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PREVENTION OF MICROSOMAL PRODUCTION OF HYDROXYL RADICALS, BUT NOT LIPID PEROXIDATION, BY THE GLUTATHIONE–GLUTATHIONE PEROXIDASE SYSTEM*

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Abstract—The glutathione–glutathione peroxidase system is an important defense against oxidative stress. The ability of this system to protect against iron-catalyzed microsomal production of hydroxyl radicals [oxidation of 4-methylmercapto-2-oxo-butyrate (KMBA)] and lipid peroxidation was evaluated. When rat liver cytosol was added to microsomes, strong inhibition against KMBA oxidation was observed. No protection was found when the cytosol was boiled or dialyzed. In the latter case, the addition of 0.5 mM glutathione restored almost complete protection, whereas in the former case protection could be restored by the addition of both glutathione and glutathione peroxidase. Cysteine could not replace glutathione, nor could glutathione *S*-transferase replace glutathione peroxidase. The glutathione–glutathione peroxidase system was also very effective in decreasing production of hydroxyl radicals stimulated by the addition of menadione or paraquat to microsomes. In the absence of cytosol, the addition of glutathione plus glutathione peroxidase was also effective; however, 5 mM glutathione was necessary to protect against KMBA oxidation. The effective concentration of glutathione required for protection was lowered when glutathione reductase was added to the system, to regenerate reduced glutathione. These results indicate that low concentrations of glutathione in conjunction with glutathione peroxidase plus reductase can be very effective in preventing microsomal formation of hydroxyl radicals catalyzed by iron and other toxic compounds. Microsomal lipid peroxidation was decreased 40% by glutathione alone, and this decrease was potentiated in the presence of glutathione reductase. In contrast to KMBA oxidation, the combination of glutathione plus glutathione peroxidase was not any more effective than glutathione alone in preventing lipid peroxidation. The differences in sensitivities of microsomal lipid peroxidation and KMBA oxidation to glutathione peroxidase suggest that these two processes can be distinguished from each other, and that free H_2O_2 and hydroxyl radicals are involved in KMBA oxidation, but not lipid peroxidation.

There is much current interest in glutathione as a protective molecule against chemical-induced cytotoxicity [1–3]. The glutathione–glutathione peroxidase system catalyzes reduction of H_2O_2 to H_2O, and also decomposes organic hydroperoxides [4–7]. Depletion of glutathione in hepatocytes results in accumulation of H_2O_2 [8], and H_2O_2 generated by cytochrome P-450-mediated drug oxidation is primarily removed by the glutathione peroxidase system [9]. Whereas depletion of glutathione need not necessarily lead to lipid peroxidation [10, 11], there are reports that depletion of glutathione below a critical level, e.g. more than 80% depletion, often results in lipid peroxidation [12, 13], and increased toxicity of certain drugs, e.g. paracetamol-induced hepatic necrosis and covalent binding, are increased after depletion of glutathione [14, 15]. Working in conjunction with the glutathione–glutathione peroxidase system is the NADPH-dependent glutathione reductase, which helps to maintain the appropriate reduced glutathione/oxidized glutathione redox status [1, 16]. The capacity of glutathione reductase to reduce the oxidized glutathione formed during hydroperoxide metabolism by glutathione peroxidase is very high [9].

Our laboratory has been studying the abilities of various iron chelates to catalyze the production of potent oxidants such as the hydroxyl radical ($^{\cdot}OH$)‡ or $^{\cdot}OH$-like species during microsomal electron transfer [17, 18]. It has long been known that H_2O_2 is formed during NADPH-dependent microsomal electron transfer [19], most likely due to dismutation of superoxide formed during auto-oxidation of the flavoprotein reductase and of the oxy-cytochrome P-450 complex [20–23]. Ferric iron can be reduced to the ferrous state directly by the reductase, by a superoxide dismutase-insensitive reaction [18, 24], and it appears that a Fenton-type of reaction between ferrous and H_2O_2 produces $^{\cdot}OH$. The addition of catalase (or omission of azide, a catalase inhibitor, from the reaction system) results in a decrease in

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‡ Abbreviations: $^{\cdot}OH$, hydroxyl radical or a species with the oxidizing power of the hydroxyl radical; and KMBA, 4-methylmercapto-2-oxo-butyrate (2-oxo-4-thiomethylbutyric acid).

microsomal production of 'OH, suggesting that H_2O_2 is the precursor of 'OH [18, 25, 26]. Since the glutathione–glutathione peroxidase system appears to be the major system responsible for removal of H_2O_2 produced by microsomes [8, 9], rather than peroxisomal catalase, experiments were carried out to evaluate the effectiveness of this system in preventing microsomal production of 'OH-like species. In other experiments, microsomal lipid peroxidation was studied since there are reports that this process is insensitive to catalase, thus indicating that H_2O_2 is not the precursor of the oxidant responsible for initiating lipid peroxidation [27–29].

MATERIALS AND METHODS

Rat liver microsomes were prepared from male, Sprague–Dawley rats weighing about 200–250 g. The rats were placed under light ether anesthesia, and livers were perfused *in situ* through the portal vein with ice-cold 0.9% NaCl to remove blood. When the livers were clear, 1:3 (w/v) homogenates were prepared in 125 mM KCl. Microsomes were isolated by differential centrifugation, resuspended in 125 mM KCl, washed and frozen at −80°. The cytosolic fraction was considered as the supernatant obtained after the first 100,000 *g* for 60 min centrifugation step. Cytosolic fractions were pooled and passed through a 60 μm filter and frozen in aliquots at −20°. In some experiments, the cytosol was dialyzed against three changes of 100 mM phosphate buffer at 4° over a 24-hr period. This resulted in a greater than 95% depletion of the glutathione concentration of the cytosolic fraction. The concentration of glutathione was determined with Ellman's reagent [30]. Standard curves were prepared using known amounts of glutathione. Although this assay measures all acid-soluble thiol compounds, glutathione is by far the major thiol found in liver. In other cases, the cytosolic fraction was boiled for 10 min or passed through an Amicon PM 10 ultrafiltration membrane. The ultrafiltrate was collected and frozen at −20°.

To evaluate microsomal production of 'OH, the oxidation of KMBA to ethylene was determined. Although this system is not specific for 'OH [31, 32], it affords a relatively rapid, sensitive procedure to assay for the presence of potent oxidants such as 'OH or 'OH-like species. Previous experiments have shown that the oxidation of KMBA is sensitive to competitive 'OH scavengers and to catalase [17, 18, 33]. Reactions were carried out at 37° using a basic reaction system consisting of 100 mM potassium phosphate, pH 7.4, 10 mM $MgCl_2$, 10 mM glucose-6-phosphate, 0.3 mM $NADP^+$, 2 units of glucose-6-phosphate dehydrogenase, 1 mM sodium azide, 10 mM KMBA, and about 2 to 2.5 mg microsomal protein in a final volume of 1 ml. Azide was added to inhibit the activity of catalase, which is present as a contaminant in isolated microsomes. Ferric-EDTA, at a final concentration of 25 μM ferric ammonium sulfate–50 μM EDTA, was added to increase microsomal production of 'OH and, subsequently, of ethylene production from KMBA [18, 26]. The reactions were initiated by the addition of the NADPH-generating system and were terminated after 10 min by the addition of 1 ml of 1 N HCl. Ethylene was determined by a head space, gas chromatography procedure [17]. All values were corrected for zero-time controls which contained the HCl added before the NADPH-generating system.

Microsomal lipid peroxidation was determined using the basic reaction system described above except that the buffer used was 50 mM Tris, pH 7.4, instead of phosphate, and the iron chelate was 25 μM ferric–500 μM ADP instead of ferric-EDTA. The latter iron chelate is known to decrease lipid peroxidation [24, 28, 29]. Reactions were carried out for 10 min, and malondialdehyde was determined by the thiobarbituric acid reaction [34].

Glutathione, glutathione peroxidase, glutathione transferase, glutathione reductase, KMBA, menadione and paraquat were from the Sigma Chemical Co., St. Louis, MO. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and $NADP^+$ were from Boehringer Mannheim, Indianapolis, IN. The water and buffers were passed through a Chelex-100 column to remove contaminating iron. Results represent the mean ± S.E.M. of three to five experiments, carried out in duplicate.

RESULTS

Effect of rat liver cytosol on microsomal oxidation of KMBA. In the presence of 25 μM ferric-EDTA, rat liver microsomes oxidized KMBA at a rate of about 5 nmoles per min per mg protein (Fig. 1). This rate is about 5- to 10-fold greater than the rate observed in the absence of ferric-EDTA [33]. The addition of the 100,000 *g* rat liver supernatant fraction (cytosol fraction) produced an inhibition of KMBA oxidation (Fig. 1). For these experiments, the cytosolic fraction was prepared in 125 mM KCl; controls contained the equivalent amount of 125 mM KCl. By itself, KCl had no effect on KMBA oxidation, even when added to a final concentration of 50 mM (400 μl to a system with a final volume of

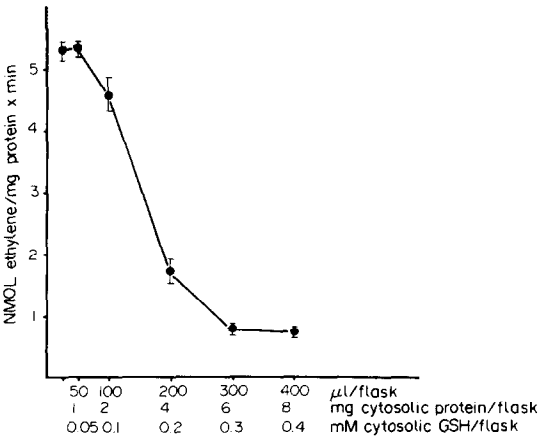

Fig. 1. Effect of rat liver cytosol fraction on microsomal oxidation of KMBA to ethylene. Experiments were carried out as described in Materials and Methods, in the presence of increasing amounts of the cytosolic fraction. Data are expressed as microliters of cytosol added per flask (final volume of 1 ml) or milligrams of cytosolic protein added per flask (per 2 to 2.5 mg microsomal protein) or the final concentration of Ellman-reactive thiol groups expressed as glutathione (derived from the cytosol) per flask.

Table 1. Effect of rat liver cytosol on microsomal oxidation of KMBA to ethylene

Reaction condition	Addition	Rate of KMBA oxidation (nmoles/min/mg microsomal protein)
Control		4.51 ± 0.12
Cytosol		0.72 ± 0.09
Ultrafiltered cytosol		3.80 ± 0.20
Dialyzed cytosol		3.40 ± 0.22
Dialyzed cytosol	5 mM Glutathione	0.7 ± 0.12
Dialyzed cytosol	5 mM Cysteine	5.55 ± 0.34
Boiled cytosol		5.53 ± 0.21
Boiled cytosol	5 mM Glutathione + 5 units glutathione peroxidase	0.87 ± 0.13
Boiled cytosol	5 mM Glutathione + 5 units glutathione transferase	4.79 ± 0.18

The production of ethylene from 10 mM KMBA by rat liver microsomes was determined as described in Materials and Methods. When present, 400 μl of cytosolic fraction (8 mg protein) was added per flask. Boiling, ultrafiltration, and dialysis of cytosol were carried out as described in Materials and Methods.

1 ml). The results of Fig. 1 show the amount of added cytosolic fraction as volume per flask, or cytosolic protein per flask, or final amount of glutathione (present in the added cytosolic fraction) per flask. At a concentration of 6 mg cytosolic protein [which is about 3-fold greater than the concentration of microsomal protein per flask (2 to 2.5 mg)], maximum inhibition of KMBA oxidation was observed. This amount of cytosolic fraction corresponded to a glutathione concentration of 0.3 mM.

Results in Table 1 show that the addition of cytosolic fraction inhibited KMBA oxidation greater than 80%. On the other hand, cytosol that was boiled for 10 min, or passed through an Amicon PM 10 ultrafiltration membrane or dialyzed for 24 hr, either failed to protect or was much less effective than control cytosol in preventing the oxidation of KMBA. When glutathione was added to the dialyzed cytosol, full protection against KMBA oxidation was restored. Cysteine could not replace glutathione in restoring this protection (Table 1). The addition of glutathione to the boiled cytosolic fraction did not result in protection against KMBA oxidation, suggesting that glutathione itself was not the protective agent and, most likely, that a heat-sensitive protein was also required. Whereas the addition of glutathione plus glutathione transferase to boiled cytosolic fraction failed to protect, the combination of glutathione plus glutathione peroxidase was fully protective (Table 1).

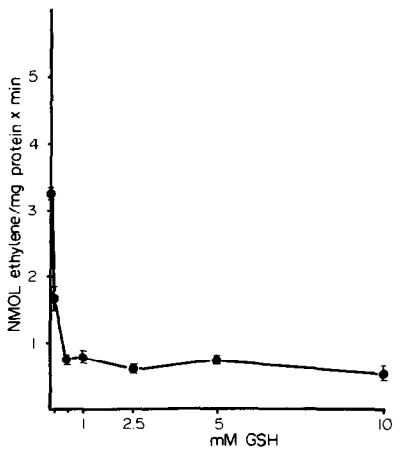

Fig. 2. Effect of glutathione added to dialyzed rat liver cytosol fraction on microsomal oxidation of KMBA to ethylene. Experiments were carried out in the presence of 400 μl (8 mg protein) of dialyzed cytosolic fraction per flask, in the absence and presence of the indicated amounts of glutathione. These amounts of glutathione, in the absence of cytosol, had no effect on KMBA oxidation.

Various levels of glutathione were added to the dialyzed cytosol fraction in order to evaluate the effective concentration necessary to restore protection against KMBA oxidation. As shown in Fig. 2, significant protection was afforded by as little as 0.1 mM glutathione, while full protection occurred at 0.5 mM glutathione.

The addition of menadione or paraquat to rat liver microsomes was found to result in an increase in the oxidation of KMBA, in the presence of ferric-EDTA (Table 2). Addition of cytosolic fraction resulted in a marked decrease in the oxidation of KMBA under all conditions, i.e. enhanced rates of KMBA oxidation promoted by the combination of menadione plus iron or paraquat plus iron were as sensitive to inhibition by the cytosolic fraction as were the control rates (Table 2).

Experiments with glutathione plus glutathione peroxidase. Since the addition of glutathione plus glutathione peroxidase to boiled cytosol was effective

Table 2. Effect of cytosol on microsomal oxidation of KMBA

Addition	Rate of KMBA oxidation (nmoles/min/mg microsomal protein) −Cytosol	+Cytosol	Effect of Cytosol (%)
Control	4.5 ± 0.1	0.7 ± 0.09	−84
0.1 mM Menadione	8.8 ± 0.9	1.0 ± 0.1	−89
1 mM Paraquat	11.8 ± 0.9	1.2 ± 0.1	−90

The production of ethylene from KMBA was assayed as decribed in Materials and Methods in the absence and presence of 400 μl of cytosolic fraction (8 mg protein) per flask. All flasks contained 25 μM ferric-EDTA.

Table 3. Effect of glutathione on microsomal lipid peroxidation

	Addition	Concentration	Rate of malondialdehyde production (nmoles/min/mg microsomal protein)	Effect of addition (%)
A.	Control		2.35 ± 0.19	
	Glutathione	5 mM	1.39 ± 0.11	−41
	Glutathione peroxidase	5 units	2.73 ± 0.25	+16
	Glutathione + glutathione peroxidase	5 mM + 5 units	1.50 ± 0.12	−36
	Glutathione transferase	5 units	2.87 ± 0.18	+22
	Glutathione + glutathione transferase	5 mM + 5 units	1.02 ± 0.11	−57
B.	Control		2.56	
	Glutathione	5 mM	1.76	−32
	Glutathione reductase	4 units	2.72	+6
	Glutathione + glutathione reductase	5 mM + 4 units	1.01	−61

The rate of malondialdehyde production was assayed as described in Materials and Methods in the presence of 25 μM ferric-ADP, and the indicated additions. Results are from either four (A) or two experiments (B).

in blocking the oxidation of KMBA, further experiments were carried out using a reconstituted system containing these two components. A glutathione peroxidase titration curve indicated that full protection against KMBA oxidation could be observed at 1–2 units of glutathione peroxidase activity (Fig. 3a). A glutathione titration curve indicated that 2.5 mM glutathione was required to observe significant protection, while 5 mM glutathione was required for full protection against KMBA oxidation (Fig. 3b). These values are considerably higher than the values needed for protection when glutathione was added back to dialyzed cytosolic fractions (0.5 mM, Fig. 2) or the values found in undialyzed cytosolic fractions (0.3 mM, Fig. 1). A possible explanation for the difference in the amounts of glutathione required for protection against KMBA oxidation in the reconstituted system versus the cytosolic fractions could be the presence of glutathione reductase in the latter, but not the former, system. In the presence of NADPH, glutathione reductase reduces oxidized glutathione back to reduced glutathione and thus helps to maintain effective levels of reduced glutathione [1, 16]. To test this possibility, glutathione reductase was added to the reconstituted system, and the glutathione titration curve was repeated. As shown in Fig. 3c, the glutathione titration curve was shifted to the left in the presence of glutathione reductase, and less glutathione was required for protection against KMBA oxidation in the presence than in the absence of glutathione reductase. In the former case, 1 mM glutathione afforded almost complete protection (Fig. 3c), whereas in the latter case 1 mM glutathione was poorly protective (Fig. 3b).

Effect of glutathione plus glutathione peroxidase on microsomal lipid peroxidation. Microsomes were incubated with an NADPH-generating system, in the presence of ferric-ADP, and lipid peroxidation was assessed via production of malondialdehyde. A ferric-ADP chelate was used for these experiments since this iron chelate, in contrast to ferric-EDTA, is very effective in promoting lipid peroxidation [28, 29, 35]. Microsomal lipid peroxidation was not affected by the addition of glutathione peroxidase but was decreased about 40% by the addition of glutathione itself (Table 3). The combination of glutathione plus glutathione peroxidase was not any

Table 4. Comparison of the oxidation of KMBA by microsomes in phosphate and Tris buffers

	Rate of KMBA oxidation and effect (nmoles/min/mg microsomal protein)			
	Phosphate		Tris	
Reaction condition	Rate	Effect (%)	Rate	Effect (%)
Control	4.38 ± 0.35		3.07 ± 0.17	
Control minus ferric-EDTA	0.89 ± 0.10	−80	0.72 ± 0.17	−77
Control plus cytosol	0.49 ± 0.07	−89	0.40 ± 0.02	−87
Control plus ethanol	1.96 ± 0.33	−55	1.49 ± 0.14	−51
Control plus DMSO	0.82 ± 0.05	−81	0.91 ± 0.03	−70

The production of ethylene from 10 mM KMBA by rat liver microsomes was determined either in 100 mM phosphate, pH 7.4, buffer or 50 mM Tris, pH 7.4, buffer. All other additions were identical. Final concentrations of ethanol and DMSO were 100 mM. Results are from three experiments.

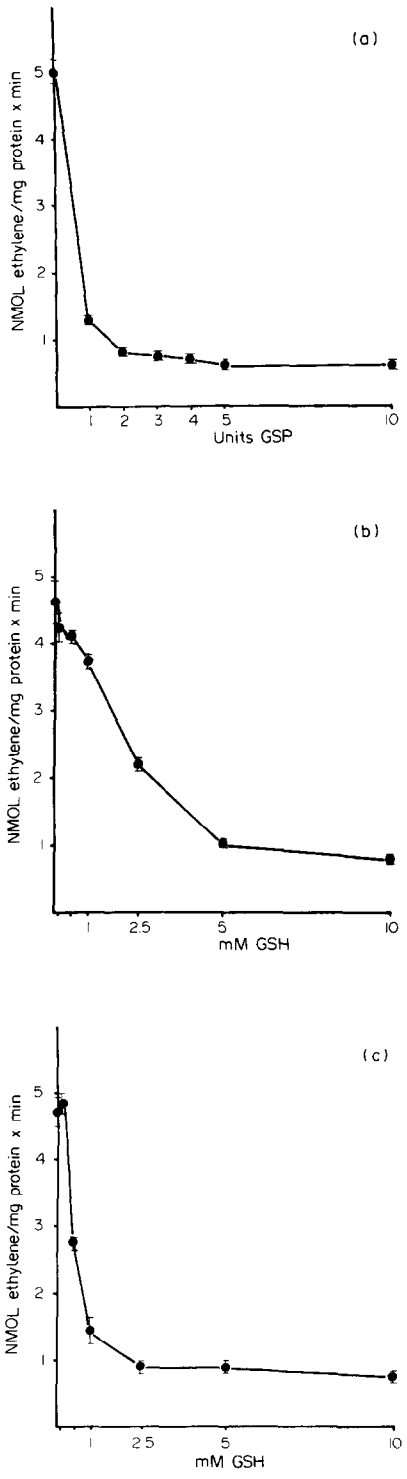

Fig. 3. Effect of reconstituted system containing glutathione and glutathione peroxidase on microsomal oxidation of KMBA. Experiments were carried out as described in Materials and Methods in the presence of various amounts of glutathione peroxidase or glutathione. (a) Each flask contained 5 mM glutathione and the indicated units of glutathione peroxidase. (b) Each flask contained 1 unit of glutathione peroxidase and the indicated concentration of glutathione. (c) Identical to b, except that all flasks also contained 4 units of glutathione reductase.

more effective than glutathione itself in lowering rates of malondialdehyde production. The ability of glutathione transferase to protect against microsomal lipid peroxidation was also evaluated. Glutathione transferase alone had no effect, whereas the combination of glutathione transferase plus glutathione was somewhat more effective than glutathione alone in protecting against microsomal lipid peroxidation (Table 3). Glutathione transferase B has been shown to decrease microsomal lipid peroxidation initiated by ferric-ADP plus NADPH [36]. Some experiments were also conducted with added glutathione reductase to evaluate whether the content of reduced glutathione could be a limiting factor as found with the KMBA experiments. Indeed, the addition of glutathione reductase increased the inhibitory effectiveness of glutathione against lipid peroxidation by 2-fold (Table 3, Exp. B).

Comparison of the effect of Tris and phosphate buffers on microsomal oxidation of KMBA. The lipid peroxidation studies were conducted in Tris buffer since very low rates were found in phosphate buffer (unpublished observations; [28, 34]). The KMBA oxidation studies were carried out in phosphate buffer, since there was concern that Tris would scavenge $^{\cdot}OH$. In view of different responses of microsomal lipid peroxidation and KMBA oxidation to the glutathione–glutathione peroxidase system, a comparison of KMBA oxidation in Tris and phosphate buffers was carried out. As shown in Table 4, the rate of KMBA oxidation in Tris buffer was about 30% lower than that found in phosphate buffer, which probably reflects some competition between KMBA and Tris for the generated $^{\cdot}OH$. The effects of various additions or omission of ferric-EDTA were identical in both buffers. In particular, the addition of cytosol produced marked inhibition of KMBA oxidation in both buffer systems, suggesting that the different responses of KMBA oxidation and lipid peroxidation to glutathione peroxidase were not a reflection of the different buffers routinely used in these assays. It should also be noted that the competitive $^{\cdot}OH$ scavengers ethanol and dimethyl sulfoxide (DMSO) produced similar extents of inhibition of KMBA oxidation in both buffer systems, i.e. there was significant $^{\cdot}OH$ remaining even in the presence of Tris to catalyze KMBA oxidation.

DISCUSSION

Results in the present manuscript demonstrate that the addition of rat liver cytosol to microsomes affords protection against the generation of $^{\cdot}OH$-like species. This protection is quite efficient as it occurred in the presence of high concentrations of ferric-EDTA, and in the presence of ferric-EDTA plus menadione or paraquat. The protective system in the cytosol can be replaced by a combination of glutathione plus glutathione peroxidase plus glutathione reductase and most likely reflects removal of H_2O_2 via glutathione peroxidase, and maintenance of reduced glutathione levels via glutathione reductase. It is of interest that significant protection against microsomal $^{\cdot}OH$ production occurred at a cytosol/microsomal protein ratio of about 2 or 3 to 1 (Fig. 1) which is about the same ratio of cytosol to

microsomes in rat liver (about 25–30 mg microsomal protein, and 75–100 mg cytosolic protein per g liver, w/w). Rat liver cytosol contains about 0.5 units of glutathione peroxidase activity per mg protein [37]. Hence, the addition of 6 mg cytosolic protein reflects the addition of about 3 units of glutathione peroxidase activity. The reconstituted studies indicate that maximal protection occurred at about 2 units of glutathione peroxidase activity (Fig. 3a), thus results with total cytosolic fraction are reasonably well-reproduced by the reconstituted system.

Results with the cytosolic fraction, dialyzed cytosolic fraction and reconstituted system indicate that, in general, glutathione concentrations below 0.5 mM were effective in protecting against microsomal 'OH production. Glutathione reductase was required to maintain glutathione in its reduced form, since in the absence of the reductase from the reconstituted system, 5 mM glutathione was required to protect against microsomal 'OH generation. Since hepatic glutathione levels are about 5 mM, it is clear that considerable depletion of glutathione, e.g. 90%, would be required to compromise the protective capacity of the glutathione–glutathione peroxidase system. This is in accord with results of others that glutathione depletion below a critical value of about 20% of the original concentration is needed in order for lipid peroxidation to occur [12, 13]. Exogenously added glutathione was shown recently to abolish ethane production by postmitochondrial supernatant fractions which had been depleted of glutathione by treatment with phorone, with an I_{50} of only 1.5 μM [12].

Rat liver microsomes contain glutathione transferase activity [38, 39] and contain selenium-independent glutathione peroxidase activity associated with the microsomal glutathione transferase activity [40]. However, the addition of glutathione alone (absence of glutathione peroxidase, Fig. 3a) or of glutathione plus glutathione transferase (Table 1) failed to protect against microsomal production of 'OH. This probably reflects the fact that the microsomal-glutathione transferase system is active with organic hydroperoxides but not hydrogen peroxide [41, 42].

Microsomal lipid peroxidation catalyzed by ferric ADP was partially prevented by glutathione alone, confirming results by Burk and co-workers [43, 44] that a glutathione-dependent protein in microsomes prevents lipid peroxidation induced by ascorbate plus ferric-ADP, NADPH plus ferric-ADP, or carbon tetrachloride. It was suggested that this glutathione-dependent microsomal protein protects against lipid peroxidation by scavenging free radicals [43, 44]. The glutathione–glutathione peroxidase system, which was so effective in protecting against microsomal 'OH production, failed to protect against microsomal lipid peroxidation (beyond the protection afforded by glutathione alone). There have been reports that a glutathione-dependent cytosolic factor could decrease microsomal lipid peroxidation, by somehow preventing the initiation of lipid peroxidation; this glutathione-dependent cytosolic factor is not glutathione peroxidase [45–47]. Thus, although very effective in decomposing H_2O_2, the glutathione–glutathione peroxidase system is not effective in decomposing lipid hydroperoxides or cannot reach the microsomal membrane sites of the lipid hydroperoxides. The differences in sensitivities of microsomal lipid peroxidation and KMBA oxidation ('OH production) to glutathione plus glutathione peroxidase indicate that these two processes can be distinct from each other in involving different precursors or initiators. The sensitivity of KMBA oxidation to glutathione plus glutathione peroxidase and to catalase [17, 48] suggests that H_2O_2 is the precursor of the oxidant responsible for the oxidation of KMBA (and other 'OH scavengers such as benzoate, *t*-butyl alcohol, dimethyl sulfoxide [17, 33]). The insensitivity of lipid peroxidation to glutathione peroxidase [45–47] or to catalase [24, 27, 28] suggests that free H_2O_2 and free 'OH are not the initiators of microsomal lipid peroxidation. Indeed, Aust and co-workers have reported that microsomal lipid peroxidation is not inhibited by competitive 'OH scavengers such as mannitol and have emphasized that 'OH may not account for the initiation of lipid peroxidation by microsomal or other systems [24, 28, 49, 50]. Initiation of lipid peroxidation may involve a chelated-reduced iron–oxygen complex, and the nature of the iron chelate could affect the rate, as well as the mechanism of lipid peroxidation [28, 29, 50]. This may explain why certain iron chelates are effective in promoting one process, but not the other, e.g. ferric-EDTA markedly increases microsomal 'OH production but not lipid peroxidation, whereas ferric-ADP is effective in stimulating lipid peroxidation, but not 'OH production [18, 28].

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