Lipid and Steroid Hydroperoxides as Substrates for the Non-Selenium-Dependent Glutathione Peroxidase

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The reduction of linoleic acid hydroperoxide catalysed by rat liver cytosol was previously shown to be catalysed by a selenium-dependent glutathione peroxidase. In contrast, the enzyme responsible in guinea-pig liver cytosol is not selenium-dependent and appears to be a glutathione transferase.

Glutathione peroxidase (EC 1.11.1.9), an enzyme that catalyses the reduction of H_2O_2 to water using glutathione as the donor substrate, was first discovered in erythrocytes (Mills, 1957). It was later found to act on lipid and steroid hydroperoxides (O'Brien & Little, 1967, 1969; Little & O'Brien, 1968; Little *et al.*, 1970) according to the following general equation:

In 1973 it was found to contain 4g-atoms of selenium per 84000 mol.wt. (Flohé *et al.*, 1973; Oh *et al.*, 1974). Recently another enzyme (isoenzyme II) with glutathione peroxidase activity of mol.wt. approx. 45000, which was active with cumene hydroperoxide but not with H_2O_2 , was found in rat brain and liver and persisted in severe selenium deficiency (Prohaska & Ganther, 1976; Lawrence & Burk, 1976). It was also found that this isoenzyme from various tissues co-purified with the glutathione transferases (Prohaska & Ganther, 1977). The much lower V_{max} and higher K_m for cumene hydroperoxide of isoenzyme II as compared with isoenzyme I makes it unlikely that the former plays any role in the normal rat liver if perfused with cumene hydroperoxide. However, as yet, the specificity of isoenzyme II with respect to other hydroperoxides, particularly those that may occur physiologically, e.g. lipid and steroid hydroperoxides, has not been investigated and consequently the physiological relevance of this peroxidase is not known. Because Lawrence & Burk (1978) have shown that the isoenzyme II is responsible for all of the glutathione peroxidase activity in the guinea-pig liver, we have investigated the enzyme responsible for the reduction of fatty acid hydroperoxides and steroid hydroperoxides in this tissue. This is the first report that glutathione peroxidase II can accept physiological peroxides as substrate.

Materials and Methods

NADPH (type I), glutathione reductase (type III) and *cis*-linoleic acid (grade II) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. $\text{Na}_2^{75}\text{SeO}_3$ was obtained from Amersham Corp., Oakville Ont., Canada.

Linoleic acid hydroperoxide was prepared and stored as described by O'Brien & Little (1969). Progesterone-17 hydroperoxide was supplied by Dr. J. E. Van Lier, Department of Biochemistry, University of Sherbrooke, Sherbrooke, Que., Canada.

Sephadex chromatography of the liver cytosol was carried out as follows. A male albino guinea pig (approx. 300g) was killed by ether anaesthesia. The liver was perfused with 1.14M-NaCl and then homogenized in 0.25M-sucrose (1:2, w/v). The homogenate was centrifuged at 100000g for 60min, and the resulting supernatant was centrifuged again at 125000g for 40min. The supernatant from the second centrifugation was decanted and frozen at -80°C . A 2.7ml portion of the 125000g supernatant was thawed and pumped on to a column (2.6cm $\times$ 26cm) of Sephadex G-100 that had previously been equilibrated with a pH8.6 buffer containing 0.05M-Tris/HCl, 0.1M- K_2HPO_4 and 1mM-disodium EDTA; the column was eluted via reverse flow. Recovery of the peroxidase activity with linoleic acid hydroperoxide was 62%. All procedures were carried out at 2°C .

The incorporation *in vivo* of ^{75}Se was carried out as follows. A male albino guinea pig (approx. 300g) was injected intraperitoneally with $75\mu\text{Ci}$ of $\text{Na}_2^{75}\text{SeO}_3$ and fed on a normal diet and water *ad lib*. for 2 weeks. A liver homogenate was prepared and the peroxidases were separated on a Sephadex G-100 column as described above. Radioactivity was determined by counting the ^{75}Se on a Beckman Gamma 310 radiation counter, up to 0.5MeV, with a sample of elution buffer as a blank.

Glutathione peroxidase activity was assayed by the method of Little & O'Brien (1968). One enzyme unit is defined as 1 μ mol of NADPH oxidized/min. The sodium salt of linoleic acid hydroperoxide was used in the assay, as ethanol was found to inhibit the enzyme.

Glutathione transferase activity was assayed as follows: 0.1 ml of enzyme source and 80 μ l of 100 mM-reduced glutathione were added to 3.78 ml of 0.1 M- KH_2PO_4 , pH 6.5. To this was added 40 μ l of 0.2 M-1-chloro-2,4-dinitrobenzene in 95% ethanol. This was mixed and the reaction mixture was left for 1 min before it was quenched with 4 ml of 4% (w/v) sulphosalicylic acid. The tubes were centrifuged at 2500 rev./min in a desk-top centrifuge for 10 min. A 0.2 ml portion of the supernatant was added to 2.7 ml of 0.2 M-Tris/HCl buffer, pH 8.5. Into this was mixed 0.1 ml of 0.2% 5,5'-dithiobis-(2-nitrobenzoic acid) in 0.1 M-Tris/HCl buffer, pH 7.6. The colour was allowed to develop for 30 s, at the end of which time the absorbance was measured at 412 nm in a Beckman DB-G spectrophotometer. One enzyme unit is defined as 1 μ mol of reduced glutathione coupled/min.

Protein determinations were done by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Results and Discussion

Guinea-pig liver cytosol had high glutathione peroxidase activity with progesterone-17 hydroperoxide and linoleic acid hydroperoxide. However, as shown in Fig. 1, this activity was eluted from Sephadex G-100 after glutathione peroxidase, assayed with H_2O_2 as a substrate, in contrast with results obtained previously with rat liver cytosol (Little & O'Brien, 1968). The apparent mol.wt. of the H_2O_2 -reducing peroxidase was 80000, whereas that of the linoleic acid hydroperoxide-reducing peroxidase was 45000. The latter enzyme was also responsible for most of the peroxidase activity with progesterone-17 hydroperoxide and the organic hydroperoxides cumene hydroperoxide and t-butyl hydroperoxide, and coincided with glutathione transferase activity (Fig. 1). The activity with progesterone hydroperoxide was too fast to measure. The order of effectiveness for the other peroxides was cumene hydroperoxide, linoleic acid hydroperoxide and t-butyl hydroperoxide.

Chromatography of the liver cytosol fraction obtained from guinea pigs after administration of ^{75}Se *in vivo* showed that most of the ^{75}Se was incorporated into proteins with a higher molecular weight than that of the linoleic acid hydroperoxide-reducing peroxidase, and that little ^{75}Se was incorporated into the linoleic acid hydroperoxide-reducing peroxidase (Fig. 2). It is concluded that in guinea-pig liver the

Fig. 1. Elution profile of guinea-pig liver cytosol fractionated on a column (2.6 cm $\times$ 26 cm) of Sephadex G-100. Fractions were assayed for glutathione peroxidase activity with: H_2O_2 ($\circ$), cumene hydroperoxide ($\blacktriangle$), linoleic acid hydroperoxide ($\bullet$) and t-butyl hydroperoxide ($\triangle$); glutathione transferase activity ($\blacksquare$) was also assayed. In the assay, the final concentration of the peroxides was 100 μ M. The void volume was 44 ml.

Fig. 2. Elution from Sephadex G-100 columns (2.5 cm $\times$ 27 cm) of liver cytosol from guinea pigs injected with ^{75}Se . Fractions were assayed for glutathione peroxidase activity with H_2O_2 ($\circ$) and linoleic acid hydroperoxide ($\triangle$); ^{75}Se ($\square$) in fraction is also shown. In the assay, the final concentration of the peroxides was 100 μ M. The void volume was 66 ml.

glutathione peroxidase responsible for the reduction of lipid and steroid hydroperoxides does not contain selenium, and that guinea pigs, unlike rats, may not have a dietary selenium requirement. It remains to be seen whether the peroxidase activity will prove to be a property of a glutathione transferase.

Although glutathione peroxidase II is unlikely to be of physiological relevance in the normal rat liver (Prohaska & Ganther, 1976), it clearly could play an important role in the normal guinea-pig liver in reducing lipid and steroid hydroperoxides. Burk *et al.* (1978) showed that it could play a role in removing the organic hydroperoxide t-butyl hydroperoxide when perfused through selenium-deficient livers.

Lipid peroxides play an important role in prostaglandin formation as activators of the cyclooxygenase and in inhibiting prostacyclin synthase (Samuelsson *et al.*, 1978). Although glutathione transferase has been shown to catalyse the formation of prostaglandin D₂ from prostaglandin H₂, its ability to reduce lipid peroxides clearly suggests that it could play another role in prostaglandin formation.

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