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Differential Regulation of Rat Liver Selenoprotein mRNAs in Selenium Deficiency

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Summary: Selenium deficiency causes a fall in the concentrations of selenoproteins but selenoprotein P and type I iodothyronine 5'-deiodinase (5'-deiodinase) are more resistant to this effect than is glutathione peroxidase. To investigate the differential regulation of these selenoproteins, a selenium-deficient diet was fed to weanling rats for 14.5 weeks and their hepatic mRNAs were measured by Northern analysis. Levels of all 3 mRNAs fell progressively with time. Selenoprotein P and 5'-deiodinase mRNAs remained higher at all time points relative to control than glutathione peroxidase mRNA. mRNA decreases were mirrored by decreases in glutathione peroxidase activity and selenoprotein P concentration. However, the decreases in the protein levels were greater than the decreases in their mRNAs, suggesting that synthesis of both proteins was limited to a similar extent at the translational level by the availability of selenium. In addition to this apparently unregulated translational effect, these results point to a pretranslational regulation, affecting mRNA levels, which could account for the differential effect of selenium deficiency on glutathione peroxidase and the other selenoproteins. This regulation might serve to direct selenium to selenoprotein P and 5'-deiodinase when limited amounts of the element are available.

The biological activity of selenium is mediated by selenoproteins. Although deficiency of the element causes a fall in selenoprotein concentrations, certain selenoproteins are maintained better than others (1). This differential regulation might be a means of directing selenium to the selenoproteins of greatest importance to the organism.

Selenoprotein P is a selenium-rich glycoprotein which is secreted by the liver into the plasma (2). Its function is unknown but there is some evidence that it serves as an antioxidant. Type I iodothyronine 5'-deiodinase (5'-deiodinase) is a selenoenzyme which plays a role in thyroid hormone metabolism (3). Cellular glutathione peroxidase is the most abundant selenoprotein in the rat and is present in virtually all tissues (4). Studies of these proteins in animals fed graded amounts of selenium from adequate to severely deficient have shown that glutathione peroxidase is the most sensitive to lack of selenium. Selenoprotein P persists in significant concentrations even when hepatic glutathione peroxidase activity is undetectable (1) and 5'-deiodinase activity is more resistant to selenium deficiency than is glutathione peroxidase activity (5). The mechanism of this differential regulation has not been established. The present study examines the hepatic concentrations of the mRNAs of these proteins during the development of selenium deficiency.

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Methods

Animals. Weanling male Sprague-Dawley rats were fed a Torula yeast-based selenium-deficient diet or the same diet with 0.5 mg of selenium added/kg as sodium selenate (6). Rats were housed in a facility with a 12-h light and dark cycle. Rats were anesthetized with pentobarbital (50 mg/kg). Then blood was removed from the aorta and treated with EDTA (1 mg/ml). Tissues were removed and immediately frozen in liquid nitrogen. They were stored at -75° C prior to RNA isolation.

RNA isolation. A 1 g portion of frozen tissue was homogenized in 4 M guanidine thiocyanate reagent for RNA isolation (7). The guanidine thiocyanate homogenate was layered onto 5.7 M CsCl and centrifuged for 16-20 h at 150,000 x g. Total RNA was isolated from the pelleted material. RNA samples were stored at -75° C until electrophoresed. RNA was isolated from tissues taken from rats fed the selenium-deficient diet for 4.5, 9.5, and 14.5 weeks and from rats fed the control diet for 14.5 weeks.

Northern analysis. RNA samples were electrophoresed on a 1% agarose gel containing 6.5% formaldehyde (7). The RNA was transferred to a nitrocellulose membrane with 20 X SSC (1 X SSC contains 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0). The RNA was attached to the nitrocellulose filter by UV-crosslinking. The nitrocellulose filter was prehybridized in 50% formamide, 10 X Denhardt's, 0.1% SDS, 5 X SSC, salmon sperm DNA (0.25 mg/ml) and polyadenylic acid (0.05 mg/ml) for 2 h at 42° C. The cDNA probe was labeled with [^{32}P] α-dCTP using a nick translation kit (NEK-004, New England Nuclear, Boston, MA). Selenoprotein P message was measured using the rat cDNA clone 16C1 (8). Glutathione peroxidase mRNA was measured with a mouse cDNA probe, pGPX PO1, that was kindly provided by Dr. Nobumasa Imura of Kitasato University, Tokyo. The rat liver cDNA probe for 5'-deiodinase (9) was provided by Dr. J. Köhrle of the Max Planck Institut für Experimentelle Endokrinologie in Hannover, Germany. The desired nick-translated probe was added to the nitrocellulose filter in the prehybridization solution. The filter was hybridized overnight at 42° C. The filter was washed sequentially in 4 X SSC at 25° for 5 min and 2 X SSC at 60° C for 1.5 h. The filter was then exposed to Kodak XAR-5 film. The autoradiographs were scanned and densities were determined. Differences in RNA loading were accounted for by normalizing the densities of 16C1, 5'-deiodinase, and pGPX P01 bands to the intensity of the corresponding 28S-ethidium bromide fluorescence band.

Selenoprotein assays. Glutathione peroxidase was assayed in 105,000 x g supernatant of liver using H_2O_2 as substrate (10). Selenoprotein P was assayed in plasma by radioimmunoassay with monoclonal antibody 8F11 (2).

Results

Northern blot analysis indicates that selenium deficiency caused a decrease in the mRNAs of all 3 selenoproteins which were measured in rat liver (figure 1). A time-course experiment was conducted to characterize these decreases. Selenium-deficient diet was fed for 14.5 weeks and quantitation of the mRNAs by Northern analysis was performed at intermediate times and at the termination of the experiment. Liver glutathione peroxidase activity and plasma selenoprotein P concentration were measured at 14.5 weeks. Table 1 shows the results of the mRNA measurements. The mRNA for glutathione peroxidase fell more rapidly and to a lower level relative to control than did the other mRNAs.

Selenium-dependent glutathione peroxidase activity in liver was 0.8% of control at 14.5 weeks and plasma selenoprotein P concentration was 4.3% of control in selenium-deficient rats at 14.5 weeks. This shows that both the selenoproteins measured fell in selenium deficiency but selenoprotein P fell less than did glutathione peroxidase. These results are qualitatively similar to the results for the mRNAs (Table 1), but the decrease in each selenoprotein was greater, relative to control, than was the decrease in its mRNA.

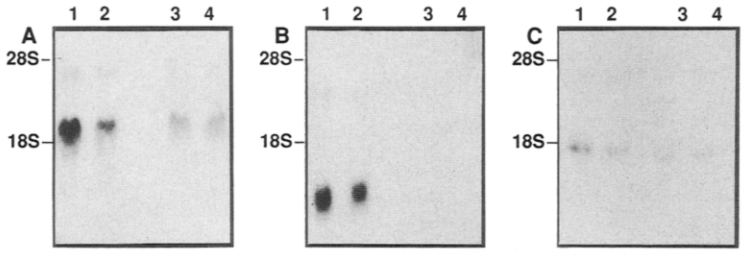

Figure 1. Northern blot analysis of rat liver RNA using cDNA probes for selenoprotein P (panel A), cellular glutathione peroxidase (panel B), and 5'-deiodinase (panel C). Panels A and B are sequential analyses of the same blot. Panel C was a separate blot using aliquots of the same RNA samples. Lanes 1 and 2 were loaded with total liver RNA from control rats and lanes 3 and 4 were loaded with total liver RNA from selenium-deficient rats. Approximately 20 μg of RNA was loaded in each lane. All rats had been fed experimental diet for 14.5 weeks beginning at weaning. Hybridization was carried out as described in the methods section. The positions of the 28S and 18S RNA bands are indicated. The size of the selenoprotein P mRNA corresponded to 2.2 kilobases, that of the glutathione peroxidase mRNA to 1.1 kilobases, and that of 5'-deiodinase to 1.9 kilobases.

Discussion

Concentrations of selenoproteins fall when the nutritional requirement for selenium is not met. One cause of this fall is generally considered to be a lack of selenium in the form of selenocysteyl-tRNA$^{[Ser]Sec}$. This form is incorporated into the primary structure of the selenoproteins during translation. This translational effect might be expected to decrease the synthesis of all selenoproteins to a similar extent.

There is also evidence that pretranslational regulation of selenoproteins occurs. Several groups have reported that levels of mRNA for cellular glutathione peroxidase decrease in selenium-deficient rat liver (11,12). In addition, a study in which decreasing levels of selenium were fed

Table 1

Selenoprotein mRNA Levels in Livers from Rats Fed a Selenium-Deficient Diet

	weeks of feeding (*% of control at 14.5 wks*[a])		
	4.5	9.5	14.5
cellular glutathione peroxidase	19	8	3 ± 2[b,c]
5'-deiodinase	36	17	13 ± 5[b]
selenoprotein P	67	34	19 ± 4[c]

[a]Values are means of 2 animals for 4.5 and 9.5 weeks and means ± S.D., n=3 for 14.5 weeks.
[b,c]Values sharing a superscript are significantly different ($p<0.05$) by Fisher PLSD.

demonstrated that liver glutathione peroxidase activity fell before selenoprotein P concentration decreased (1). This indicated that selenoprotein P was synthesized in preference to liver glutathione peroxidase when selenium was limiting. Such differential effects are often pretranslational.

The present experiments provide evidence that the decrease in selenoproteins which occurs in selenium deficiency is caused both by translational and by pretranslational mechanisms. The decrease in the protein relative to control was greater than the corresponding decrease in mRNA for both selenoproteins which were assessed. Moreover, the ratios of mRNA to protein were approximately 4:1 in each case, indicating that limited availability of selenium at the ribosomal level restricted synthesis of the selenoproteins to approximately the same extent.

There was evidence of a pretranslational effect as well and it was different in magnitude for different selenoproteins. Table 1 shows that selenoprotein P and 5'-deiodinase mRNAs were more resistant to selenium deficiency than was glutathione peroxidase mRNA. This demonstrates that the organism regulates selenoprotein mRNA levels in a differential manner. The purpose of this regulation might be to direct the limited available selenocysteyl-tRNA$^{[Ser]Sec}$ to the synthesis of certain selenoproteins. Glutathione peroxidase, which is very abundant and highly sensitive to decreased selenium supply, might store selenium for release when the element is scarce. The selenium released from glutathione peroxidase under these conditions would be available for synthesis of selenoproteins of greater importance to the survival of the organism.

The mechanisms of selenoprotein mRNA regulation are not known. It was observed several years ago that selenium deficiency does not affect transcription of liver glutathione peroxidase mRNA (13). This suggests that the regulation is exerted through changes in mRNA degradation. Further work will be needed to clarify the regulation of selenoprotein mRNAs.

Acknowledgment

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