Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats

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Regulation of synthesis of the selenoenzymes cytosolic glutathione peroxidase (GSH-Px), phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px) and type-1 iodothyronine 5'-deiodinase (5'IDI) was investigated in liver, thyroid and heart of rats fed on diets containing 0.405, 0.104 (Se-adequate), 0.052, 0.024 or 0.003 mg of Se/kg. Severe Se deficiency (0.003 mg of Se/kg) caused almost total loss of GSH-Px activity and mRNA in liver and heart. 5'IDI activity decreased by 95% in liver and its mRNA by 50%; in the thyroid, activity increased by 15% and mRNA by 95%. PHGSH-Px activity was reduced by 75% in the liver and 60% in the heart but mRNA levels were unchanged; in the thyroid, PHGSH-Px activity was unaffected by Se depletion but its mRNA increased by 52%. Thus there is

differential regulation of the three mRNAs and subsequent protein synthesis within and between organs, suggesting both that mechanisms exist to channel Se for synthesis of a particular enzyme and that there is tissue-specific regulation of selenoenzyme mRNAs. During Se depletion, the levels of selenoenzyme mRNA did not necessarily parallel the changes in enzyme activity, suggesting a distinct mechanism for regulating mRNA levels. Nuclear run-off assays with isolated liver nuclei showed severe Se deficiency to have no effect on transcription of the three genes, suggesting that there is post-transcriptional control of the three selenoenzymes, probably involving regulation of mRNA stability.

INTRODUCTION

The micronutrient selenium (Se) is present as Se-cysteine in a number of proteins, including cytosolic glutathione peroxidase (GSH-Px), phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px) and type-1 iodothyronine 5'-deiodinase (5'IDI) [1]. GSH-Px and PHGSH-Px are involved in the regulation of intracellular H₂O₂ and lipid hydroperoxide concentrations [2] whereas 5'IDI is involved in the metabolism of thyroid hormones [3]. The Se content of diets modifies the activity and concentrations of selenoproteins, and, under conditions of limited Se supply, there are differential effects on GSH-Px, PHGSH-Px and 5'IDI activities in the liver [4,5]. This ability of tissues to control synthesis of individual selenoproteins is vital for efficient utilization of limited amounts of the element for its most essential functions, especially as the metabolic role and relative importance of the three enzymes vary between tissues [6]. Thus it is important to compare the dependence of GSH-Px, PHGSH-Px and 5'IDI activities on dietary Se supply, and to investigate the mechanisms that regulate selenoprotein gene expression and the synthesis of these enzymes in different tissues under conditions of limited Se supply.

Se is incorporated into selenoproteins by recognition of the stop codon UGA as a codon for selenocysteine and this requires specific stem-loop structures [7]. Reduced availability of Se leads to termination of translation but it is unclear how the differential effects on expression of the various selenoenzymes arise and whether there is also other transcriptional and translational control. Although previous studies have investigated selenoenzymes in single tissues, the aims of this work were to carry out a comprehensive study to compare three selenoenzyme activities and mRNA levels in liver, heart and thyroid from rats fed on

diets of different Se content, and to assess the importance of transcriptional regulation using nuclear run-off assays with isolated liver nuclei.

MATERIALS AND METHODS

DNA probes and chemicals

The GSH-Px probe, a gift from Dr. P. Harrison, Beatson Institute, Glasgow, Scotland, U.K., was a 0.7 kb *EcoRI* fragment from the 1.5 kb cDNA of the mouse gene cloned into pUC12 [7]. The 5'IDI probe, a 0.55 kb *HindIII* fragment of the rat cDNA, was a gift from Dr. M. Berry and Dr. P. R. Larsen, Harvard Medical School, Boston, MA, U.S.A. [8]. The PHGSH-Px probe [5] was a 0.8 kb *EcoRI* restriction fragment of the rat cDNA, and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe [9] was a 0.78 kb *PstI-XbaI* fragment of the human foetal liver cDNA obtained from American Tissue Culture Collection (accession number 57090). The 18S rRNA cDNA [10] was obtained from Dr. R. Fulton, Beatson Institute, Glasgow, U.K., and a 1.4 kb *BamHI* fragment derived from it was used for hybridization. Multiprime labelling kits, Hyperfilm-MP and [³²P]dCTP were purchased from Amersham International, Amersham, Bucks., U.K. Genescreen nylon membrane was purchased from NEN-Dupont, and other chemicals were of either Analar or molecular-biology grade.

Animals and experimental design

Male Hooded Lister rats of the Rowett strain were used throughout. Animals were randomly allocated to groups of six and fed on a semisynthetic diet containing different amounts of

Abbreviations used: GSH-Px, cytosolic glutathione peroxidase; PHGSH-Px, phospholipid hydroperoxide glutathione peroxidase; 5'IDI, type-1 iodothyronine 5'-deiodinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 1 × SSC, 0.15 M NaCl/0.015 M sodium citrate; T₄, thyroxine.

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Se for 6 weeks from weaning. The basal diet [4] contained 0.003 mg of Se/kg (severely Se-deficient) and the other diets contained 0.024 or 0.052 (Se-deficient), 0.104 (Se-adequate) and 0.405 (supra-optimal Se) mg of Se/kg as sodium selenite. Se content of the diets was analysed by acid digestion and fluorimetric analysis [11].

Animals were anaesthetized with ether, blood samples were taken by cardiac puncture and the liver perfused with sterile 0.15 M KCl via the portal vein to remove residual blood. Liver, heart and thyroids were then rapidly removed, frozen in liquid nitrogen and stored at -70°C . Half of each thyroid was used for RNA extraction and the remaining half for determination of enzyme activity.

Polyribosome preparation

Polyribosomes were prepared from liver by the method of Blobel and Sabatini [12] immediately after perfusion. Tissue was homogenized in 3.5 vol. of Tris-buffered sucrose (50 mM Tris, pH 7.6, 0.25 M sucrose, 25 mM KCl, 5 mM MgCl_2 and 5 mM 2-mercaptoethanol) and centrifuged, first at 800 *g* for 10 min to remove nuclei and cell debris, and then at 10000 *g* for 10 min to sediment the mitochondria. The postmitochondrial supernatant was filtered through two layers of muslin, and sodium deoxycholate added to a final concentration of 0.5%. Polyribosomes were then separated by centrifugation through a sucrose cushion (1 M sucrose in homogenization buffer containing additional KCl to a final concentration of 500 mM) at 100000 *g* for 5 h. The pelleted polyribosomes were rinsed in homogenization buffer and RNA was extracted immediately.

RNA extraction and hybridization

Tissues were rapidly broken up while frozen and immediately homogenized in buffer composed of 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl and 0.1 M 2-mercaptoethanol using an Ultra-Turrax homogenizer. Polyribosome pellets were dissolved in the same mixture. Total RNA was then extracted by the acid/guanidinium/phenol/chloroform procedure [13], and the purity of the preparation assessed by the A_{260}/A_{280} ratio. RNA species were then separated by electrophoresis through a denaturing 2.2 M formaldehyde/1.2% agarose gel [14] and transferred to nylon membrane (Genescreen) by capillary blotting. RNA was fixed to the membrane by exposure to UV light and the membranes were stored dry until required.

Hybridization assays were carried out as described previously [15]. Membranes were prehybridized overnight at 42°C with 0.1 mg/ml denatured salmon sperm DNA in buffer containing 50% formamide, 10% dextran sulphate, 0.2% BSA, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.1% sodium pyrophosphate, 1% SDS and 50 mM Tris/HCl, pH 7.5. Then 50–100 ng of the DNA probes was labelled with [^{32}P]dCTP by random priming (Multiprime kit) and the labelled DNA was separated from free nucleotides by gel filtration on Sephadex G-50; probe specific radioactivities were approx. 10^8 c.p.m./ μg of DNA. The labelled probes were added to the prehybridization mix and hybridized at 42°C for 24 h. The membranes were washed to remove non-specifically bound probe as follows: two washes in $2\times\text{SSC}$ ($1\times\text{SSC} = 0.15\text{ M NaCl}/0.015\text{ M sodium citrate}$) at room temperature for 5 min, followed by two washes at 65°C for 1 h in either $1\times\text{SSC}/1\%$ SDS (GSH-Px, PHGSH-Px, 5'IDI and GAPDH) or $0.2\times\text{SSC}/1\%$ SDS (18S rRNA), and a final wash in $0.1\times\text{SSC}$ at room temperature. Specific hybridization was then detected by both direct imaging using a Packard Instantimager and autoradiography using Hyperfilm-

MP at -70°C . After analysis, membranes were washed in 0.1% SDS for 5–7 min at 95°C before rehybridization to other probes.

Quantification of the bound probe was carried out using the Instantimager, and results for each probe were expressed per unit of hybridization achieved with the 18S rRNA probe; this allowed correction for any variation between loading of RNA on the gel or transfer to the nylon membrane. For liver and heart, samples from three animals of each group were analysed on duplicate filters (for thyroids there was sufficient RNA for only one filter) and samples from the other three animals in each group processed in parallel on a second pair of filters. Results for each filter were expressed as a percentage of the mean for animals on the Se-adequate (0.104 mg/kg) diet and the data combined, thus giving means of six (thyroid) or 12 (heart, liver) values from six animals.

Nuclear run-off assays

After perfusion with sterile 0.15 M KCl, to remove blood, the liver was minced in buffer composed of 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 14 mM 2-mercaptoethanol, 0.5 mM EGTA, 2 mM EDTA and 15 mM Hepes, pH 7.5, and nuclei isolated by differential centrifugation by previously described methods [16]. Nuclei were resuspended in 20 mM Tris/HCl buffer, pH 7.9 containing 75 mM NaCl, 0.5 mM EDTA, 0.85 mM dithiothreitol, 0.125 mM PMSF and 50% glycerol at $5\times 10^7/200\mu\text{l}$ and aliquots frozen at -70°C before use. Run-off assays were performed in a final volume of 400 μl with 200 μl of reaction buffer [17] with 5×10^7 nuclei and 140 μCi of [$\alpha\text{-}^{32}\text{P}$]UTP (800 $\mu\text{Ci}/\text{mmol}$; NEN-Dupont); the reaction was for 15 min at 28°C . The reaction was stopped by the addition of 20 μl of 20 mM CaCl_2 and 20 μl of 10 units/ μl DNase 1 and incubation continued for 5 min. Total RNA was isolated [13] and after denaturation (100°C ; 10 min) equal amounts of labelled RNA (3×10^7 c.p.m.) from each nuclear preparation were then incubated with separate nylon membranes on to which appropriate cDNA probes had been deposited and fixed by UV irradiation. Whole plasmids (5 $\mu\text{g}/\text{slot}$) containing both vector and insert were used to detect specific labelled transcripts and the vectors without the specific cDNA inserts were used as controls: GSH-Px, PHGSH-Px and 5'IDI cDNAs were all in Bluescript vector. In addition to the selenoenzyme cDNAs, the following controls were also used: for data normalization GAPDH (in pBR322), a non-selenoprotein control, β -actin (in pUC18), and, as a further negative control, the bacterial threonine B in pSVpolyA. Membranes were prehybridized at 65°C for 30 min in 5 ml of Church buffer (0.5 M Na_2PO_4 , pH 7.1, 7% SDS, 0.1 mM EDTA) and hybridization was carried out at 65°C for 18 h. Non-specific hybridization products were removed by washing in $2\times\text{SSC}$ (1 h; 65°C), $2\times\text{SSC}$ containing 15 $\mu\text{g}/\text{ml}$ RNase A (37°C ; 30 min) and then $2\times\text{SSC}$ for 1 h at 37°C . Under these conditions the signal obtained was linear with increasing quantities of labelled RNA. The specifically bound transcripts were detected by autoradiography and the amounts bound quantified using an Instantimager. Results were corrected for non-specific binding by subtraction of counts bound to the appropriate vector and normalized between blots by calculating the data as the ratio of c.p.m. bound to selenoenzyme cDNA/c.p.m. bound to the GAPDH cDNA.

Enzyme activities and thyroid hormone concentrations

GSH-Px was measured by the method of Beckett et al. [18], PHGSH-Px by the method of Weitzel et al. [19] and 5'IDI by release of iodine from ^{125}I -labelled reverse tri-iodothyronine [20]. There was not enough thyroid tissue to allow determination of

PHGSH-Px activity as well as the mRNA and other enzymes. PHGSH-Px activity was therefore determined in thyroids from an additional two groups of six rats offered the basal diet or the diet supplemented to 0.104 mg of Se/kg for 6 weeks from weaning. Plasma free and total thyroxine (T_4) were determined using Amerlite MAB and Amerlite total T_4 kits from Kodak Clinical Diagnostics, Amersham, Bucks. U.K.

RESULTS

Enzyme activities and thyroid hormone concentrations

Hepatic GSH-Px activity decreased in a dose-dependent manner as the dietary Se supply was reduced from an Se-adequate level of 0.104 mg/kg to 0.052, 0.024 and 0.003 mg/kg (Table 1). In severe Se deficiency, GSH-Px activity was reduced by over 99 %, 5'IDI activity by 95 % and PHGSH-Px activity by 75 % (Table 1); the PHGSH-Px and 5'IDI activities also decreased in a dose-dependent manner with decreasing dietary Se. In heart, GSH-Px activity decreased by 90 % and PHGSH-Px activity by 60 % in severe Se deficiency (Table 2); the GSH-Px activity was more sensitive to reduced Se supply, as shown by the 50 % decrease in heart GSH-Px activity compared with the unchanged PHGSH-Px activity in animals fed on the diet containing 0.052 mg of Se/kg. No 5'IDI activity or mRNA could be detected in this organ. In contrast, in the thyroid, 5'IDI activity increased by 15 %, PHGSH-Px activity was unaffected and GSH-Px activity decreased by only 50 % in severe Se deficiency (Table 3); thyroid GSH-Px activity was reduced to approximately the same extent in all the Se-deficient animals. The changes in GSH-Px activity confirmed the different Se status of the three groups of Se-deficient animals. Plasma total and free T_4 concentrations increased as dietary Se decreased from 0.104 to 0.003 mg/kg, reflecting decreased hepatic and renal 5'IDI activity (Table 4).

mRNA analysis

Hybridization of Northern blots showed that all four probes reacted to a single mRNA species in preparations of total RNA extracted from heart, liver and thyroid (e.g. thyroid in Figure 1); the approximate sizes of the mRNAs detected corresponded closely to the known sizes of GSH-Px (1.1 kb [7]), PHGSH-Px (1.0 kb [5]) and 5'IDI (1.9 kb [8]) mRNAs.

Visual inspection of both autoradiographs and images from the Instantimager showed that, in the liver of the severely Se-deficient rats, GSH-Px mRNA concentrations were greatly reduced, 5'IDI mRNA concentrations moderately decreased and PHGSH-Px mRNA showed little or no change. Quantification of the results and expression of the extent of hybridization per unit of RNA are shown in Table 1. The mean GSH-Px mRNA abundance in liver from severely Se-deficient animals was reduced by approx. 90 % compared with animals fed on the Se-adequate diet, that of 5'IDI was lowered by 50 %, whereas there was no significant change in that of PHGSH-Px. Both 5'IDI and GSH-Px mRNAs decreased in a dose-dependent manner as the Se supply was reduced, but GSH-Px mRNA more sensitive; in liver from animals fed on the diet containing 0.052 mg of Se/kg, GSH-Px mRNA was reduced by 40 % whereas 5'IDI mRNA was unchanged.

In the heart the pattern of mRNA expression was affected differently by Se deficiency; the abundance of PHGSH-Px mRNA was not changed but that of GSH-Px was reduced by 72 % (Table 2). Interestingly, GSH-Px mRNA in the liver was more sensitive to Se supply than that in the heart: in the liver it was reduced by 40 % in rats fed on the 0.052 mg of Se/kg diet and by 80 % in those fed on the 0.024 mg of Se/kg diet, whereas in heart it was unchanged in those animals fed on the former diet and reduced by only 60 % in those fed on the latter diet.

In contrast, in the thyroid there was no significant change in GSH-Px mRNA levels in Se deficiency, whereas PHGSH-Px and 5'IDI mRNA levels increased (Table 3). Analysis of the data showed a 52 and 95 % increase in PHGSH-Px mRNA and 5'IDI mRNA abundance respectively in the tissue from rats fed on the severely Se-deficient diet (0.003 mg of Se/kg) compared with animals fed on the Se-adequate diet (0.104 mg of Se/kg); statistically significant increases in these two mRNAs were also observed in thyroids from animals fed on the diet containing 0.024 mg of Se/kg.

There was no effect of Se deficiency on the abundance of GAPDH mRNA (GAPDH does not contain Se) in liver or thyroid and this was confirmed by analysis of the data (Tables 1-3); in heart there was a small increase in GAPDH mRNA in the severely Se-deficient rats.

The effect of dietary Se supply on the extent of mRNA translation was assessed by measuring mRNA abundance in liver polyribosomes as well as in total tissue RNA. As shown in Figure 2, GSH-Px, 5'IDI and PHGSH-Px mRNAs were all reduced in

Table 1 Selenoenzyme activities and mRNA abundance in liver from rats fed on diets of different Se content

Samples of total RNA were loaded on to duplicate gels and after transfer to nylon membranes all samples were hybridized under identical conditions. Filters were hybridized successively with probes for the three selenoenzyme mRNAs, for GAPDH and finally for 18S rRNA. For each probe, the degree of hybridization was determined by direct quantification of bound DNA using a Packard Instantimager, and the ratio of hybridization with each probe to that with the 18S probe was calculated. Results were expressed in arbitrary units and the data from the duplicate filters combined. Results are shown as means $\pm$ S.E.M. for the six animals in each group. Results were compared with those obtained for animals on the Se-adequate diet by a Mann-Whitney *U* test: ***P* < 0.01; ****P* < 0.001.

Se content of diet (mg/kg)	Enzyme activity			mRNA (arbitrary units)			
	GSH-Px (units/mg of protein)	PHGSH-Px (m-units/mg of protein)	5'IDI (pmol/min per mg of protein)	GSH-Px	PHGSH-Px	5'IDI	GAPDH
0.003	0.01 $\pm$ 0.00***	0.90 $\pm$ 0.11**	276 $\pm$ 43***	1.02 $\pm$ 0.19***	8.23 $\pm$ 2.21	1.11 $\pm$ 0.36***	31.19 $\pm$ 7.00
0.024	0.03 $\pm$ 0.00***	1.35 $\pm$ 0.12**	1311 $\pm$ 112**	1.60 $\pm$.023***	6.83 $\pm$ 1.45	1.66 $\pm$ 0.44**	25.45 $\pm$ 4.92
0.052	0.32 $\pm$ 0.03***	2.74 $\pm$ 0.21	2894 $\pm$ 399**	5.12 $\pm$ 1.03***	7.90 $\pm$ 1.75	2.12 $\pm$ 0.49	30.67 $\pm$ 7.74
0.104	1.58 $\pm$ 0.08	3.45 $\pm$ 0.16	5989 $\pm$ 512	9.07 $\pm$ 1.15	7.10 $\pm$ 1.17	2.22 $\pm$ 0.40	24.68 $\pm$ 3.86
0.405	2.02 $\pm$ 0.12	4.89 $\pm$ 0.54	4843 $\pm$ 402	7.60 $\pm$ 1.18	7.40 $\pm$ 1.22	2.22 $\pm$ 0.51	23.71 $\pm$ 5.16

Table 2 Selenoenzyme activities and mRNA abundance in heart from rats fed on diets of different Se content

Samples of total RNA were loaded on to duplicate gels and after transfer to nylon membranes all samples were hybridized under identical conditions. Filters were hybridized successively with probes for the three selenoenzyme mRNAs, for GAPDH and finally for 18S rRNA. For each probe, the degree of hybridization was determined by direct quantification of bound DNA using a Packard Instantimager, and the ratio of hybridization with each probe to that with the 18S probe calculated. Results were expressed in arbitrary units and the data from the duplicate filters combined. Results are shown as means $\pm$ S.E.M. for the six animals in each group. Results were compared with those obtained for animals on the Se-adequate diet by a Mann-Whitney *U* test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Se content of diet (mg/kg)	Enzyme activity		mRNA (arbitrary units)		
	GSH-Px (units/mg of protein)	PHGSH-Px (m-units/mg of protein)	GSH-Px	PHGSH-Px	GAPDH
0.003	0.04 $\pm$ 0.01**	1.42 $\pm$ 0.29**	3.32 $\pm$ 0.53***	46.67 $\pm$ 2.94	89.74 $\pm$ 5.45*
0.024	0.11 $\pm$ 0.02**	3.04 $\pm$ 0.31	5.44 $\pm$ 0.49***	44.22 $\pm$ 2.98	85.13 $\pm$ 7.83
0.052	0.25 $\pm$ 0.02**	3.80 $\pm$ 0.36	11.24 $\pm$ 0.81	44.70 $\pm$ 2.84*	79.33 $\pm$ 7.16*
0.104	0.54 $\pm$ 0.05	3.81 $\pm$ 0.26	11.60 $\pm$ 0.86	36.43 $\pm$ 4.99	64.82 $\pm$ 8.41
0.405	0.56 $\pm$ 0.05	3.13 $\pm$ 0.37	14.05 $\pm$ 1.18	40.11 $\pm$ 2.53	63.75 $\pm$ 4.92

Table 3 Selenoenzyme activities and mRNA abundance in thyroids from rats fed on diets of different Se content

Samples of total RNA were loaded on to duplicate gels and after transfer to nylon membranes all samples were hybridized under identical conditions. Filters were hybridized successively with probes for the three selenoenzyme mRNAs, for GAPDH and finally for 18S rRNA. For each probe, the degree of hybridization was determined by direct quantification of bound DNA using a Packard Instantimager, and the ratio of hybridization with each probe to that with the 18S probe calculated. Results were expressed in arbitrary units and the data from the duplicate filters combined. Results are shown as means $\pm$ S.E.M. for the six animals in each group. Results were compared with those obtained for animals on the Se-adequate diet by a Mann-Whitney *U* test: **P* < 0.05; ***P* < 0.01.

Se content of diet (mg/kg)	Enzyme activity			mRNA (arbitrary units)			
	GSH-Px (units/mg of protein)	PHGSH-Px (m-units/mg of protein)	5'-IDI (pmol/min per mg of protein)	GSH-Px	PHGSH-Px	5'-IDI	GAPDH
0.003	0.07 $\pm$ 0.01*	1.60 $\pm$ 0.19	921 $\pm$ 50	6.64 $\pm$ 1.15	32.68 $\pm$ 6.83*	28.19 $\pm$ 4.07**	2.47 $\pm$ 0.87
0.024	0.06 $\pm$ 0.01**		800 $\pm$ 80	7.66 $\pm$ 1.56	33.93 $\pm$ 4.55*	21.51 $\pm$ 3.24*	2.06 $\pm$ 0.60
0.052	0.07 $\pm$ 0.01*		824 $\pm$ 56	8.00 $\pm$ 2.19	31.59 $\pm$ 8.33	16.44 $\pm$ 2.94	2.25 $\pm$ 1.10
0.104	0.12 $\pm$ 0.01	1.72 $\pm$ 0.28	800 $\pm$ 88	6.76 $\pm$ 1.49	21.34 $\pm$ 4.28	14.46 $\pm$ 1.91	1.82 $\pm$ 0.79
0.405	0.14 $\pm$ 0.01		684 $\pm$ 49	7.97 $\pm$ 1.95	28.61 $\pm$ 3.98	2.18 $\pm$ 2.12*	2.25 $\pm$ 1.06

Table 4 Total and free T₄ concentrations in plasma from rats fed on diets of different Se content

Results are means $\pm$ S.E.M. from six animals in each group.

Se content of diet (mg/kg)	Total T ₄ (nM)	Free T ₄ (pM)
0.003	85.70 $\pm$ 4.05	37.00 $\pm$ 5.30
0.024	72.70 $\pm$ 1.73	20.80 $\pm$ 0.48
0.052	58.00 $\pm$ 2.21	15.70 $\pm$ 1.33
0.104	51.50 $\pm$ 1.93	13.20 $\pm$ 0.54
0.405	56.30 $\pm$ 3.29	17.00 $\pm$ 1.63

liver from Se-deficient animals compared with animals fed on a Se-adequate diet. Quantification of the gels confirmed this observation and showed the effect to be greatest on GSH-Px mRNA. mRNA abundance in polyribosomes from the severely Se-deficient animals, expressed as a percentage of the mean of the Se-adequate control group, was: GSH-Px, 8 $\pm$ 1 % (*n* = 5); PHGSH-Px, 65 $\pm$ 6 %; 5'IDI, 63 $\pm$ 5 %. In contrast, there was no significant difference in GAPDH mRNA abundance in polyribosomes prepared from liver of animals fed on Se-deficient or

Figure 1 Northern-blot hybridization of total thyroid RNA showing the effect of Se deficiency on abundance of selenoenzyme mRNAs in the thyroid

All lanes were loaded with 10 μ g of total RNA and filters were hybridized successively with GSH-Px, PHGSH-Px and 5'IDI probes. The three corresponding RNAs were detected by autoradiography. For a given probe, samples from Se-adequate (0.104 mg/kg) and Se-deficient (0.003 mg/kg) animals were hybridized under identical conditions. The positions of migration of 18S rRNA (2.0 kb) and GAPDH mRNA (1.4 kb) are indicated.

Figure 2 Northern-blot hybridization showing levels of selenoenzyme mRNAs in polyribosomes isolated from liver of rats fed on Se-adequate or Se-deficient diets

All lanes were loaded with 20 μ g of polyribosomal RNA and filters were hybridized successively with GSH-Px, PHGSH-Px, 5'ID1 and GAPDH probes. The four corresponding RNAs were detected by autoradiography. For a given probe, samples from Se-adequate and Se-deficient animals were hybridized under identical conditions.

Se-adequate diets (mean values 100 ± 6 and $114 \pm 7\%$ respectively). Thus the decreases in GSH-Px and 5'ID1 mRNAs were similar in total RNA and in polyribosomes, whereas, although PHGSH-Px mRNA was unchanged in total RNA, it was reduced in polyribosomes; this suggests that, although translation of this mRNA was reduced, its total concentration was maintained.

In a further experiment transcription of the selenoenzyme genes was assessed in nuclei isolated from livers of animals fed on either the severely Se-deficient diet or the Se-adequate diet. As shown in Figure 3, although deficiency again caused a reduction in the levels of GSH-Px and 5'ID1 mRNAs in the liver, nuclear run-off assays showed no apparent effect of deficiency on transcription of the three selenoenzyme genes. Quantification of the amounts of specifically bound transcripts confirmed that deficiency had no effect on transcription of GSH-Px, PHGSH-Px or 5'ID1 genes (Table 5).

Figure 3 Nuclear-run off assays showing transcription of selenoenzyme genes in nuclei isolated from liver of rats fed on Se-adequate or Se-deficient diets

A pair of identical membranes were hybridized for 18 h with RNA from nuclei from liver of an Se-adequate (+) or Se-deficient (—) rat. Nuclei were incubated with [32 P]UTP for 15 min at 28 °C, RNA was isolated and in each case 3×10^7 c.p.m. of RNA incubated with the nylon membrane for 18 h. After being washed, the bound RNA was detected by autoradiography for 3 days. Membranes were loaded (using a slot-blot apparatus) with 5 μ g of the following plasmids: A, Bluescript 2SK with GSH-Px cDNA; B, Bluescript with PHGSH-Px cDNA; C, Bluescript with 5'ID1 cDNA; D, pBR322 with GAPDH cDNA; E, Bluescript vector; F, pUC18 with β -actin cDNA; G, pSVpolyA with threonine B; H, pBR322 vector; I, pUC18 vector; J, pSVpolyA vector. Binding to vectors without plasmid or to the negative control of the bacterial threonine b cDNA was negligible.

Table 5 Quantification of nuclear run-off transcription assays

The amount of bound mRNA was quantified using an Instantimager, the c.p.m. bound were corrected for any binding to the vector without plasmid insert and the data then normalized between filters by calculating a ratio of c.p.m. bound to selenoenzyme cDNA/c.p.m. bound to GAPDH cDNA. Results are means $\pm$ S.E.M.

Gene	mRNA synthesized (% of mean of Se-adequate group)	
	Se-adequate	Se-deficient
GSH-Px	100 ± 20	112 ± 20
PHGSH-Px	100 ± 25	138 ± 17
5'ID1	100 ± 24	91 ± 23

DISCUSSION

These results clearly show that dietary Se supply regulates in a differential manner both the activity of the selenoenzymes and the abundance of their respective mRNAs. The data confirm earlier observations that the abundance of GSH-Px mRNA is greatly reduced in liver of Se-deficient rats [21,22] and that the abundance of PHGSH-Px mRNA is little affected [5]. However, in addition, the present results demonstrate that there is individual regulation of GSH-Px, PHGSH-Px and 5'ID1 such that there are differences in both the sensitivity of the synthesis of each enzyme, and the abundance of its mRNA, to Se supply within a given tissue and also differences in regulation between tissues. In severely Se-deficient animals, 5'ID1 activity was maintained or increased in the thyroid with increased mRNA levels, whereas it was decreased in the liver. PHGSH-Px activity was reduced in both heart and liver, whereas the mRNA levels were preserved. In contrast, under the same conditions both activity and mRNA level of cytosolic GSH-Px were decreased by approx. 90 %. In all three tissues, cytosolic GSH-Px activity was more sensitive than 5'ID1 and PHGSH-Px to Se depletion. Furthermore this differential effect of Se deficiency on selenoenzyme activity was accompanied by differences in the response of RNA abundance to Se depletion.

The differential regulation between liver, thyroid and heart appears to be physiologically important in that it allows, under

conditions of limiting Se supply, a preferential maintenance of some selenoenzymes within and between tissues [6,21,23]; for example, thyroid 5'IDI activity, and thus thyroid hormone metabolism, is maintained under conditions of low Se supply [24] and this may ameliorate some, but not all, adverse effects on thyroid hormone metabolism. The observed changes in selenoenzyme synthesis were accompanied by increases in plasma T_4 and free T_4 concentrations as dietary Se decreased (Table 4), indicating that the biochemical changes within tissues were associated with some hormonal imbalances which have the potential to impair biochemical function.

Se-cysteine is incorporated into proteins by recognition of a UGA (stop) codon [7], and therefore when Se is limiting there is likely to be premature termination of translation with production of inactive abnormal protein products [8,25]. The differential regulation of the three selenoenzymes observed in the present experiments suggests that recruitment of Se-cysteine and translation of the three mRNAs for GSH-Px, PHGSH-Px and 5'IDI are differentially controlled. Analysis of liver polyribosomes showed that, indeed, the extent of translation of GSH-Px, PHGSH-Px and 5'IDI mRNAs was different in severe Se depletion. Thus there appears to be a mechanism whereby Se-cysteine is preferentially channelled for synthesis of one protein rather than another. Furthermore this channelling is regulated differently in the thyroid and the liver so that 5'IDI activity is maintained in the former and PHGSH-Px activity in the latter: in the thyroid this may be affected by thyroid-stimulating hormone, as this hormone is known to induce 5'IDI and its circulating level also increases during Se deficiency; furthermore, in isolated human thyrocytes, Se supply changes 5'IDI activity in the presence but not in the absence of thyroid-stimulating hormone [26].

Although the data show that low Se supply affects both mRNA abundance and activity of GSH-Px, PHGSH-Px and 5'IDI, the alterations in selenoenzyme activity caused by Se deficiency are not necessarily paralleled by changes in mRNA abundance. Furthermore, the nuclear run-off experiments show that, in the liver, there was no change in transcription of the GSH-Px, PHGSH-Px or 5'IDI genes during severe Se deficiency. This indicates that the decreases in GSH-Px and 5'IDI mRNA levels in the liver are not due to alterations in transcription. The observed changes in mRNA, at least in the liver, presumably therefore reflect differences in, and control of, mRNA stability. In severe Se deficiency, GSH-Px activity and mRNA abundance are reduced to similar extents (Table 1) and this is consistent with the hypothesis that there is increased degradation of the mRNA after release from polyribosomes due to premature termination of translation in the absence of Se-cysteine. However, the data in Table 1 also show that GSH-Px activity is more sensitive to low Se supply than its mRNA concentration, suggesting that the fall in mRNA concentration is not simply a consequence of premature release from polyribosomes and subsequent degradation but that a second mechanism exists; perhaps this involves the ability of Se to stabilize GSH-Px mRNA.

In severe Se deficiency, the changes in PHGSH-Px and 5'IDI activities do not parallel changes in their mRNA levels: in the liver, although PHGSH-Px activity decreased and this was reflected in lower PHGSH-Px mRNA abundance in polyribosomes, total mRNA abundance was maintained, suggesting that a proportion of this mRNA remained untranslated under conditions of severe Se deficiency; similarly, in the heart, PHGSH-Px activity was decreased whereas mRNA abundance was maintained; in the thyroid, PHGSH-Px mRNA levels

increased whereas activity was unchanged, and 5'IDI mRNA concentration increased by a much greater extent (95%) than enzyme activity (15%). These differences could reflect a compensatory control mechanism regulating gene transcription or regulation of mRNA stability. In view of the lack of effect of Se deficiency on selenoenzyme gene transcription in the liver, we suggest that the differences in the responses of the GSH-Px, PHGSH-Px and 5'IDI mRNAs in Se deficiency are due to different stabilities of the mRNAs and differential regulation of their stability (by Se and other factors) in liver, thyroid and heart.

In conclusion, the present data indicate that there is differential control of GSH-Px, PHGSH-Px and 5'IDI expression both within a tissue and between liver, thyroid and heart, implying that some mechanism exists to allow synthesis of particular selenoproteins in different tissues; in the liver, at least, this involves post-transcriptional regulation.

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REFERENCES

- Sunde, R. A. (1994) in *Selenium in Biology and Human Health* (Burk, R. F., ed.), pp. 45–77, Springer-Verlag, New York
- Weitzel, F. and Wendel, A. (1993) *J. Biol. Chem.* **268**, 6288–6292
- Arthur, J. R. and Beckett, G. J. (1994) in *Selenium in Biology and Human Health* (Burk, R. F., ed.), pp. 93–115, Springer-Verlag, New York
- Arthur, J. R., Nicol, F., Hutchinson, A. R. and Beckett, G. J. (1990) *J. Inorg. Biochem.* **39**, 101–108
- Sunde, R. A., Dyer, J. A., Moran, T. V., Evenson, J. K. and Sugimoto, M. (1993) *Biochem. Biophys. Res. Commun.* **193**, 905–911
- Beech, S. G., Walker, S. W., Dorrance, A. M. et al. (1993) *J. Endocrinol.* **136**, 361–370
- Chambers, I., Frampton, J., Goldfarb, P., Affara, N., McBain, W. and Harrison, P. R. (1986) *EMBO J.* **5**, 1221–1227
- Berry, M. J., Banu, L. and Larsen, P. R. (1991) *Nature (London)* **349**, 438–440
- Tso, J. Y., Sun, X. H., Koo, T., Reece, K. S. and Wu, R. (1985) *Nucleic Acids Res.* **13**, 2485–2502
- Erickson, J. M., Rushford, C. L., Dorney, D. J., Wilson, G. N. and Schmickel, R. D. (1981) *Gene* **16**, 1–9
- Boyne, R. and Arthur, J. (1986) *J. Nutr.* **116**, 816–822
- Blobel, G. and Sabatini, D. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 390–394
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Whitelaw, P. F. and Hesketh, J. E. (1992) *Biochem. J.* **281**, 143–147
- Shibler, U., Hagenbuchle, O., Wellauer, P. K. and Pittet, A. C. (1983) *Cell* **33**, 501–508
- Toyoda, H., Himeno, S. and Imura, N. (1990) *Biochim. Biophys. Acta* **1049**, 213–215
- Beckett, G. J., Beddows, S. E., Morrice, P. C., Nicol, F. and Arthur, J. R. (1987) *Biochem. J.* **248**, 443–447
- Weitzel, F., Ursini, F. and Wendel, A. (1990) *Biochim. Biophys. Acta* **1036**, 88–94
- Nicol, F., Lefranc, H., Arthur, J. R. and Trayhurn, P. (1994) *Am. J. Physiol.* **267**, R144–R149
- Hill, K. E., Lyons, P. R. and Burk, R. F. (1992) *Biochem. Biophys. Res. Commun.* **185**, 260–263
- Yoshimura, S., Takekoshi, S. and Fujii-Kuriyama, Y. (1988) *Biochem. Biophys. Res. Commun.* **154**, 1024–1028
- Behne, D., Hilmert, H., Scheid, S., Gessner, H. and Elger, W. (1988) *Biochim. Biophys. Acta* **966**, 12–21
- Beckett, G. J., Nicol, F., Rae, P. W. H., Beech, S., Guo, Y. and Arthur, J. R. (1993) *Am. J. Clin. Nutr.* **57**, S240–S243
- Leinfelder, W., Forchhammer, K., Zinoni, F., Sawers, G., Mandrand-Berthelot, M.-A. and Bock, A. (1988) *J. Bacteriol.* **170**, 540–546
- Beech, S. G., Walker, S. W., Arthur, J. R., Nicol, F. and Beckett, G. J. (1993) in *Trace Elements in Man and Animals* (Anke, M., Meissner, D. and Mills, C. F., eds.), vol. 8, pp. 1062–1066, Verlag Medica Touristik, Gersdorf