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DIFFERENCES IN THE OCCURRENCE OF GLUTATHIONE TRANSFERASE ISOENZYMES IN RAT LUNG AND LIVER

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Cytosolic GSH transferases have been purified from rat lung by affinity chromatography followed by chromatofocusing. On the criteria of order of elution, substrate specificity, apparent subunit M_r, sensitivity to inhibitors, and reaction with antibodies, transferase subunits equivalent to subunits 2, 3, and 4, in the binary combinations occurring in liver, were identified. However, subunit 1 (and therefore transferases 1-1 and 1-2) was not detected. The most conspicuous difference is the presence in lung of a new form, eluting at pH 8.7, which is not detected in rat liver. This isoenzyme (transferase "pH 8.7") is characterized by its low apparent subunit M_r and high efficiency in the conjugation of glutathione with anti-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide, considered the ultimate carcinogen of benzo(a)-pyrene.

Glutathione transferases (EC 2.5.1.18) are important detoxication enzymes which catalyze the conjugation of GSH with a variety of electrophilic compounds (1-3). These enzymes have been most extensively characterized in the rat liver, but have been detected in many other species and tissues (2-4). A tissue of particular interest is the lung, a major site of exposure to xenobiotics and capable of metabolizing these compounds to reactive electrophilic species (5). In view of the difference in susceptibility of lung and liver to carcinogens such as polycyclic hydrocarbons, and of the possible role of GSH transferases in the protection against carcinogens, it is of

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Abbreviations: GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; BPDE, (±)anti-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide; DTE, dithioerythritol; SDS, sodium dodecylsulfate.

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interest to compare the GSH transferases in these two tissues. Further, as multiple forms of GSH transferase are known (2-4; see ref. 6 for current nomenclature of the rat liver enzymes), it is of particular importance to identify the isoenzymes present.

Even though several forms of GSH transferase have been separated from rat lung (7-9), the identity of the different isoenzymes has not been clearly established. The present report describes the purification of cytosolic GSH transferases from rat lung by use of affinity chromatography on S-hexylglutathione Sepharose 6B, followed by separation of the isoenzymes using chromatofocusing, and their comparison with hepatic forms on the basis of several identifying criteria.

MATERIALS AND METHODS

Δ^5-Androstene-3,17-dione was a generous gift of Dr. P. Talalay, The Johns Hopkins University School of Medicine, Baltimore, MD, USA; BPDE of the Cancer Research Program of the National Cancer Institute, Bethesda, MD, USA. S-Hexylglutathione was synthesized and coupled to epoxy-activated Sepharose 6B to give an affinity matrix (10). Chromatography materials were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Enzymatic activities with various substrates were determined as described (11-14). Inhibition studies were performed as described (15). SDS-Polyacrylamide gel electrophoresis was done essentially as described by Laemmli (16). Immunological reactivity was tested by Ouchterlony double diffusion. Protein concentrations were measured by the method of Lowry et al. (17) and Kalckar (18).

Purification of GSH transferases from rat lung. Untreated male Sprague-Dawley rats (220-250 g) were killed by decapitation. The lungs were excised and homogenized (20 %, w/v) in cold 0.25 M sucrose, containing 1 mM EDTA, with an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen, FRG) followed by 5 strokes in a teflon/glass homogenizer. The homogenate was centrifuged at 18,000 x g for 20 min and the resulting supernatant fraction at 105,000 x g for 60 min. The microsome-free supernatant fraction was passed through a Sephadex G-25 column equilibrated with 10 mM Tris/HCl, 0.2 mM DTE (pH 7.8) and applied to a column of the affinity matrix packed in the same buffer. The column was rinsed with 0.2 M NaCl in the Tris buffer, and the GSH transferases were eluted with 5 mM S-hexylglutathione in the salt-fortified buffer. The transferase-containing effluent was collected, desalted on a column of Sephadex G-25 equilibrated with 25 mM triethanolamine/acetic acid (pH 8.0), and concentrated by ultrafiltration. The isoenzymes of GSH transferase were separated with the Fast Protein Liquid Chromatography system (Pharmacia). The sample was applied to a chromatofocusing column (Mono P HR 5/20) equilibrated with 25 mM triethanolamine/acetic acid (pH 8.0). A pH gradient was developed by elution with a mixture of 3 ml Polybuffer 96 and 7 ml Polybuffer 74 diluted with water and adjusted to pH 5.0 with acetic acid to give a final volume of 200 ml. The activity not retained on the Mono P column was collected and concentrated by ultrafiltration. After adjustment to pH 9 with triethylamine base, the sample was applied to a Mono P column equilibrated with 25 mM triethylamine/HCl (pH 10.5). The sample was eluted in two steps. First, with Pharmalyte (pH 8-10.5) diluted 1:170 with water. Second, by a mixture of 0.75

ml Pharmalyte (pH 8-10.5) plus 3.75 ml Polybuffer 96 diluted with water and adjusted to pH 7.5 with HCl to give a final volume of 150 ml.

The isoenzyme in peak IV (Fig. 1) was further purified by cation-exchange chromatography. The sample was passed through a column of Sephadex G-50 packed in 5 mM sodium phosphate, 0.2 mM DTE (pH 5.8) and then applied to a Mono S HR 5/5 column equilibrated with the same buffer. Elution was done with a linear gradient of 0-0.1 M NaCl in the same buffer. All solutions and samples applied to the Mono P and Mono S columns were first passed through 0.22 µm pore size filters. GSH transferase activity was monitored with CDNB as electrophilic substrate throughout the purification.

RESULTS AND DISCUSSION

The initial steps in the purification of GSH transferases from rat lung cytosol are summarized in Table 1. Approx. 80 % of the GSH transferase activity with the substrate CDNB was retained on the S-hexylglutathione affinity column. This percentage of total activity bound to the affinity gel is less than that found with rat liver cytosol (≥95 %). At least one hepatic GSH transferase (form 5-5) is known to be poorly adsorbed to the affinity gel (19). This form is characterized by its high activity with the substrate 1,2-epoxy-3-(p-nitrophenoxy)propane. In the present study approx. 80 % of the cytosolic activity with this substrate was not adsorbed to the affinity gel, indicating the possible presence of GSH transferase 5-5.

The various isoenzymes of GSH transferase were subsequently resolved on a chromatofocusing column. When applied to the Mono P column at pH 8.0, approx. 10 % of the activity was retained. This value is higher than the 5 %

Table 1. Purification of GSH transferases from rat lung

Fraction	Volume (ml)	Total activity[a] (µmol/min)	Specific activity (µmol/min/mg)[b]	Yield (%)
Supernatant	210	291	0.13	(100)
Sephadex G-25	330	268	0.11	92
S-Hexylglutathione Sepharose 6B + Sephadex G-25	85	226	16.4	78
Pass + wash of affinity matrix	1020	49	NT	17

[a]Activity with CDNB. [b]Protein concentrations calculated from absorbance at 260 and 280 nm (18); NT = not determined.

found for rat liver preparations (20) but much lower than the 50 % detected with rat testis (21). The activity adsorbed at pH 8.0 was resolved into four peaks after chromatofocusing in the pH interval 8-5 (results not shown). These isoenzymes with acidic or near-neutral isoelectric points have not yet been fully characterized. However, the presence of an isoenzyme eluting at pH 6.3 is noteworthy. This form is characterized by its relatively high activity with ethacrynic acid. A similar isoenzyme has also been detected in rat testis and in rat kidney (Guthenberg et al., unpublished results).

The activity not retained at pH 8.0 was reapplied to the chromatofocusing column at pH 10.5. A pH gradient of 10.5 to 7.5 resolved six peaks of activity towards CDNB (Fig. 1). Less than 3 % of the applied activity was released after further elution with 2 M NaCl and the total recovery of activity was 87 %. The isolated GSH transferases were characterized by the pH of elution, substrate specificity (Table 2), precipitation with specific antibodies, sensivity to inhibitors, and apparent subunit M_r, in order to allow possible identification with the isoenzymes earlier isolated from rat liver (3,15,20,22).

The pattern of basic GSH transferases shows obvious similarities to that in rat liver. The enzyme in peaks I and II has properties characteristic

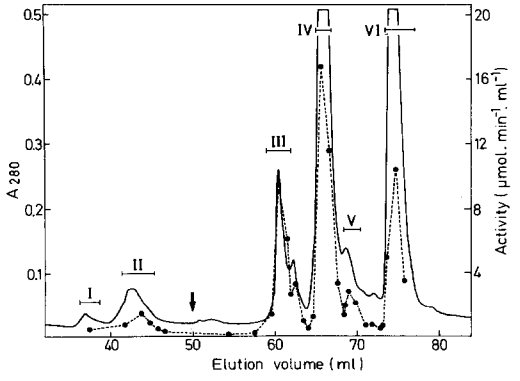

Fig. 1. Separation of basic cytosolic GSH transferases from rat lung by chromatofocusing. The absorbance profile was recorded at 280 nm (———) and activity determined with CDNB as substrate (●---●). The bars indicate the fractions pooled and retained for further characterization. The arrow indicates the change in elution buffer (see Materials and Methods).

Table 2. Relative substrate specificities of basic GSH transferases from rat lung[a]

Substrate	Peak (pH of elution from Mono P column)					
	I (9.25)	II (9.2)	III (8.95)	IV (8.7)	V (8.6)	VI (8.3)
CDNB	100 (22)[b]	100 (35)	100 (72)	100 (21)	100 (36)	100 (16)
1,2-Dichloro-4-nitrobenzene	ND	0.3	9.2	1.5	6.8	0.3
trans-4-Phenyl-3-buten-2-one	NT	NT	0.2	0.4	0.7	10.1
Cumene hydroperoxide	38.4	38.4	1.3	0.8	0.1	7.3
Δ^5-Androstene-3,17-dione	0.8	ND	0.1	NT	NT	0.2
BPDE	ND	ND	0.03	2.3 (9.5)[c]	1.7	2.6

[a]Values are given as percentage of the specific activity determined with CDNB as substrate for which the absolute values are given in parentheses as $\mu mol \cdot min^{-1} \cdot mg^{-1}$.
ND = no detectable activity under the assay conditions used; NT = not tested.
[b]Protein concentration in peak I calculated from absorbance at 280 and 260 nm (18).
[c]After further purification by ion-exchange chromatography.

of isoenzyme 2-2. Low activity was found with 1,2-dichloro-4-nitrobenzene and relatively high activity with cumene hydroperoxide. The concentrations of the inhibitors triethyltin bromide and bromosulfophthalein giving 50 % inhibition of the activity with CDNB and the apparent subunit M_r were very similar to that of form 2-2 (see ref. 3), and precipitin lines occurred with antibodies to form 2-2, but not to form 1-1, in Ouchterlony immunodiffusion assays. However, the activities with Δ^5-androstene-3,17-dione and BPDE were lower than those obtained with transferase 2-2 isolated from rat liver (3,23). Although the relationship between peaks I and II is unclear, we have earlier observed the occurrence of more than one peak having properties of form 2-2 in some preparations from rat liver. In contrast to rat liver, forms 1-1 and 1-2 are absent from rat lung, (and rat testis, ref. 21).

The isoenzymes eluted in peaks III and VI are by similar criteria clearly identified as isoenzymes 3-3 and 4-4, respectively (see ref. 3). The heterodimer 3-4, which is readily isolated from rat liver (22), cannot be unambiguously identified in rat lung. Peak IV eluted at the pH expected for transferase 3-4, contained small amounts of a protein with an apparent subunit M_r of 26,500, and precipitin lines occurred with antibodies to both

transferases 3-3 and 4-4 in immunodiffusion assays. As peak IV contained an additional isoenzyme (see below), accurate characterization of the minor component on the basis of substrate specificity and sensitivity to inhibitors was not possible.

A form present in peak V, is similar to a less well characterized isoenzyme eluting immediately after transferase 3-4 in experiments with hepatic cytosol (20). This form is immunoprecipitated by antibodies to form 3-3 and has a substrate specificity similar to form 3-3.

The most conspicuous feature of the isoenzyme pattern in rat lung is the presence of a major form eluting in peak IV at pH 8.7. This isoenzyme has a lower apparent subunit M_r (approx. 24,000) than those of the previously identified hepatic GSH transferases. This isoenzyme has not been demonstrated in normal rat liver, but available data suggest that an identical protein is present in rat kidney (Guthenberg et al., unpublished results).

Of particular interest is the high activity of the isoenzymes in peak fractions IV, V and VI (Table 2) in catalyzing the conjugation of GSH with BPDE, generally considered as the ultimate carcinogen of benzo(a)pyrene (24). In studies with the hepatic GSH transferases, highest activity was found to be associated with subunit 4 and the lowest with subunit 3 (23). Similar results were obtained here with transferases 3-3 and 4-4 isolated from rat lung. However, high activity was also found with the GSH transferases present in peaks IV and V, neither of which have been assayed previously with BPDE as substrate. After further purification by ion-exchange chromatography, the major isoenzyme present in peak IV gave a relative activity with BPDE of 9.5 % of the CDNB activity, 3.6 times greater than that obtained with transferase 4-4 (peak VI).

In conclusion, the isoenzyme patterns in rat lung and liver have been shown to differ in that GSH transferases 1-1 and 1-2 are not detected in the lung. Another major difference is that the rat lung contains an isoenzyme not found in the liver. This form (transferase "pH 8.7") is distinguished by its low apparent subunit M_r and its high activity with the substrate BPDE.

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