Studies of endogenous inhibitors of microsomal glutathione *S*-transferase

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Glutathione *S*-transferase is present in rat liver microsomal fraction, but its activity is low relative to the transferase activity present in the soluble fraction of the hepatocyte. We have found, however, that the activity of microsomal glutathione *S*-transferase is increased 5-fold after treatment with small unilamellar vesicles made from phosphatidylcholine. The increase in activity is due to the removal of an inhibitor of the enzyme from the microsomal membrane. The inhibitor is present in the organic layer of a washed Folch extract of the microsomal fraction. When this fraction of the microsomal extract is reconstituted in the form of small unilamellar vesicles, it inhibits microsomal glutathione *S*-transferase that had been activated by prior treatment with small unilamellar vesicles of pure phosphatidylcholine, but does not affect the activity of unactivated microsomal glutathione *S*-transferase. The inhibitor did not seem to be formed during the isolation of the microsomal fraction, and hence may be a physiological regulator of microsomal glutathione *S*-transferase. In this regard, both free fatty acid (palmitate) and lysophosphatidylcholine were shown to inhibit the enzyme reversibly. The results indicate that the activity of microsomal glutathione *S*-transferase is far greater than appreciated until now, and that this form of the enzyme may be an important factor in the hepatic metabolism of toxic electrophiles.

The glutathione *S*-transferases (EC 2.5.1.18) are a family of enzymes that catalyse reactions between GSH and electrophiles (Jakoby, 1978). Several forms of these enzymes are present in the soluble portion of the liver cell. Recently, two groups of investigators have found glutathione *S*-transferase activity in the microsomal fraction (referred to below simply as 'microsomes') of the liver cell. The enzyme in this location is attached firmly to the microsomal membrane, and its properties are different from those of the soluble forms (Friedberg *et al.*, 1979; Morgenstern *et al.*, 1979, 1980). Since the activity of the microsomal enzyme is low compared with the activities of the soluble enzymes (Morgenstern *et al.*, 1979), little physiological importance has been attributed to the former enzyme. Of interest, however, are observations made by Morgenstern *et al.* (1979, 1980), who have shown that microsomal glutathione *S*-transferase can be activated 8-fold by treatment with *N*-ethylmaleimide. The results reported in the present paper reveal another mechanism for increasing the activity of microsomal glutathione *S*-transferase. This enzyme can be activated

5-fold by treatment with small unilamellar vesicles (referred to below simply as 'vesicles') made from phosphatidylcholine. This activation is due to removal from microsomes of an inhibitor of the enzyme. The putative inhibitor appears to be present *in vivo*. In addition, the activity of the microsomal enzyme is shown to be susceptible to modulation by a variety of physiological substances.

Materials and methods

Materials

1-Chloro-2,4-dinitrobenzene was purchased from BDH Chemicals, Poole, Dorset, U.K. Glucose 6-phosphate dehydrogenase, *Naja naja* venom, GSH, egg phosphatidylcholine, dioleoyl phosphatidylcholine, dimyristoyl phosphatidylcholine, *N*-ethylmaleimide and bovine serum albumin (fatty acid-free) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Phosphatidyl[*N*-Me-¹⁴C]choline was purchased from Amersham Corp., Arlington Heights, IL, U.S.A. Bio-Beads SM-2 were purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A. Chloroform and methanol were redistilled before use.

Abbreviation used: GSH, reduced glutathione.

Preparation of microsomes and 100000g supernatant

Fed male Sprague-Dawley rats, 250–300g body wt. (Simonsen Laboratories, Gilroy, CA, U.S.A.), were killed by a blow to the head. The livers were removed rapidly and placed in chilled 0.25 M-sucrose. All subsequent steps were performed at 0–4°C. The microsomes were isolated as described previously (Zakim & Vessey, 1973) either in 0.25 M-sucrose or in 0.25 M-sucrose/10 mM-Tris/HCl buffer, pH 8.0, containing 2–5 mM-EDTA. The post-microsomal supernatant, containing the soluble forms of glutathione *S*-transferase, was collected (100000g supernatant) and stored at –80°C until used. Washed microsomes were stored in 0.25 M-sucrose at a final protein concentration of 30–40 mg/ml. Microsomes either were used fresh (day of preparation) or were frozen at –80°C. Frozen microsomes were used within 30 days of preparation. Studies with fresh or frozen microsomes gave similar results. Fresh microsomes always were used when studies were performed with *N*-ethylmaleimide (Morgenstern *et al.*, 1979). Contaminating cytosolic proteins were removed from microsomes by washing with 0.1 M-Tris/HCl buffer, pH 8. This treatment was sufficient to remove all soluble glutathione *S*-transferase. Microsomes washed with 0.1 M-Tris/HCl buffer were used within 24 h of preparation.

Preparation of lipid vesicles

Vesicles were prepared from the specified phospholipids (Boyer *et al.*, 1980) and used only at temperatures above their phase-transition temperatures (Szoka & Papahadjopoulos, 1980). When vesicles were made from a chloroform/methanol extract of microsomes (Folch *et al.*, 1957), 0.001% butylated hydroxytoluene was added to the solvents to minimize lipid peroxidation. The concentration of phospholipids was determined gravimetrically, or by measuring inorganic phosphorus (Duck-Chong, 1979).

Enzyme assays

Glutathione *S*-transferase activity was measured with 1-chloro-2,4-dinitrobenzene and GSH in 0.1 M-potassium phosphate buffer, pH 6.5. Assays were performed at 25°C by monitoring the appearance of product *S*-(2,4-dinitrophenyl)glutathione at 340 nm (Habig *et al.*, 1974). Non-enzymic rates of synthesis of *S*-(2,4-dinitrophenyl)glutathione were determined for each concentration of substrates, and were subtracted from the rates measured in the presence of enzyme. Preliminary investigations demonstrated that the relative activation of microsomal glutathione *S*-transferase by vesicles was the same irrespective of the concentrations of 1-chloro-2,4-dinitrobenzene (0.1–1 mM) or GSH (0.2–5 mM)

present in the assay mixture. Most of the assays were performed at 0.3 mM-1-chloro-2,4-dinitrobenzene and 5 mM-GSH (standard assay conditions). This concentration of 1-chloro-2,4-dinitrobenzene was 60-fold greater than the reported apparent K_m of 5 μ M for microsomal glutathione *S*-transferase (Morgenstern *et al.*, 1979). The latter was selected because it is near the physiological concentration of GSH present in the rat liver (Beatty & Reed, 1980). Protein was measured by the biuret method (Gornall *et al.*, 1949).

Treatment of microsomes and 100000g supernatant with vesicles and N-ethylmaleimide

Microsomes or 100000g supernatant and vesicles were mixed together in an assay cuvette containing 0.1 M-phosphate buffer, pH 6.5, and kept at room temperature for the prescribed time period. Control assay mixtures containing no vesicles were kept at room temperature for identical time periods. 1-Chloro-2,4-dinitrobenzene and GSH then were added to initiate the enzyme reaction. In separate experiments, vesicles (egg phosphatidylcholine) that had been centrifuged at 120000g for 1 h were mixed with Tris/HCl-washed microsomes in 0.1 M-phosphate buffer, pH 6.5, and the mixture was kept at room temperature for 10 min. The mixture then was centrifuged at 120000g for 30 min at 4°C to sediment the microsomes. The supernatant was discarded. The microsomes were resuspended in 0.25 M-sucrose and used in a standard assay. Control microsomes were treated in an identical fashion except that vesicles were omitted. To ascertain how much of the added vesicle lipid remained with the sedimented microsomes, vesicles were prepared with phosphatidyl[¹⁴C]choline and then mixed with microsomes and centrifuged as above. In this experiment 3.0% of the vesicle lipid remained with the microsomes. The necessity of contact between vesicles and microsomes for activation to occur was tested for by placing 1.3 mg (0.5 ml) of Tris/HCl-washed microsomes within dialysis tubing (12000–14000-mol.wt. cut-off) and dialysing the microsomes against 10 ml of 0.1 M-Tris/HCl buffer, pH 8, at 4°C that either did or did not contain 50 mg of egg phosphatidylcholine in the form of vesicles. After 3 h, the microsomes were removed and activity of glutathione *S*-transferase was determined. Microsomes were treated with *N*-ethylmaleimide as previously described (Morgenstern *et al.*, 1979).

Treatment of microsomes with albumin and chromatography on Bio-Beads SM-2

Tris/HCl-washed microsomes were mixed with different concentrations of albumin in 0.1 M-Tris/HCl buffer, pH 7.4, at 25°C. The mixture was kept at room temperature for 10 min and was then

centrifuged at 120000 *g* for 30 min to sediment the microsomes. The microsomes were resuspended and washed twice with 0.1 M-Tris/HCl buffer to remove residual albumin, and finally homogenized in 0.25 M-sucrose for use in the standard assay. Control microsomes and microsomes exposed to vesicles were treated in an identical manner.

Tris/HCl-washed microsomes were chromatographed on Bio-Beads SM-2. The Bio-Beads were washed with methanol (Holloway, 1973) before being packed in the column (1 cm $\times$ 21 cm). The column was equilibrated and eluted with 0.1 M-Tris/HCl buffer, pH 8, at 4°C. A 13 mg portion of microsomal protein were loaded on to the column; fractions containing the microsomes were collected at a flow rate of 0.2–0.3 ml/min. The microsomes were recovered by centrifugation and were resuspended in 0.25 M-sucrose before use.

Lipid peroxidation and treatment with phospholipase A₂

Lipid peroxidation was generated enzymically in Tris/HCl-washed microsomes as described previously (Gibson *et al.*, 1980). The incubation was continued for 10 min at 37°C and peroxidation was monitored by measuring malondialdehyde production (Buege & Aust, 1978). After 10 min, the microsomes were recovered by centrifugation and glutathione *S*-transferase activity was measured. Control microsomes were incubated in the Tris/HCl buffer for an identical time period and were recovered by centrifugation.

Tris/HCl-washed microsomes (17 mg) were digested on ice with phospholipase A₂ prepared from *Naja naja* venom for 30 min as described previously (Zakim & Vessey, 1973). The reaction was stopped by the addition of EDTA, and microsomes were recovered and assayed for activity as described above.

Results

Activation of microsomal glutathione S-transferase by egg-phosphatidylcholine vesicles

The activity of microsomal glutathione *S*-transferase was increased by the addition to microsomes of vesicles made from egg phosphatidylcholine. Activation was not instantaneous but followed the time course shown in Fig. 1. A careful analysis of the time course revealed that a rapidly occurring activation was followed by a second, slower, process that apparently was first-order (Fig. 2). The rate of the latter depended on the amount of vesicle lipid added to the assay medium in that the first-order process was slower with smaller amounts of lipid.

The maximum obtainable activation of glutathione *S*-transferase after a single treatment with vesicles was 3.7-fold (Fig. 3). A ratio of approx.

Fig. 1. Effects of time of incubation with vesicles on the activity of microsomal glutathione *S*-transferase

Each assay mixture contained 5 mM-GSH, 0.3 mM-1-chloro-2,4-dinitrobenzene, 0.1 M-phosphate buffer, pH 6.5, and 0.173 mg of microsomal protein (Tris/HCl-washed microsomes) in a volume of 1 ml. □, No lipid present; ○, 0.66 mg of lipid (egg-phosphatidylcholine vesicles) present; △, 3.3 mg of lipid (egg-phosphatidylcholine vesicles) present. The microsomes were incubated with or without lipid in the presence of 0.1 M-phosphate buffer, pH 6.5, for the prescribed time period at 23°C. The reaction was initiated by adding 1-chloro-2,4-dinitrobenzene and GSH. Rates are μ mol of *S*-(dinitrophenyl)-glutathione formed/min per mg of microsomal protein.

18 mg of lipid vesicles/mg of microsomal protein was required for this degree of activation. The activity of glutathione *S*-transferase in microsomes treated with a single large amount of vesicles could be increased further, however, if the liposomes were removed and the microsomes treated subsequently with a fresh volume of vesicles. For example, microsomes were treated with vesicles (18 mg of vesicle lipid/mg of microsomal protein), as in the experiment reported in Fig. 3. The microsomes and vesicles then were separated by sedimenting the former in a high-speed centrifuge. About 97% of the vesicles were removed from the microsomes by centrifugation. Removal of the vesicle phospholipid did not affect the activity of the resuspended microsomes, which remained 3.8-fold greater than the activity of glutathione *S*-transferase in untreated control microsomes. Addition of vesicles of egg phosphatidylcholine to these already-vesicle-treated washed microsomes increased the activity of gluta-

Fig. 2. Time course of activation of microsomal glutathione *S*-transferase

The conditions of the assay were similar to those for Fig. 1 except that the assay mixture contained 0.126 mg of microsomal protein and 0.61 mg of lipid (egg-phosphatidylcholine vesicles). v_0 is the enzyme activity measured in the absence of liposomes, v_t is enzyme activity measured at a specified time t after the addition of liposomes, and v_∞ is the enzyme activity measured at a time when it no longer increased after addition of the liposomes, in this case 20 min.

thione *S*-transferase to a value 4.5-fold greater than that of untreated control microsomes. A third treatment with vesicles, after the microsomes had again been washed free from vesicles, stimulated glutathione *S*-transferase to 5-fold greater than control activity. This increase in activity was stable for at least 8 h, when the microsomes were maintained at 4°C. Vesicles composed of dimyristoyl or dioleoyl phosphatidylcholine were similar to egg-

Fig. 3. Effects of amount of lipid in assay on activity of microsomal glutathione *S*-transferase

The conditions of the assay were similar to those for Fig. 1, except that the assay mixture contained 0.34 mg of microsomal protein. The microsomes and vesicles were mixed together in 0.1 M-phosphate buffer, pH 6.5. After this mixture had been kept at 23°C for 10 min, the reaction was initiated by the addition of 1-chloro-2,4-dinitrobenzene and GSH. Relative activity is the enzymic activity measured for each ratio of vesicle lipid to microsomal protein divided by activity obtained in the assay without lipid (0.1 μ mol/min per mg of protein).

phosphatidylcholine vesicles in their ability to increase the activity of microsomal glutathione *S*-transferase (Fig. 4).

Direct contact between vesicles and microsomes appeared to be necessary for activation to occur. When, as described in the Materials and methods section, Tris/HCl-washed microsomes were placed inside a dialysis bag and vesicles were on the outside of the bag, the specific activity of the microsomal enzyme was unaltered after 3 h of dialysis. Removing the microsomes from the bag at the end of dialysis and mixing them with the vesicles led to prompt activation.

The activities of glutathione *S*-transferase as a function of variable concentrations of substrates were compared in microsomes treated with vesicles made from egg phosphatidylcholine and subsequently washed to remove exogenous lipid and control microsomes. Treatment with vesicles caused an increase in both the activity at $V_{\max}$ and the apparent K_m values for 1-chloro-2,4-dinitrobenzene and GSH (Table 1).

Fig. 4. Effects of different phospholipids on the activity of microsomal glutathione *S*-transferase

The conditions of the assay were similar to those for Fig. 1 except that assays were performed at 30°C. The microsomes and vesicles were mixed together; 10 min later, the reaction was initiated by the addition of 1-chloro-2,4-dinitrobenzene and GSH. The relative activities were obtained by dividing the activity at each ratio of liposomal lipid to microsomal protein by the activity obtained in the assay without liposomes (0.15 $\mu\text{mol}/\text{min}$ per mg of protein). Lipids, when present, were dioleoyl phosphatidylcholine (O) or dimyristoyl phosphatidylcholine (Δ).

Fig. 5. Effect of vesicles made from a microsomal extract on the activity of microsomal glutathione *S*-transferase

The lipid-washed microsomes were prepared by mixing 150 mg of vesicles (egg phosphatidylcholine) with 6 mg of Tris/HCl-washed microsomes in 0.1 M-phosphate buffer, pH 6.5. The mixture was kept at 23°C for 10 min. The microsomes were then sedimented by centrifugation at 4°C and the vesicles were decanted off. Microsomes were resuspended in 0.25 M-sucrose. Control microsomes were treated in an identical fashion except that vesicles were omitted. The conditions of the assay were as described for Fig. 1 except that each assay mixture contained 0.05 mg of microsomal protein. The microsomes, lipid-washed (O) or control (●), were mixed with vesicles made from the microsomal extract in a cuvette containing buffer. After the mixture had been kept at room temperature for 15 min, the reaction was initiated by the addition of 1-chloro-2,4-dinitrobenzene followed by GSH.

Table 1. Effect of vesicles on the kinetic properties of microsomal glutathione *S*-transferase

The lipid-washed microsomes were prepared by mixing 11 mg of vesicles (egg phosphatidylcholine) with 1 mg of Tris/HCl-washed microsomes in 0.1 M-phosphate buffer, pH 6.5. The mixture was kept at 23°C for 10 min. The microsomes were then sedimented by centrifugation at 4°C and the vesicles were decanted off. Microsomes were resuspended in 0.25 M-sucrose. Control microsomes were treated in an identical fashion except that the vesicles were omitted. The kinetic values were obtained from double-reciprocal plots of rate data that were obtained in experiments performed in triplicate. The apparent K_m for 1-chloro-2,4-dinitrobenzene was determined at 5 mM-GSH and the apparent K_m for GSH at 0.1 mM-1-chloro-2,4-dinitrobenzene.

	Apparent K_m (μM)		V_{max} ($\mu\text{mol}/\text{min}$ per mg)
	GSH	Chlorodinitrobenzene	
Microsomes			
Control	700	5.4	0.15
Lipid-washed	3300	15.0	0.59

Studies of the mechanism of vesicle-induced activation

The activation of microsomal glutathione *S*-transferase by vesicles could be due either to the

transfer of phospholipids from vesicles to microsomes or to the vesicle-induced removal of an inhibitor from microsomes. The former seemed to be unlikely, on the basis of the known low rates of transfer of phospholipids between membranes (Wirtz *et al.*, 1980). To explore the latter possibility, we determined whether lipids extracted from microsomes would inhibit glutathione *S*-transferase previously activated with vesicles. If the vesicles removed an inhibitor from the microsomal membrane, then these lipid-washed microsomes should be susceptible to inhibition by an extract of native microsomes. Vesicles made from a chloroform/methanol extract of microsomes were added to assays containing Tris/HCl-washed microsomes or

microsomes that had been washed with both Tris/HCl buffer and a 25-fold excess (mg of lipid/mg of protein) of vesicles made from egg phosphatidylcholine. Vesicles made from microsomal phospholipids decreased the activity of microsomal glutathione *S*-transferase that was activated previously by vesicles made from egg phosphatidylcholine, but similar treatment of control microsomes had no effect on activity (Fig. 5). Inhibition occurred in a time-dependent fashion, with a rapid loss of activity over the first 10 min. Prolonging the incubation to 30 min led to little further loss of activity. These findings are compatible with the conclusion that rat liver microsomes contain an inhibitor of glutathione *S*-transferase.

In order to examine the possible specificity of lipid-induced activation, microsomes were treated with non-lipid agents that have high affinity for lipophilic materials, i.e. albumin and Bio-Beads. Tris/HCl-washed microsomes were mixed with

albumin at ratios of 5:1 and 20:1 (mg of albumin/mg of microsomal protein) as described in the Materials and methods section. Washing of the microsomes with large amounts of albumin caused a small increase in activity of the microsomal enzyme, about 12%. Chromatography on Bio-Beads caused a slight increase in enzymic activity (Table 2).

Inhibitors of microsomal glutathione S-transferase

The inhibitor of microsomal glutathione *S*-transferase could be present *in vivo* or could have developed during the isolation procedure. Endogenous phospholipase A activity or lipid peroxidation could occur *in vitro* or *in vivo* and yield products that might inhibit the microsomal enzyme. To test for these possibilities, microsomes were treated with purified phospholipase A₂ or under conditions that promote lipid peroxidation, as described in the Materials and methods section. Both treatments caused a complete loss of enzyme activity. After peroxidation, enzyme activity in the microsomes could not be recovered by treating the microsomes with vesicles (Table 3). In contrast, the enzyme activity in the phospholipase A₂-treated microsomes was recovered by mixing the microsomes with vesicles made from egg phosphatidylcholine (Table 3). Thus it appeared that endogenous phospholipase A₂ activity, either *in vivo* or during isolation of the microsomes, could lead to the formation of inhibitors of microsomal glutathione *S*-transferase. The activity of endogenous microsomal phospholipase A₂ is decreased greatly by EDTA (Zakim & Vessey, 1973). Livers were therefore homogenized and microsomes were isolated in solutions containing up to 5 mM-EDTA. This treatment failed to alter significantly the specific activity of the microsomal enzyme. Hence it appears unlikely, on the basis of these results, that phospholipase A₂-catalysed hydrolysis of phospholipids during the isolation of the microsomes is responsible for the inhibitor present in rat liver microsomes.

Table 2. *Effect of chromatography with Bio-Beads on the activity of microsomal glutathione S-transferase*
Tris/HCl-washed microsomes were applied to a column of Bio-Beads SM-2. The column was equilibrated with 0.1 M-Tris/HCl buffer, pH 8.0, at 4°C and eluted with the same buffer. The turbid fractions containing the microsomes were collected and the microsomes were recovered as described in the Materials and methods section. Control microsomes were treated in an identical fashion except that they were not passed down the column. Vesicles were made from egg phosphatidylcholine. Each assay mixture contained 5 mM-GSH, 0.3 mM-1-chloro-2,4-dinitrobenzene and 0.1 M-phosphate buffer, pH 6.5.

Source of microsomes	Protein (mg)	Addition to assay mixture	Activity (μmol/min per mg)
Control	0.067	None	0.082
Control	0.067	Vesicles (1 mg)	0.248
Bio-Beads	0.056	None	0.113
Bio-Beads	0.056	Vesicles (1 mg)	0.303

Table 3. *Effects of lipid peroxidation and treatment with phospholipase A₂ on the activity of microsomal glutathione S-transferase*

The microsomes underwent lipid peroxidation or treatment with phospholipase A₂ as described in the Materials and methods section. The microsomes were recovered by centrifugation. Each assay mixture contained 5 mM-GSH, 0.3 mM-1-chloro-2,4-dinitrobenzene and 0.1 M-phosphate buffer, pH 6.5. The lipid was added in the form of vesicles. The amount of microsomal protein was 0.05–0.06 mg/assay.

Phosphatidylcholine added (mg)	Activity (μmol/min per mg)	
	Peroxidation	Phospholipase A ₂
0	0.007	0
2.5–2.8	0.1	0.108
5.2–5.6	0.006	0.192

Table 4. *Effects of lysophosphatidylcholine and palmitic acid on the activity of microsomal glutathione S-transferase*

The microsomes were incubated with the lysophosphatidylcholine or palmitic acid for 20 min before the addition of substrates. Each assay mixture contained 5 mM-GSH, 0.3 mM-1-chloro-2,4-dinitrobenzene, 0.1 M-phosphate buffer, pH 6.5, and 0.036–0.044 mg of microsomal protein. N.D., Not determined.

Addition	Amount (nmol)	Inhibition (%)	
		Lipid-washed microsomes	Tris/HCl-washed microsomes
Lysophosphatidylcholine	50	38.7	39.7
	100	59.3	50.0
	1000	84.8	N.D.
Palmitic acid	5	29.5	18.0
	10	45.4	30.2
	20	55.3	46.8

Table 5. *Effects of N-ethylmaleimide and vesicles on the activity of microsomal glutathione S-transferase*

Each assay mixture contained 5 mM-GSH, 0.3 mM-1-chloro-2,4-dinitrobenzene, 0.1 M-phosphate buffer, pH 6.5, and 0.17 mg of microsomal protein. Treatment of the enzyme with *N*-ethylmaleimide and vesicles was as described in the Materials and methods section. All assays were performed in duplicate.

Addition(s) to assay mixture	Activity ($\mu\text{mol}/\text{min per mg}$)
None	0.081
<i>N</i> -Ethylmaleimide	0.60
Vesicles (1.48 mg)	0.26
<i>N</i> -Ethylmaleimide and vesicles	1.22

The products of phospholipase A_2 -treated microsomes are lysophosphatides and free fatty acids. We determined the effect of lysophosphatidylcholine and palmitic acid on the activity of the microsomal enzyme. Lysophosphatidylcholine decreased the enzymic activity of both lipid-washed and Tris/HCl-washed microsomes (Table 4). Palmitic acid also decreased enzymic activity. However, it appeared to be a somewhat better inhibitor than lysophosphatidylcholine (Table 4). The inhibition caused by lysophosphatidylcholine and palmitic acid was reversed by adding vesicles made from egg phosphatidylcholine.

Effect of liposomes on the activity of soluble glutathione S-transferase

Addition of liposomes (egg phosphatidylcholine) to an assay mixture containing soluble glutathione

S-transferase caused a decrease in activity. For example, when the ratio of soluble protein to liposomal lipid was 1:1 (mg/mg), enzymic activity (with 0.3 mM-1-chloro-2,4-dinitrobenzene and 5 mM-GSH) was decreased by 37% (1.05 to 0.66 $\mu\text{mol}/\text{min per mg}$). At a protein/lipid ratio of 1:7.4, activity was decreased by 50% (1.05 to 0.52 $\mu\text{mol}/\text{min per mg}$). These inhibitors of activity can be accounted for simply by sequestration of the substrate by the liposomes. The presence of liposomes does not affect the activity of purified glutathione *S*-transferases A, B and C at saturating concentrations of 1-chloro-2,4-dinitrobenzene (Boyer *et al.*, 1982). It is possible to conclude, therefore, that liposomes have no direct effects on the properties of soluble glutathione *S*-transferase.

Treatment of microsomal glutathione S-transferase with N-ethylmaleimide

Morgenstern *et al.* (1979) reported that treatment of microsomes with *N*-ethylmaleimide leads to an 8-fold increase in the activity of microsomal glutathione *S*-transferase. We were therefore interested in whether activation by vesicles would alter the response of the enzyme to *N*-ethylmaleimide. The data in Table 5 show that treatment with vesicles and *N*-ethylmaleimide have an additive effect on enzyme activity. The order of addition of *N*-ethylmaleimide and vesicles did not affect the results in Table 5. The mechanism of activation of microsomal glutathione *S*-transferase by vesicles therefore appears to be different from that due to activation of the enzyme by *N*-ethylmaleimide.

Discussion

The activity of glutathione *S*-transferase in rat liver microsomes can be increased several-fold by treating microsomes with a physiological substance, namely phosphatidylcholine. The findings are compatible with the conclusion that activation reflects removal of an inhibitor of microsomal glutathione *S*-transferase. It is unproven as yet whether the inhibitor occurs *in vivo*, but this appears to be likely. However, even if the inhibitor is produced only *in vitro*, the results presented are important because they indicate that the activity of microsomal glutathione *S*-transferase is greater than previously believed. For example, the reported activity of glutathione *S*-transferase in rat microsomes is in the range 0.05–0.15 $\mu\text{mol}/\text{min per mg}$ (Morgenstern *et al.*, 1979; Friedberg *et al.*, 1979). The activity of the lipid-activated enzyme exceeds these activities by as much as 10-fold.

The presence of the inhibitor in the chloroform layer of a washed Folch extract is consistent with it being a non-polar molecule. The failure of albumin and Bio-Beads to remove the inhibitor suggests

either that the inhibitor is bound poorly by these substances or that the inhibitor is tightly bound to the membrane and they do not interact with the microsomes in a manner that allows for removal of the inhibitor. On the other hand, phosphatidylcholine vesicles, which do remove the inhibitor, are known to remove the tightly bound molecule oestrone from microsomes (Boyer *et al.*, 1980). In the current investigation, contact between microsomes and vesicles appears to be necessary for activation to occur.

An alternative and perhaps related possibility for the lipid-induced activation of microsomal glutathione *S*-transferase is that the vesicles exchange phospholipids with the microsomes, causing a change in the phospholipid milieu of the microsomal enzyme and thus an increase in activity. We consider that this is an unlikely mechanism, because the spontaneous transfer of phospholipids between membranes appears not to occur, or occurs very slowly (Wirtz *et al.*, 1980), whereas activation after exposure to vesicles was relatively rapid (Fig. 2). Also, the effects on activity of repeated treatment of microsomes with vesicles is explained more readily by the idea of an inhibitor than by that of a rapid exchange of phospholipids. Thus the putative lipid-soluble inhibitor will partition between liposomes and microsomes, and no amount of liposomes can remove all the inhibitor from the microsomes.

The activity of microsomal glutathione *S*-transferase is susceptible to inhibition by a variety of non-polar and amphipathic molecules. Bile acids have been shown to decrease the enzymic activity (Vessey & Zakim, 1981). However, washed microsomes do not contain inhibitory concentrations of bile acids (D. Vessey, T. D. Boyer & D. Zakim, unpublished work).

In the current study, palmitic acid and lyso-phosphatidylcholine also decreased the enzymic activity. Thus the activity of this enzyme may vary, depending on the nutritional state of the animal, or it may be decreased in certain pathological conditions that lead to lipid peroxidation, an increase in bile acids or an increase in phospholipase A activity.

The microsomal enzyme is in an ideal location for the metabolism of toxic electrophiles that are products of microsomal Phase I reactions. The microsomal enzyme is in an environment where

these non-polar electrophiles are formed and concentrated, thus enhancing rather than limiting the enzyme's activity. The location of microsomal glutathione *S*-transferase and the current demonstration that its activity is much greater than previously appreciated suggest that this may be an important enzyme in the prevention of toxic liver injury.

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