Activation of Microsomal Glutathione *S*-Transferase in *tert*-Butyl Hydroperoxide-Induced Oxidative Stress of Isolated Rat Liver

Yoko Aniya[1,2] and Ai Daido[1]

[1]*Laboratory of Physiology and Pharmacology, School of Health Sciences,* [2]*Research Center of Comprehensive Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan*

Received May 10, 1994 Accepted June 17, 1994

ABSTRACT—The activation of microsomal glutathione *S*-transferase in oxidative stress was investigated by perfusing isolated rat liver with 1 mM *tert*-butyl hydroperoxide (*t*-BuOOH). When the isolated liver was perfused with *t*-BuOOH for 7 min and 10 min, microsomal, but not cytosolic, glutathione *S*-transferase activity was increased 1.3-fold and 1.7-fold, respectively, with a concomitant decrease in glutathione content. A dimer protein of microsomal glutathione *S*-transferase was also detected in the *t*-BuOOH-perfused liver. The increased microsomal glutathione *S*-transferase activity after perfusion with *t*-BuOOH was reversed by dithiothreitol, and the dimer protein of the transferase was also abolished. When the rats were pretreated with the antioxidant α-tocopherol or the iron chelator deferoxamine, the increases in microsomal glutathione *S*-transferase activity and lipid peroxidation caused by *t*-BuOOH perfusion of the isolated liver was prevented. Furthermore, the activation of microsomal GSH *S*-transferase by *t*-BuOOH in vitro was also inhibited by incubation of microsomes with α-tocopherol or deferoxamine. Thus it was confirmed that liver microsomal glutathione *S*-transferase is activated in the oxidative stress caused by *t*-BuOOH via thiol oxidation of the enzyme.

Keywords: Enzyme activation, Glutathione *S*-transferase, Liver perfusion, Oxidative stress, *tert*-Butyl hydroperoxide

Glutathione (GSH) *S*-transferases (EC 2. 5. 1. 18) are multifunctional proteins that function as the transferase catalyzing the conjugation of GSH with xenobiotics and reactive metabolites, and also as GSH peroxidase and binding proteins (1-4). Rat liver microsomal GSH *S*-transferase contains one cysteine residue per subunit (5, 6) and is activated by modification of the sulfhydryl group (7-9). Recently we reported that oxidants or reduced oxygen species cause an oxidation of the sulfhydryl group of microsomal GSH *S*-transferase followed by a formation of the dimeric protein or a mixed-disulfide bond, resulting in an activation of the GSH *S*-transferase (10-12). In addition, oxidative stress-induced activation of microsomal GSH *S*-transferase was also demonstrated by ischemia/reperfusion or perfusion with hydrogen peroxide of isolated rat liver (13). Furthermore, organic hydroperoxides including linoleic hydroperoxide were shown to activate the microsomal GSH *S*-transferase via the thiol oxidation of the enzyme (14). Since hydroxyalkenals and lipid hydroperoxides, which are toxic products from lipid peroxidation, serve as substrates for microsomal GSH *S*-transferase and GSH peroxidase (15, 16), it was expected that microsomal GSH *S*-transferase is oxidatively activated by reactive oxygen species or metabolic products formed during oxidative stress, and simultaneously detoxifies these toxic products as its own substrates.

On the other hand, Lundqvist and Morgenstern (17) and Haenen et al. (18) reported that microsomal GSH *S*-transferase is activated by covalent modification of the sulfhydryl group in the enzyme, but not through oxidation by reactive oxygen species.

To obtain a clear understanding of the activation mechanism of microsomal GSH *S*-transferase, we examined the activation of the enzyme in oxidative stress caused by *tert*-butyl hydroperoxide (*t*-BuOOH) perfusion of the liver because radical-mediated oxidative stress by this agent has been well established (19-21). In the present study, we confirmed that the microsomal GSH *S*-transferase is activated by thiol oxidation in the oxidative stress caused by *t*-BuOOH perfusion of the isolated rat liver and discussed the relationship between oxidative acti-

vation and covalent modification of the enzyme.

MATERIALS AND METHODS

Chemicals

Reduced and oxidized glutathione, glutathione reductase, cumene hydroperoxide, *t*-BuOOH, deferoxamine mesylate and dithiothreitol were purchased from Sigma Chemicals (St. Louis, MO, USA). 1-Chloro-2,4-dinitrobenzene, 2,4-dinitrofluorobenzene, iodo acetic acid and thiobarbituric acid were obtained from Wako Pure Chemicals (Osaka). NADPH and hydrogen peroxide were obtained from Oriental Yeast (Tokyo) and Santoku-Kagaku (Tokyo), respectively. Sodium pentobarbital and DL-α-tocopherol were from Abbott Laboratories (Chicago, IL, USA) and Nacalai Tesque (Kyoto), respectively. All other chemicals used were of analytical reagent grade.

Pretreatment of animals

Male Sprague-Dawley rats (170–230 g) were used. In cases where pretreatment of rats with an antioxidant was necessary, α-tocopherol (100 mg/kg/5 ml) and deferoxamine (500 mg/kg/5 ml) were injected intraperitoneally 15 and 0.5 hr before killing, respectively. α-Tocopherol was dissolved in ethanol and diluted with 0.9% sodium chloride solution containing 16% (v/v) Tween 80, whereas deferoxamine was dissolved in 0.9% sodium chloride solution. Control rats were given the same volume of each vehicle.

Perfusion of the isolated liver with t-BuOOH

Rats fasted overnight were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally), and the isolated liver was perfused, as described previously (14), with Krebs-Henseleit buffer in the presence or absence of 1 mM *t*-BuOOH for the indicated times. After perfusion, the liver microsomes and cytosol were prepared as described previously (10).

Treatment of microsomes with agents in vitro

Liver microsomes (4–6 mg/ml) prepared from non-treated rats were suspended in 0.05 M potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose and 0.3 mM EDTA and were incubated with 0.1 mM *t*-BuOOH in the presence or absence of 0.01 mM GSH in 0.05 M potassium phosphate buffer (pH 7.4). Effects of dithiothreitol, α-tocopherol, and deferoxamine on GSH *S*-transferase activity were examined under the conditions described in each legend.

Assay

The GSH content of liver homogenates was measured by the method of Reed et al. (22) using high performance liquid chromatography. GSH *S*-transferase and peroxidase activities were assayed by the method of Habig et al. (23) with the substrates 1-chloro-2,4-dinitrobenzene and GSH and the method of Reddy et al. (24) with cumene hydroperoxide, respectively. Lipid peroxidation in liver homogenates and microsomes was determined as thiobarbituric-acid-reactive substances (TBARS) by the method of Buege and Aust (25). Protein concentration was determined by the method of Lowry et al. (26).

Gel electrophoresis and immunoblotting

SDS-polyacrylamide gel electrophoresis and immunoblotting were carried out by using anti-microsomal GSH *S*-transferase antibody, which was prepared in our laboratory, as described previously (11).

Statistical analyses

Data are expressed as means±S.D. Significance of difference was evaluated by Student's *t*-test, and P values <0.05 were taken as significant.

RESULTS

Time course effect of t-BuOOH perfusion on enzyme activity and lipid peroxidation

Figure 1 shows the time course of the effect of *t*-BuOOH perfusion of isolated liver on GSH *S*-transferase activity, GSH content and lipid peroxidation. When the isolated liver was perfused with 1 mM *t*-BuOOH from 7 to 60 min, microsomal GSH *S*-transferase activity was increased 1.3-fold at 7 min and 1.7-fold at 10 min of perfusion, followed by a decrease to 80% of the control after a 60 min perfusion. However, cytosolic GSH *S*-transferase activity was decreased to 86% of the control at 7 min, to 83% at 10 min and to 20% at 60 min of perfusion. GSH content was decreased time dependently: 73% of the control at 7 min, 64% at 10 min and was depleted at 60 min of perfusion. Oxidized GSH was not detected in the liver during *t*-BuOOH perfusion. Lipid peroxidation in the liver was also increased with increasing time of perfusion, showing a moderate elevation of lipid peroxide at 10 min and a marked increase at 60 min of perfusion.

Although microsomal GSH *S*-transferase activity was significantly increased after perfusion of the isolated liver with *t*-BuOOH for 7 min, a small increase (114%) in microsomal GSH peroxidase activity was observed. Cytosolic GSH peroxidase activity was not altered by the *t*-BuOOH perfusion (Table 1).

Gel electrophoresis and immunoblotting of liver microsomes after t-BuOOH perfusion

As shown in Fig. 2, the microsomal GSH *S*-transferase

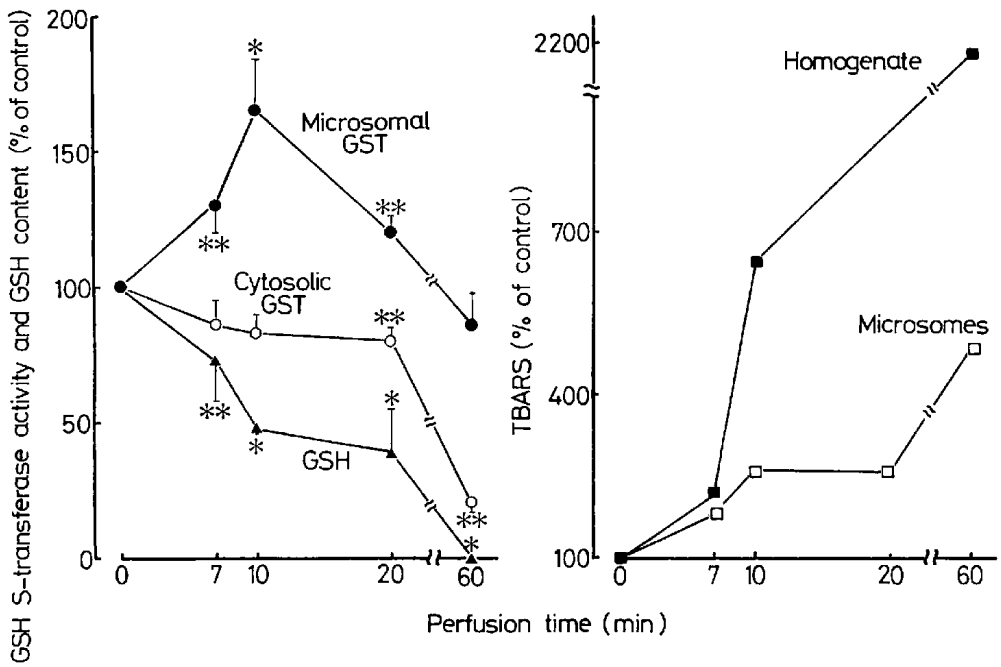

Fig. 1. Time course effect of *t*-BuOOH perfusion on GSH *S*-transferase (GST) activity, GSH content and lipid peroxidation of isolated rat liver. Isolated rat liver was perfused with or without 1 mM *t*-BuOOH for the indicated time at 37℃; and GST activity, GSH content and lipid peroxidation (TBARS) were measured. Each point shows the mean±S.D. for 3 to 5 rats. *P<0.05, **P<0.01: significant difference from the control. The activity of GST in the control group (μmol/mg protein/min) was as follows: cytosolic GST (○), 1.388±0.232–1.594±0.174; microsomal GST (●), 0.100±0.007–0.138±0.020. The content of GSH and lipid peroxidation in the control group (nmol/mg protein) were as follows: GSH (▲), 8.58±3.86–16.49±2.56; homogenate lipid peroxidation (■), 0.018±0.002–0.023±0.003; microsomal lipid peroxidation (□), 0.085±0.003–0.109±0.039.

Table 1. Alteration of GSH *S*-transferase and GSH peroxidase activities after *t*-BuOOH perfusion of isolated liver

Treatment	GSH *S*-transferase		GSH peroxidase	
	cytosol (μmol/mg/min)	microsome (μmol/mg/min)	cytosol (μmol/mg/min)	microsome (μmol/mg/min)
Control	1.572±0.265	0.126±0.015	0.364±0.123	0.058±0.003
t-BuOOH	1.349±0.119 (86)	0.164±0.018** (130)	0.339±0.052 (93)	0.066±0.011 (114)

Isolated rat liver was perfused with or without 1 mM *t*-BuOOH for 7 min at 37℃. The activities of GSH *S*-transferase and GSH peroxidase were measured as described in Materials and Methods. Values are presented as means±S.D. for 5 rats. The value in the parenthesis shows percent of control. **P<0.01, vs. control.

activity was increased to 137% of the control upon perfusion with 1 mM *t*-BuOOH for 7 min and was decreased to 112% by treatment of the microsomes with dithiothreitol. In the microsomes prepared from the *t*-BuOOH-perfused liver, the native microsomal GSH *S*-transferase (Mr 17,000) and a protein with a Mr of 34,000 were detected; and the later protein was abolished by treatment of microsomes with dithiothreitol, showing the dimer of microsomal GSH *S*-transferase. In the control liver, the native GSH *S*-transferase in microsomes and the protein with a Mr of 50,000, assigned as a trimer of GSH *S*-transferase, were observed in spite of the presence or absence of dithiothreitol. Thus it was clarified that microsomal GSH *S*-transferase was activated with a concomitant formation of the dimeric protein by *t*-BuOOH perfusion of the liver.

Effect of α-tocopherol and deferoxamine on t-BuOOH-induced activation of microsomal GSH S-transferase

When the liver pretreated with α-tocopherol or with

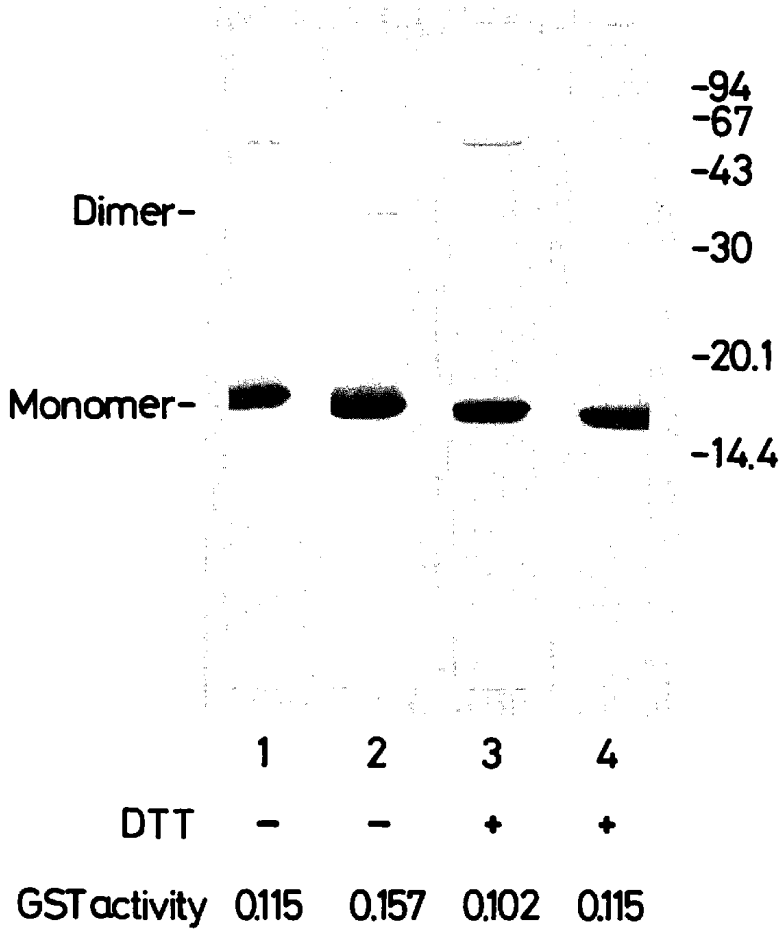

Fig. 2. Gel electrophoresis and immunoblotting of microsomal proteins of *t*-BuOOH-perfused liver. Isolated rat liver was perfused with or without 1 mM *t*-BuOOH for 7 min at 37°C. GSH *S*-transferase activity (μmol/mg/min) in the liver microsomes was measured, and microsomal proteins from the control (33 μg protein) and *t*-BuOOH perfusion (44 μg protein) were applied to separate lanes of 15% SDS-polyacrylamide gels. Electrophoresis and immunoblotting were carried out as described previously (11). Lanes 1 and 3, control microsomes; lanes 2 and 4, *t*-BuOOH-perfused microsomes; lanes 3 and 4, microsomes incubated with 5 mM dithiothreitol in 0.05 M potassium phosphate buffer (pH 7.4) for 10 min at room temperature. Protein standards were bovine milk α-lactalbumin, Mr 14,400; soybean trypsin inhibitor, Mr 20,100; bovine erythrocyte carbonic anhydrase, Mr 30,000; egg white ovalbumin, Mr 43,000; bovine serum albumin, Mr 67,000; and phosphorylase b, Mr 94,000.

Table 2. Effect of antioxidants on GSH *S*-transferase (GST) activity, GSH content and lipid peroxidation (TBARS) of *t*-BuOOH-perfused liver

Treatment	GST (μmol/mg/min)		GSH (nmol/mg)	TBARS (nmol/mg)	
	cytosol	microsome		homogenate	microsome
Control	1.334±0.201	0.110±0.010	15.20±3.86	0.022±0.03	0.080±0.010
t-BuOOH	1.014±0.334	0.183±0.027*	7.90±3.25**	0.111±0.084	0.222±0.085
V.E → *t*-BuOOH	1.382±0.266	0.129±0.021#	8.37±0.42**	0.030±0.003**	0.083±0.010
DFO → *t*-BuOOH	1.230±0.168	0.139±0.027	9.15±4.02*	0.035±0.004**	0.102±0.039

α-Tocopherol (V.E, 100 mg/kg) and deferoxamine (DFO, 500 mg/kg) were given intraperitoneally to rats 15 hr and 0.5 hr before killing, respectively, and then the livers were isolated and perfused with *t*-BuOOH (1 mM) for 10 min under the same conditions described in Fig. 1. Values are presented as means±S.D. for 4 to 6 rats. **$P<0.01$, *$P<0.05$, vs. control; #$P<0.05$, vs. *t*-BuOOH.

deferoxamine was perfused with 1 mM *t*-BuOOH for 10 min, microsomal GSH *S*-transferase activity was increased only to 117% and to 126% of the control, respectively, compared to 166% by the perfusion with *t*-BuOOH alone. The decreased GSH *S*-transferase activity in the cytosol (76% of the control) after the *t*-BuOOH perfusion was recovered to the control level by pretreatment of rats with the agents. GSH content in the liver homogenates after perfusion with *t*-BuOOH was decreased to 52%, and the decrease was not improved by pretreatment with α-tocopherol and deferoxamine. An increase in lipid peroxidation in the homogenates and microsomes of the liver after *t*-BuOOH perfusion was depressed by pretreatment of rats with α-tocopherol or deferoxamine (Table 2).

When microsomes were incubated with *t*-BuOOH or with *t*-BuOOH and GSH in vitro in the presence of the iron chelator deferoxamine, the increase in GSH *S*-transferase activity by *t*-BuOOH (1.3-fold by *t*-BuOOH alone and 1.5-fold by *t*-BuOOH and GSH) was completely prevented by the chelator. On the other hand, in α-tocopherol (0.2 mM)-treated microsomes, about a 10% increase in microsomal GSH *S*-transferase activity was seen by *t*-BuOOH alone even though there was no increase in the activity by *t*-BuOOH and GSH (Table 3).

Effect of dithiothreitol and N-ethylmaleimide on t-BuOOH-activated GSH S-transferase in vitro

As shown in Table 4, microsomal GSH *S*-transferase activity, which was increased to 1.8-fold by *t*-BuOOH alone and to 2.7-fold by *t*-BuOOH and GSH, showed a 1.5-fold and a 1.2-fold increase, respectively, after treatment with dithiothreitol. Although *N*-ethylmaleimide caused a 5.3-fold increase in GSH *S*-transferase activity in

Table 3. Effect of antioxidants on microsomal GSH *S*-transferase (GST) activity in vitro

Microsomes	Treatment	GST (μmol/mg/min)	%
Control	None	0.089±0.002	100
	t-BuOOH	0.117±0.001**	131
	GSH	0.090±0.004	101
	t-BuOOH+GSH	0.131±0.003**	147
Deferoxamine	None	0.092±0.002	100
	t-BuOOH	0.090±0.001##	98
	GSH	0.087±0.001	94
	t-BuOOH+GSH	0.089±0.001##	97
Control	None	0.090±0.002	100
	t-BuOOH	0.110±0.004**	122
	GSH	0.085±0.002	94
	t-BuOOH+GSH	0.145±0.004**	161
α-Tocopherol	None	0.091±0.002	100
	t-BuOOH	0.100±0.002##	110
	GSH	0.082±0.002	90
	t-BuOOH+GSH	0.092±0.001##	101

Microsomes were incubated in 0.05 M potassium phosphate buffer (pH 7.4) with *t*-BuOOH (0.1 mM) or *t*-BuOOH (0.1 mM) and GSH (0.01 mM) in the presence or absence of deferoxamine (1 mM) at room temperature (26–30℃) for 30 min, and then GST activity was measured as described in the Materials and Methods. In the case of α-tocopherol treatment, microsomes were preincubated with α-tocopherol (0.2 mM) at room temperature for 60 min, followed by centrifugation at 105,000×*g* for 60 min, and the resultant pellets were suspended and incubated with *t*-BuOOH or *t*-BuOOH plus GSH under the same conditions as used in deferoxamine treatment. Values are presented as means±S.D. for three incubations. **P<0.01, vs. none treatment; ##P<0.01, vs. *t*-BuOOH or *t*-BuOOH−GSH in the control group.

Table 4. Effect of dithiothreitol and *N*-ethylmaleimide on *t*-BuOOH-activated GSH *S*-transferase (GST) in liver microsomes

Treatment	DTT 5 mM	GST (μmol/mg/min)		
		none	NEM	% increase
Control	−	0.089±0.02	0.477±0.009	536
	+	0.068±0.002	0.452±0.006	665
t-BuOOH	−	0.164±0.001	0.197±0.004	120
	+	0.101±0	0.421±0.002	416
t-BuOOH+GSH	−	0.241±0.003	0.239±0.003	99
	+	0.086±0.01	0.445±0.001	517

After incubation with *t*-BuOOH (0.1 mM) or *t*-BuOOH (0.1 mM) and GSH (0.01 mM) for 30 min, the microsomes were further incubated with or without dithiothreitol (DTT, 5 mM) for 15 min at room temperature, followed by centrifugation at 105,000×*g* for 60 min. The GST activity in the resultant pellets was measured in the absence or presence of *N*-ethylmaleimide (NEM, 1 mM, 2 min). Values are presented as means±S.D. of triplicate determinations.

the control microsomes, the increase was only 1.2-fold in *t*-BuOOH-treated microsomes, and no increase in the activity was seen in *t*-BuOOH and GSH-treated microsomes, showing consumption of the sulfhydryl group of the enzyme by *t*-BuOOH. After the incubation of these microsomes with dithiothreitol, the activation of GSH *S*-transferase by *N*-ethylmaleimide was 6.6-fold in the control-, 4.1-fold in *t*-BuOOH- and 5.2-fold in *t*-BuOOH with GSH-treated microsomes. Thus it was clarified that the activation of microsomal GSH *S*-transferase by *t*-BuOOH alone is partially recovered by dithiothreitol, suggesting that an irreversible modification of GSH *S*-transferase is also attributed to the activation, whereas *t*-BuOOH and GSH causes a reversible activation of the GSH *S*-transferase via the disulfide bond formation.

DISCUSSION

In the present study, it was demonstrated that microsomal GSH *S*-transferase activity is significantly increased in oxidative stress caused by perfusion of the liver with *t*-BuOOH. The increase in the GSH *S*-transferase activity was seen during the perfusion period from 7 min to 20 min, in which the GSH content was decreased to about half of the control level and the lipid peroxidation was moderately enhanced. In this case, a dimer protein of microsomal GSH *S*-transferase was also detected. The increase in GSH *S*-transferase activity caused by *t*-BuOOH perfusion was reversed by dithiothreitol and the dimer protein was abolished by the agent. Although GSH *S*-transferase with a mixed-disulfide bond could not be distinguished by the immunoblot analysis, it may be involved as an active form of microsomal GSH *S*-transferase as seen previously (11, 12). Thus it was confirmed that in the oxidative stress caused by *t*-BuOOH perfusion, the liver microsomal GSH *S*-transferase is activated via disulfide bond formation of the enzyme. Furthermore, the fact that the increases in the microsomal GSH *S*-transferase activity and lipid peroxidation after *t*-BuOOH perfusion of the liver were prevented by pretreatment of rats with α-tocopherol or deferoxamine clarified the oxidative stress mediated-activation of microsomal GSH *S*-transferase. Studies have suggested that *t*-BuOOH is converted catalytically by both heme- and nonheme-iron complexes to radicals, which in turn attack cellular components resulting in lipid peroxidation and cellular damages (19–21) and that protein thiols are oxidized via thiyl radicals after administration of organic hydroperoxides to rats (27). α-Tocopherol can scavenge free radicals that are produced within membranes (28, 29), and deferoxamine is an iron chelator (30, 31). Thus it is clear that radicals formed from *t*-BuOOH through iron-mediated reactions lead to the oxidation of the thiol in microsomal GSH *S*-transferase via thiyl radical, resulting in disulfide bond formation with a concomitant increase in the enzyme activity and that deferoxamine disturbs *t*-BuOOH radical formation by chelating irons and α-tocopherol scavenges *t*-BuOOH radicals; consequently, both agents prevent *t*-BuOOH-induced activation of GSH *S*-transferase and lipid peroxidation.

These findings were confirmed by in vitro studies. Although incubation of microsomes with *t*-BuOOH alone and *t*-BuOOH with GSH increased GSH *S*-transferase activity in vitro, it was completely prevented in the

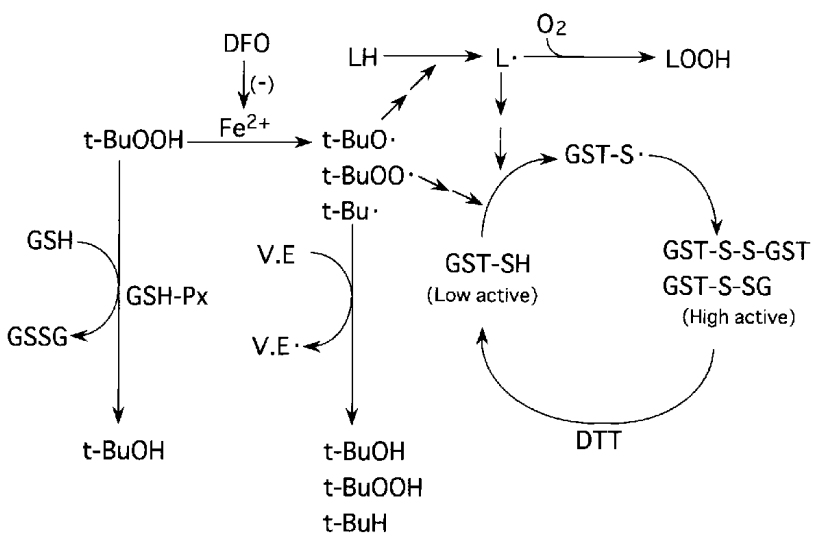

Fig. 3. Activation mechanism of microsomal GSH *S*-transferase by *t*-BuOOH perfusion of isolated liver. LH, lipid; LOOH, lipid hydroperoxide; GSH-Px, GSH peroxidase; DFO, deferoxamine; V.E, α-tocopherol; GST-SH, microsomal GSH *S*-transferase and DTT, dithiothreitol.

presence of deferoxamine. Thus the results show that any radicals responsible for oxidation of the thiol in the microsomal GSH S-transferase were not formed from t-BuOOH in the presence of deferoxamine. In α-tocopherol-treated microsomes, the activation of microsomal GSH S-transferase by t-BuOOH and t-BuOOH with GSH was inhibited, but about a 10% increase in GSH S-transferase activity remained. It is, therefore, suggested that since the cysteine residue in microsomal GSH S-transferase is located in the cytoplasmic phase, t-BuOOH radicals formed in microsomal membranes were scavenged by α-tocopherol, but the radicals formed in aqueous phase may attack the enzyme thiol. All these data indicate that microsomal GSH S-transferase is activated in oxidative stress caused by t-BuOOH via thiyl radical, resulting in disulfide bond formation. The activation mechanism of microsomal GSH S-transferase by t-BuOOH perfusion is summarized in Fig. 3.

We further examined the possibility of covalent binding or irreversible modification of microsomal GSH S-transferase in vitro. As shown in Table 4, the increased GSH S-transferase activity by t-BuOOH alone was partially recovered by dithiothreitol, whereas in t-BuOOH with GSH-treated microsomes, the increase in the activity was almost completely reversed to the control level by dithiothreitol. Thus it is considered that in addition to disulfide bond formation, some irreversible modifications of microsomal GSH S-transferase are involved in the activation of the enzyme by t-BuOOH alone, whereas only a disulfide bond formation contributes to the activation when t-BuOOH was incubated with microsomes in the presence of GSH. Since the SH blocker N-ethylmaleimide activates microsomal GSH S-transferase by covalent binding to the protein thiol, it was used to evaluate the SH modification of microsomal GSH S-transferase. Thus a slight activation of microsomal GSH S-transferase by N-ethylmaleimide after treatment by t-BuOOH alone confirmed that the sulfhydryl group of the enzyme is modified by the action of the hydroperoxide. Furthermore, a decrease in the activation of t-BuOOH-treated microsomal GSH S-transferase by N-ethylmaleimide after the reduction of disulfide bond by dithiothreitol suggests that an activation mechanism other than disulfide bond formation is involved in the t-BuOOH-induced activation of microsomal GSH S-transferase. It may be a covalent modification of the protein thiol by t-BuOOH radicals or by products of lipid peroxidation or an irreversible oxidation of the protein thiol. It is therefore expected that in oxidative stress, radicals including reactive oxygen species attack the thiol of microsomal GSH S-transferase followed by formations of dimeric protein and mixed disulfide bond and that under severe oxidative stress such as GSH depletion an irreversible modification of the enzyme by metabolic products of lipid peroxidation may occur.

In contrast to the prevention by α-tocopherol and deferoxamine of the increase in GSH S-transferase activity and lipid peroxidation in t-BuOOH perfused liver, the decrease in GSH content in the liver after t-BuOOH perfusion was not recovered by pretreatment of rats with the agents. Since GSH in the liver is consumed by a nonenzymatic reaction with radicals or by GSH peroxidase that catalyzes the reduction of t-BuOOH to its alcohol accompanying oxidation of GSH, it is suggested that the liver GSH, which is present in the cytoplasm, is quickly consumed by cytosolic GSH peroxidases with t-BuOOH before the t-BuOOH is decomposed to radicals, and the oxidized glutathione formed from the action of GSH peroxidase is reduced by GSH reductase. Thus the t-BuOOH that escaped from the action of GSH peroxidase can form its radicals which can attack cellular components and be modulated by radical scavengers. Consequently, GSH content in the liver after t-BuOOH perfusion may be kept at a low level regardless of pretreatment with α-tocopherol or with deferoxamine.

In severe oxidative stress such as a 60-min perfusion of the liver with t-BuOOH, 80% of the microsomal GSH S-transferase activity still remained in spite of a marked decrease in cytosolic GSH S-transferase activity. This means that the remaining activity of microsomal GSH S-transferase plays an important role in the detoxication of toxic metabolites formed during oxidative stress. Considering not only that microsomal GSH S-transferase can detoxify fatty acid hydroperoxides or 4-hydroxyalkenals, which are its own substrates (15), but also that lipid hydroperoxides can activate the microsomal GSH S-transferase (14), it is likely that the microsomal GSH S-transferase is activated under oxidative stress by toxic metabolites from lipid peroxidation, and then this activated transferase can defoxify them.

Although we showed in the previous report that microsomal GSH S-transferase is activated by ischemia/reperfusion or perfusion with hydrogen peroxide of isolated rat liver (13), in those cases, we determined neither lipid peroxidation as a marker of oxidative stress nor an antioxidant effect against oxidative stress. Thus, in the present study, it was concluded that the microsomal GSH S-transferase is activated by disulfide bond formation of the enzyme under oxidative stress by t-BuOOH, and an activation by an irreversible modification of the enzyme does not contribute in this case. In the in vitro activation of microsomal GSH S-transferase by t-BuOOH, in addition to the activation by disulfide bond formation, an irreversible modification of the GSH S-transferase is involved in the absence of GSH.

Acknowledgments

We thank Ms. N. Murayama for typing the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1 Jakoby WB and Habig WH: Glutathione transferase. *In* Enzymatic Basis of Detoxication, Edited by Jakoby WB, Vol **2**, pp 63–94, Academic Press, New York (1980)
2 Habig WH: Glutathione *S*-transferase: Versatile enzymes of detoxication. *In* Radioprotectors and Anticarcinogens, Edited by Nygaard OF and Simic MG, pp 169–190, Academic Press, New York (1983)
3 Morgenstern R and DePierre JW: Microsomal glutathione *S*-transferase. Rev Biochem Toxicol **7**, 67–104 (1985)
4 Mannervik B and Danielson UH: Glutathione transferases — structure and catalytic activity. CRC Crit Rev Biochem **23**, 283–337 (1988)
5 Morgenstern R and DePierre JW: Microsomal glutathione transferase: Purification in unactivated form and further characterization of the activation process, substrate specificity and amino acid composition. Eur J Biochem **134**, 591–597 (1983)
6 Morgenstern R, DePierre JW and Jornvall H: Microsomal glutathione transferase; Primary structure. J Biol Chem **260**, 13976–13983 (1985)
7 Morgenstern R, DePierre JW and Ernster L: Activation of microsomal glutathione *S*-transferase activity by sulfhydryl reagents. Biochem Biophys Res Commun **87**, 657–663 (1979)
8 Aniya Y and Anders MW: Regulation of rat liver microsomal glutathione *S*-transferase activity by thiol/disulfide exchange. Arch Biochem Biophys **270**, 330–334 (1989)
9 Morgenstern R, DePierre JW and Ernster L: Reversible activation of rat liver microsomal glutathione *S*-transferase activity by 5′,5-dithiobis (2-nitrobenzoic acid) and 2,2′-dipyridyl disulfide. Acta Chem Scand **B34**, 229–230 (1980)
10 Aniya Y and Anders MW: Activation of rat liver microsomal glutathione *S*-transferase by reduced oxygen species. J Biol Chem **264**, 1998–2002 (1989)
11 Aniya Y and Anders MW: Activation of rat liver microsomal glutathione *S*-transferase by hydrogen peroxide: Role for protein-dimer formation. Arch Biochem Biophys **296**, 611–616 (1992)
12 Aniya Y and Anders MW: Activation of hepatic microsomal glutathione *S*-transferase by hydrogen peroxide and diamide. *In* Oxygen Radicals, Edited by Yagi K, Kondo M, Niki E and Yoshikawa T, pp 737–740, Elsevier, Amsterdam (1992)
13 Aniya Y and Naito A: Oxidative stress-induced activation of microsomal glutathione *S*-transferase in isolated rat liver. Biochem Pharmacol **45**, 37–42 (1993)
14 Aniya Y and Daido A: Organic hydroperoxide-induced activation of liver microsomal glutathione *S*-transferase of rats in vitro. Jpn J Pharmacol **62**, 9–14 (1993)
15 Mosialou E and Morgenstern R: Activity of rat liver microsomal glutathione transferase toward products of lipid peroxidation and studies of the effect of inhibitors on glutathione-dependent protection against lipid peroxidation. Arch Biochem Biophys **275**, 289–294 (1989)
16 Mosialou E, Ekstrom G, Adang AE and Morgenstern R: Evidence that rat liver microsomal glutathione transferase is responsible for glutathione-dependent protection against lipid peroxidation. Biochem Pharmacol **45**, 1645–1651 (1993)
17 Lundqvist G and Morgenstern R: Mechanism of activation of rat liver microsomal glutathione transferase by noradrenaline and xanthine oxidase. Biochem Pharmacol **43**, 1725–1728 (1992)
18 Haenen GRMM, Jansen FP, Vermeulen NPE and Bast A: Activation of the microsomal glutathione *S*-transferase by metabolites of α-methyldopa. Arch Biochem Biophys **287**, 48–52 (1991)
19 Hrycay EG and O'Brien PJ: Microsomal electron transport. II. Reduced nicotinamide adenine dinucleotide-cytochrome b_5 reductase and cytochrome P-450 as electron carriers in microsomal NADH-peroxidase activity. Arch Biochem Biophys **160**, 230–245 (1974)
20 Thornalley PJ, Trotta RJ and Stern A: Free radical involvement in the oxidative phenomena induced by *tert*-butyl hydroperoxide in erythrocytes. Biochim Biophys Acta **759**, 16–22 (1983)
21 Davies MJ: Detection of peroxy and alkoxyl radicals produced by reaction of hydroperoxides with heme-proteins by electron spin resonance spectroscopy. Biochim Biophys Acta **964**, 28–35 (1988)
22 Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW and Potter DW: High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide and related thiols and disulfide. Anal Biochem **106**, 55–62 (1980)
23 Habig WH, Pabst MJ and Jakoby WB: Glutathione *S*-transferase. J Biol Chem **249**, 7130–7139 (1974)
24 Reddy CC, Tu C-PD, Burgess JR, Ho C-Y, Scholz RW and Massaro EJ: Evidence for the occurrence of selenium-independent glutathione peroxidase activity in rat liver microsomes. Biochem Biophys Res Commun **101**, 970–978 (1981)
25 Buege JA and Aust SD: Microsomal lipid peroxidation. Methods Enzymol **52**, 302–310 (1977)
26 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem **193**, 265–275 (1951)
27 Maples KR, Kennedy CH, Jordan SJ and Mason RP: In vivo thiyl free radical formation from hemoglobin following administration of hydroperoxides. Arch Biochem Biophys **277**, 402–409 (1990)
28 Takenaka Y, Miki M, Yasuda H and Mino M: The effect of α-tocopherol as an antioxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites. Arch Biochem Biophys **285**, 344–350 (1991)
29 Maiorino M, Coassin M, Roveri A and Ursini F: Microsomal lipid peroxidation: Effect of vitamin E and its functional interaction with phospholipid hydroperoxide glutathione peroxidase. Lipids **24**, 721–726 (1989)
30 Younes M and Strubelt O: The role of iron and glutathione in *t*-butyl hydroperoxide-induced damage towards isolated perfused rat livers. J Appl Toxicol **10**, 319–324 (1990)
31 Younes Y and Wesss A: The role of iron in *t*-butyl hydroperoxide-induced lipid peroxidation and hepatotoxicity in rat. J Appl Toxicol **10**, 313–317 (1990)