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PURIFICATION OF GLUTATHIONE S-TRANSFERASES FROM RAT LUNG BY AFFINITY CHROMATOGRAPHY. EVIDENCE FOR AN ENZYME FORM ABSENT IN RAT LIVER

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SUMMARY: Glutathione S-transferases from rat lung cytosol were purified about 200-fold in one step by chromatography on S-hexylglutathione bound to epoxy-activated Sepharose 6B. Further purification on hydroxyapatite resolved the lung transferases into five peaks of activity as measured with 1-chloro-2,4-dinitrobenzene as substrate. Three of the peaks were identified with transferases A, B, and C of rat liver on the basis of chromatographic properties, immunochemical reactivity, and substrate specificity. The other two activity peaks were not detectable in liver; one originated from the lung tissue and one appeared to result from blood in the lung.

The glutathione S-transferases are important detoxification enzymes which are widely distributed in animal species (1). These enzymes catalyze the conjugation of glutathione (GSH) with a variety of electrophilic compounds. In mammals GSH S-transferases have been demonstrated in the cytosol of cells from various organs (1,2) and several enzymes have been highly purified from liver (3-6). The liver is exposed to many toxic substances, as are the kidney, intestine, and lung. The existence of GSH S-transferase activity in these organs is well documented, but the occurrence of multiple forms of enzyme has been well established only for the liver. In view of the differences in susceptibility of lung and liver to the effects of carcinogens such as polycyclic hydrocarbons, and of the possible role of GSH S-transferases in protection of cells against some carcinogens, it was of interest to characterize the activity in lung tissue and compare it with that of the liver.

The present report describes a partial purification of GSH S-transferases from rat lung and rat liver by use of affinity chromatography on S-hexylglutathione Sepharose 6B and a comparison of the activity profiles of lung and liver GSH S-transferases obtained after chromatography on hydroxyapatite. Some of the results have been reported earlier (7).

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MATERIALS AND METHODS

S-Hexylglutathione was synthesized by method A of Vince et al. (8). The affinity gel, previously used for glyoxalase I (9), was made by coupling the amino group of the glutathione derivative via a spacer to an agarose matrix; epoxy-activated Sepharose 6B (Pharmacia) was used to immobilize the ligand (cf. ref. 10). The sources of other chemicals and materials used in the present investigation have been reported previously (3,4). Enzyme activities with various substrates were determined as earlier described (3,4). Protein concentration was calculated from the absorbance at 260 and 280 nm (11) or measured by the method of Bradford (12). Antisera were obtained from rabbits immunized with purified GSH S-transferases A, B, and C.

Purification of GSH S-transferases. Enzymes were prepared from rat lungs and from rat livers by the same procedure. Male specific-pathogen-free Sprague-Dawley rats were killed by decapitation and the lungs and livers were excised. For one preparation the blood was removed from the lungs in situ by means of perfusion; 0.9 % NaCl was injected through the right ventricle of the heart. Supernatant fractions were prepared as described earlier (4). They were passed through a suitable bed (size: 5 times the volume of the sample) of Sephadex G-25 equilibrated with 10 mM Tris/HCl (pH 7.8) and then applied to a column (2 cm x 15 cm) containing the affinity matrix, which had been equilibrated with the same buffer. The amount of liver supernatant used was less than the amount of lung supernatant in order to obtain transferase activities of similar magnitudes from the two sources. After sample application the matrix was washed with the same buffer fortified with 0.2 M NaCl until no protein was eluted from the column. The GSH S-transferases were eluted with 5 mM S-hexylglutathione dissolved in 10 mM Tris/HCl buffer (pH 7.8) containing 0.2 M NaCl. The active fractions were desalted on a column of Sephadex G-25 packed in 10 mM sodium phosphate (pH 6.8) containing 1 mM EDTA and subsequently applied to a column (2 cm x 9 cm) of hydroxyapatite equilibrated with the same buffer. After washing the column with 3 vol. of the starting buffer the GSH S-transferases were eluted by use of a linear concentration gradient (10-350 mM; total volume 320 ml) of potassium phosphate (pH 6.8).

RESULTS

Cytosol fractions obtained from homogenized lungs and livers of male Sprague-Dawley rats were chromatographed on S-hexylglutathione coupled to Sepharose 6B. The results are summarized in Table 1. The specific elution of the GSH S-transferase activity achieved with S-hexylglutathione, which has been shown to be an inhibitor of two of the liver enzymes (4), indicates that the chromatography is biospecific. The specific activity of the enzymes is about 20-fold lower in lung than in liver cytosol and the purification factor after affinity chromatography was consequently much higher for the enzymes from lung than for those from liver (Table 1). In fact, the specific activities obtained after affinity chromatography are within 50 % of the maximal activities obtained for homogeneous enzymes.

Table 1

Purification by affinity chromatography of GSH S-transferases from rat lung and liver

Fraction	Volume (ml)	Total activity[a] (μmol/min)	Specific activity (μmol/min per mg protein)	Yield (%)
Lung supernatant	325	185	0.051	(100)
Sephadex G-25	453	172	0.067	93
S-Hexylglutathione Sepharose 6B + Sephadex G-25	72	125	13.8	68
Liver supernatant	35	1100	1.04	(100)
Sephadex G-25	54	1040	0.97	95
S-Hexylglutathione Sepharose 6B + Sephadex G-25	81	823	26.7	75

[a]Activity measured at 30°C and pH 6.5 using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH as substrates.

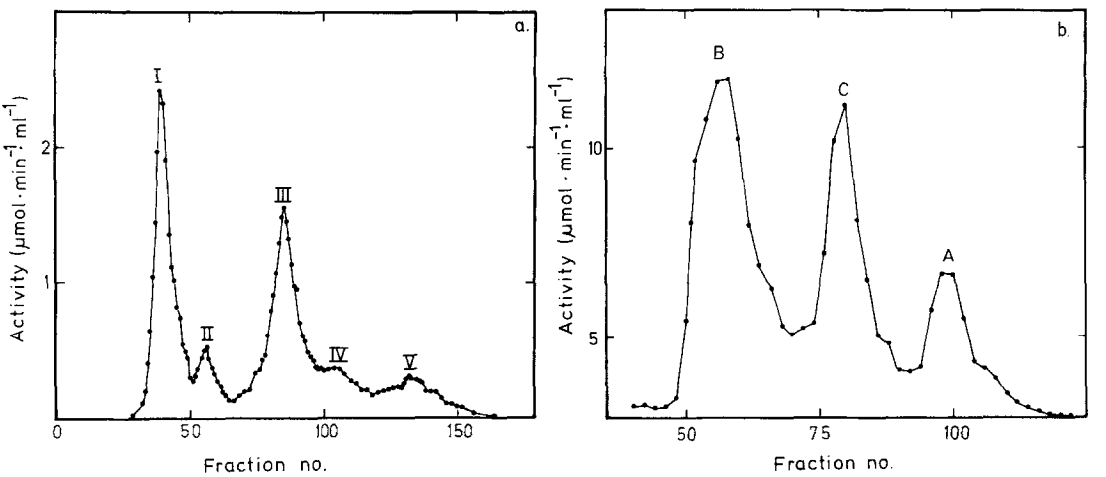

Fig. 1. Separation of multiple forms of GSH S-transferase on hydroxyapatite. The elution was made with a linear gradient of potassium phosphate (pH 6.8). The enzymatic activity was assayed with 1-chloro-2,4-dinitrobenzene and GSH. The samples applied had been purified by affinity chromatography (see Table 1): (a) lung, (b) liver.

The enzymes were further purified and the multiple forms separated by chromatography on hydroxyapatite. The material from unperfused lungs showed five peaks of activity (Fig. 1a) and that from liver three peaks after elution (Fig. 1b). The liver enzymes were GSH S-transferases B, C, and A, respectively, as verified by precipitin formation with antibodies (Ouchterlony diffusion) and by their substrate specificities (assayed with 3,4-dichloro-1-nitrobenzene, 1-chloro-2,4-dinitrobenzene, and trans-4-phenyl-3-buten-2-one). (Note that the order of elution of transferases B and C is reversed as compared with the order of elution from CM-cellulose (3)). The elution profile for the lung enzymes was reproduced several times and always showed five peaks of activity. However, after perfusion of the lungs only a trace of the second peak remained, whereas the other four activity peaks remained unchanged. We therefore conclude that the second peak is due to an enzyme in blood, which is different from those present in lung (or liver) tissue. A distinct form of GSH S-transferase has previously been demonstrated in human erythrocytes (13). The specific activities of

the peak fractions I to V of the enzymes obtained from rat lung (Fig. 1a) were, respectively, 24, 11, 18, 22, and 25 μmol/min per mg protein (as assayed with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH at pH 6.5 and 30°C). The corresponding specific activities of the liver transferases B, C, and A (Fig. 1b) were: 25, 30, and 31 μmol/min per mg protein.

An attempt was made to identify the transferases obtained from lung with those of liver by immunological techniques and by comparison of activities versus different substrates. Peaks I and II gave no precipitates with any of the antibodies used, whereas peak III gave a precipitin line with anti-transferase B antibodies. Peaks IV and V both reacted with antibodies raised against transferase A and transferase C. We have confirmed with the purified liver transferases the earlier report (3) that forms A and C cross-react immunologically and the distinction between the two forms must accordingly be based on other criteria. The order of elution from the hydroxyapatite column indicated that peak IV of the lung preparation should be the same as form C of liver and that peak V should be the same as form A. This assignment was corroborated by the relative activities obtained with various substrates. As expected for forms A and C (3), both peaks were active with 3,4-dichloro-1-nitrobenzene, but showed an approximately 10-fold lower specific activity than with 1-chloro-2,4-dinitrobenzene. Form A could be distinguished from form C by the use of trans-4-phenyl-3-buten-2-one, because form C is 20-fold more active than form A with this substrate (3). Peak IV gave about 10-fold higher activity with this substrate than did peak V, thus confirming the earlier assignment. The substrate specificity of peak III further supported the identification of this peak with transferase B. Peaks I and II, like peak III, were similar to transferase B in giving an activity with 1-chloro-2,4-dinitrobenzene, which was about three orders of magnitude higher than that obtained with 3,4-dichloro-1-nitrobenzene as substrate (3).

DISCUSSION

The use of affinity chromatography on immobilized S-hexylglutathione for the purification of GSH S-transferases from lung tissue gave about 200-fold purification of the enzymes in one step. The purification factor for the liver enzymes was lower, because the enzymes are about 20-fold more abundant in liver than in lung (cf. ref. 14) and represent 5-10 % of the soluble proteins of the liver cytosol (1). Other affinity adsorbents which we have tested were less suitable than that based on S-hexylglutathione. For example, use of the conjugate of bromosulfophthalein and GSH as a ligand gave a very tight binding of the transferases, which caused difficulties in the subsequent elution of the enzymes from the adsorbent. The ligand S-hexylglutathione, on the other hand, provided a good compromise between good binding and elution properties. We conclude that affinity chromatography is very useful for the purification of GSH S-transferases, especially from sources in which they are less abundant than in liver.

The results of the purification show the presence in rat lung of the GSH S-transferases A, B, and C, previously purified from rat liver (3,4). Under identical conditions, two activity peaks from the lung preparation were found, which could not be detected in the liver preparation. The first peak originated from the lung tissue, whereas the second peak appeared to represent a GSH S-transferase of the blood (cf. ref. 13). Both these activities had substrate specificities similar to that of liver transferase B, but they were clearly different enzymes judging from their lack of immunological reactivity with antibodies directed towards transferase B. Inhibition experiments with tributyltin acetate (cf. ref. 15), which showed that peaks I and II are significantly less sensitive than transferases A, B, and C, also support the conclusion that peaks I and II represent new enzymes. Accordingly, the lung contains at least one transferase, which is not present in liver. The biological roles of the different forms of the transferases and their importance for the biotransformation of xenobiotics remain to be established.

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