GLUTATHIONE PEROXIDASE ACTIVITY IN SELENIUM-DEFICIENT RAT LIVER[1]

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SUMMARY: Glutathione peroxidase activity in the liver supernatant from rats fed a Se-deficient diet for 2 weeks was 8% of control when measured with H_2O_2 but 42% of control when assayed with cumene hydroperoxide. Two peaks of glutathione peroxidase activity were present in the Sephadex G-150 gel filtration chromatogram of rat liver supernatant when 1.5 mM cumene hydroperoxide was used as substrate. Only the first peak was detected when 0.25 mM H_2O_2 was used as substrate. The first peak was absent from chromatograms of Se-deficient rat liver supernatants; but the second peak, which eluted at a position corresponding to M.W. = 39,000, appeared unchanged. The second peak thus represents a second glutathione peroxidase activity which catalyzes the destruction of organic hydroperoxides but has little activity toward H_2O_2 and which persists in severe selenium deficiency.

INTRODUCTION: Glutathione peroxidase (glutathione: H_2O_2 oxidoreductase, EC 1.11.1.9) was discovered by Mills (1) who partially purified and characterized it (2). This enzyme has since been obtained in homogeneous form from several sources (3,4,5,6). It contains Se which is tightly bound (5,7,8) and its activity in various tissues from several species is dependent on dietary Se intake (9). It has been shown that Se-deficient rat liver has no glutathione peroxidase activity toward H_2O_2 (10), the substrate used for its assay by most investigators. Since glutathione peroxidase can catalyze the destruction of a variety of organic hydroperoxides (11), some workers have used other substrates such as cumene hydroperoxide. With this substrate substantial glutathione peroxidase activity has been reported in Se-deficient rat

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liver. (12). Using cumene hydroperoxide we have observed glutathione peroxidase activities that were 30% of control in rats fed a Se-deficient diet for 14 months. This finding indicated the possible existence of an additional glutathione peroxidase which might not be Se-dependent. Since glutathione peroxidase activity is used as a means of assessing nutritional or functional Se status, a non-Se-dependent glutathione peroxidase activity could be an important source of error. In addition, studies of the ability of an animal to resist stress from oxidative agents should take into account such an activity, which might not be measured by assays with H_2O_2. For these reasons we have fractionated rat liver 105,000 x g supernatants on Sephadex G-150 and measured the glutathione peroxidase activity of the fractions using both cumene hydroperoxide and H_2O_2 as substrates.

METHODS: Weanling male Holtzman rats were fed a Torula yeast-based Se-deficient diet (13) or the same diet supplemented with 0.5 mg Se as sodium selenite per Kg (controls). The rats were exsanguinated under ether anesthesia. The livers were perfused with 1.14 M NaCl and homogenized in 0.25 M sucrose. The supernatant fraction was prepared by centrifugation at 105,000 x g for 1 hour.

Enzyme activity was measured by a modification of the coupled assay procedure of Paglia and Valentine (14). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 E.U./ml GSSG-reductase, 1 mM GSH, 1.5 mM cumene hydroperoxide or 0.25 mM H_2O_2 in a total volume of 1 ml. All ingredients except enzyme source and peroxide were combined at the beginning of each day. Enzyme source (0.1 ml) was added to 0.8 ml of the above mixture and allowed to incubate 5 min. at room temperature before initiation of the reaction by the addition of 0.1 ml peroxide solution. Absorbance at 340 nm was recorded for 5 min. and the activity was calculated from the slope of these lines as μmoles NADPH oxidized per minute. Blank reactions with enzyme source replaced by distilled water were subtracted from each assay. Protein was measured by the method of Lowry et al. (15).

RESULTS AND DISCUSSION: Table 1 shows glutathione peroxidase activity in liver supernatants from rats fed Se-deficient and control diets for 2 weeks. The activity measured with H_2O_2 had decreased in the Se-deficient rats to 8% of control by 2 weeks but the activity measured with cumene hydroperoxide had decreased much less, to 42% of control.

Figure 1 shows gel filtration chromatograms of liver supernatants from rats fed the experimental diets for 3 months. Two peaks of glutathione peroxidase activity appeared in the chromatogram of the supernatant from the

TABLE I: Effect of Dietary Selenium Supplementation on Rat Liver Glutathione Peroxidase Activity[1]

	GSH-Peroxidase Activity[2] ± S.E.	
Dietary Se	H_2O_2	Cumene·OOH
0 ppm	28 ± 7.5	314 ± 18
0.5 ppm	354 ± 65	748 ± 81

[1]Rats were fed the respective diets from weaning for 2 weeks before killing and processing as described in the text.

[2]Results are expressed as µmoles NADPH oxidized per min. per mg protein. There were 4 rats per group.

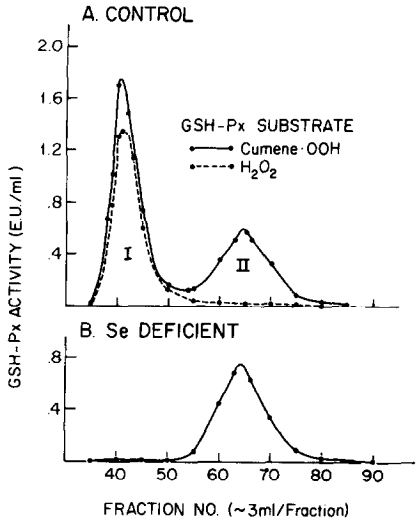

Fig. 1 Sephadex G-150 gel filtration of rat liver supernatant. Gel filtration was performed on a 5 x 22.5 cm column of Sephadex G-150 eluted with 0.05 M tris buffer (pH 8.6) containing 1 mM EDTA and 0.1 M K_2HPO_4 at a flow rate of 60 ml/hr. The sample was applied in a 10 ml volume.

Se-adequate rat (Fig. 1A) when cumene hydroperoxide was used as substrate but the second peak was not detected when H_2O_2 was used as substrate. This indicates the presence of 2 glutathione peroxidases capable of destroying cumene

hydroperoxide, only 1 of which destroys H_2O_2 under these assay conditions. The chromatogram of the Se-deficient supernatant (Fig. 1B) showed no peak I activity but persistence of peak II activity. Peak I thus appears to be the Se-dependent glutathione peroxidase. Peak II seems to be a different enzyme on the basis of its molecular weight, substrate specificity, and persistence in Se deficiency.

Our assay procedure measures the oxidized glutathione formed in the glutathione peroxidase reaction by coupling it to the oxidation of NADPH via glutathione reductase. There was a possibility, therefore, that peak II catalyzed the oxidation of NADPH directly rather than through oxidation of GSH. We have also been able to measure this activity by direct measurement of GSH remaining as described by Hafeman, et al. (10) indicating that peak II catalyzes the oxidation of GSH.

Table II shows results of a heat denaturation experiment. Peak II

TABLE II: Effect of Heating at 60° C on GSH-Px Activity[1]

Time (min.)	Peak I % of Control	Peak II % of Control
0	100[2]	100[2]
10	50	0
20	20	0
30	10	0

[1]Aliquots of the combined fractions having peak I and peak II activity from Sephadex G-150 chromatography were heated in a thermostated water bath at 60° C. At the times indicated 0.1 ml samples were withdrawn, added to 0.8 ml ice cold reaction mixture, then allowed to incubate at room temperature for 5 minutes and assayed for GSH-Px activity as described in the text.

[2]Contained 0.2 enzyme units expressed as change in O.D. at 340 nm per minute for peak I and 0.38 enzyme units for peak II per ml.

activity is completely destroyed by heating for 10 minutes at 60° C but peak I activity is still detectable after 30 minutes at 60° C. This experiment provides further evidence of a difference between peak I and peak II and of the protein nature of peak II.

The peak II enzyme activity eluted at a volume corresponding to M.W. = 39,000 when chromatographed on the Sephadex G-150 superfine column as shown in Fig. 2. This is about one-half the molecular weight determined for the Se-dependent glutathione peroxidase by other investigators (3,4,5,6). Since the Se-dependent glutathione peroxidase has been found to contain 4 subunits (3,4,5,6) and the peak II enzyme has a molecular weight about one-half that of the peak I enzyme, the peak II enzyme could be a dimer of the peak I

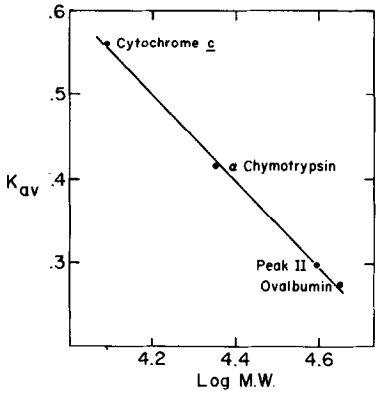

Fig. 2 Molecular weight determination of glutathione peroxidase peak II. Rat liver supernatant was chromatographed on a 2.6 x 29.4 cm column of Sephadex G-150 superfine eluted with the same buffer as Fig. 1 at a flow rate of 6 ml/hr. The sample was applied in a 1.5 ml volume. This column was calibrated using cytochrome c (M.W. = 12,384), α-chymotrypsin (M.W. = 22,500) and ovalbumin (M.W. = 45,000).

enzyme subunits that retains activity toward organic hydroperoxides but not toward H_2O_2. We have rechromatographed peak I fractions on Sephadex G-150 and have observed no activity in the peak II region, indicating that peak I does not dissociate to give peak II under these conditions. Also, since peak II activity is not diminished by severe Se deficiency, it probably does not

contain Se as does the peak I enzyme. Se analysis of purified peak II enzyme has not yet been performed, however. The evidence available at this time suggests that these are 2 different enzymes. Even if they should be structurally related, this would not detract from the importance of the observed physiological differences between them.

The onset of dietary liver necrosis, which occurs in rats fed diets low in Se, vitamin E and sulfur-containing amino acids, can be delayed by supplementation of these diets with cysteine or methionine (16). The existence of this enzyme may explain this effect of the sulfur-containing amino acids. By increasing the GSH concentration they could provide substrate for this peak II enzyme which is not decreased in Se deficiency. In addition, species such as the sheep which do not develop dietary liver necrosis in Se and vitamin E deficiency may, as our preliminary results indicate, have much higher peak II enzyme activity than peak I enzyme activity in the liver in contrast to the rat which has higher peak I activity than peak II activity.

The existence of the peak II enzyme poses a problem for the assay of glutathione peroxidase in tissues where both enzymes are present as is demonstrated by Table I. Assays using H_2O_2 as substrate at the usual concentrations should still give a reliable estimate of peak I or Se-dependent glutathione peroxidase activity, since the peak II enzyme activity cannot be detected at these concentrations of H_2O_2. No substrate specific for the peak II enzyme has been found so assay of its activity must be done after separation from the peak I enzyme.

REFERENCES:

1. Mills, G.C. (1957) J. Biol. Chem. 229, 189-197.
2. Mills, G.C. (1959) J. Biol. Chem. 234, 502-506.
3. Schneider, F., and Flohe, L. (1967) Hoppe-Seyler's Z. Physiol. Chem. 348, 540-552.
4. Awasthi, Y.C., Beutler, E., and Srivastava, S.K. (1975) J. Biol. Chem. 250, 5144-5149.
5. Oh, S.H., Ganther, H.E., and Hoekstra, W.G. (1974) Biochem. 13, 1825-1829.

6. Nakamura, W., Hosoda, S., and Hayashi, K. (1974) Biochem. Biophys. Acta 358, 251-261.
7. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. (1973) Science 179, 588-590.
8. Flohe, L., Gunzler, W.A., and Schock, H.H. (1973) FEBS Lett. 32, 132-134.
9. Ganther, H.E., Hafeman, D.G., Lawrence, R.A., Serfass, R.E., and Hoekstra, W.G. (1976) in Trace Elements in Human Health and Disease (Prasad, A.S., Ed.) in press, Academic Press, N.Y.
10. Hafeman, D.G., Sunde, R.A., and Hoekstra, W.G. (1974) J. Nutr. 104, 580-587.
11. Little, C., and O'Brien, P.J. (1968) Biochem. Biophys. Res. Commun. 31, 145-150.
12. Chow, C.K., and Tappel, A.L. (1974) J. Nutr. 104, 444-451.
13. Burk, R.F., and Masters, B.S.S. (1975) Arch. Biochem. Biophys. 170, 124-131.
14. Paglia, D.E., and Valentine, W.N. (1967) J. Lab. Clin. Med. 70, 158-169.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
16. Schwarz, K. (1965) Fed. Proc. 24, 58-67.