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RESPONSIVENESS OF SELENOPROTEINS TO DIETARY SELENIUM[1,2]

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ABSTRACT

Selenocysteine-containing enzymes that have been identified in mammals include the glutathione peroxidase family (GPX1, GPX2, GPX3, and GPX4), one or more iodothyronine deiodinases and two thioredixin reductases. Selenoprotein P, a glycoprotein that contains 10 selenocysteine residues per 43 kDa polypeptide and selenoprotein W, a 10 kDa muscle protein, are unidentified as to function. Levels of all of these selenocysteine-containing proteins in various tissues are affected to different extents by selenium availability. Increased amounts of selenoproteins observed in response to selenium supplementation were shown in several studies to correlate with increases in the corresponding mRNA levels. In general, selenoprotein levels in brain are less sensitive to dietary selenium fluctuation than the corresponding selenoprotein levels in other tissues.

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INTRODUCTION

The eventual recognition that the micronutrient selenium[3] is essential in the diet of mammals (34, 37, 38) followed a long period in which this trace element was known only for its toxicity and as a possible potent carcinogen. In contrast, a negative correlation between the amount of selenium in the diet and the incidence of a variety of cancers began to emerge. In domestic animals, important disease syndromes such as white muscle disease and excessive fragility of vascular and erythrocyte membranes were shown to be manifestations of selenium deficiency. In some studies of large human populations with low dietary intakes of selenium, a higher-than-normal incidence of cancer—including lung, liver, stomach, and bladder—was reported (14). There now are numerous studies in which dietary selenium requirements of man and animals have been assessed, not only in terms of relationship to cancer development but also as related to protection from oxidant damage, survival of AIDS infection (3), and amelioration of other disease conditions. The exact mechanism of the apparent protective effects of selenium supplementation against development of certain types of cancer is not known. Formation of selenium metabolites that indirectly affect cancer growth (11, 43), expression of selenium-containing enzymes, and/or changes in other selenium-dependent regulatory components of cells are among the possibilities cited by various investigators.

[3]Many recent publications on regulation of selenoenzyme synthesis by selenium have not distinguished true regulation—which includes effects of selenium on gene expression, mRNA stability, enzyme activity, and stability; rates of enzyme turnover; and various related processes—from the obvious requirement of selenium as an actual chemical component of selenoproteins. In this review we emphasize the regulatory aspects of selenium per se and exclude reference to most reports dealing solely with selenium incorporation in selenoproteins as a function of selenium supplementation of deficient diets or culture media known to be low in selenium.

SPECIFIC OCCURRENCE OF SELENOCYSTEINE IN PROTEINS

Most of the essential selenoproteins currently known, particularly those of mammalian origin, contain selenocysteine residues (39) in positions corresponding to UGA codons in the respective RNA messages (mRNAs). This specific incorporation of selenocysteine as directed by UCA instead of the normal usage of the codon as termination of protein synthesis, requires a unique complex of translation components that has been defined in detail in *Escherichia coli* (Table 1) (21, 39). In eukaryotes, the nucleotide selenocysteine insertion sequence (SECIS) that forms a hairpin secondary structure is located in the 3′-untranslated region of the mRNA (8), whereas in *E. coli* the stem loop structure is immediately 3′ to the UGA in the mRNA. Although recently the human SECIS binding protein was identified and cloned (35), additional protein components essential for insertion of selenocysteine at UGA remain to be detected. Also, the exact mechanism of conversion of seryl-tRNA^{Sec} to selenocysteyl-tRNA^{Sec} in eukaryotes has not been established.

TYPES OF ESSENTIAL SELENIUM-CONTAINING COMPOUNDS IN CELLS

The requirement for the trace element selenium in the diets of animals and in media used to culture eukaryotic cell lines and several types of bacteria is

Table 1 Factors required for UGA-directed selenocysteine incorporation in selenoproteins of prokaryotes[a]

Gene	Identity of gene product	Function
SelC	tRNA^{Sec}; anticodon UCA	Accepts serine; delivers selenocysteine contranslationally to UGA
SelA	Selenocysteine synthase; pyridoxal phosphate dependent	Converts seryl-tRNA^{Sec} to PLP-2,3-aminoacryl-tRNA^{Sec}; addition of selenophosphate forms selenocysteyl-tRNA^{Sec}
SelD	Selenophosphate synthetase	Forms selenophosphate ($SeP0_3H_3$) from ATP and selenide
SelB	Specific elongation factor	Specific complex formation with selenocysteyl-tRNA^{Sec} and a SECIS[b] element (mRNA secondary stem loop structure) immediately 3′ to the UGA; binding of the complex to the ribosome and delivery of selenocysteine at UGA

[a]For details concerning the structures and functions of these gene products, see References 21 and 39.
[b]SECIS, Selenocysteine insertion sequence.

primarily for biosynthesis of essential selenium-containing macromolecules. These macromolecules consist mainly of selenium-dependent enzymes and some selenoproteins of unknown function that contain selenocysteine residues in highly specific locations encoded by UGA (39). Also, in several anaerobic and facultative bacteria, a 2-selenouridine residue occurs in the "wobble position" of the anticodons of certain tRNAs. To date, about 21 specific selenoproteins have been described in mammals and bacteria, and the biological functions of 18 of these have been identified. In mammals these include four different glutathione peroxidases, two deiodinases, two thioredoxin reductases, and one selenophosphate synthetase. Highly reactive protein-bound selenosulfides, $RSSe^-$, and selenophosphate, the oxygen-labile biosynthetic intermediate required for synthesis of selenocysteyl-$tRNA^{Sec}$, probably exist in the cell in finite amounts, but these compounds may be used in situ as generated. Likewise, the specific selenocysteine–acylated $tRNA^{Sec}$ species used for selenoprotein synthesis presumably is a transient intermediate.

Methylated selenium species (15) such as dimethylselenide and trimethylselenium, which are important detoxification products in humans, are rapidly excreted either in the breath or the urine, respectively, and have not been shown to accumulate.

DIETARY SELENIUM

Utilization of Selenium Compounds in Natural Products as Dietary Sources of Selenium

Mammalian and other animal tissues that contain selenium-dependent enzymes are sources of the trace element in the readily available form of selenocysteine in proteins. Utilization of selenomethionine present in dietary selenium sources such as cereal grains, derived grain products (bread, pastas, breakfast cereals, etc), and high-selenium yeast supplements involves reaction steps that produce selenium analogs of the well-known sulfur compounds (scheme 1) homocysteine, cystathionine, and cysteine. Thus, sulfur pathway enzymes can also produce selenohomocysteine, selenocystathionine, and selenocysteine. In certain plants tolerant to high selenium levels in arid soils, selenocysteine also occurs as Se-methyl selenocysteine, often in high and toxic concentrations (28). This methyl ether derivative of selenocysteine has been used as a selenium source in various cancer prevention studies (22). It is produced by a specific methylase, with *S*-adenosylmethionine as the methyl donor (27). Se-adenosylselenomethionine, also produced by *S*-adenosylmethionine synthetase from ATP and selenomethionine, presumably can function as an alternative methyl donor in selenium-tolerant plants.

Amount of Selenium in Proteins from Plants, Yeast, and Yogurt is Dependent on the Extent of Nonspecific Incorporation of Selenomethionine and Selenocysteine

Nonspecific incorporation of selenocysteine, presumably in place of cysteine, has been reported to occur normally in *Saccharomyces cereviseae* (25) and in plants such as corn and cereal grains, especially when grown in seleniferous soils. *E. coli* and *Salmonella typhimurium* strains lacking selenophosphate synthetase activity because of a mutated *selD* gene, and therefore unable to synthesize selenocysteyl-tRNA^{Sec}, nevertheless incorporated selenocysteine into proteins nonspecifically (23, 40). Misacylation of tRNA^{Cys} with free selenocysteine produced by these mutants and its insertion in place of cysteine is a likely explanation. The extent of replacement depended on the ratio of effective Se and S donors available. For example, when synthetic media lacking cysteine were supplemented with [^{75}Se] selenite, incorporation of [^{75}Se] selenocysteine in proteins was much higher than when rich media that contain yeast extracts and protein hydrolysates were used. It now is evident that a dilution effect of added cysteine on selenocysteine incorporation in proteins is diagnostic of a nonspecific insertion mechanism.

Strains of lactic acid bacteria that traditionally have been used to produce fermented milk products such as yogurt incorporate high concentrations of selenium in their biomass (9). *Lactobacillus delbrueckii* subspecies *bulgaricus* used selenite and selenide but not selenate as a selenium source and incorporated 900 μg of Se per 3.75 g biomass per liter of culture medium when grown in media containing 2 mg of Se as selenite per liter. Thus, 45% of the added Se was incorporated in biomass. Analysis of ^{75}Se-labeled cells revealed the presence of numerous labeled proteins, but only labeled selenocysteine was detected in protein hydrolysates. Incorporation of Se in biomass from added selenite was markedly decreased when 2 mM L-cysteine was added to the medium, indicating that incorporation of selenocysteine in proteins was nonspecific. Selenium incorporation was unaffected by addition of 2 mM L-methionine under these conditions, indicating a defect in its utilization as a cysteine source (Figure 1). From these experiments it appears that, in contrast to selenized yeast as a dietary supplement that may furnish equivalent amounts of selenomethionine and selenocysteine, a fermented milk product containing selenium exclusively as selenocysteine may serve as a more readily available source of this trace element.

It has been known for many years that methionine can be nonspecifically inserted into proteins by the usual methionine incorporation process (12, 26). This type of substitution occurs widely in biological systems. In humans and other animals, selenomethionine from ingested foods can be incorporated randomly

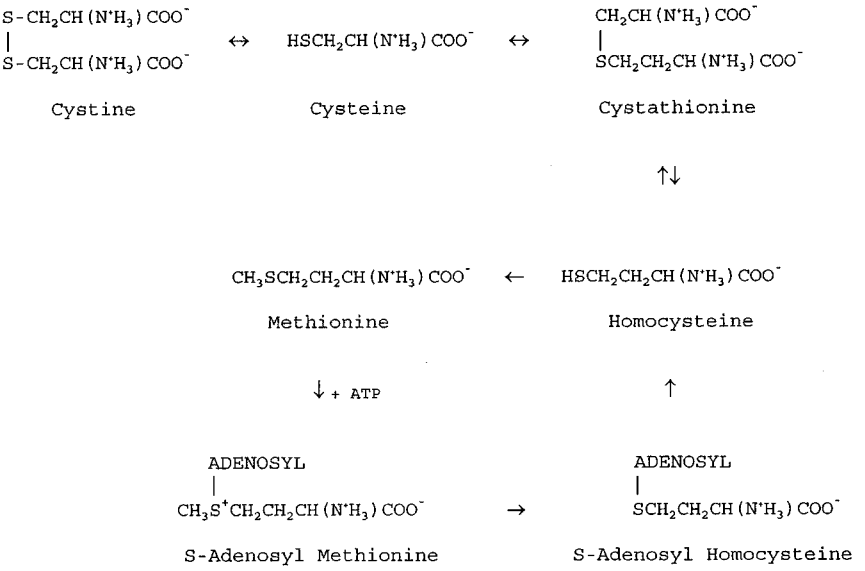

Figure 1 Sulfur amino acid intermediary metabolism. Interconversion of cysteine and methionine.

into cellular proteins. In studies with bacteria, several proteins especially rich in methionine became conspicuously radiolabeled because of nonspecific [^{75}Se] selenomethionine substitution throughout the polypeptide chain, even when a relatively low level of labeled substrate was provided (36). To date, no specific incorporation of selenomethionine in proteins has been documented. In contrast to the selenol group of selenocysteine, which usually is ionized and highly reactive at physiological pH compared with a normal cysteine thiol group with a pK_a value of 8.3, no catalytic advantage of a selenoether over a thioether in a protein would be expected (48).

GLUTATHIONE PEROXIDASES AND DEIODINASES

The first mammalian protein identified as a selenoprotein was cytosolic glutathione peroxidase (GPX-1). Additional glutathione peroxidase enzymes have been identified, including a glycosylated plasma glutathione peroxidase (GPX-2), a gastrointestinal glutathione peroxidase (GPX-3), and a phospholipid-hydroperoxide glutathione peroxidase (GPX-4). Except for GPX-4, these peroxidases exist as tetramers, with each subunit containing one selenocysteine residue. The tetrameric forms catalyze the reduction of a variety of hydroperoxides, including hydrogen peroxide, cumene hydroperoxide, *t*-butyl

hydroperoxide, and fatty acid hydroperoxides (13, 18). In contrast, GPX-4, a monomer that contains one selenocysteine residue, catalyzes the reduction of both fatty acid hydroperoxides and cholesterol hydroperoxides (44). After reaction with a peroxide substrate, regeneration of the reduced active form of the enzyme requires GSH (reduced glutathione). However, because GSH levels in the plasma are low, the regeneration of GPX-2 requires the thioredoxin/thioredoxin reductase system. The second mammalian selenoenzyme identified was type I iodothyronine deiodinase (5′DI) (8, 18). This enzyme, a homodimer that contains one selenocysteine at each active site, catalyzes the 5′-deiodination of L-thyroxine to tri-iodo-L-thyronine (18).

Selenium-Dependent Expression

In mammals, selenium preferentially accumulates in tissues such as endocrine glands, brain, and reproductive organs (4). Under selenium-deficient conditions, a tissue-specific regulation of selenoprotein expression is observed. Several studies have been performed that demonstrate this regulation. The selenium-dependent expression of the selenocysteine-containing proteins GPX-1 and 5′DI was examined in the porcine kidney epithelial cell line LLC-PK_1 cultured in serum-free media (18). A selenite concentration dependence on the expression of both GPX-1 and 5′DI proteins was demonstrated. Initially, cells grown in serum-free media exhibited marginal GPX-1 and 5′DI activities. When selenite was added to the media, 5′DI activity increased rapidly after selenite concentrations exceeded 0.5 nM, whereas GPX-1 activity started to increase at 1 nM. Both enzyme activities increased linearly as selenite concentrations reached 20 nM; when the selenite concentration approached 50 nM, 5′DI activity began to decrease compared with GPX-1 activity, which showed little change. Northern-blot analysis performed to monitor mRNA levels showed that GPX-1 and 5′DI mRNAs were low but detectable in the cells grown in selenium-deficient serum-free media. The levels of mRNA corresponding to GPX-1 increased with the continued addition of selenite, but 5′DI mRNA, which was barely detectable at low selenite concentrations, did not increase appreciably until the selenite concentration reached 47 nM. At selenite concentrations above 47 nM, the level of 5′DI mRNA decreased at a rate consistent with the decrease observed in enzyme activity.

Expression of GPX-1 and 5′DI was investigated further in selenite repletion and deletion experiments. The LLC-PK_1 cells grown initially for 4 days in serum-free media in the absence of selenium were supplemented with 47 nM selenite and incubated an additional 4 days. The activity of GPX-1 increased linearly for 3 days after selenite supplementation compared with the 5′DI activity, which exhibited a biphasic increase, with a transition between days 1 and 2. In a similar experiment after 4 days of growth in selenite-containing media,

LLC-PK_1 cells were transferred to media without selenite and incubated for an additional 4 days. The activity of GPX-1 decreased steadily over the 4-day growth period in the selenium-deficient media with an activity half-life of 1 day. In contrast, 5′DI activity decreased during the first day of depletion at a similar rate to GPX-1 inactivation but then showed an increase in activity.

These experiments with the porcine kidney epithelial cell line LLC-PK_1 suggest a difference in the regulation of the expression of selenoproteins 5′DI and GPX-1, with 5′DI being preferred over GPX-1 when selenite conditions are limited or depleted. A comparison of the enzyme activity to mRNA levels suggests that selenium affects the expression of selenoproteins by indirectly affecting mRNA stability or mRNA transcription.

Tissue-Specific Regulation

Another study examined the tissue-specific regulation of the selenoenzymes GPX-1, GPX-4, and 5′DI in the heart, liver, and thyroid of rats fed a diet with varying concentrations of selenium, ranging from adequate to severely selenium deficient (7). When selenium concentrations were severely deficient, activities of GPX-1 and GPX-4 in the heart decreased 90% and 60%, respectively. In the liver, GPX-1 activity was reduced by 99%, GPX-4 by 75%, and 5′DI by 95%. In the thyroid, GPX-1 activity decreased 50%, GPX-4 was unaffected, and 5′DI increased 15%. Measurement of mRNA levels in the various organs showed that in the liver, GPX-1 mRNA was reduced 90%, 5′DI levels decreased by 50%, and no GPX-4 mRNA was detected. In the heart, GPX-1 mRNA dropped 72%, and no change was observed in GPX-4 mRNA. In the thyroid, GPX-1 mRNA levels were unchanged, and GPX-4 mRNA and 5′DI mRNA increased 50% and 90% respectively. No 5′DI activity or mRNA was detected in the heart.

These results demonstrate that the three selenoproteins GPX-1, GPX-4, and 5′DI are regulated individually within a given tissue during selenium deficiency and that the extent of regulation varies from organ to organ. The observed differential regulation of these selenoenzymes may be physiologically important in creating a hierarchy of expression of selenoproteins during selenium limitation. The lack of correlation of mRNA levels with enzyme activities under conditions of selenium depletion suggest that regulation was not under transcription control but perhaps reflected mRNA stability.

Correlation of mRNA Stability to Enzyme Activity

A recent study with H4 hepatoma cells showed that in selenium deficiency, the stability of translated mRNA was an important factor in regulation of selenoprotein levels (6). H4 hepatoma cells grown in selenium-deficient medium showed an 80% decrease in GPX-1 activity after 2–4 days of culture, whereas GPX-4 activity decreased by 50% after 3 days of growth. Northern blot analysis

showed that there was a large reduction in the GPX-1 mRNA but not of the GPX-4 mRNA. Stability of the two mRNAs was determined by Northern blotting performed over a 12-h period after transcription was inhibited by the addition of actinomycin D. Analysis of mRNA extracted from the selenium-deficient cells at 0, 4, 8, and 12 h revealed that both GPX-1 mRNA and GPX-4 mRNA were degraded. However, degradation of GPX-4 mRNA occurred at the same rate in cells grown in selenium-supplemented media. The rate of GPX-1 mRNA degradation was much more rapid, with a determined half-life of 8 h in selenium-deficient cells and 13 h in cells grown in selenium-sufficient media. Thus, under these conditions, in selenium-deficient H4 hepatoma cells, the stability of the mRNA for GPX-4 is maintained compared with the mRNA for GPX-1. The report that GPX-4 mRNA remains bound to polysomes compared with GPX-1 mRNA, which is released more readily and degraded (5), correlates well with the observed higher activity of GPX-4 compared with GPX-1.

MAMMALIAN THIOREDOXIN REDUCTASE

The selenocysteine-containing enzyme thioredoxin reductase (TR) catalyzes the NADPH-dependent reduction of thioredoxin (16, 17, 42). The thioredoxin/thioredoxin reductase system is widely used in biological systems to reduce ribonucleotides to deoxyribonucleotides, which are essential in the synthesis of DNA, to maintain redox balance in cells, to regulate activity of transcription factors, and to regenerate antioxidant systems. As expected, protein expression and activity levels of TR in cultured cells are dependent on the availability of selenium in media (14). Levels of selenite in the growth media required for optimal TR activity in A549 lung cancer, MCF-7 breast cancer, and HT-26 colon cancer cells differed with cell types. The increase in TR activity at 1 μM selenite, compared with TR activity in cells grown in the absence of selenite, was on the order of 8- to 37-fold higher. Moreover, the level of TR protein also increased in the presence of added selenite. However, the increase in protein levels was not as high as the increase in TR activity. The increase in TR protein, in the cell lines studied, was on the order of 1.8- to 2.8-fold. Thus, the increase in TR activity is not directly related to a greater amount of protein produced but rather to an increase in the specific activity of the enzyme (from 0.06 $nmol^{-1}$ min^{-1} mg with no selenium to 3.0 $nmol^{-1}$ min^{-1} mg with 1 μM selenite added). The increase in specific activity is also correlated to an increase in the amount of selenium found within TR. The amount of ^{75}Se incorporated into TR increased from 0.01 at 27 nM selenium to 0.98 at 1.03 μM selenium. It has previously been shown that TR isolated from human placental cells contained one selenocysteine/monomer, consistent with

the isolated enzyme from cells grown in media with 1 μM selenite. In view of the fact that proteins have not been subjected to complete amino acid analysis, it is not known whether the lower specific activity of TR is due to termination at the UGA where selenocysteine is to be inserted (17). A truncated protein formed when translation is terminated at UGA is also consistent with a TR that contains less ^{75}Se as well as a lower specific activity.

SELENIUM DEFICIENCY AND SKIN CANCER

There is suggestive evidence linking selenium to decreased skin damage, tumor formation, and overall mortality after exposure to UVB radiation (29, 41). In humans, it has been demonstrated that selenium deficiency is associated with up to a fourfold increase in the development of skin cancer (10). In a recent study, the effects of UVB radiation on the expression of [^{75}Se] selenoproteins in cultured human skin cells, primarily melanocytes, fibroblasts, and keratinocytes, was examined (32). Among the selenoproteins detected were 21-kDa and 60-kDa selenoproteins that were identified as GPX-4 and TR, respectively. These selenoenzymes showed distinctly different profiles in the human skin cell types. Expression of both GPX-4 and TR was greatest in fibroblasts and lowest in keratinocytes. There was no detectable change in the expression of any ^{75}Se-labeled selenoproteins in the three cell types during the first 24 h after UVB exposure. However, 48 h after exposure of keratinocytes to UVB (960 J/m^2) and melanocytes to UVB (720 J/m^2), 80% cell death was observed. A marked protective effect from UVB radiation–induced cell death was observed by pretreatment of cells with selenite (1–200 nM) or selenomethionine (10 nM–1 μM), provided that selenium addition was 24 h prior to UVB exposure. However, when selenium was added immediately after exposure, only modest protection was observed and higher concentrations of selenite and selenomethionine were required. The greater protection observed by selenium pretreatment prior to UVB exposure suggests an indirect effect requiring selenoprotein synthesis rather than a direct antioxidant effect of the added selenium compound. Furthermore, these experiments show that the form of selenium supplementation is an important factor in preventing cell death. A selenite concentration as low as 1 nM was sufficient to increase cell protection compared with selenomethionine, where no protection was observed at concentrations below 10 nM. Selenomethionine can be more effectively utilized by cells and taken up into proteins because of its nonspecific incorporation in place of methionine. However, selenomethionine in place of methionine offers no additional catalytic activity within a protein (47). Selenite can readily be reduced to selenide, which is a precursor of selenophosphate, the universal selenium donor in vivo, whereas selenomethionine is a less available metabolic source of selenium (scheme 1). In these studies, skin cancer has been found to occur in

melanocytes and keratinocytes only. However, in previous reports, fibroblasts were shown to be protected from UVB radiation by selenium supplementation (33).

MAMMALIAN SELENOPROTEINS OF UNKNOWN CATALYTIC ACTIVITY

Selenoprotein P and selenoprotein W are selenocysteine-containing mammalian proteins of unknown function (39). Evaluation of the effects of selenium supplementation on the biosynthesis of these proteins is limited to responses in protein and mRNA levels only, because protein activity is not measurable.

Selenoprotein P

Selenoprotein P, one of two selenium-containing glycoproteins in plasma, accounts for about one third, 0.4 μmol/liter, of the total plasma selenium in humans (1). The other selenium-containing glycoprotein in plasma is the extracellular form of glutathione peroxidase, which accounts for about 0.2 μmol of plasma selenium per liter. By sodium dodecyl sulfate–polyacrylamide gel electrophoresis, purified human selenoprotein P migrates as two bands apparently of 61 and 55 kDa (1). When selenoprotein P is enzymatically N-deglycosylated, multiple new bands are observed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, which suggests there are different polypeptide forms of selenoprotein P (1). Selenoprotein P contains up to 10 selenocysteine residues per 43-kDa polypeptide chain. The protein also is rich in cysteine and histidine, which suggests a metal binding function. Because of the abundance of selenocysteine residues and the fact that it is the major selenium component in plasma, it has been suggested that selenoprotein P may be a useful biological marker for selenium status (31).

An important starting point for determining the dietary response of selenoprotein P to selenium supply is to measure changes in protein levels as a function of selenium levels in the diet. Supplementation with selenium does generally increase plasma levels of selenoprotein P up to some limiting saturation concentration. Persson-Moschos et al (30) used a radioimmunoassay to measure selenoprotein P levels in 50 healthy Finnish males after supplementation with different forms of selenium. Two trials, one in 1981 (24) and the other in 1987 (2), were conducted on the same study group. Frozen plasma samples from the original study, which was designed to follow the effects of selenium supplementation on platelet glutathione peroxidase activity, were used later for selenoprotein P determinations. The test group was given supplemental selenium orally at 200 μg/day for 11 weeks as selenium-enriched yeast, sodium selenate, or selenium-enriched wheat, and the control group received a placebo. It was estimated that the average amount of selenium received per day without

supplementation was about 40 μg. Selenoprotein P levels increased substantially within the first 2 weeks, reached a maximum after 4 weeks, and then leveled off during the remaining 5 weeks of the supplementation period. The maximum increase in selenoprotein P levels was about 40% over the baseline for all three selenium sources, and there were no differences in rates of increase of selenoprotein P.

A study similar to the Finnish one that involved numerous groups of people was made in China in 1987 (20). Boys received 100 μg of selenite orally per day for 2 weeks and men received 200 μg/day. The original blood samples from this study also were stored frozen, and a radioimmunoassay was used for the analysis of selenoprotein P after its discovery. Similar to the Finnish study, selenoprotein P levels increased with supplementation of selenium. Gender and age had no effect on selenoprotein P levels in subjects who had an adequate selenium intake; however, children between the ages of 2–5 tended to have lower selenoprotein P levels than did older people when levels of selenium in the diet were inadequate.

The response of selenoprotein P to available selenium also was investigated in two cultured liver cell lines, HepG2 and H4IIE (19). After growth of cells for 48 h without added selenium in a serum-free medium, cultures were supplemented with sodium selenite, 10 ng/ml, and incubated an additional 48 h. The HepG2 cell line showed a 90% reduction in selenoprotein P levels under conditions of selenium depletion compared with the selenium-supplemented cells. In contrast, the H4IIE cells showed only a 60% reduction in selenoprotein P levels under conditions of selenium depletion.

The selenoprotein P mRNA levels in the two cell lines showed marked differences in response to conditions of selenium repletion. There was no significant increase in selenoprotein P mRNA in the H4IIE cells, whereas in the HepG2 cells the mRNA increased by about 50%. Thus, both protein levels and mRNA levels in the HepG2 cells were more sensitive to selenium supply compared with the H4IIE cell line. In the case of the HepG2 cell line, the mRNA levels did not correspond to protein amounts, which suggests that a posttranscriptional regulatory process may be involved in selenoprotein P biosynthesis in these cells.

Selenoprotein W

Selenoprotein W was isolated from rat muscle (45), a partial amino acid sequence was determined, and a selenium content of about 1 g atom of selenium as selenocysteine per 1 g mol of protein was established. The cDNA sequence confirmed the known protein primary sequence, including a selenocysteine residue encoded by TGA (46). Four different forms of selenoprotein W were purified from rat muscle. The molecular masses ranged from 9,500–10,000. The differences in molecular masses were later shown by matrix-assisted laser

desorption ionization mass spectrometry to arise from varying amounts of bound glutathione and an unidentified low-molecular-weight moiety (5).

Numerous studies have shown that selenoprotein W levels also increase in response to dietary supplementation with selenium. Reliable data that relate the specific tissue distribution and responses of selenoprotein W to dietary selenium are available. In one study (50), a complicated set of supplementation experiments was carried out to determine not only the tissue distribution of selenium as selenoprotein W, but also the specific level of supplementation required to affect selenoprotein W synthesis within each tissue studied. Compared with those fed a basal diet without selenium, rats fed 0.1 mg of selenite per kg of body weight for 6 weeks had significant increases in selenoprotein W levels in muscle, spleen, and testes, as measured by Western blotting. When the supplementation was increased to 0.4 mg of selenite per kg of body weight, higher levels of selenoprotein W were found in muscle, brain, and spleen tissue compared with to 0.1-mg supplementation levels. The levels of selenoprotein W in the testes, however, did not increase further with the higher supplementation levels. Significant increases in the amount of selenoprotein W mRNA also were observed in muscle tissue. The intensities of the bands in the Northern blots increased with each increase in dietary selenium.

To refine the selenium supplementation effects on selenoprotein W levels, rats were fed seven different levels of selenium, varying from 0.01 to 4.0 mg/kg of body weight. In muscle, selenoprotein W began to increase at 0.06 mg/kg; it showed the largest increase at 1.0 mg/kg and then plateaued. In the brain and spleen, linear increases of selenoprotein W were seen up to 0.1 mg of selenium per kg in the diet, and with higher selenium supplementation amounts, the amounts remained the same. In the testes, the effects were very different. At only 0.01 mg of selenium per kg in the diet, the selenoprotein W levels were increased significantly and an inflection was reached. These results clearly indicate that selenoprotein W regulation in response to dietary selenium in rats is different within individual tissues.

The response of selenoprotein W expression to dietary selenium also has been measured in sheep[4] (49). In these experiments, sheep were fed either 0.02 or 3 mg of selenite/kg of body weight for 10.5 weeks. Selenoprotein W levels were measured in muscle tissue from biopsies initially and at 3.5, 7.0, and 10.5 weeks, at which time the sheep were sacrificed to measure selenoprotein W levels in

[4]In ruminants, ingested foods first are partially fermented in the rumen. During this preliminary digestion period, varying amounts of the nutrients are incorporated into cell material of the microbial population. The abundant methane bacteria in the rumen synthesize an unusually large number of selenocysteine-containing enzymes, and this can be a potential problem under conditions of selenium deficiency. The availability of the element to the animal then depends on subsequent digestion of bacterial proteins in the stomach.

other tissues. Selenoprotein W levels were determined in tissues by Western blots with a polyclonal antibody. Levels of selenoprotein W in sheep muscle increased from about 25 ng/mg of protein to 115 ng/mg of protein after supplementation, whereas those fed a selenium-deficient diet showed decreases to about 15 ng/mg of protein. The increase was approximately linear, as was the decrease.

Selenoprotein W was found in all the tissues tested at the end of the supplementation period: muscle, heart, tongue, brain, lung, spleen, kidney, and liver. The results from measurements of selenoprotein W in different tissues were generally what would be expected, namely that the levels increased with supplementation. In the brain, however, a different result was observed. The level of selenoprotein W in the brain was the highest in selenium-starved animals compared with the other tissues tested, and brain levels remained constant, even with supplementation of 3 mg/kg. In addition, at the end of the supplementation period, total selenium levels in the brain increased about 50% compared with sheep on a basal diet. Thus, the brain was the only tissue tested that did not respond to dietary selenium even when additional selenium became available. It is evident from this study that selenoprotein W tissue distribution does not correlate with selenium distribution and that selenoprotein W is the most conserved in the brain.

CONCLUSIONS

Expression of essential mammalian selenoenzymes and selenoproteins of unknown function clearly depends on availability of selenium, an obligatory component usually occurring in the form of selenocysteine residues in the polypeptide chains. When the supply of selenium is suboptimal for growth, levels of various selenoproteins are affected to different extents. Whereas levels of some may fall precipitously, those of others may be little affected. Moreover, changes in levels of selenoproteins as a function of dietary selenium depletion and repletion can vary markedly from tissue to tissue. In general, selenoproteins in brain are less prone to fluctuate with changes in dietary selenium than are those in other tissues. In some cases, a correlation of mRNA level with level of the corresponding translation product is observed, and mRNA instability under conditions of selenium deficiency has been documented. More precise information on rates of synthesis and degradation of the various selenoenzymes and identification of other factors, in addition to selenium availability, that determine these rates should contribute to our understanding of cellular regulation of selenoenzymes.

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