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POSSIBLE REGULATION MECHANISM OF MICROSOMAL GLUTATHIONE *S*-TRANSFERASE ACTIVITY IN RAT LIVER

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Abstract—After rats were injected with the reduced glutathione (GSH) depletor phorone (diisopropylidene acetone, 250 mg/kg, i.p.), there was a significant increase in microsomal glutathione *S*-transferase activity in the liver. The maximum activity was observed 24 hr after injection and was about 2-fold that of the control activity. Diethylmaleate (500 mg/kg, i.p.) had the same effect. Twenty-four hours after phorone injection (250 mg/kg, i.p.), the concentrations of GSH and oxidized glutathione (GSSG) in the liver were increased about 2-fold. Under the same conditions, the level of mixed disulfides with microsomal proteins (GSS-protein) was aiso increased. Further, the activity of microsomal glutathione *S*-transferases was increased by the *in vitro* addition of disulfide compounds such as GSSG, cystine and homocystine, and the activity increased by GSSG was reduced to control levels by incubating with the corresponding sulfhydryl compounds such as GSH, cysteine and homocysteine respectively. Thus, microsomal glutathione *S*-transferase activity appears to be regulated by the formation and/or cleavage of a mixed disulfide bond between the sulfhydryl group present in the enzyme and GSSG. Therefore, the increase of microsomal glutathione *S*-transferase activity after phorone injection may be due to the formation of a mixed disulfide bond between the sulfhydryl group in the enzyme and GSSG.

Glutathione *S*-transferases are enzymes that catalyze the reaction between GSH and a large variety of compounds bearing an electrophilic site [1]. Most of the enzymes are present in the cytosol fraction, although it was demonstrated recently that the enzyme activity is also located in the microsomal fraction of rat liver [2–4]. The properties of the microsomal enzymes are different from those of the cytosol enzymes, e.g. the activation by *N*-ethylmaleimide (NEM) [2, 4], lack of drug inducibility [3, 4], molecular weight [5], immunochemical properties [6], and dependence on detergent for the activity [5, 7]. However, there was no information on the alteration and regulation of microsomal glutathione *S*-transferase activity *in vivo*. In the present study, we found that microsomal glutathione *S*-transferase activity was increased markedly in the liver of rats injected with phorone, a GSH-depleting agent. The increase of the enzyme activity appears to be due to the increased formation of mixed disulfide bonds between sulfhydryl groups present in the enzymes and GSSG.

MATERIALS AND METHODS

Phorone was obtained from the Aldrich Chemical Co. Inc., Milwaukee, WI, U.S.A. 1-Chloro-2,4-dinitrobenzene (CDNB) was purchased from the J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A. GSH and GSSG were from the Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals used were of analytical grade.

Male Wistar rats (5-weeks old) were used for the experiments. The rats were decapitated at approximately the same time (9:00–10:00). The liver was removed and perfused with cold saline, and was homogenized in 4 vol. of 0.25 M sucrose. The homogenate was centrifuged at 10,000 *g* for 20 min, and the resulting supernatant fraction was centrifuged at 105,000 *g* for 60 min. The microsomal pellets obtained were washed with 0.15 M Tris–HCl (pH 8.0) equivalent to 10-fold volumes of original tissue weight, and recentrifuged at 105,000 *g* for 60 min to remove cytosolic contamination. The resulting pellets were suspended in 0.15 M Tris–HCl (pH 8.0) and were used as the microsomal fraction.

Glutathione *S*-transferase activity was measured with CDNB as a substrate using the method of Habig *et al.* [8]. The assay was performed in 1.0 mM CDNB and 5.0 mM GSH in 0.1 M potassium phosphate buffer (pH 6.5) at 25°.

For the assay of microsomal GSS-protein level, the microsomal protein pellet was prepared by precipitating microsomal suspension with an equal volume of 12% perchloric acid. The pellet was suspended in Tris–HCl buffer (pH 7.4) and then reduced by 2% sodium borohydride in the presence of 8 M urea according to the method of Modig [9]. After reduction, excess borohydride was removed by acidification, and the sample was then centrifuged at 25,000 *g* for 15 min. The supernatant fraction was neutralized and assayed for GSH.

GSH and GSSG were determined by the fluorometric assay using *o*-phthalaldehyde according to the method of Hissin and Hilf [10].

Protein was assayed by the method of Lowry *et al.* [11].

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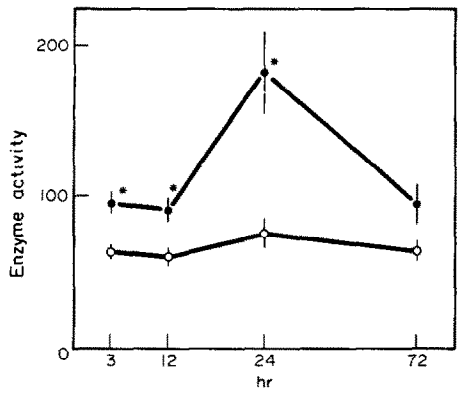

Fig. 1. Time course of microsomal glutathione *S*-transferase activity in the livers of phorone (250 mg/kg, i.p.) treated rats. Enzyme activity is expressed as nmoles CDNB conjugated/min/mg protein. Points and bars represent the mean and S.E.M. of three to four rats respectively. An asterisk (*) indicates significantly different from the control ($P < 0.01$). Key: (○) control (sesame oil), and (●) phorone.

RESULTS

Figure 1 shows the time course of the microsomal glutathione *S*-transferase activity in the liver from rats injected with phorone (250 mg/kg, i.p.). The enzyme activity increased 3 hr after phorone injection and reached a peak, about 2-fold of the control level, at 24 hr. The increased activity returned almost to the control activity 72 hr after phorone. However, such alteration was not observed in the cytosolic glutathione *S*-transferase activity in the liver [12].

The increase of the enzyme activity by phorone was found to be dose dependent (Table 1). Phorone is thought to be useful as a GSH-depleting agent [13]. Thus, to know whether the effect is due to GSH depletion, the effect of diethylmaleate, the most widely used GSH depletor [13], was examined. Diethylmaleate (500 mg/kg, i.p.) also had the same effect. Microsomal glutathione *S*-transferases are known to be activated by the *in vitro* addition of NEM [2, 4], and this activation has been attributed to the alkylation of the sulfhydryl group present in the enzymes with NEM [7]. Thus, we investigated the influence of various sulfhydryl compounds and the corresponding disulfides including GSH and GSSG on microsomal glutathione *S*-transferase activity. As shown in Table 2, all three of the disulfides markedly increased the activity of microsomal glutathione *S*-transferases. The corresponding reduced forms of cystine and homocystine were inactive, but GSH caused a slight decrease of the enzyme activity. The concentration dependence of the activation by GSSG was also examined (Table 3). The enzyme activity was increased by 0.1, 1.0 and 10.0 mM GSSG to 1.4-, 2.6-, and 3.5-fold that of the control level, respectively. Under the conditions, NEM (1 mM) activated the enzyme activity to 5.2-fold that of the control level.

Next, the effects of sulfhydryl compounds on the microsomal glutathione *S*-transferase activity activated by GSSG were measured (Table 4). Enzyme activity activated by GSSG was decreased by preincubation with any of the sulfhydryl compounds. In addition, GSH (3 mM) reduced activity almost to initial levels, and the degree of the inactivation depended upon the concentration of GSH used, whereas the effects of cysteine and homocysteine were moderate. These findings may indicate that microsomal glutathione *S*-transferases are activated by the formation of a disulfide bond between GSSG and the sulfhydryl group present in the enzyme, and that the activated enzymes are inactivated by the cleavage of the disulfide bond by sulfhydryl compounds.

Both diethylmaleate and phorone resulted in the severe depletion of tissue GSH levels a few hours after injection, but at 12–24 hr after injection, GSH levels were increased markedly by the stimulation of GSH synthesis [13, 14]. Since the dramatic alteration of GSH level by these compounds is thought to be accompanied by a change of GSSG level, the perturbation of GSH–GSSG status in the liver could

Table 1. Effects of phorone and diethylmaleate on microsomal glutathione *S*-transferase activity

Treatment	Enzyme activity (nmoles/min/mg protein)
Control	57 ± 7
Phorone, 100 mg/kg, i.p.	80 ± 5*
250 mg/kg, i.p.	140 ± 20†
Diethylmaleate, 500 mg/kg, i.p.	101 ± 6†

Rats were killed 24 hr after injection.
Each value represents the mean ± S.E.M. of four rats.
* $P < 0.05$.
† $P < 0.01$.

Table 2. Effects of sulfhydryl and disulfide compounds on microsomal glutathione *S*-transferase activity in rat liver

Treatment	Enzyme activity (nmoles/min/mg protein)	%
None	87	100
Cysteine	90	102
Homocysteine	85	98
GSH	68	77
Cystine	225	258
Homocystine	221	253
GSSG	177	203

Microsomal enzyme was incubated with various compounds (1.0 mM) for 20 min. Each value represents the mean of duplicate experiments.

Table 3. Effect of GSSG on microsomal glutathione *S*-transferase activity in rat liver

Treatment	Enzyme activity (nmoles/min/mg protein)	%
None	51	100
GSSG, 0.1 mM	72	143
1.0 mM	135	267
10.0 mM	178	352
NEM, 1.0 mM	262	519

Microsomal enzyme was incubated with GSSG or NEM for 20 min. Each value represents the mean of duplicate experiments.

Table 4. Effects of sulfhydryl compounds on microsomal glutathione S-transferase activity activated by GSSG

Treatment	Enzyme activity (nmoles/min/mg protein)	%
None + none	51	
GSSG + none	108	100
GSSG + cysteine, 1.0 mM	94	87
GSSG + homocysteine, 1.0 mM	99	92
GSSG + GSH, 1.0 mM	79	73
GSSG + GSH, 3.0 mM	65	60

Microsomal enzyme activated by preincubation with GSSG (1.0 mM) for 10 min was incubated with various sulfhydryl compounds for 10 min. All additions were made sequentially to the same mixture. Each value represents the mean of duplicate experiments.

not be ruled out as a cause of the increase of microsomal glutathione S-transferase activity. To evaluate this possibility, the effect of phorone on the GSH–GSSG status in the liver was investigated (Table 5). Twenty-four hours after phorone (250 mg/kg, i.p.), both GSH and GSSG levels increased about 2-fold over the control values, but the molar ratio of GSH to GSSG was not altered. Further, under the same conditions, GSS-protein level in the microsomes from phorone-injected rats also increased about 2-fold over that of control rats. The increase of microsomal GSS-protein levels is likely to result from the increase of GSSG levels in the liver. In this regard, there is a positive correlation between GSSG and GSS-protein levels in tissues [15]. These findings strongly suggest that the increase in microsomal glutathione S-transferase activity after phorone injection is due to the increased formation of mixed disulfide bonds between sulfhydryl groups present in the enzymes and GSSG.

DISCUSSION

Microsomal glutathione S-transferase activity was increased by the treatment of the hepatic microsomes with disulfide compounds such as GSSG, cystine and homocystine, and the enzymes activated with GSSG were restored to the initial activity by incubation with the corresponding sulfhydryl compounds. In particular, the effect of GSH was concentration dependent. The activation of microsomal glutathione S-transferases is known to occur by the incubation of the microsomes with NEM [2, 4] and lipid vesicles [16]. According to Morgenstern and DePierre [7], the activation with NEM involves the binding of one molecule of NEM to the single cysteine residue present in each polypeptide chain of the enzyme. In contrast, the activation with lipid vesicles may be due to the elimination of an endogenous inhibitory factor present in the microsomal membrane [16]. Since the disulfide compounds used are known to react with sulfhydryl groups in protein to form a mixed disulfide bond, the activation of microsomal glutathione S-transferase activity with the disulfide compounds is assumed to occur by the formation of the mixed disulfide bond between the sulfhydryl group present in glutathione S-transferases and the disulfide compounds. This idea is further supported by the finding that the activity of glutathione S-transferases activated with GSSG was decreased to the initial level by the sulfhydryl compounds which degrade the mixed disulfide bond. Further, the native glutathione S-transferase activity was decreased slightly by incubating with GSH. These findings suggest that, under normal conditions, a small amount of microsomal glutathione S-transferases in rat liver may exist as an active form with a mixed disulfide bond, possibly as Enz-SSG, although most of the glutathione S-transferases may exist as an inactive form with a free sulfhydryl group (Enz-SH), and that when GSSG level in the cells increases, the inactive form of glutathione S-transferases may be converted to the active form with a mixed disulfide bond.

In fact, under the conditions where hepatic GSSG was increased to about 2-fold of the control level 24 hr after phorone (250 mg/kg, i.p.), GSS-protein in the microsomes was also increased. This supports the idea that the increase of microsomal glutathione S-transferase activity by the injection of phorone may be due to the formation of the mixed disulfide bond between free sulfhydryl groups present in the microsomal glutathione S-transferases and GSSG.

Table 5. Effect of phorone administration on GSH, GSSG and microsomal GSS-protein in rat liver

Treatment	GSH	GSSG (nmoles/mg protein)	Microsomal GSS-protein
Control	24.5 ± 2.5	1.38 ± 0.11	2.59 ± 0.14
Phorone, 250 mg/kg, i.p.	45.5 ± 3.1*	2.38 ± 0.39*	4.25 ± 0.37†

Each value represents the mean ± S.E.M. of four rats.
Rats were killed 24 hr after phorone injection.
* $P < 0.05$.
† $P < 0.01$.

With respect to the increase of glutathione *S*-transferase activity 3 hr after phorone, a relative predominance of GSSG owing to the severe depletion of GSH possibly results in the conversion of the inactive enzyme with free sulfhydryl to the active enzyme with the mixed disulfide bond. These findings suggest that the relative balance between GSH and GSSG levels in the liver plays an important role in the regulation of microsomal glutathione *S*-transferase activity.

The specific activity of microsomal glutathione *S*-transferases with a variety of substrates under normal conditions is less than 10% of that of the cytosolic enzymes. However, Wolf *et al.* [17] have demonstrated recently that the hepatic microsomal fraction plays a major role in the conjugation of hexachloro-1:3-butadiene with GSH, the reaction rate in the microsomal fraction being up to twice that found in the cytosol fraction. Thus, considering the biological role of microsomal glutathione *S*-transferases in microsomal drug metabolism, the present finding that the activity of the microsomal glutathione *S*-transferases is regulated *in vivo* through the relative balance of the GSH–GSSG status in the liver is of great interest.

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