DECOMPOSITION OF HYDROPEROXIDES DERIVED FROM MICROSOMES OR LIPOPROTEIN BY GLUTATHIONE PEROXIDASE AND GLUTATHIONE *S*-TRANSFERASE*

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(Received January 24, 1983)

Protective effects of glutathione peroxidase, glutathione *S*-transferase purified from human liver and superoxide dismutase against lipid peroxidation were investigated. In the presence of glutathione, lipid hydroperoxides found in microsomal membrane were decomposed by glutathione peroxidase and cationic glutathione *S*-transferase, but anionic glutathione *S*-transferase had no effect on them. Superoxide dismutase exhibited antioxidant effect by preventing accumulation of lipid hydroperoxides. Serum lipid hydroperoxides existing in low density lipoprotein fraction were also decomposed by glutathione peroxidase and cationic glutathione *S*-transferase. These findings suggest that the hydroperoxide level, which has high toxicity, could be controlled by these glutathione-dependent glutathione peroxidase and cationic glutathione *S*-transferase.

Keywords—glutathione peroxidase; glutathione *S*-transferase; superoxide dismutase; lipid peroxidation; microsomes; lipoprotein

INTRODUCTION

There has recently been great interest generated in the chemistry and the physiological action of lipid hydroperoxides. The formation and inhibition of lipid hydroperoxides had been investigated by Poyer and McCay,¹⁾ Fong *et al.*,²⁾ and Svingen *et al.*,³⁾ and the theory that lipid peroxidation proceeds by initiation step and propagation step of radical, which was presented by Svingen *et al.*³⁾ is coming to be accepted as the most viable mechanism of lipid peroxidation.

Lipid hydroperoxides cause some diseases, for example, diabetes,⁴⁾ retinopathy,⁵⁾ liver diseases,⁶⁾ and the pathogenesis is studied in relation to the serum lipid hydroperoxide level. Serum lipid hydroperoxides are thought to exist in lipoprotein fraction^{7,8)} and the correlations between its level and many diseases are now being studied by numerous investigators. For example, many studies have been carried out concerning atherosclerosis in respect to high density lipopro-

tein (HDL), low density lipoprotein (LDL) level in sera and lipid peroxide level in these fractions.^{9–11)} But the findings indicating that albumin fraction contains as much lipid hydroperoxides as lipoprotein fraction were presented by Miki,¹²⁾ therefore, which fraction is most responsible for carrying lipid peroxide remains to be studied.

Lipid peroxidation of biomembrane in some tissues and some organella was studied from the viewpoint of initiator and propagator of lipid peroxidation¹³⁾ or quencher and scavenger of active oxygens.¹⁴⁾ Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH peroxidase) are thought to be protective enzymes against lipid peroxidation and the action of SOD has been well studied.¹⁴⁾ GSH peroxidase, catalyzing the reduction of various peroxide compounds^{15,16)} can be speculated to inhibit lipid peroxidation by reducing membrane lipid hydroperoxides. Recently, McCay *et al.*¹⁷⁾ and

* This paper forms Part 207 of "Studies on Enzymes," by M. Sugiura.

Burk *et al.*¹⁸⁾ have reported that GSH peroxidase is not useful for prevention of microsomal lipid peroxidation, but that glutathione *S*-transferase (GSH *S*-transferase) is more effective in the prevention of lipid peroxidation in rats. Moreover, the GSH peroxidase activity of GSH *S*-transferase is chemically explained by Prohaska.¹⁹⁾

In this paper we assert that human hepatic GSH peroxidase and GSH *S*-transferase can reduce microsomal lipid hydroperoxides and lipid peroxides in human serum lipoprotein fraction.

MATERIALS AND METHODS

Materials—Cumene hydroperoxide and *tert*-butyl hydroperoxide were purchased from

Nakarai Chemicals Co. Other reagents were of reagent grade. Linolic acid hydroperoxide was prepared by rotating linolic acid as thin film as described by O'Brien.²⁰⁾

Enzymes—GSH peroxidase was purified from human liver as described in the previous report.²¹⁾ Cationic and anionic GSH *S*-transferase were purified from human liver according to the method of Awasthi *et al.*²²⁾ and SOD was purified from human erythrocytes by the method of McCord and Fridovich.²³⁾ Each enzyme activity was determined as described in the reports, respectively.

Preparation of Microsomes and Lipoprotein—Microsomes were prepared from the liver

FIG. 1. *Sephadex G-150 Gel Filtration of Soluble Fraction and Effect of Fractions on Malondialdehyde and Lipid Hydroperoxide Production in the NADPH-microsomal Lipid Peroxidation System*

Rat liver soluble fraction (condensed 10-fold by $(\text{NH}_4)_2\text{SO}_4$ precipitation) was applied to a Sephadex G-150 column (3×110 cm) equilibrated by 20 mM potassium phosphate, pH 7.0, containing 0.1 M NaCl. Eluate of 0.3 ml was added to the microsomal lipid peroxidation system, containing 2.5 mM GSH and incubated at 37°C for 40 min. Final volume was 4 ml.

of Wistar rats (200 g) according to the method of Burk *et al.*¹⁸⁾ and serum lipoprotein was prepared by ultracentrifugation from normal human serum which was adjusted to the density of 1.063 g/ml according to the method of DeLalla and Gofman.²⁴⁾

Determination of Lipid Peroxides — Lipid peroxidation was determined by measuring the malondialdehyde formation or by iodometric assay²⁵⁾ when microsomes were used, and in the experiment of serum lipoprotein peroxidation, thiobarbituric acid (TBA) method described by Yagi²⁶⁾ was used.

NADPH-microsomal Lipid Peroxidation System — This system was prepared according to the method of Burk *et al.*¹⁸⁾ with minor modifications. Details are as follows: 12 μ M FeCl₂, 4 mM ADP, 300 μ M NADPH, 5 mM glucose-6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase and microsomes from 0.4 g of liver were added to 50 mM Tris-HCl, pH 7.5, containing 140 mM NaCl to make a final volume of 4 ml and incubated at 37°C in the air. Aliquots were taken at the indicated times and malondialdehyde formation and lipid peroxide content were determined.

Lipoprotein Peroxidation System — Human serum lipoprotein 3 mg (determined for protein by the method of Lowry *et al.*²⁷⁾) was suspended in 5 ml of 50 mM Tris-HCl, pH 7.5, containing 140 mM NaCl, and incubated at 37°C in the air. Aliquots were taken and lipid hydroperoxide formation was determined by TBA method.

RESULTS AND DISCUSSION

Inhibitory Effect of Rat Liver Soluble Fraction on Lipid Peroxidation

Rat liver soluble fraction, which was fractionated by Sephadex G-150 gel filtration was investigated for its preventive effect on the production of malondialdehyde and lipid hydroperoxides in the NADPH-microsomal lipid peroxidation system. As shown in Fig. 1, the highest preventive effect was observed in fraction No. 62, almost coinciding with SOD activity, but we could not determine which particular enzyme

activity is responsible for prevention of malondialdehyde formation. These results were somewhat different from the report of Burk *et al.*,¹⁸⁾ who used the same peroxidation system and rat liver soluble fraction and reported that overlapping fractions of GSH peroxidase and GSH *S*-transferase activity have preventive effect in this system. The formation of malondialdehyde progress *via* lipid endoperoxide and lipid peroxy radicals, on the other hand, lipid hydroperoxides are made from lipid peroxy radicals by abstracting hydrogen atoms, so the formation of malondialdehyde and lipid hydroperoxides are differentiated in this process of radical reaction. However, by using iodometric assay for the determination of lipid hydroperoxides, the result was the same as in the malondialdehyde experiment. Nevertheless this result does not always refuse the mechanism of decomposition of lipid peroxides by GSH peroxidase and GSH *S*-transferase; and there are

FIG. 2. Effect of Enzymes on Lipid Hydroperoxide Production in the NADPH-microsomal Lipid Peroxidation System

The peroxidation system was incubated for 60 min prior to the additions of GSH and the following enzymes: —○— 2.5 mM GSH; —□— 2.5 mM GSH + 2 U GSH reductase; —△— 2.5 mM GSH + 2 U GSH reductase + 3.2 U GSH peroxidase; —●— 2.5 mM GSH + 2 U GSH reductase + 6 U anionic GSH *S*-transferase; —■— 2.5 mM GSH + 2 U GSH reductase + 6 U cationic GSH *S*-transferase; —×— none.

Final volume was 4 ml. Data were gathered from an average of two series of experiments.

two possible reasons for this: 1) whether the concentration of the enzyme overcomes the formation of hydroperoxides or not, 2) whether the enzymes decompose radical species or not. A tendency of lipid peroxidation stimulation effect is found in fraction No. 73, but this reason is not yet clear.

Effect of Preventive Enzymes on Peroxidized Microsomes

Rat enzymes did not show a precise antioxidant effect, and the enzymatic characteristics are different between rats and humans. In order to investigate the effect of human GSH peroxidase and GSH *S*-transferase on lipid peroxidation in detail, purified enzymes were applied to the microsomal lipid peroxidation system. As shown in Fig. 2, glutathione alone could prevent lipid peroxidation to a certain degree, but when either GSH peroxidase or cationic GSH *S*-transferase

was presented with glutathione, these enzymes could decompose the lipid hydroperoxides. Anionic GSH *S*-transferase was not effective in lipid peroxidation prevention. Furthermore, SOD alone showed preventive action on lipid peroxidation as shown in Fig. 3. From these results we can deduce that SOD effectively scavenges superoxide radical and prevent Fenton's reaction and also it deters other radical reaction on lipid peroxidation from commencing.

The theory that GSH peroxidase is responsible for prevention of lipid peroxidation²⁸⁾ has been denied in rats by some recent reports,^{17,18)} but the result in this paper shows that high concentration of human GSH peroxidase or cationic GSH *S*-transferase can decompose hydroperoxides from microsomes. And it can be speculated that high concentration of rat GSH peroxidase will prevent

FIG. 3. *Effect of Superoxide Dismutase on Lipid Hydroperoxide Production in the NADPH-microsomal Lipid Peroxidation System*

The peroxidation system was incubated for 40 min with the indicated amount of superoxide dismutase.

FIG. 4. *Effect of GSH-dependent Enzymes on the Peroxidation Occurring in Human Serum Lipoprotein*

Three mg of human serum lipoprotein was incubated with GSH-dependent enzymes in 5 ml of 50 mM Tris-HCl, pH 7.5, containing 140 mM NaCl. —○— 3 mM GSH, —□— 3 mM GSH + 5 U GSH reductase, —△— 3 mM GSH + 5 U GSH reductase + 5 U GSH peroxidase, —●— 3 mM GSH + 5 U GSH reductase + 5 U anionic GSH *S*-transferase, —■— 3 mM GSH + 5 U GSH reductase + 5 U cationic GSH *S*-transferase, —×— none.

TABLE I. K_m Value and Substrate Specificity of Glutathione Peroxidase and Glutathione Peroxidase Activity of Cationic and Anionic Glutathione *S*-Transferase

Substrate	Glutathione peroxidase		Anionic glutathione <i>S</i> -transferase		Cationic glutathione <i>S</i> -transferase	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
H ₂ O ₂	83.3 μ M	224 U/mg	n.d.	n.d.	n.d.	n.d.
Linolic acid hydroperoxide	63.5 μ M	217 U/mg	n.d.	n.d.	1.4 mM	7.9 U/mg
<i>tert</i> -Butyl hydroperoxide	33.3 μ M	183 U/mg	n.d.	n.d.	n.d.	n.d.
Cumene hydroperoxide	27.8 μ M	137 U/mg	n.d.	n.d.	0.54 mM	8.6 U/mg
1-Chloro-2,4-dinitrobenzene			0.83 mM	25.0 U/mg	0.46 mM	13.9 U/mg

n.d.: not detectable.

GSH concentration of 4 mM was used to determine the K_m value of hydroperoxides.

microsomal lipid peroxidation.

Effects of Protective Enzymes on Serum Lipoprotein Peroxidation System

Other substrate, hydroperoxides in lipoprotein was used in order to investigate the effect of the above enzymes on it. The pathogenesis of some diseases are thought to have some correlations with serum lipid hydroperoxide level. The origin of serum lipoprotein and lipoprotein's carrier action on lipid hydroperoxides is becoming clear. Whether the increase of serum lipid hydroperoxide level is the result or the cause of some diseases is now under discussion. Therefore, we isolated serum lipoprotein and elucidated the effect of glutathione-dependent enzymes on lipid hydroperoxides existing in this lipoprotein fraction. As shown in Fig. 4, the content of lipid hydroperoxides increase with incubation time, but the addition of GSH peroxidase or cationic GSH *S*-transferase prevented the increase of lipid hydroperoxides although the decomposition of

lipid hydroperoxides was also found. However, anionic GSH *S*-transferase had no effect on this system.

K_m and V_{max} of Enzymes

Cationic GSH *S*-transferase reduced the synthetic substrates with relatively low molecular weight, but anionic GSH *S*-transferase exhibited no activity on these substrates as shown in Table I. Furthermore, the ability of cationic GSH *S*-transferase to decompose substrates decreased with the size, and is smaller than that of GSH peroxidase. While in this experiment macromolecules such as microsomal membrane and lipoprotein were decomposed by two GSH dependent enzymes, SOD did not attack the lipid hydroperoxides, since its substrate specificity shows no reactivity. In the human body, GSH concentration is physiologically controlled at the 3–6 mM level,²⁹⁾ and K_m 's of GSH peroxidase and cationic GSH *S*-transferase were 3–5 mM and 0.3 mM, respectively.^{21,22)} The results of these

experiments suggest that both enzymes can be responsible for decomposition of hydroperoxides in human, which are generated under physiological conditions. Thus, further investigation is being undertaken to determine the content of GSH peroxidase and cationic GSH *S*-transferase in human tissue and serum.

Acknowledgement The authors wish to thank S.J. Kay for her critical reading of this manuscript.

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