,17-dione was maximal (0.88 unit/mg) when no GSH was added to the assay system, whereas the steroid isomerase activity of GT-10.3 was stimulated by GSH, reaching 0.15 unit/mg in the presence of 1 mM-GSH. Thus GT-10.3 resembled human and rat liver GSH transferases exhibiting GSH-stimulated steroid isomerase activities [43].

Immunological reactivities of the isoenzymes were examined by precipitation with rabbit antibodies as described above. Precipitation with anti-GT-8.7, anti-GT-9.3 and anti-GT-9.0 γ -globulins revealed three immunologically distinct classes of isoenzymes. Anti-GT-8.7 and anti-GT-9.3 antibodies reacted only with the class Mu transferases and not with GT-9.0 or GT-10.6. Use of anti-GT-9.0 antibody showed no immunological relationship of GT-9.0 to any of the other isoenzymes.

Correlations with hepatic GSH transferases from other mouse sources

GT-8.7, the major GSH transferase isoenzyme from livers of female CD-1 mice, appeared to be closely related to the class Mu isoenzyme MIII (N1-1) from NMRI

Table 2. Catalytic activities of GSH transferases towards selected substrates

Substrate	Class ...	Specific activity (units/mg of protein)						
		GT-8.7 Mu	GT-8.8a Mu	8.8b Mu	GT-9.3 Mu	GT-9.0 Pi	GT-10.3 Alpha	GT-10.6 Alpha
1-Chloro-2,4-dinitrobenzene		95	86	109	22.2	69	6.0	14.5
1,2-Dichloro-4-nitrobenzene		1.8	2.6	2.7	0.08	0.05	0.04	0.02
Sulphobromophthalein		0.43	0.60	0.60	0.009	< 0.001	< 0.001	< 0.001
<i>p</i> -Nitrobenzyl chloride		8.9	10.6	10.5	0.50	0.42	0.92	0.24
Ethacrynic acid		0.04	0.07	0.06	0.012	4.28	0.23	0.07
Cumene hydroperoxide		0.02	0.04	0.04	0.02	0.01	1.21	40.6
<i>trans</i> -4-Phenylbut-en-2-one		0.05	0.07	0.07	0.007	0.015	0.011	0.015

Fig. 2. Effects of GSH (●) and 2-mercaptoethanol (○) on the rates of isomerization of androst-5-ene-3,17-dione by GT-9.0 (a) and GT-10.3 (b)

The assay systems contained either thiol compound at the concentrations shown on the abscissae. The initial velocities of enzymic isomerization of androst-5-ene-3,17-dione, shown on the ordinates, are expressed (a) as percentages of the activity observed when neither thiol compound was added, or (b) as percentages of the activity observed at the highest GSH concentration tested.

mice [23] and to isoenzyme P5 from LACA mice [24]. GT-8.7 may also be related to transferase F3 from DBA/2J mice [22], although their activities towards *p*-nitrobenzyl chloride differed almost 100-fold. No isoenzyme homologous to GT-9.3 was found in studies on other mice. GT-9.0, which we previously found to be abundant also in the small-intestinal mucosa of CD-1 mice [44], resembled the class Pi GSH transferase MII (N3-3) from NMRI mice and isoenzymes P1/P2 from LACA mice [23,24]. GT-10.3 appeared not to have a counterpart among the hepatic GSH transferases identified from other mice. On the basis of its Ya subunit type and its high activity towards cumene hydroperoxide, GT-10.6 appeared to be closely related to isoenzyme P4 from LACA mice. Substantial differences in amino acid composition and a 3.5-fold difference in specific activity towards the characteristic substrate cumene hydroperoxide indicate that GT-10.6 and the class Alpha isoenzyme MI (N4-4) from NMRI mice [23] are not identical, although they may be related. GT-8.7, GT-9.3, GT-9.0 and GT-10.6 would be designated 'C1-1', 'C2-2', 'C3-3' and 'C4-4' according to a recently proposed system for uniform nomenclature [23].

H.p.l.c. analysis of GSH transferase subunits

H.p.l.c. elution profiles of purified GSH transferases are shown in Fig. 3. Each enzyme was run individually to establish its retention time. GT-8.7, GT-9.3, GT-9.0 and GT-10.6 each yielded a single predominant peak. GT-8.8a and GT-8.8b (results not shown) co-chromatographed with GT-8.7. The peak in this position has been designated GT-8.7 in these analyses. All GT-10.3 preparations examined contained two distinct subunits, designated *a* and *b*, as well as smaller and variable amounts of GT-10.6. The GT-10.3 elution profile shown in Fig. 3 was obtained by rechromatography of recombined *a* and *b* subunits after removal of GT-10.6 from the preparation. Calibration curves were prepared by chromatographing five to seven different amounts of each subunit, in the range 1–4.5 μg. A linear relationship

Fig. 3. Determination of retention times and peak areas for GSH transferase polypeptides

Purified GSH transferases were run separately and then as a pooled sample on the Vydak column with a linear 50 min gradient as described in the text. Trace A, GT-10.3a (2 μg) and GT-10.3b (later peak, 1 μg); trace B, GT-9.0 (2.6 μg); trace C, GT-10.6 (1.4 μg); trace D, GT-9.3 (6.25 μg); trace E, GT-8.7 (1.9 μg); trace F, pooled sample containing GT-10.3a/b (3.1 μg), GT-9.0 (2.6 μg), GT-10.6 (2.8 μg), GT-9.3 (5.5 μg) and GT-8.7 (3.5 μg).

between peak area and micrograms of protein was found for each subunit. r values < 0.98 were obtained for the linear regression of peak area versus micrograms of protein. The slope of the regression line was different for each subunit. Significant variations in ϵ_{214} have been noted for the rat subunits [40].

Specificities of BHA and BEX as inducers of GSH transferase isoenzymes

Reverse-phase h.p.l.c. was used to analyse the subunit compositions of affinity-purified hepatic GSH transferases from male and female mice that had received either BHA or BEX at 0.5% (w/w) of the diet for 14 days, with return to the control diet 1 day before they were killed, and from mice that had concurrently received the unsupplemented control diet. The elution profile of affinity-purified GSH transferases from female CD-1 mice induced with BEX is shown in Fig. 4 for illustrative purposes. Fig. 5 summarizes the distribution of GSH transferase subunits in livers from induced and non-induced mice.

BHA and BEX exhibited quite different inducer specificities. The major effect of BEX was on GT-9.3 subunits, which constituted only 0.0–0.2% of total liver cytosol protein in control mice and rose to 5.9% (in females) and 7.5% (in males) of liver cytosol protein. A major effect of BHA was on GT-8.7 subunits, which were 0.7–1% of liver cytosol protein in untreated control mice compared with 7.8% in BHA-induced females and 4.5% in BHA-induced males. GT-9.3 was also induced by BHA but to a smaller extent than by BEX. Pearson *et al.* [21] raised the question whether GT-9.3 mRNA was expressed in livers of non-induced CD-1 mice. Our results show GT-9.3 to be present in livers of control mice, but only at very low concentrations. The dependence of GT-9.3 upon induction would explain the failure to detect an isoenzyme of this type in studies on non-induced mice [22–24].

GT-9.0 induction by BHA and BEX was gender-

Fig. 5. Quantification of the hepatic concentrations of GSH transferase polypeptides in uninduced mice and in BHA-induced and BEX-induced mice by reverse-phase h.p.l.c.

GSH transferases were affinity-purified from liver cytosols and the polypeptides were then quantified by measuring areas of the peaks in the reverse-phase h.p.l.c. elution profile in comparison with peak areas for the standards as described in the text. The error bars represent S.E.M. ($n = 4$). Where error bars are not shown, the results are the means of two determinations. (a) Females; (b) males.

dependent. This class Pi isoenzyme predominates in livers of male mice and is much less abundant in female livers [45]. In male CD-1 mice, GT-9.0 was 3.9% of the cytosol protein and was not induced by BHA or BEX. In females, BHA and BEX each elicited an approx. 4-fold increase in GT-9.0, from 0.3% to 1.2–1.4% of liver cytosol protein. Three types of class Alpha subunits were measurable in liver cytosols. GT-10.6 subunits were about 0.8–1% of liver cytosol protein, and were relatively unresponsive to induction except for a 2-fold increase in BEX-treated male mice. Pearson *et al.* [21] reported that the clone pGT41 detected a class Alpha isoenzyme that was present at a very low concentration in control female

Fig. 4. H.p.l.c. elution profile of affinity-purified GSH transferases from livers of BEX-treated female CD-1 mice

The hepatic GSH transferases desorbed from an *S*-hexyl-GSH-Sepharose column were analysed by reverse-phase h.p.l.c. as described in the text. The equivalent of 105 μ g of liver cytosol protein was applied to the column.

CD-1 mouse liver and greatly induced by BHA. This pattern is inconsistent with the abundance of GT-10.6 and its response to BHA. Possibly one of the GT-10.3 subunits may be encoded by this probe. The less-hydrophobic *a* subunit of GT-10.3 was detected only after induction, whereas the more-hydrophobic *b* subunit was present, albeit in very small amounts, in livers from non-induced mice. After induction by BHA or BEX, GT-10.3's *a* and *b* subunits were present in approximately equal amounts, totalling 0.3% to 0.9% of cytosol protein.

Clearly BHA and BEX have very different effects on hepatic GSH transferase isoenzyme profiles. A greater understanding of the contributions of GSH transferase induction to anticarcinogenesis by BHA and BEX will require isoenzyme analysis of extrahepatic target tissues as well as expanded knowledge of the roles of the individual GSH transferase isoenzymes in carcinogen metabolism.

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