Biochemical Pharmacology, Vol. 45, No. 8, pp. 1645–1651, 1993.
Printed in Great Britain.

0006–2952/93 $6.00 + 0.00

EVIDENCE THAT RAT LIVER MICROSOMAL GLUTATHIONE TRANSFERASE IS RESPONSIBLE FOR GLUTATHIONE-DEPENDENT PROTECTION AGAINST LIPID PEROXIDATION

ERIFILI MOSIALOU,* GUNILLA EKSTRÖM,† ANTON E. P. ADANG‡ and RALF MORGENSTERN

Department of Toxicology and †Department of Physiological Chemistry, Karolinska Institutet, Box 60400, S-10401 Stockholm, Sweden; and ‡Department of Organic Chemistry, Division of Toxicology, Center for Bio-Pharmaceutical Sciences, University of Leiden, Leiden, The Netherlands

(*Received* 8 *April* 1992; *accepted* 7 *January* 1993)

Abstract—Evidence that rat liver microsomal glutathione transferase is responsible for the glutathione-dependent inhibition of lipid peroxidation in liver microsomes has been obtained. Activation of the microsomal glutathione transferase in microsomes by cystamine renders this organelle even more resistant to lipid peroxidation in the presence of glutathione compared with untreated microsomes. Upon examining the effect of seven glutathione analogues on lipid peroxidation, it was found that only those that serve as good substrates for the microsomal glutathione transferase (Glutaryl-L-Cys-Gly and α-L-Glu-L-Cys-Gly) can inhibit lipid peroxidation. The lack of inhibition by the other five analogues (α-D-Glu-L-Cys-Gly, γ-D-Glu-L-Cys-Gly, β-L-Asp-L-Cys-Gly, α-L-Asp-L-Cys-Gly and α-D-Asp-L-Cys-Gly) shows the specificity of the protection and rules out any non-enzymic component. Inhibitors of selenium-dependent glutathione peroxidase (mercaptosuccinate at 50 μM) and phospholipid hydroperoxide glutathione peroxidase (iodoacetate, 1 mM + glutathione, 0.5 mM) do not inhibit the glutathione-dependent protection of rat liver microsomes against lipid peroxidation. Purified microsomal glutathione transferase, NADPH-cytochrome P450 reductase and cytochrome P450 were reconstituted in microsomal phospholipid vesicles by cholate dialysis. The resulting membranes contained functional enzymes and did display enzymic lipid peroxidation induced by 75 μM NADPH and 10 μM Fe-EDTA (2:1). This model system was used to investigate whether microsomal glutathione transferase could inhibit lipid peroxidation in a glutathione-dependent manner. The results show that 5 mM glutathione did inhibit lipid peroxidation when functional microsomal glutathione transferase was included. This was not the case when the enzyme had been pre-inactivated with diethylpyrocarbonate. Furthermore, the protective effect of glutathione could be partly reversed by an inhibitor (100 μM bromosulphophtalein) of the enzyme. Apparently, rat liver microsomal glutathione transferase has the capacity to inhibit lipid peroxidation in a reconstituted system.

It has long been known that rat liver microsomes contain a factor that can protect these vesicles against lipid peroxidation in a glutathione-dependent manner [1]. The protection is evident as a marked prolongation of the lag phase before onset of lipid peroxidation. The factor has been shown to be sensitive to heat treatment [2], proteases [2], sulphydryl reagents [3] and a number of glutathione transferase inhibitors [4, 5], as well as being largely dependent on the presence of vitamin E in the membrane [6]. On the basis of the above observations it has been concluded that the protective factor is a protein and two hypotheses for its function have been put forward: (1) the protein functions as a glutathione-dependent vitamin E radical reductase [7] and (2) the protein is identical to the microsomal glutathione transferase [8], the glutathione peroxidase activity of which can reduce lipid hydroperoxides [5]. These mechanisms can conceivably protect membranes from lipid peroxidation by (1) maintaining the vitamin E level and (2) eliminating hydroperoxides which support further lipid peroxidation. In the latter case vitamin E is required to form hydroperoxides by reacting with peroxyl radicals. The latter mechanism also has precedence in the fact that the phospholipid hydroperoxide glutathione peroxidase does protect membranes in this way [9].

Our earlier findings that a range of inhibitors for the microsomal glutathione transferase can inhibit glutathione-dependent protection against lipid peroxidation in rat liver microsomes and that the microsomal glutathione transferase functions as a glutathione peroxidase with phosphatidylcholine dilinoleoylhydroperoxide and linoleic acid hydroperoxide, as well as a glutathione transferase with hydroxynonenal, strongly indicated the capacity of this protein to protect against lipid peroxidation [5]. In this study we describe additional experiments that lend support to this conclusion. A preliminary report of part of these data has appeared [10].

MATERIALS AND METHODS

Glutathione analogues were synthesized as

* Corresponding author. Tel. (46) 8 7287555; FAX (46) 8 334467.

described [11]. NADPH, *N*-ethylmaleimide, diethylpyrocarbonate and glutathione were obtained from the Sigma Chemical Co. (MO, U.S.A.). Bromosulphophtalein and 1-chloro-2,4-dinitrobenzene were from Merck (Darmstadt, Germany). All other chemicals were of highest purity and obtained from common commercial sources.

Rat liver microsomes and microsomal glutathione transferase were prepared as described earlier [12, 13]. The specific activity of microsomal glutathione transferase with 0.5 mM 1-chloro-2,4-dinitrobenzene was measured as described [13].

Lipid peroxidation in microsomes was started by the addition of ascorbate (0.5 mM) and Fe^{3+}-ADP (6 μM–2 mM) to freshly prepared microsomes (0.3–0.9 mg protein/mL) in 50 mM Tris–HCl, 0.14 M NaCl, pH 7.4, at 37° as described by Burk [2], and assayed by determination of thiobarbituric acid (TBA) reactive substances [14] expressed as malonaldehyde equivalents ($\varepsilon_{535} = 156\ mM^{-1}\ cm^{-1}$). Glutathione and glutathione analogues at the concentrations indicated in the figures were added before Fe^{3+}-ADP and all experiments were performed at least twice.

In order to inhibit the selenium-dependent glutathione peroxidase, mercaptosuccinate (50 μM) [15] was added before Fe^{3+}-ADP as described above ± glutathione (5 mM) and lipid peroxidation was followed for 60 min at 10 min intervals. Phospholipid hydroperoxide glutathione peroxidase was inhibited by preincubation of microsomes (10–20 mg/mL) in 50 mM Tris–HCl, 0.14 M NaCl with 1 mM iodoacetate and 0.5 mM glutathione [16] on ice for 10–100 min before being added to incubations and lipid peroxidation was followed for 60 min at 10 min intervals. This treatment did not result in activation of the microsomal glutathione transferase activity. These experiments were performed twice.

Activation of the microsomal glutathione transferase activity in microsomes by *N*-ethylmaleimide: microsomes (10–20 mg/mL in 50 mM Tris–HCl, 0.14 M NaCl, were incubated with 0.2 or 1 mM *N*-ethylmaleimide on ice for 10–100 min before being added to incubations. This consistently resulted in a 4–6-fold increase of the activity towards 1-chloro-2,4-dinitrobenzene.

Activation of the microsomal glutathione transferase activity in microsomes by cystamine: microsomes (10–20 mg/mL) in 50 mM Tris–HCl, 0.14 M NaCl, were incubated with 10 mM cystamine on ice for 10–20 min and subsequently diluted 5-fold with 0.15 M Tris–HCl, pH 8, and centrifuged at 100,000 *g* for 30 min to remove excess cystamine. The pellet was rinsed and resuspended in the original volume of 50 mM Tris–HCl, 0.14 M NaCl, before being added to the incubations. This treatment consistently resulted in a 4–6-fold increase of the activity towards 1-chloro-2,4-dinitrobenzene.

Thiol content in control and treated microsomes (20 mg/mL) was measured with 2,2′-dipyridyl disulphide in 1% sodium dodecyl sulphate, 0.1 M sodium phosphate at pH 7 [17]. The amount of thiol was 50 nmol/mg protein in control microsomes.

Microsomal phospholipids were extracted from rat liver microsomes and separated on a silica gel column [18]. The lipids were stored in sealed tubes under nitrogen at −18°.

Electrophoretically homogenous preparations of NADPH-cytochrome P450 reductase (20–22 nmol flavin/mg) and cytochrome P450 (CYP2B1) (10.5–13 nmol/mg) were prepared from liver microsomes of phenobarbital-treated rabbits according to methods of Yasukochi and Masters [19] and Haugen and Coon [20], respectively. The activity of NADPH-cytochrome P450 reductase was determined at 30° in 0.33 M potassium phosphate buffer, pH 7.6, by monitoring the reduction of cytochrome *c* at 550 nm. Cytochrome P450 was determined according to Omura and Sato [21]. Oxidation of NADPH was quantified spectrophotometrically at 340 nm using the absorption coefficient $6.2\ mM^{-1}\ cm^{-1}$.

Activation of purified microsomal glutathione transferase with 5 mM *N*-ethylmaleimide was performed for 5 min on ice and the reaction was terminated by the addition of an equimolar amount of glutathione (activation was usually 10-fold). Inactivation of microsomal glutathione transferase was performed with 2 mM diethylpyrocarbonate for 20 min on ice (inactivation was more than 90%).

Membrane vesicles were prepared in the following way: 20 mg of microsomal phospholipid dissolved in chloroform–methanol (2:1) was dried under a stream of nitrogen, 0.2 mL of 20% cholate was added and the mixture was sonicated until the solution became transparent. Buffer (1.8 mL) containing 10 mM potassium phosphate, pH 7.0, 20% glycerol, 0.1 mM EDTA, 50 mM potassium chloride (referred to as buffer E) and 2 mg of purified activated or inactivated (we chose to use inactivated enzyme as a control in view of the effect that protein content could have on lipid peroxidation) microsomal glutathione transferase in 10 mM potassium phosphate, 0.1 mM EDTA, 1% Triton X-100, 1 mM glutathione, 20% glycerol, 0.1 M KCl (vol. 2–4 mL) was added. The enzyme–phospholipid solution was then dialysed for 72 hr against buffer E containing 1 mM glutathione and 0.05% cholate (2 changes/24 hr) and then reductase (5000 U) and cytochrome P450 (10 nmol) were added. The dialysis was continued for an additional 48 hr against buffer E (2 changes/24 hr) and subsequently against 10 mM potassium phosphate, pH 7, 0.05 M KCl (48 hr with 2 changes/24 hr). The proteoliposome solution was kept under nitrogen and stored at 4°. Recovery of the cytochrome P450 system was >80% and the activity of microsomal glutathione transferase was approximately 5 μmol/min mg (2-fold activation). The latter value is lower than what was expected from the initial activation but reasonable in view of the slow gradual decline of activity during dialysis and storage. Inactivated microsomal glutathione transferase activity was not measurable after dialysis.

Reaction mixtures contained membranes corresponding to 0.25 nmol cytochrome P450 and 10 μM Fe:EDTA (2:1) in 50 mM potassium phosphate buffer, pH 7.4 in a final volume of 1 mL. The incubations were started by the addition of 75 μM NADPH at 30°. Aliquots of the incubation mixtures (37.5 μL) were taken at defined time points and mixed with 25 μL 40% trichloroacetic acid, 12.5 μL 5 M HCl and 25 μL 2% TBA [20]. In control

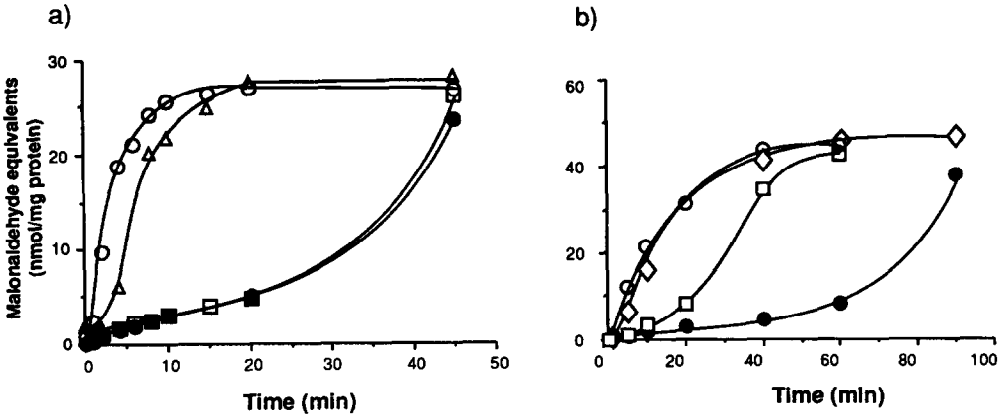

Fig. 1. Malonaldehyde equivalent formation in rat liver microsomes undergoing non-enzymic lipid peroxidation. (a) *N*-Ethylmaleimide (0.2 mM)-pretreated microsomes in the absence of added glutathione (○); in the presence of 0.1 mM (△) or 5 mM (□) glutathione. Untreated microsomes in the presence of 5 mM (●) glutathione. (b) *N*-Ethylmaleimide (1 mM)-pretreated microsomes in the absence of added glutathione (○); in the presence of 5 mM (□) glutathione. Untreated microsomes in the absence of (◊) or presence of 5 mM (●) glutathione. Experimental details are described in Materials and Methods.

incubations, NADPH was added after HCl and the absorbance was subtracted from the corresponding sample. The results are representative of two to three experiments.

Protein was determined by the method of Peterson [22] with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

To determine whether a certain activity (in this case glutathione-dependent protection against lipid peroxidation) can be attributed to a specific protein, in a complex system such as microsomes, two approaches can be adopted. First, one can compare known characteristics of the purified enzyme to the properties of the microsomal activity. Second, one can elucidate whether the mechanism of the activity is compatible with the function of the protein in question. In the present study we have taken the former approach to investigate if known properties of the microsomal glutathione transferase (sulphydryl reagent activation and limited substrate specificity towards glutathione analogues) correspond to those of the glutathione-dependent factor that protects against lipid peroxidation.

It was shown earlier that sulphydryl reagents and proteolysis abolish the protective effect of glutathione against lipid peroxidation [2, 3]. This would seem to rule out a role for the microsomal glutathione transferase in mediating this protection, since this enzyme is activated by sulphydryl reagents and proteolysis. However, the antioxidant capacity of membrane sulphydryls (which is lost upon treatment with sulphydryl reagents or proteases) is critical as such, as shown by Takenaka *et al.* [23].

Reexamination of the effect of treating microsomes with 1 mM *N*-ethylmaleimide revealed that a substantial part (but not all as claimed earlier [3]) of the protection against lipid peroxidation afforded by 5 mM glutathione is removed (Fig. 1). *N*-Ethylmaleimide pretreatment at a lower concentration (0.2 mM) does not abolish the glutathione-dependent protection against lipid peroxidation, and there is some protection even at a low glutathione concentration (0.1 mM) (Fig. 1a).

Since both concentrations of *N*-ethylmaleimide effectively activate the microsomal glutathione transferase the results are consistent with a balance between activating this enzyme and the blocking of other sulphydryls that serve an antioxidant role or are essential for the activity of another enzyme.

The results prompted us to try another sulphydryl reagent with more hydrophilic characteristics [cystamine, that has a more limited access to sulphydryls within the membrane (see below)] to examine whether, indeed, the factor responsible for protection could be activated. As is evident in Fig. 2, there is a marked increase in glutathione-dependent protection against lipid peroxidation by pretreating microsomes with cystamine at a concentration that activates the microsomal glutathione transferase.

The lack of activation of the protection by *N*-ethylmaleimide versus the effectiveness of cystamine could be a consequence of different access to membrane sulphydryls (under our incubation conditions 0.2, 1 mM *N*-ethylmaleimide and 10 mM cystamine react with 20%, 60% and 14% of the microsomal thiol groups, respectively) but also of the different type of bonds formed (thioether vs mixed disulphide).

The glutathione peroxidase activity of the purified microsomal glutathione transferase is increased by sulphydryl reagents [5, 13], and it is therefore reasonable to assume that activation of the enzyme can improve its ability to protect membranes against oxidative stress.

In order to test whether phospholipid hydroperoxide glutathione peroxidase (which is known to associate to some degree with intracellular

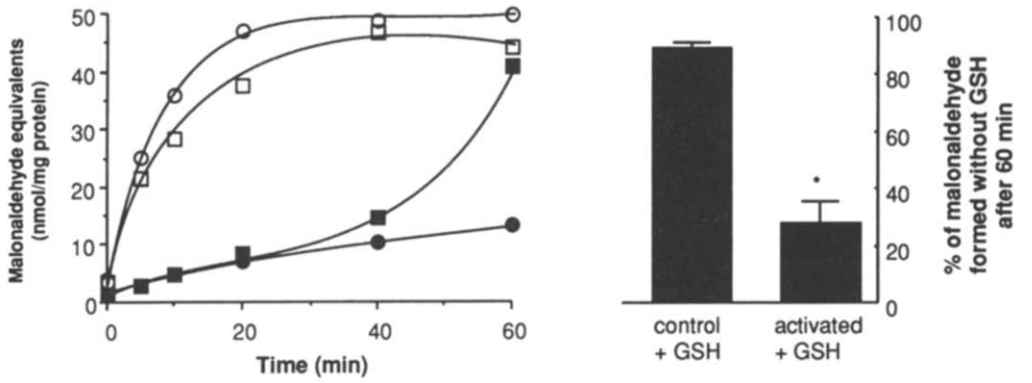

Fig. 2. Malonaldehyde equivalent formation in rat liver microsomes undergoing non-enzymic lipid peroxidation. Untreated microsomes without (□) and with (■) 5 mM glutathione added. Cystamine-treated microsomes without (○) and with (●) 5 mM glutathione added. The bar diagram shows the mean % (± SEM, N = 3) of malonaldehyde equivalents formed in the presence of glutathione compared to the amount formed in the absence of glutathione at 60 min of incubation (which is 100%) for the control (untreated) and cystamine-activated microsomes (*$P < 0.001$ using Student's t-test). Experimental details are described in Materials and Methods.

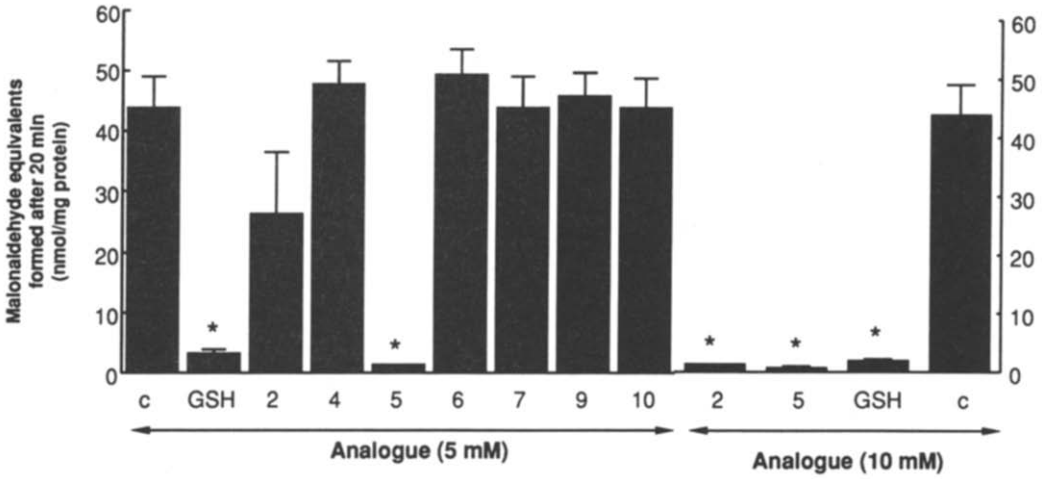

Fig. 3. Malonaldehyde equivalent formation after 20 min in rat liver microsomes undergoing non-enzymic lipid peroxidation in the presence of glutathione and glutathione analogues. Glutathione (GSH), Glutaryl-L-Cys-Gly (2), γ-D-Glu-L-Cys-Gly (4), α-L-Glu-L-Cys-Gly (5), α-D-Glu-L-Cys-Gly (6), β-L-Asp-L-Cys-Gly (7), α-L-Asp-L-Cys-Gly (9) and α-D-Asp-L-Cys-Gly (10) were included at 5 and 10 mM concentrations as indicated. Control without added analogue or GSH (c). Values are means ± SEM, N = 3 (5 and 10 mM). An * denotes $P < 0.001$ using Students t-test. Experimental details are described in Materials and Methods. The specific activity of microsomal glutathione transferase with 0.5 mM 1-chloro-2,4-dinitrobenzene and 5 mM analogue is 5, 1, 0.08 and 4 μmol/min mg towards Glutaryl-L-Cys-Gly (2), α-L-Glu-L-Cys-Gly (5), α-D-Glu-L-Cys-Gly (6) and glutathione, respectively (the other analogues had no detectable activity) [32].

membranes [24]) is also involved in protecting microsomes against lipid peroxidation under these conditions, we examined the effect of pretreating microsomes with iodoacetate together with glutathione [16]. Also, any possible involvement of the classical selenium-dependent glutathione peroxidase was tested by the incorporation of mercaptosuccinate during assay of glutathione-dependent protection against lipid peroxidation. None of the inhibitors had any effect on glutathione-dependent protection (not shown) which is in agreement with the findings of Bast and Haenen [25]. Based on experiments with microsomes contaminated to different degrees with cytosol, Burk [2] also concluded that contaminating cytosolic factors could be ruled out. In this context, however, it is pertinent to point out that the addition of several cytosolic factors (including selenium-dependent phospholipid hydroperoxide glutathione

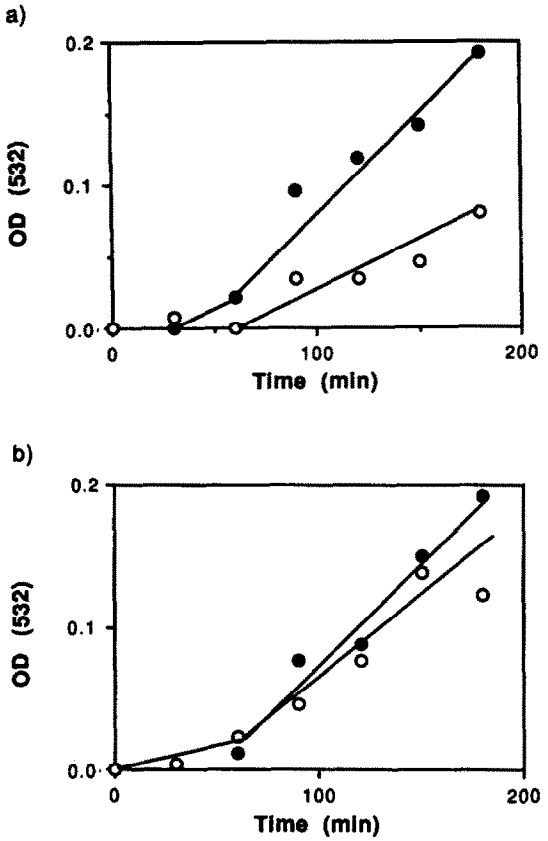

Fig 4. TBA-reactive material produced during enzymic lipid peroxidation induced by 75 μM NADPH and 10 μM Fe-EDTA (2:1) in reconstituted membranes with NADPH-cytochrome P450 reductase and cytochrome P450 together with activated (a) and inactivated (b) microsomal glutathione transferase (●). The effect of including 5 mM glutathione (○). Details are described in Materials and Methods.

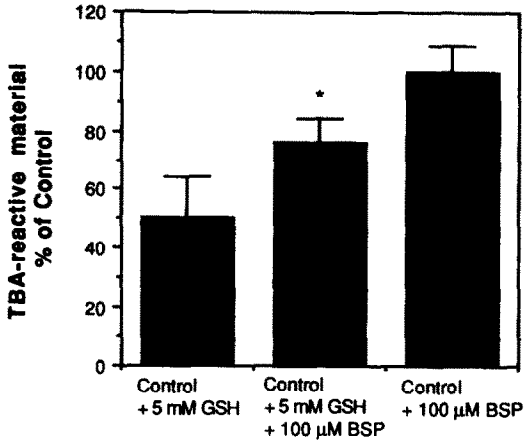

Fig. 5. The effect of 100 μM bromosulphophtalein on glutathione-dependent protection against lipid peroxidation in reconstituted membranes. Conditions are the same as described for Fig. 1, with native microsomal glutathione transferase. The incubation time was 60 min [with 6.8 ± 0.68 (N = 3) nmol TBA-reactive substances being formed/nmol P450 min]. * Denotes that the inhibitory effect on protection seen by bromosulphophtalein is statistically significant (Student *t*-test, $P < 0.05$).

peroxidase [9], selenium-dependent glutathione peroxidase [26] and cytosolic glutathione transferase [27]) can protect membranes from lipid peroxidation in a glutathione-dependent manner either alone (selenium-dependent phosphoplipid hydroperoxide glutathione peroxidase) or in conjunction with phospholipase A_2. Thus, in the intact cell, the glutathione-dependent protection against lipid peroxidation most likely includes multiple defence systems.

The protection against lipid peroxidation in rat liver microsomes has been shown to be specific for glutathione insofar as cysteine, mercaptoethanol and dithiothreitol cannot substitute for this compound [2]. This is another observation that speaks against a role for the phospholipid hydroperoxide glutathione peroxidase (in this system) since this enzyme displays a very broad specificity towards thiols [9].

The structural features of the γ-glutamyl residue in glutathione that are essential for its function in protection against lipid peroxidation were compared with the requirements of the purified microsomal glutathione transferase (Fig. 3). Clearly, the two analogues that can inhibit lipid peroxidation in microsomes are also good substrates (see legend to Fig. 3) for the microsomal glutathione transferase. The ineffective analogues are not substrates, except for α-D-Glu-L-Cys-Gly, which is a very poor substrate. This lends strong support to the conclusion that microsomal glutathione transferase inhibits lipid peroxidation. Furthermore, the subtle alterations (having little effect on chemical reactivity [11]) made in the five ineffective analogues show that there is apparently no non-enzymic component involved in the protection by glutathione.

As can be seen (Fig. 3) α-L-Glu-L-Cys-Gly is effective at inhibiting lipid peroxidation both at 5 and 10 mM, whereas Glutaryl-L-Cys-Gly is less effective at 5 mM. This is somewhat surprising in view of the higher specific activity seen with the latter. It must be remembered, however, that we are comparing, on the one hand, the ability of the analogues to inhibit lipid peroxidation in microsomes, where the capacity to serve as a substrate for the microsomal glutathione transferase as a glutathione peroxidase is presumably critical, and on the other hand, the capacity of the analogue to serve as a substrate for the purified microsomal glutathione transferase in the conjugation of 1-chloro-2,4-dinitrobenzene. Therefore, more subtle comparison is as yet premature. Work to characterize phospholipid hydroperoxides and glutathione analogues as substrates in relationship to each other and glutathione is under way in our laboratory.

The work described here does in no way exclude the existence of a glutathione-dependent vitamin E radical reductase. However, if this were the sole enzyme responsible for glutathione-dependent

protection against lipid peroxidation in microsomes, it would have to share with the microsomal glutathione transferase the property of being activated by cystamine, its restricted use of glutathione analogues and inhibitor sensitivity [5, 10]. Also, recent work using ESR techniques gave no evidence for the existence of a vitamin E radical reductase that utilizes glutathione [28].

Another set of experiments involving reconstitution of microsomal glutathione transferase into liposomes together with cytochrome P450 and NADPH-cytochrome P450 reductase (as an enzymic lipid peroxidation system [29, 30]) were performed to investigate whether the enzyme can inhibit lipid peroxidation. Incubation of membrane vesicles containing cytochrome P450, NADPH-cytochrome P450 reductase, microsomal glutathione transferase and microsomal phospholipids with Fe-EDTA and NADPH resulted in a time-dependent production of TBA-reactive products (Fig. 4). Two different sets of membranes were prepared, one containing inactivated microsomal glutathione transferase and one containing activated enzyme. The rate of reaction was independent of the state of activity of the glutathione transferase in the absence of added glutathione (Fig. 4). When 5 mM glutathione was added to the reaction mixture, a prolonged lag phase was observed only with the membranes containing the active form of microsomal glutathione transferase (Fig. 4a). This demonstrates the ability of rat liver microsomal glutathione transferase to inhibit lipid peroxidation and is relevant to the *in vivo* situation in the respect that enzymatic lipid peroxidation was employed utilizing a microsomal cytochrome P450 system and phospholipids. To further confirm the involvement of microsomal glutathione transferase, the effect of bromosulphophtalein, an inhibitor of the enzyme [31], was investigated. It was shown that the compound could reverse the glutathione-dependent protection against lipid peroxidation in this system (Fig. 5) whereas it did not affect the extent of lipid peroxidation without added glutathione. This finding is in agreement with those of Yonoha and Tampo [4] and Mosialou and Morgenstern [5] who showed that bromosulphophtalein inhibits glutathione-dependent protection of lipid peroxidation in microsomes.

In summary, the results reported here together with earlier findings [5] indicate strongly that rat liver microsomal glutathione transferase can inhibit lipid peroxidation and remove harmful products of lipid peroxidation. These functions of the enzyme put it in the category of proteins that can protect the organism under conditions of oxidative stress.

Acknowledgements—This work was supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council and funds from the Karolinska Institute, Lars Hiertas Minne and Alex och Eva Wallströms stiftelse. Support by stiftelsen Bengt Lundqvists Minne (of E. M.) is gratefully acknowledged.

REFERENCES

1. Cristophersen BO, The inhibitory effect of reduced glutathione on the lipid peroxidation of the microsomal fraction and mitochondria. *Biochem J* **106**: 515–522, 1968.
2. Burk RF, Glutathione-dependent protection by rat liver microsomal protein against lipid peroxidation. *Biochim Biophys Acta* **757**: 21–28, 1983.
3. Haenen GRMM, Tai Tin Tsoi JNL, Vermeulen NPE, Timmerman H and Bast A, 4-hydroxy-2,3-*trans*-nonenal stimulates microsomal lipid peroxidation by reducing the glutathione-dependent protection. *Arch Biochem Biophys* **259**: 449–456, 1987.
4. Yonoha M and Tampo Y, Studies on the protection by glutathione against lipid peroxidation in rat liver microsomes. Effect of bromosulfophtalein. *Chem Pharm Bull* **10**: 4195–201, 1986.
5. Mosialou E and Morgenstern R, Activity of rat liver microsomal glutathione transferase towards products of lipid peroxidation and studies on the effect of inhibitors on glutathione-dependent protection against lipid peroxidation. *Arch Biochem Biophys* **275**: 289–294, 1989.
6. Hill KE and Burk RF, Influence of vitamin E and selenium on glutathione-dependent protection against microsomal lipid peroxidation. *Biochem Pharmacol* **33**: 1065–1068, 1984.
7. Haenen GRMM and Bast A, Protection against lipid peroxidation by a glutathione-dependent labile factor. *FEBS Lett* **159**: 24–28, 1983.
8. Morgenstern R and DePierre JW, Microsomal glutathione transferases. In: *Reviews in Biochemical Toxicology* (Eds. Hodgson E, Bend JR and Philpot RM), pp. 67–84. Elsevier Scientific Publishing, New York, 1985.
9. Ursini F, Maiorino M and Gregolin C, The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim Biophys Acta* **839**: 62–70, 1985.
10. Morgenstern R, Lundqvist G, Mosialou E and Andersson C, Membrane-bound glutathione transferase: function and properties. In: *Glutathione S-Transferases and Drug Resistance* (Eds. Hayes JD, Pickett BC and Mantle TJ), pp. 57–64. Taylor & Francis, London, 1990.
11. Adang AEP, Duindam AJG, Brussee J, Mulder GJ and Van Der Gen A, Synthesis and nucleophilic reactivity of a series of glutathione analogues, modified at the γ-glutamyl moiety. *Biochem J* **255**: 715–720, 1988.
12. Morgenstern R, Meijer J, DePierre JW and Ernster L, Characterization of rat liver microsomal glutathione transferase activity. *Eur J Biochem* **104**: 167–174, 1980.
13. 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.
14. Aust SD, *CRC Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald E). CRC Press, Boca Raton, Florida, 1985.
15. Chaudiere J, Wilhelmsen EC and Tappel AL, Mechanism of selenium-glutathione peroxidase and its inhibition by mercaptocarboxylic acids and other mercaptans. *J Biol Chem* **259**: 1043–1050, 1984.
16. Maiorino M, Roveri A, Coassin M and Ursini F, Kinetic mechanism and substrate specificity of glutathione peroxidase activity of ebselen (PZ51). *Biochem Pharmacol* **37**: 2267–2271, 1988.
17. Grassetti DR and Murray Jr JF, Determination of sulfhydryl groups with 2,2′- or 4,4′-dithiopyridine. *Arch Biochem Biophys* **119**: 41–49, 1967.
18. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917, 1959.
19. Yasukochi Y and Masters BSS, Some properties of detergent-solubilized NADPH-cytochrome *c* (cyto-

chrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344, 1976.
20. Haugen DA and Coon MJ, Properties of electrophoretically homogenous phenobarbital-inducible and β-naphtoflavone-inducible forms of liver microsomal cytochrome P-450. *J Biol Chem* **251**: 7929–7939, 1976.
21. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. 1. Evidence for its hemoprotein nature. *J Biol Chem* **234**: 2370–2378, 1964.
22. Peterson GL, A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Anal Biochem* **83**: 346–356, 1977.
23. Takenaka Y, Miki M, Yasuda H and Mino M, The effect of alpha-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.
24. Ursini F, Maiorino M and Gregolin C, A glutathione peroxidase active on phospholipid hydroperoxides protects phosphatidylcholine liposomes and biomembranes from peroxidation. In: *Oxy Radicals and their Scavenger Systems* (Eds. Greenwald RA and Cohen G), pp. 224–229. Elsevier Publishing Co., New York, 1983.
25. Bast A and Haenen GRMM, Cytochrome P-450 and vitamin E free radical reductase: formation and protection against free radicals. In: *Free Radicals, Lipoproteins, and Membrane Lipids* (Eds. Crastes de Poulet A, Douste-Blazy L and Paoletti R), pp. 359–370. Plenum Press, New York, 1990.
26. Sevanian A, Muakkassah-Kelly S and Montestruque S, The influence of phospholipase A2 and glutathione peroxidase on the elimination of membrane lipid peroxides. *Arch Biochem Biophys* **223**: 441–452, 1983.
27. Tan KH, Meyer DJ, Belin J and Ketterer B, Inhibition of lipid peroxidation by glutathione and glutathione transferases B and AA. *Biochem J* **220**: 243–252, 1984.
28. Packer L, Maguire JJ, Melhorn RJ, Serbinova E and Kagan VE, Mitochondria and microsomal membranes have a free radical reductase activity that prevents chromanoxyl radical accumulation. *Biochem Biophys Res Commun* **159**: 229–235, 1989.
29. Ekström G and Ingelman-Sundberg M, Mechanisms of lipid peroxidation dependent upon cytochrome P-450 LM_2. *Eur J Biochem* **158**: 195–201, 1986.
30. Ekström G and Ingelman-Sundberg M, Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450. *Biochem Pharmacol* **38**: 1313–1319, 1989.
31. Mosialou E and Morgenstern R, Inhibition studies on rat liver microsomal glutathione transferase. *Chem Biol Interact* **74**: 275–280, 1990.
32. Andersson C, Mosialou E, Adang AEP, Mulder GJ, Vandergen A and Morgenstern R, Studies on the activity and activation of rat liver microsomal glutathione transferase with a series of glutathione analogues. *J Biol Chem* **266**: 2076–2079, 1991.