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Glutathione *S*-transferases of mouse lung

Selective binding of benzo[*a*]pyrene metabolites by the subunits which are preferentially induced by t-butylated hydroxyanisole

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Six isoenzymes of glutathione S-transferase (GST) present in mouse lung have been purified and characterized. GST I (pI 9.8) is a dimer of M_r-26500 subunits and GST II is a heterodimer of M_r-26500 and -22000 subunits, and GST III (pI 7.9) and IV (pI 6.4) are dimers of M_r-24500 subunits. GST V (pI 5.7) is a heterodimer of M_r-24500 and -23000 subunits, whereas GST VI (pI 4.9) is a dimer of M_r-23000 subunits. Immunological studies indicate that the M_r-24500 subunits present in GST III (pI 7.9) are distinct from those present in GST IV (pI 6.4) and V (pI 5.7). Structural and immunological studies provide evidence that at least five distinct types of subunits in their different binary combinations give rise to various GST isoenzymes of mouse lung. These isoenzymes express varying degrees of catalytic activities towards a wide range of electrophilic substrates including benzo[*a*]pyrene 7,8-oxide and benzo[*a*]pyrene 4,5-oxide. The dietary antioxidant t-butylated hydroxyanisole (BHA) preferentially induces GST II and III. Also, these two isoenzymes selectively bind benzo[*a*]pyrene (B[*a*]P) metabolites, indicating that they play an important physiological role in the detoxification of B[*a*]P metabolites. The preferential induction of the GST isoenzymes involved in the detoxification of activated B[*a*]P metabolites indicates that the anti-neoplastic activity of BHA against B[*a*]P-induced neoplasia in mouse lung [Wattenberg (1973) J. Natl. Cancer Inst. **50**, 1541–1544] may be due to the enhanced detoxification of B[*a*]P metabolites.

INTRODUCTION

Dietary antioxidants such as t-butylated hydroxytoluene (BHT) and t-butylated hydroxyanisole (BHA) have been shown to retard the incidence of chemical carcinogenesis induced in mouse lung by polycyclic aromatic hydrocarbons (PAH) such as benzo[*a*]pyrene (B[*a*]P) (Wattenberg, 1972, 1973, 1975). It has been suggested (Benson *et al.*, 1978; Nakagawa *et al.*, 1981; Awasthi *et al.*, 1983; Partridge *et al.*, 1983) that the anti-neoplastic effect of these antioxidants may be due to the induction of glutathione S-transferases (GSTs; EC 2.5.1.18), a family of multifunctional enzymes which can detoxify PAH metabolites through catalytic conjugation to GSH as well as by non-catalytic binding (Booth *et al.*, 1961; Chasseaud, 1979; Litwack *et al.*, 1971; Jakoby, 1978). However, the evidence for the enhanced detoxification of PAH metabolites mediated by GST in the lung of antioxidant-treated mice is lacking. Even though GST activity towards the commonly used substrate 1-chloro-2,4-dinitrobenzene (CDNB) is increased in mouse lung on the administration of BHA (Benson *et al.*, 1979), it is not known whether the activity of this enzyme system towards the PAH epoxides is also affected. This is pertinent because the multiple forms of GST characterized from rat (Partridge *et al.*, 1985; Singh *et al.*, 1984) or human lung (Partridge *et al.*, 1984) differ significantly among each other in their binding properties and substrate specificities, and in rat tissues these isoenzymes are differentially induced by BHT (Awasthi *et al.*, 1984; Singh *et al.*, 1985*a*). It should also be pointed out that, owing to the inter-species differences in the composition and properties of GST isoenzymes in mammalian lung (Partridge *et al.*, 1984, 1985; Singh *et al.*, 1984), the characteristics of rat lung GST cannot be extrapolated to the mouse model that has been used to study the protective role of antioxidants against chemical carcinogenesis. Therefore, to address the question whether or not GSTs are involved in the anti-neoplastic activity of these antioxidants, it is essential that the isoenzyme/subunit composition of mouse lung GST be determined, the activities of the isoenzymes towards PAH metabolites be estimated, and the effect of the antioxidant on each of the isoenzymes be investigated.

During the present investigations we have purified six isoenzymes of GST present in mouse lung to an apparent homogeneity and studied the structural, kinetic, and immunological interrelationships among these isoenzymes. Substrate specificities of these isoenzymes towards B[*a*]P-7,8-epoxide and B[*a*]P-4,5-epoxide as well as the binding to B[*a*]P metabolites have been investigated. In addition, the induction of each of these isoenzymes by BHA has been studied and the protective role of GST in the detoxification processes in lung is discussed.

MATERIALS AND METHODS

Materials

Unless otherwise stated, the sources of the chemicals used have been described previously (Partridge *et al.*, 1985; Singh *et al.*, 1985*b*). BHA, B[*a*]P and NADPH were

Abbreviations used: GST, glutathione *S*-transferase; BHA, t-butylated hydroxyanisole; B[*a*]P, benzo[*a*]pyrene; BHT, t-butylated hydroxytoluene; PAH, polycyclic aromatic hydrocarbons; CDNB, 1-chloro-2,4-dinitrobenzene; PAGE, polyacrylamide-gel electrophoresis.

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purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and semipurified AIN-76 diet was purchased from ICN Biochemicals, Cleveland, OH, U.S.A. [^{14}C]B[*a*]P was obtained from New England Nuclear, Boston, MA, U.S.A. ^{3}H-labelled and unlabelled B[*a*]P-4,5-oxide and B[*a*]P-7,8-oxide were obtained from the Cancer Research Program of the National Cancer Institute, Division of Cancer Cause and Prevention, Bethesda, MD, U.S.A.

Enzyme assays

GST activity towards CDNB and other substrates was determined by the method of Habig *et al.* (1974). GST activities with B[*a*]P-4,5-oxide and B[*a*]P-7,8-oxide were determined by the method of Mukhtar & Bend (1977). GSH peroxidase activity was determined by the method of Awasthi *et al.* (1975), with cumene hydroperoxide as substrate. One unit of enzyme utilized 1 μmol of substrate/min at 25 °C for GST and at 37 °C for GSH peroxidase. Protein content was determined by the method of Bradford (1976), bovine serum albumin being used as standard.

Purification of mouse lung GST

Female CD-1 mice were killed by cervical dislocation and lungs were taken out and washed with ice-cold 10 mM-potassium phosphate buffer, pH 7.0, containing 1.4 mM-2-mercaptoethanol (buffer A). All subsequent purification steps were performed at 4 °C. The tissue was minced and homogenized in buffer A in a Sorvall Omnimixer. The homogenate (10%, w/v) was centrifuged at 14000 *g* for 1 h and the supernatant was dialysed against 22 mM-potassium phosphate, pH 7.0, containing 1.4 mM-2-mercaptoethanol (buffer B). The dialysed supernatant was subjected to affinity chromatography over a column (1 cm × 10 cm) of GSH linked to epoxy-activated Sepharose 6B by the method of Simons & Vander Jagt (1977). The affinity column was pre-equilibrated with buffer B at a flow rate of 10 ml/h. The enzyme was eluted with 5 mM-GSH in 50 mM-Tris/HCl, pH 9.6, containing 1.4 mM-2-mercaptoethanol. The eluted enzyme was dialysed against buffer A and subjected to isoelectric focusing in an LKB-8100-1 column with Ampholines in the pH range 3.5–10 in a 0–50% (w/v) sucrose density gradient. The isoelectric focusing was carried out at 1600 V for 18 h, and 0.8 ml fractions were collected and monitored for pH and GST activity with CDNB as electrophilic substrate. The six isoenzymes separated by isoelectric focusing were dialysed against buffer A for kinetic and immunological studies and against distilled water for structural studies.

The M_r values of an affinity-purified mixture of GST isoenzymes and individual GST isoenzymes of mouse lung were determined by gel permeation over a 2.0 cm × 85 cm column of Sephadex G-100. The column was eluted with buffer A. Fractions (4.4 ml each) were collected and monitored for protein and GST activity with CDNB as substrate.

Effect of BHA on mouse lung GST

Female CD-1 mice were housed in groups of four in hanging steel-wire cages (without bedding) with free access to diet and water. The animals were given AIN-76 semi-purified diet for at least 3 days before starting the feeding experiments. The experimental group of mice were fed on AIN-76 diet containing 0.76% BHA, whereas the control group of mice were fed on the AIN-76 semi-purified diet alone. After 8 days of BHA feeding, four mice from both groups were killed and lungs were excised and washed with 10 mM-potassium phosphate, pH 7.0, containing 1.4 mM-2-mercaptoethanol and 150 mM-NaCl (buffer C). The GST from the pooled lungs of control and BHA-fed mice were purified and analysed separately as described in this section.

Binding of B[*a*]P metabolites to lung GST

A 10% (w/v) homogenate of mouse lung (0.5 g wet weight of lung tissue, pooled from three mice, representing about 3 nmol of GST protein) was prepared in buffer A. A 30 nmol portion of ^{14}C-labelled (specific radioactivity 10000 c.p.m./nmol) B[*a*]P, representing a 10-fold molar excess over the estimated amount of GST, was dissolved in 30 μl of acetone and added to the homogenate. The reaction mixture was incubated in the presence of 10 mM-NADPH or an NADPH-generating system (containing 2.5 μmol of NADP, 50 μmol of glucose 6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 0.05 M-potassium phosphate buffer, 5 mM-$MgCl_2$ and 0.15 M-KCl; Pantarotto *et al.*, 1982) for 1 h at 37 °C, followed by an additional overnight incubation at 4 °C. The incubation mixture was dialysed against buffer A (2 litres; two changes) and centrifuged at 10000 *g* for 30 min. Radioactivity and GST activity were determined in the pellet as well as in the supernatant. The isoenzymes of mouse lung GST were separated by affinity chromatography and isoelectric focusing as described above. The enzyme activity, protein and radioactivity associated with each isoenzyme were quantified.

Immunological studies

The antibodies against mouse lung GST II (pI 8.7) and III (pI 7.9) were raised in male New Zealand albino rabbits by the method described previously (Awasthi *et al.*, 1980). Immunotitration studies were performed as described previously (Awasthi *et al.*, 1980).

Electrophoretic analysis

Urea/SDS/2-mercaptoethanol/PAGE was performed with the buffer system described by Laemmli (1970). The concentration of urea in both resolving and stacking gel was 6 M. The resolving gel comprised 12.5% (w/v) polyacrylamide and the concentration of cross-linker (*NN'*-methylenebisacrylamide) was 0.35% (w/v). The stacking gel comprised 5.9% (w/v) of polyacrylamide and 0.15% (w/v) of cross-linker. The standard polypeptide markers used were: phosphorylase *b* (M_r 94000) bovine serum albumin (M_r 67000), ovalbumin (M_r 43000), carbonic anhydrase (M_r 30000), soya-bean trypsin inhibitor (M_r 20100) and α-lactalbumin (M_r 14400).

RESULTS

Mouse lung has 10.8 ± 2.4 ($n = 8$) units of GST activity/g wet wt. towards CDNB. GST activity in the 14000 *g* supernatant of mouse lung towards several other substrates was also determined and the results (not shown) suggest that mouse lung GST can catalyse the conjugation of a variety of compounds to GSH. By using affinity chromatography over a column of GSH linked to epoxy-activated Sepharose 6B, mouse lung GSTs were purified in an yield of about 50% (Table 1). When GSH-affinity-purified GSTs were subjected to Sephadex G-100 gel permeation, a symmetrical coincident peak of

Table 1. Purification of mouse lung GSTs

	GST activity		Protein	Specific activity (units/mg of protein)	Purification	Yield
	(units/ml)	(total units)	(mg)		(fold)	(%)
14000 *g* supernatant	1.229	54.0	343.2	0.157	—	100
Affinity chromatography	0.881	26.4	1.56	16.9	107.7	49
Isoelectric focusing*						
GST I (pI 9.8)	—	0.22	0.18	1.27	—	26.3†
GST II (pI 8.7)	—	7.57	0.60	12.7	—	
GST III (pI 7.9)	—	2.67	0.45	5.9	—	
GST IV (pI 6.4)	—	0.15	0.07	2.13	—	
GST V (pI 5.7)	—	0.25	0.17	1.45	—	
GST VI (pI 4.9)	—	0.11	0.13	0.84	—	

* Out of 26.4 units, only 20.2 units were applied to the isoelectric-focusing column.
† Combined yield of the activity towards CDNB of all GST isoenzymes.

GST activity and protein was observed (results not shown). The M_r of the enzyme was approx 50000, which, taken together with the results of urea/SDS/2-mercaptoethanol/PAGE (Figs. 1*a* and 1*b*), indicate that these enzymes are dimers. On isoelectric focusing, the affinity-purified GST separated into six peaks of enzyme activity. A representative profile of the results of isoelectric focusing is presented below (Fig. 3*a*). Comparable isoelectric-focusing profiles were obtained in all the eight different purifications performed during the period of these studies. We have designated these isoenzymes in order of their decreasing isoelectric points as mouse lung GST I (pI 9.8), II (pI 8.7), III (pI 7.9), IV (pI 6.4), V (pI 5.7) and VI (pI 4.9). Means (±S.D.) of ratios of activity of different peaks in eight different purifications were: GST I, 2±0.05%; II, 69±0.80%; III, 24±0.60%; IV, 1.5±0.08%; V, 2.5±0.80%; and VI, 1.0±0.02%. During isoelectric focusing and subsequent dialysis the GST protein was almost quantitatively recovered in different peaks of enzyme activity. More than 50% of GST activity was, however, lost during isoelectric focusing and subsequent dialysis, and most of this loss was encountered on the dialysis of pooled peaks. The overall yield of purified GST after isoelectric focusing was therefore about 25%. Isoelectric focusing of the 14000 *g* supernatant of mouse lung homogenate (results not shown) also gave an isoenzyme profile similar to that of the affinity-purified enzyme, indicating that during the purification none of the isoenzymes was selectively lost. With respect to the protein content, the isoenzymes I–VI account for approx. 11, 37, 28, 5, 11 and 8% of the total GST protein of lung. The cationic isoenzymes, GST I (pI 9.8), II (pI 8.7) and III (pI 7.9), account for about 95% of the total GST activity of mouse lung towards CDNB. It is interesting to note that the anionic isoenzymes, GST IV (pI 6.4), V (pI 5.7) and VI (pI 4.9), which represent only about 5% of the total GST activity of lung towards CDNB, account for about 25% of the total GST protein.

Structural studies

Purified GST isoenzymes were subjected to urea/SDS/2-mercaptoethanol PAGE, the buffer system described by Laemmli (1970) being used. The results presented in Fig. 1 indicate that GST I is a dimer of M_r-26500 subunits (Fig. 1*a*, lane 2), whereas GST III and IV are dimers of M_r-24500 subunits (Fig. 1*a*, lanes 3 and 4 respectively). GST II was a heterodimer of M_r-26500 and -22000 subunits (Fig. 1*b*, lane 2). GST V (pI 5.7) was found to be a heterodimer of M_r-24500 and -23000 subunits (Fig. 1*a*, lane 5). GST VI (pI 5.9) was a dimer of M_r-23000 subunits (Fig. 1*a*, lane 6). The striking difference in the intensities of M_r-24500 and M_r-23000 bands of GST V appears to be inconsistent with the idea that this isoenzyme is a dimer of these subunits. It is possible that differential affinities of M_r-24500 and -23000 subunits for Coomassie Blue stain in SDS/polyacrylamide gels may account for the uneven staining of the two subunits of GST V. Differential staining of the subunit of mouse lung GST by Coomassie Blue stain in SDS-containing gels is evident from the results in Fig. 1(*a*). In lanes 2–6 of Fig. 1(*a*), equal amounts (30 μg) of protein dissolved in a 50 μl volume of buffer were applied. As can be seen in Fig. 1(*a*), the bands representing the subunits of GST V and GST I are stained lightly, whereas those representing GST III subunits are stained comparatively more darkly. The M_r-24500 subunits present in the homodimer GST IV and the heterodimer GST V also showed light staining. Differential staining of the GST subunits of human brain GST in SDS-containing gels has been documented previously (Theodore *et al.*, 1985). In SDS-free gels the subunits are almost evenly stained (results not shown).

Immunological properties

Attempts to raise antibodies against individual isoenzymes of mouse lung GST were successful in obtaining the antibodies against GST II (pI 8.7) and III (pI 7.9). In immunotitration experiments (results not shown) the antibodies raised against mouse lung GST II (pI 8.7) cross-reacted with the isoenzymes I (pI 9.8) and II (pI 8.7) and did not precipitate any of the other isoenzymes. The antibodies raised against GST III (pI 7.9) did not precipitate any other isoenzyme except GST III. The results of immunotitration studies revealed that GST I and II share common immunogenic determinants and that GST III is immunologically distinct from the other isoenzymes.

Substrate specificities

The specific activities of the purified isoenzymes towards a variety of substrates including the epoxides of B[*a*]P are given in Table 2. GST II (pI 8.7) had the

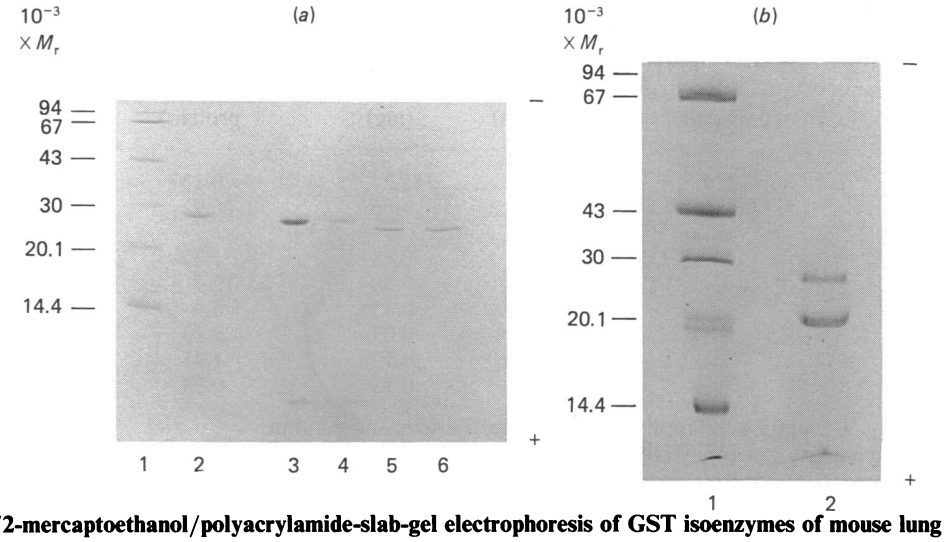

Fig. 1. Urea/SDS/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis of GST isoenzymes of mouse lung

Protein (30 μg of each isoenzyme) was dialysed against distilled water and concentrated to 50 μl by using Amicon Centricon-10 microconcentrator tubes. The electrophoresis was performed by the method of Laemmli (1970) using 12.5% (w/v) polyacrylamide gels. Gels were stained with Coomassie Blue R in methanol/acetic acid/water (5:1:4, by vol.) and destained with methanol/acetic acid/water (5:1:4, by vol.). (*a*) Lane 1, standards; lane 2, GST I (pI 9.8); lane 3, GST III (pI 7.9), lane 4, GST IV (pI 6.4); lane 5, GST V (pI 5.7); and lane 6, GST VI (pI 4.9). (*b*) Lane 1, standards (50 μg); lane 2, GST II (pI 8.7).

Table 2. Substrate specificities of mouse lung GST isoenzymes

		Specific activity (units/mg of protein)					
Substrate	Isoenzyme... / pI...	I 9.8	II 8.7	III 7.9	IV 6.4	V 5.7	VI 4.9
3,4-Dichloronitrobenzene		0.015	ND†	0.22	0.14	0.03	ND
p-Nitrobenzyl chloride		ND	0.34	1.20	0.70	0.72	0.47
1,2-Epoxy-3-(*p*-nitrophenoxy)propane		0.11	0.38	0.40	1.20	0.77	0.85
Bromosulphophthalein		0.01	0.04	ND	1.00	0.10	0.05
Ethacrynic acid		0.17	0.98	0.16	ND	0.97	0.41
B[*a*]P-4,5-oxide		0.06	0.04	0.07	0.06	0.14	0.05
B[*a*]P-7,8-oxide		0.015	0.008	0.017	0.014	0.032	0.012
Cumene hydroperoxide*		1.35	0.15	ND	ND	ND	ND

* GSH peroxidase II activity was determined with cumene hydroperoxide as substrate.
† ND, not detected.

highest activity towards CDNB. GST III (pI 7.9) of mouse lung had highest activity towards *p*-nitrobenzyl chloride and 3,4-dichloronitrobenzene among all the isoenzymes of mouse lung. GST IV (pI 6.4) expressed highest activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane and bromosulphophthalein. Only GST I (pI 9.8) and GST II (pI 8.7) expressed GSH peroxidase II activity measured with cumene hydroperoxide as substrate. All the isoenzymes of mouse lung GST were found to be active towards the two B[*a*]P epoxides used as substrates during the present study. Among all the isoenzymes, GST V (pI 5.7) had the highest specific activity towards these epoxides (Table 2). Specific activities of these isoenzymes towards both the B[*a*]P epoxides used in this study were in the order V > III > I ⩾ IV > VI > II. The GST isoenzymes II and III have comparatively lower specific activities towards B[*a*]P-7,8-oxide as compared with B[*a*]P-4,5-oxide.

Binding of B[*a*]P metabolites to GST isoenzymes

Binding of B[*a*]P metabolites to GST isoenzymes of mouse lung was studied as described in the Materials and methods section and the results are presented in Table 3. Approx. 50% of the radioactivity was lost during dialysis of the incubation mixture. About 43000 c.p.m. was present in the supernatant, of which about 15000 c.p.m. was associated with the affinity-purified mixture of lung GSTs. When affinity-purified GST was subjected to Sephadex G-100 gel filtration, a single coincident peak of protein content and radioactivity corresponding to M_r 50000 was obtained (results not shown), indicating that B[*a*]P or B[*a*]P metabolite(s) were specifically bound to GST. The different isoenzymes of mouse lung GST were separated by subjecting the mixture of affinity-purified GST to isoelectric focusing (Fig. 2), and the amount of radioactivity associated with each of the six peaks was

Table 3. Binding of B[*a*]P to GST of mouse lung

Enzyme activity was determined with CDNB as substrate. Experimental details are given in the text. Abbreviation: ND, not detected.

Fractions obtained from 0.5 g of lung tissue	Radioactivity (c.p.m.)
Whole homogenate	300000
Homogenate after incubation and dialysis	151000
10000 *g* supernatant	43100
10000 *g* pellet	107760
Total GST obtained by affinity chromatography*	15000
Isoelectric focusing†	
GST I (pI 9.8)	ND
GST II (pI 8.7)	7200
GST III (pI 7.9)	5000
GST IV (pI 6.4)	ND
GST V (pI 5.7)	ND
GST VI (pI 4.9)	ND

* The yield of enzyme activity during the affinity step was about 50%. During affinity chromatography the recovery of total radioactivity in the absorbed and unabsorbed fractions was 94%.

† Total radioactivity of material subjected to isoelectric focusing was 14000 c.p.m.

estimated (Table 3). The radioactivity was found to be associated only with the enzyme-activity peak at pH 8.7 and 7.9, indicating that only mouse lung GST II and III bind B[*a*]P or its metabolites. When purified mouse lung GST II or III (3 nmol) was incubated with 30 nmol of B[*a*]P (specific radioactivity 10000 c.p.m./nmol) under conditions similar to those used for lung homogenate, after dialysis of the incubation mixture against buffer A (2 litres; two changes), no radioactivity was found to be associated with GST. This suggests that B[*a*]P needs activation by microsomal enzymes (Miller & Miller, 1969) before binding with GST. When GST II and GST III preparations having bound radioactive metabolites of B[*a*]P were subjected to dialysis against 1% SDS (1000 vol., eight changes over 72 h), the dialysed enzymes retained more than 30% of the radioactivity, indicating tight binding between GST and B[*a*]P metabolites. The precise nature of this binding remains to be determined.

Induction of mouse lung GST by BHA

As described in the Materials and methods section, the effect of BHA on mouse lung GST was studied. In time-course studies the maximum level of induction of lung GST was achieved in 8 days (results not presented). Therefore these studies were performed on mice after 8 days of BHA feeding. During three separate experiments in which the female CD-1 mice were given 0.76% (w/w) BHA mixed in their diet for 8 days, a 1.6 ± 0.13-fold increase in the total GST activity was observed in the lungs of BHA-treated mice over that of the controls. This finding is somewhat similar to that reported by Benson *et al.* (1979). In order to examine the effect of BHA on individual isoenzymes, the GSTs of mouse lung were purified by affinity chromatography from control and BHA-fed mice (results not shown) and the isoenzymes were separated by isoelectric focusing as described in the Materials and methods section. From 0.75 g of lung tissue pooled separately from four control and four BHA-fed mice, about 5.8 and 9.2 units of purified GST activity and 350 μg and 600 μg of purified GST protein respectively were obtained. The overall yields of GST from control and BHA-fed mice were fairly close, but the lungs of BHA-fed mice had 1.7-fold more GST protein as

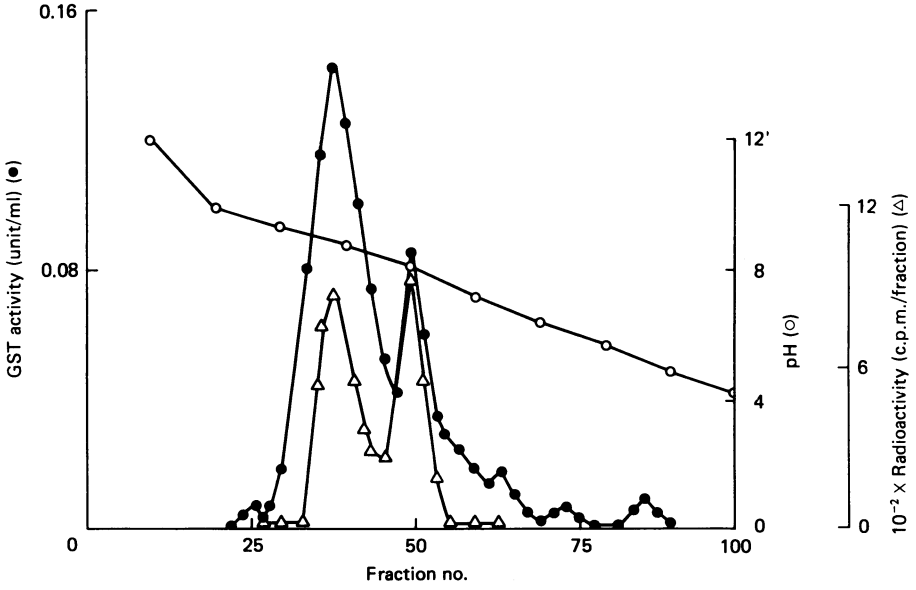

Fig. 2. Binding of B[*a*]P metabolites to isoenzymes of mouse lung GST

Experimental details are given in the text. A total of 14000 c.p.m. was present in the sample applied to the isoelectric-focusing column. Fractions (0.8 ml) were collected and monitored for pH, radioactivity and GST activity (with CDNB as substrate).

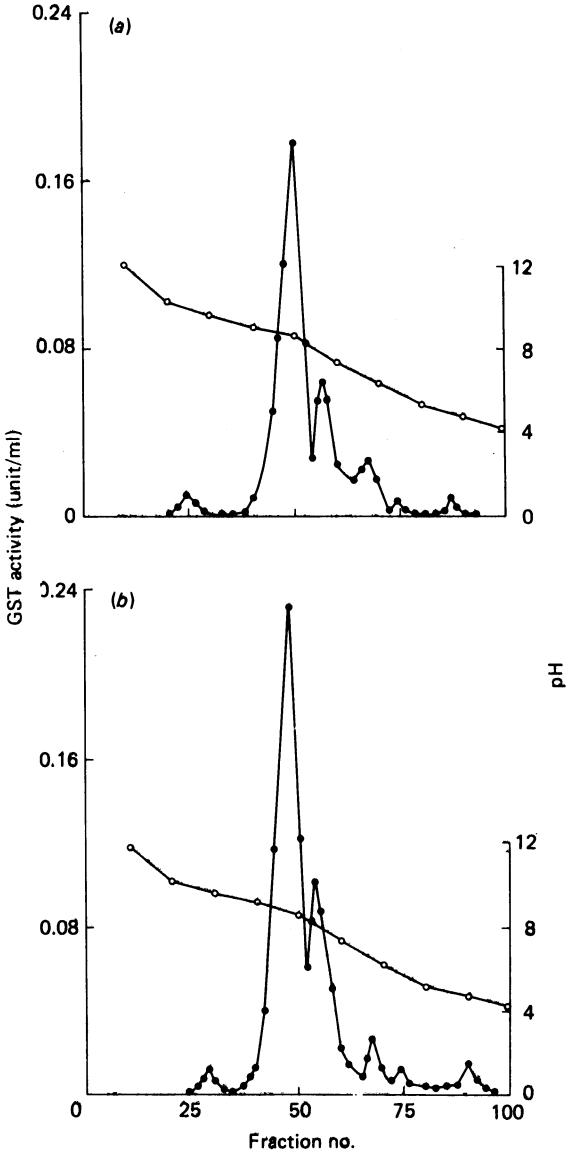

Fig. 3. Isoelectric-focusing profile of affinity-purified GST from the lungs of (*a*) control mice and (*b*) BHA-fed mice

○, pH gradient; ●, GST activity towards CDNB. Experimental details are given in the text.

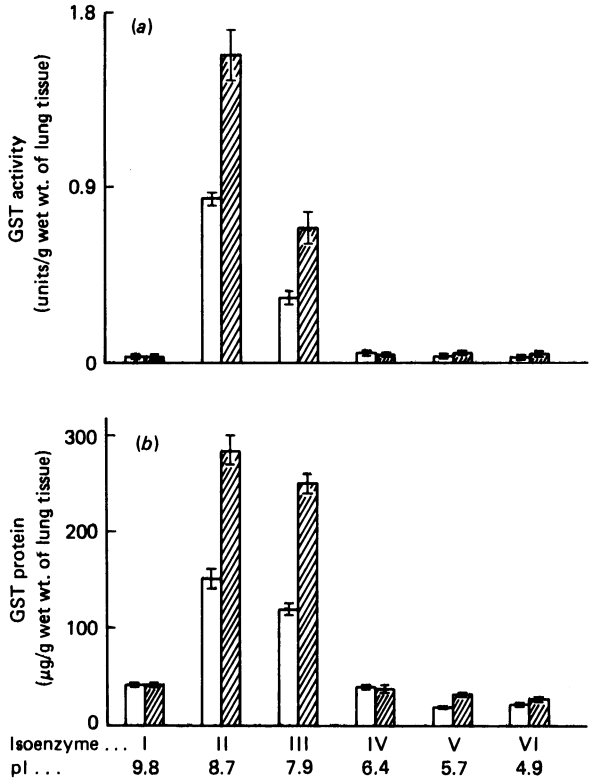

Fig. 4. Effect of BHA on GST activity (*a*) and GST protein (*b*) of individual isoenzymes of mouse lung

For experimental details, see the text. □, Control; ▨, BHA-fed.

compared with that of control lung. The isoenzyme patterns of the affinity-purified GST of control (Fig. 3*a*) and the BHA-fed mice (Fig. 3*b*) were comparable, except that some of the isoenzymes were induced in the lung of BHA-fed mice. The GST activity towards CDNB as well as the protein content of each of the isoenzymes in control and BHA-fed mouse lung were quantified and the results are presented in Fig. 4. The extent of induction for the different isoenzymes was in the order GST III > GST II > GST V > GST VI. It may be pointed out that the isoenzymes II and III account for the major portion of GST protein of lung, and these two isoenzymes also account for the major portion of increased protein due to the induction of enzyme by BHA. The possibility of the GST isoenzymes in BHA-treated mice being different from those of the controls was considered. Owing to the paucity of purified GST isoenzymes from BHA-treated mice, detailed chemical comparison of the isoenzymes from control and BHA-treated mice could not be done. However, the specific activities towards CDNB, binding characteristics towards B[*a*]P metabolites, mobilities on gels (results not shown) and pI values of the isoenzymes of BHA-treated mice were comparable with those of the corresponding isoenzymes of controls.

To examine whether the preferential induction of GST II (pI 8.7) and III (pI 7.9) by BHA resulted in an enhanced binding of B[*a*]P metabolites in the lungs of BHA-fed mice, the amounts of B[*a*]P metabolites bound by GST present in equal amounts of lung tissues of control and BHA-fed mice were quantified and compared. It was found that the amount of B[*a*]P metabolites bound to GST present in 0.5 g (wet weight) of lung tissue from BHA-fed mice was approx. 80% more than that bound by the GST present in 0.5 g of lung tissue from control mice (results not shown). Both in BHA-fed mice and in the controls, B[*a*]P metabolites were bound only by GST II and III.

DISCUSSION

The present study indicates that six GST isoenzymes of mouse lung arise from different homo- or hetero-dimeric combinations of subunits of apparently four

different sizes. Several different-sized subunits have also been described in mouse liver (Lee *et al.*, 1981; Pearson *et al.*, 1983; Hayes & Mantle, 1986; Warholm *et al.*, 1986), and it is likely that some of the subunits of mouse lung and liver GST may be similar. Lee *et al.* (1981) described four isoenzymes, designated as Fl (pI 6.5 ± 0.5), F2 (pI 8.2), F3 (pI 8.8) and F4 (pI 9.5 ± 0.5) in the liver of DBA/2J mice. Of these isoenzymes, Fl and F2 both were homodimers of M_r-22000 subunits and were identical proteins, whereas F3 was a homodimer of M_r-25000 subunits. In another study (Pearson *et al.*, 1983), two isoenzymes, GT 8.7 and GT 9.3, were isolated from liver of female CD-1 mouse and both these isoenzymes were shown to be homodimers of M_r-24000 subunits. In the liver of NMRI male mice, Warholm *et al.* (1986) have recently described three GST isoenzymes, M I (pI 9.7), M II (pI 8.7) and M III (pI 8.5), which are homodimers of M_r-25000, M_r-23000 and M_r-26500 subunits respectively. These studies suggest that M III is closely related to GT 8.7 (Pearson *et al.*, 1983) and F3 (Lee *et al.*, 1981) isoenzymes. It is possible that the mouse lung isoenzyme GST I (pI 9.8) characterized in the present study is closely related to isoenzyme M I (Warholm *et al.*, 1986) of liver, because they have similar pI values and subunit structure, and both express GSH peroxidase II activity. The pI of mouse lung GST II (8.7) is close, or similar, to that of liver isoenzymes GT 8.7 (Pearson *et al.*, 1983), F3 (Lee *et al.*, 1981), M II and M III (Warholm *et al.*, 1986), but the subunit composition of GST II (pI 8.7) of mouse lung is different from those of these isoenzymes. It may be pointed out that the electrophoretic mobility and, therefore, the estimated M_r values of polypeptide subunits of mammalian GST may vary with the concentration of cross-linker, *NN'*-methylenebisacrylamide, in the resolving gel (Hayes & Mantle, 1986), and caution must be exercised in comparing the M_r values determined in SDS/polyacrylamide gels in different laboratories. Further studies, such as amino acid sequence analyses, are therefore needed to compare the structures of these isoenzymes.

On the basis of structural and kinetic properties, other GST isoenzymes characterized in the present study, GST III (pI 7.9), GST IV (pI 6.4), GST V (pI 5.7) and GST VI (pI 4.9), could not be related to any known mouse GST isoenzyme. It is possible that some of the subunits/isoenzymes may be specific to lung, because tissue-specific expression of GST subunits has been previously suggested in rat and human tissues (Tu *et al.*, 1983; Awasthi & Singh, 1985).

Preferential induction of GST II and III may be physiologically important, because these two isoenzymes selectively bind the B[*a*]P metabolites in lung. It is possible that these two isoenzymes play an important role in the non-catalytic scavenging or 'chemical removal' (Jakoby, 1978) of these xenobiotics from lung and that their induction by BHA results in an enhanced detoxication of B[*a*]P metabolites in the lungs of BHA-treated mice. The isoenzymes II and III also have relatively high activity towards B[*a*]P-oxides. Therefore a relatively higher induction of these two enzymes by BHA should enhance not only the non-catalytic binding of B[*a*]P metabolites but also the GST-conjugating activity of lung enzymes towards B[*a*]P metabolites. Taken together, these results suggest that the anti-neoplastic activity of BHA observed against B[*a*]P-induced carcinogenesis in mouse lung may, at least in part, be due to the induction of GST. Our studies also show that the catalytic activity of isoenzymes II and III is not significantly affected by the non-catalytic binding of B[*a*]P metabolites, suggesting that these isoenzymes can simultaneously participate in the detoxication of B[*a*]P metabolites through catalytic, as well as non-catalytic, mechanisms.

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