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SELENOPROTEIN P: An Extracellular Protein with Unique Physical Characteristics and a Role in Selenium Homeostasis

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■ **Abstract** Selenoprotein P is an abundant extracellular glycoprotein that is rich in selenocysteine. It has two domains with respect to selenium content. The *N*-terminal domain of the rat protein contains one selenocysteine residue in a UxxC redox motif. This domain also has a pH-sensitive heparin-binding site and two histidine-rich amino acid stretches. The smaller *C*-terminal domain contains nine selenocysteine and ten cysteine residues. Four isoforms of selenoprotein P are present in rat plasma. They share the same *N* terminus and amino acid sequence. One isoform is full length and the three others terminate at the positions of the second, third, and seventh selenocysteine residues. Selenoprotein P turns over rapidly in rat plasma with the consequence that approximately 25% of the amount of whole-body selenium passes through it each day. Evidence supports functions of the protein in selenium homeostasis and oxidant defense. Selenoprotein P knockout mice have very low selenium concentrations in the brain, the testis, and the fetus, with severe pathophysiological consequences in each tissue. In addition, those mice waste moderate amounts of selenium in the urine. Selenoprotein P binds to endothelial cells in the rat, and plasma levels of the protein correlate with prevention of diquat-induced lipid peroxidation and hepatic endothelial cell injury. The mechanisms of these apparent functions remain speculative and much work on the mechanism of selenoprotein P function lies ahead. Measurement of selenoprotein P in human plasma has shown that it is depressed by selenium deficiency and by cirrhosis. Selenium supplementation of selenium-deficient human subjects showed that glutathione peroxidase activity was optimized before selenoprotein P concentration was optimized, indicating that plasma selenoprotein P is the better index of human selenium nutritional status.

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INTRODUCTION

The beneficial effects of selenium as a micronutrient were first recognized in the 1950s. It was 1973, however, before the first animal selenoprotein, glutathione peroxidase, was identified in rat liver (62). With that discovery, the known biological effects of selenium appeared to be explained (40).

Research on selenium continued, however, and another selenium-containing protein was identified in 1977, this time in blood plasma (31). That protein was further characterized (9) and shown in 1982 to contain its selenium as selenocysteine (57). Because no function could be attributed to it, the letter P was used in its name in reference to its location. Thus, selenoprotein P was the second animal selenoprotein to be identified. Its discovery fostered efforts to find additional selenoproteins and to determine how selenoproteins are produced.

An elegant series of studies by Böck and his collaborators in the late 1980s characterized selenoprotein synthesis in prokaryotes (6). Building on the prokaryotic results, other workers have provided many of the details of eukaryotic selenoprotein synthesis. A description of the process is beyond the scope of this review (see 24, 47, 50), but the open reading frame of the selenoprotein mRNA contains a UGA at the position of each selenocysteine residue in the protein, and its 3′ untranslated region contains a stem loop structure with conserved features. Thus, the DNA of a selenoprotein has identifiable features.

Twelve selenoproteins had been identified by conventional means before genomic databases became available (12). Those proteins included four glutathione peroxidases, three iodothyronine deiodinases, and a thioredoxin reductase.

By the late 1990s, it had become obvious that selenoproteins have a wide variety of metabolic roles.

Efforts were made to search available databases for additional selenoproteins. Early efforts sought stem loop structures and identified more selenoproteins (46, 48). They also demonstrated that some enzymes are selenoproteins in one species but contain cysteine in place of selenocysteine in another species.

The availability of databases of complete genomes made it possible to search for all the selenoproteins. Recently a strategy that requires candidate genes to be present in both murine and human genomes has identified 25 selenoprotein genes in the human genome and 24 in the mouse genome (44). This genome-wide search has probably identified virtually all the genes that code for selenoproteins through conventional selenoprotein mRNAs. It is possible, though, that selenoproteins with forms of selenium other than selenocysteine will be found as they have been in prokaryotes (30).

Although 25 selenoprotein genes are present in the human genome, alternative splicing and other strategies to produce more than 1 protein per gene will raise the number of selenoproteins to more than 25. A few of the selenoproteins have been extensively characterized, but little beyond the genomic data is known about many of them (44). Biochemical and physiological characterization will be required for each.

As the second animal selenoprotein identified, selenoprotein P has been the subject of a number of studies over the past 20 years. It is an abundant protein that has several striking properties not present in other selenoproteins and it appears to play a major role in whole-body selenium metabolism. In this review, we discuss the genetic and physical characteristics of selenoprotein P as well as its metabolism and physiological properties. Our aims are to draw attention to unusual and unexplained properties of this complex protein that may serve as clues to its biological activity and to provide suggestions of how it contributes to selenium homeostasis.

GENE

Structure

Selenoprotein P genomic sequences have been reported in DNA from the mouse (71) and the human being (82). Each gene contains five exons with the coding sequence being restricted to exons 2–5. The first in-frame TGA (coding for selenocysteine) is in the second exon. The other nine in-frame TGAs and the two selenocysteine insertion sequence (SECIS) elements are in the fifth exon. The mouse gene is 10.3 kb in size and the human gene is 12 kb. Because their exons are the same size, this implies a difference in the size of some introns between the two species.

No evidence has been found for multiple copies of the mouse or human genes. However, two selenoprotein P genes are implied by the presence of two cDNAs in the zebrafish-expressed sequence tag database (45). One cDNA implies a protein

of 367 amino acids that contains 17 selenocysteine residues and has two SECIS elements in its 3′ untranslated region. The second cDNA implies a protein of 265 amino acids that contains only one selenocysteine residue and one SECIS element. The shorter implied protein has similarity with the *N*-terminal region of the longer one and with the *N* terminus (and shorter isoforms) of rat selenoprotein P (see "Isoforms" section below and Figure 1, color insert). Thus, selenoprotein P genes encode proteins containing from 1 to 17 selenocysteine residues.

Selenoprotein P appears to be expressed only in vertebrates. It was not found in the genomes of *Caenorhabditis elegans* or *Drosophila melanogaster* (44).

Regulation

Nuclear run-on experiments were carried out in rat liver to assess the effect of selenium deficiency on selenoprotein P gene transcription (11). No effect was found. Thus, transcription of the selenoprotein P gene in liver is not sensitive to the selenium status of the animal.

The promoters of the human and mouse selenoprotein P genes have been sequenced (23, 71). They contain binding sites for numerous transcription factors, including tissue-specific ones that presumably account for the extreme variation in expression among tissues.

Transfection experiments with the human promoter region using a luciferase reporter gene showed inhibition of expression by tumor necrosis factor-alpha (TNFα), interferon γ, and interleukin 1β. This suggests that production of selenoprotein P would be inhibited in an acute phase response. Transforming growth factor-beta (TGFβ) also inhibits expression through Smad 3 and 4 (56). However, effects of cytokines and growth factors have not been evaluated in vivo.

The spatial and temporal expression of the two selenoprotein P mRNAs in zebrafish has been assessed during embryogenesis (74). The shorter isoform was expressed primarily in the liver and the longer one primarily in the brain, kidney, and intestine. This shows a differential regulation of these genes, which indicates that the functions of the short and long isoforms are different.

PROTEIN STRUCTURE

Not enough is known about the structure of selenoprotein P to allow construction of three-dimensional representations. Similarities with other proteins have not been reported and efforts to produce crystals of it for X-ray crystallography have not yet been successful (K.E. Hill, unpublished observations). Mass spectrometry and other techniques have revealed a number of characteristics of rat selenoprotein P, however, and they are shown in Figure 1 and discussed in this section.

Primary Structure

The highly unusual nature of this protein became obvious as soon as it was subjected to analysis. As purified, rat selenoprotein P had multiple selenocysteine

residues per polypeptide (61) compared with a single selenium per polypeptide in glutathione peroxidase (28).

Once the selenoprotein P cDNA had been cloned from a rat liver library, an implied amino acid sequence of 366 residues (Figure 1) and a preceding 19-amino-acid signal peptide were revealed (33). The implied mature protein contained 10 selenocysteine and 17 cysteine residues. A further unusual feature of the amino acid sequence was the presence of two histidine-rich regions. One was 14 residues long (residues 185–198) and contained eight histidine and two lysine residues; the other was 10 residues long (residues 225–234) and contained eight histidine and two lysine residues. These histidine-rich regions are likely to be involved in binding other molecules (see below).

Based on its selenium content, the protein was recognized to have two domains. The first and larger one extends from the *N* terminus through the residue just before the second selenocysteine (residues 1–244). It contains one selenocysteine and seven cysteine residues as well as the two histidine-rich sequences. The smaller domain extends from the second selenocysteine to the *C* terminus (residues 245–366). It contains nine selenocysteine and ten cysteine residues. Thus, 90% of the selenocysteines and 59% of the cysteines are concentrated in this shorter stretch of residues. The selenium-rich *C*-terminal domain has some features that suggest it might be on the inside of the molecule. Chemical modification of basic amino acid residues in the native protein did not label residues distal to the lysine at residue 261 (41), and limited trypsin proteolysis occurred after residue 264 but not more distally (R.F. Burk, unpublished observation). This lack of reactivity of the *C*-terminal domain suggests that it is shielded in the native protein.

Selenoprotein P sequences from mammalian species have overall identities in the 70%–80% range, although some of them have unique features. The seven sequential proline-histidine repeats in the bovine sequence beginning at residue 196 are an example of this (63).

Isoforms

Selenoprotein P isoforms were recognized to occur in plasma after the immunoaffinity-purified protein preparation was observed to bind to heparin at pH 7 but not at pH 8.5 (21). Application of a pH gradient to the heparin column eluted several selenoprotein P peaks of different masses. Conventional peptide sequencing and mass spectrometry were used to characterize those isoforms of rat selenoprotein P (39, 53).

In addition to the full-length selenoprotein P, three shorter isoforms were identified that shared the same *N* terminus but had *C* termini at the positions of the second, third, and seventh UGAs (53). The shorter isoforms were given the names Se-P_1, Se-P_2, and Se-P_6, while the full-length isoform was named Se-P_{10}. The subscript indicates the number of selenium atoms in the isoform. These three shorter isoforms appeared to arise from the same mRNA as the full-length protein

because they shared the same sequence. Moreover, only one selenoprotein P mRNA is known. Therefore, it was postulated that three of the in-frame UGAs code alternatively for insertion of selenocysteine or for termination of translation. It is not known whether cells are programmed as to which isoform(s) they produce or whether they are able to switch among isoforms according to need.

Because the isoforms terminate precisely at UGAs, it seems unlikely that they are produced from the full-length protein by proteolysis. For that to occur, a protease that cleaves at selenocysteine residues would be needed, and no such enzyme is known. Another possible mechanism of isoform production would be inefficiency of UGA translation as selenocysteine. Such an occurrence would be predicted to take place at all UGAs, however, and not just at specific ones. Moreover, it would be expected that products of premature termination would be eliminated by the quality-control mechanism of the endoplasmic reticulum. Thus, this appears to be the first demonstration of alternative use of the UGA codon to produce different isoforms of a protein.

The presence of genes in the zebrafish that code for proteins analogous to Se-P_1 and Se-P_{10} suggests that the organism needs both the shorter and the longer forms of selenoprotein P. It can be speculated that higher vertebrates have developed a mechanism for producing shorter and longer forms from one gene to accomplish what the zebrafish does with two genes.

Glycosylation

The average mass of Se-P_{10} was estimated to be 50,474 by matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry (53). Yet, its predicted peptide weight was only 41,096 (33). This large discrepancy indicated that the protein contained post-translational modifications. Purified selenoprotein P took stains for carbohydrate and treatment of it with glycosidases increased its migration on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (61). This indicated that at least part of the modification was carbohydrate.

Se-P_{10} was analyzed for glycosylation using mass spectrometry (52a). Peptides from protease digests of Se-P_{10} had carbohydrate attached. Figure 1 shows the three occupied *N*-glycosylation sites and the one *O*-glycosylation site. All sites displayed microheterogeneity but, on average, they accounted for 9330 mass units. Addition of this value to the predicted peptide weight yields 50,426, which is close to the measured mass of Se-P_{10}. Thus, additional small modifications cannot be ruled out, but carbohydrate appears to account for the nonpeptide mass of Se-P_{10}. Similar calculations were made for the shorter isoforms, based on the carbohydrate present in Se-P_{10}, and similar results were obtained for them.

Disulfide and Selenenylsulfide Linkages

Se-P_1 was analyzed by mass spectrometry for disulfide linkages (52a). The selenocysteine at residue 40 and the cysteine at residue 43 formed a selenenylsulfide bond (Figure 1) that has characteristics of a redox center (see "Enzyme Activity" section

below). Two disulfide linkages were found between cysteine residues 149–167 and 153–156. Thus, the *N*-terminal domain has two disulfide bonds that appear to be structural and a potential redox site that contains selenocysteine.

A fragment made up of residues 298–324 was isolated from a trypsin digest of Se-P_{10} (51). Mass spectrometric analysis revealed that the fragment contained two selenenylsulfide linkages (Figure 1) (52b). This is the only structural information available on the *C*-terminal domain of selenoprotein P.

BIOCHEMICAL PROPERTIES

The study of biochemical properties of selenoproteins is often difficult because most of them, including selenoprotein P, cannot be expressed in bacteria. Thus, only the amounts of selenoprotein P that can be purified are available for study and site-directed mutagenesis cannot be employed. Initial success in expression of recombinant rat selenoprotein P in a human cell line has been reported (76). Further development of this technique will allow more extensive characterization of this protein. However, the studies reported to date have used only selenoprotein P purified from plasma.

Enzyme Activity

In general, selenoproteins serve as enzymes that catalyze redox reactions. Examples are the glutathione peroxidases, in which the selenium atom accepts electrons from GSH and transfers them to a hydroperoxide, and the thioredoxin reductases, in which the selenium atom is paired with a sulfur atom (in a cysteine residue) and this redox pair transfers electrons from the enzyme to thioredoxin (84). The primary structure of selenoprotein P (Figure 1) contains many potential redox centers in the form of cysteine and selenocysteine residues. One of them, at residues 40–43, has the classic CxxC sequence (29), with the first C being a U, or selenocysteine.

Early efforts to detect glutathione peroxidase activity of selenoprotein P were unsuccessful (81). However, Takahashi's group was able to demonstrate that their human selenoprotein P preparation weakly catalyzed the oxidation of GSH by a phosphatidylcholine hydroperoxide (64). It thus functioned as a phospholipid hydroperoxide glutathione peroxidase. Thiols such as dithiothreitol substituted effectively for GSH, but neither H_2O_2 nor small organic hydroperoxides could serve as substrates. This indicated that the peroxidase activity of selenoprotein P was highly selective, but it appeared to be suitable for acting on membrane hydroperoxides.

Further experiments revealed that an *N*-terminal fragment containing the UxxC sequence was responsible for the peroxidase activity (65). Moreover, thioredoxin was approximately 500-fold more effective as the reducing substrate than GSH (73). These findings point toward the UxxC of selenoprotein P being an enzyme active site. The nature of its endogenous substrates is uncertain, however.

Heparin Binding

A number of extracellular proteins have heparin-binding properties that allow them to bind to specific structures and thereby focus their biological activities (22). Selenoprotein P is a heparin-binding protein (2, 31). Its heparin-binding properties are highly unusual, however, because they are pH sensitive within the physiological pH range (21). Thus, rat selenoprotein P binds to heparin at pH 7.0 and becomes unbound as the pH is raised toward 8.5. This pH sensitivity was exploited to separate selenoprotein P isoforms from one another (see "Isoforms" section above). It also implies that the heparin-binding site of the protein is not typical.

Heparin binding depends on positive charge, and the basic amino acids arginine and lysine, with pK_as of >9, are almost always the constituents of heparin-binding sites. Histidine, with a pK_a in the range of 6.5–7.0, becomes positively charged as pH nears 7. In theory, it could be a factor in heparin binding at low pH. Selenoprotein P has two histidine-rich sequences (residues 185–198 and 225–234; see Figure 1) that are candidates for being heparin-binding sites.

A heparin-binding site has been identified on rat selenoprotein P using conventional chemical modification methods (41). The site was not one of the two histidine-rich sequences but was localized to residues 80–95 (Figure 1). Six positively charged residues, three lysines and three histidines, reside within that sequence. Part of the sequence, LKHAHL, is arranged in a typical heparin-binding motif xBBxBx, where B is a basic residue, and x is any other one (22). Thus, the heparin-binding site is typical in consisting of basic amino acids in a recognized motif, but it is atypical in containing histidine residues as some of the basic residues. These histidine residues are probably responsible for the pH sensitivity of heparin binding because they would become protonated only as pH fell toward 7.

The histidine-rich sequences might contribute to heparin binding under some circumstances. Peptides derived from them met some, but not all, of the criteria of heparin-binding sites (41). A separate study that used surface plasmon resonance to evaluate heparin binding of human selenoprotein P indicated that high-affinity and low-affinity binding sites were present (3). Because in vivo binding sites are heparan sulfate proteoglycans, and not heparin itself, the actual binding behavior of selenoprotein P in the organism is likely to be different from that observed in vitro. The in vivo binding should be pH sensitive, however. This allows the speculation that selenoprotein P will bind in areas of inflammation that typically have low pH. It would likely bind only to host cells in those areas because bacteria do not have heparan sulfate proteoglycans.

Mercury Binding

Interactions of selenium and mercury have been reported in a substantial literature dating back 30 years. Selenium has generally been shown to protect against the toxicity of mercury while paradoxically increasing tissue mercury concentrations.

In some instances selenium accumulated along with the mercury (8, 43); in others only mercury accumulated (10). These disparate results indicate that different mechanisms underlie each of them.

In 1997, Suzuki and colleagues reported that selenoprotein P bound a mercury-selenium complex following administration of high doses of mercuric chloride and selenite to rats (83). Our group had observed this binding much earlier in association with mutual detoxification of the elements but had not been able to identify the protein that bound the elements (8).

Suzuki reproduced the binding in vitro by adding selenite and mercuric chloride to plasma containing added GSH (83). That work indicated that reaction of selenite with GSH produced selenide, which, in turn, reacted with mercuric ion to produce a polymer that contained mercury and selenium in equimolar amounts. That this polymer bound only to selenoprotein P in plasma indicates how unusual that protein is. Competition experiments suggested that the binding was to the histidine-rich regions (72).

Although the chemistry of this binding is fascinating, there is little evidence that the phenomenon has relevance under physiological conditions. Administration of mercuric chloride alone did not result in its binding to selenoprotein P. Thus for selenoprotein P to sequester mercury, simultaneous doses of selenite and mercuric chloride would have to be administered. Such a situation is unlikely to occur outside the laboratory.

Because of its high histidine and cysteine content, selenoprotein P would appear to be suited to bind heavy metals. Evidence that it does so under conditions that are physiologically relevant is not yet available.

PHYSIOLOGICAL PROPERTIES

Expression, Turnover, and Localization

Selenoprotein P is present in extracellular fluids (11) and bound to endothelial cells (14). It is also present in milk (69). No evidence has been presented that intracellular pools of it exist, aside from those associated with its synthesis and degradation. The relative amounts of bound and free selenoprotein P have not been determined, but its concentration in plasma is high—30 mg protein per liter in the rat (61) and 5–6 mg protein per liter in the human being (67, 78). Using these figures and the amounts of selenium removed from plasma by immunoprecipitation of selenoprotein P, values of 5–6 selenium atoms per molecule of selenoprotein P are calculated. This indicates that some circulating selenoprotein P molecules contain fewer than 10 selenium atoms. Potential explanations of this are that significant amounts of the shorter isoforms are circulating or that some of the Se-P_{10} in plasma has lost selenium atoms. Although both of these are possible (39, 51), the presence of isoforms in rat plasma has been documented and so is at least part of the explanation. Methods to quantitate isoforms have not been developed, so the amounts of each of them in the plasma are not known.

Although the liver appears to be the source of 75% of plasma selenoprotein P (20), virtually all tissues express its mRNA and therefore presumably secrete it. In addition to liver, tissues with high concentrations of the mRNA are kidney and heart (11). Lesser concentrations have been detected in lung, brain, skeletal muscle, and testis. Expression of the protein by a tissue implies that it is secreted into the interstitial space of that tissue. This distinguishes selenoprotein P from proteins such as albumin that are produced solely in the liver. Proteins secreted only by the liver have limited access to the interstitial space of extrahepatic tissues. Based on its localization, a primary function of selenoprotein P in the interstitial space can be expected.

Several studies have assessed the fate of the selenium that is incorporated into selenoprotein P (15, 16, 18, 57). Absorbed selenium is removed from portal vein blood by the liver (42). Some of it is then released into the blood in the form of selenoprotein P. How selenoprotein P is degraded is not known, but the fate of its selenium has been examined in the rat. Selenoprotein P selenium has a plasma half-life of 3–4 hours and appears in peripheral tissues with a preference for testis and brain (18). Moreover, brain up-regulates its mechanism for acquiring selenium from selenoprotein P in selenium deficiency. This implies that testis and brain have receptors for selenoprotein P and that function of the putative brain receptor is regulated by the selenium status of the animal. Such a mechanism would explain how the brain conserves selenium in selenium deficiency.

Based on selenoprotein P plasma concentration and estimated turnover rate (18), 38 μg of it is synthesized per hour in a selenium-replete rat weighing 250 grams. As an indication of the magnitude of this process, this figure is just over 1% of the albumin synthesis rate (27). Moreover, it corresponds to secretion into the plasma of 440 ng of selenium per hour or just over 1% of the whole-body selenium content. This means that approximately 25% of the amount of whole-body selenium circulates through the plasma as selenoprotein P in a day. Thus, it seems likely that the functioning of selenoprotein P leads to its degradation.

Feeding a selenium-deficient diet to an animal produces selenium deficiency. The effect of selenium deficiency on rat selenoprotein P concentration has been assessed (80). Under deficient conditions, plasma glutathione peroxidase activity decreased to approximately 1% of its activity under selenium-replete conditions; selenoprotein P, however, fell only to 5%–10%. This shows that, even under severely deficient conditions, a considerable amount of the available selenium in the animal is used to synthesize selenoprotein P.

Whole-Body Selenium Metabolism

The liver is the central organ of selenium metabolism. Most ingested selenium enters specific selenium metabolism pathways there after uptake from portal vein blood or removal from selenomethionine via the transsulfuration pathway (26). This "new" selenium is used for synthesis of selenoproteins or is released as metabolites that are excreted in the urine. The selenoproteins that are synthesized

by the liver include selenoenzymes that support liver function and selenoprotein P that is exported to the blood.

Insights into the role of selenoprotein P in whole-body selenium metabolism have been gained by study of mice with deletion of the selenoprotein P gene (*Sepp*). Deletion of selenoprotein P resulted in sharp decreases in brain and testis selenium concentrations, increase or no change in liver selenium concentration, and modest decreases in selenium concentrations in kidney and other tissues (37, 68). Increased urinary excretion of ingested selenium was also noted (17). These findings suggest that the $Sepp^{-/-}$ liver directs the selenium that would have been used to synthesize selenoprotein P into the production of urinary metabolites. This results in a loss of selenium from the body and a modest decrease of selenium in most tissues. The large decreases in brain and testis selenium indicate that selenoprotein P has a more direct role in supply of selenium to those tissues.

Clinical phenotypes are associated with the low concentrations of brain and testis selenium. $Sepp^{-/-}$ mice fed normal or low-selenium diets developed spasticity and progressed to paralysis and death within a few weeks of weaning (38). Feeding a high-selenium diet from weaning prevented this sequence of events. Institution of a high-selenium diet after the spasticity had appeared prevented progression of the condition but did not reverse the abnormalities that had already developed (38). These observations indicate that low selenium concentrations in the brains of mice lacking selenoprotein P led to irreversible neurological injury. They support the hypothesis that selenoprotein P supplies the brain with selenium. It is clear, however, that other forms of selenium can be taken up by the brain because feeding a high-selenium diet prevents the development of spasticity (38).

Male $Sepp^{-/-}$ mice have such low fertility that they cannot be used for breeding purposes (37, 68). Moreover, feeding a high-selenium diet does not correct the infertility (58a). The morphology of their sperm resembles that of selenium-deficient mice. There is a 180° kink at the junction of the midpiece and the principal piece. Female $Sepp^{-/-}$ mice are fertile if they are fed a high-selenium diet (35) or are given selenium in the drinking water (69).

These studies in $Sepp^{-/-}$ mice imply that selenoprotein P is essential for adequate selenium supply to the brain, testis, and fetus. Moreover, the evidence that uptake of selenium from selenoprotein P by brain increases in selenium deficiency (see above; Reference 18) is compatible with receptor-mediated uptake of the protein. Thus, a second protein, a selenoprotein P receptor, might be involved in selenium supply to these tissues. Mutations in selenoprotein P or its putative receptor could impair selenium delivery and play a role in diseases affecting these tissues.

Selenoprotein P is secreted by cells and therefore has been suggested to play a role in regulating cell selenium content. However, $Sepp^{-/-}$ mice fed a high-selenium diet did not accumulate more selenium in tissues than did $Sepp^{+/+}$ mice (37). This indicates that selenoprotein P is not necessary for cells to export selenium.

Selenium Supply to Cells

Cultured cells require selenium for growth (54). They can acquire it from serum or from forms such as selenite. Several reports have indicated that the serum component providing selenium to cultured cells is selenoprotein P. One group purified selenoprotein P from bovine serum using as a marker its activity as a survival-promoting factor for cultured rat central neurons (79). In their system, selenium in the form of their purified bovine selenoprotein P was six times more effective than was selenium as selenite. Moreover, boiling the purified protein did not abolish the protection, which suggests that the native structure of the selenoprotein was not required for the effect.

Takahashi's group addressed directly the question of selenium supply to cultured cells (66). Glutathione peroxidase activity was used as an index of the selenium status of Jurkat cells. Human selenoprotein P was approximately five times more effective in supplying selenium than was plasma glutathione peroxidase, selenocystine, and selenite. Selenomethionine was even less effective.

In a follow-up study, this group digested human selenoprotein P into *N*-terminal and *C*-terminal fragments (65). They showed that the *C*-terminal fragment, but not the *N*-terminal fragment, could supply selenium to cultured cells. However, the effectiveness of the *C*-terminal fragment was only about one-tenth that of the full-length protein. These findings suggest the selenium supplied by selenoprotein P is that in its *C* terminus, but the *N* terminus enhances uptake. This would speak for the binding properties of the *N*-terminal portion of the molecule (see "Heparin Binding" section above) playing a role in the selenium donation.

Defense Against Oxidative Injury

Several intracellular selenoproteins metabolize reactive oxygen intermediates and thereby defend the cell against oxidative injury. Selenoprotein P has been postulated to defend against oxidative injury in the extracellular space. Its phospholipid hydroperoxide thiol peroxidase activity and its ability to bind to cells are properties that might support such a function. In addition, in vitro incubation experiments showed that adding selenoprotein P delayed free radical–induced oxidative damage to low-density lipoproteins (75) and prevented nitrosylation by peroxynitrite (70).

In 1980, it was reported that selenium-deficient rats developed massive liver necrosis and lipid peroxidation following administration of small doses of the redox-cycling compound diquat (19). Injection of selenium ten hours before diquat injection, but not two hours before it, prevented the injury. It was postulated that synthesis of a selenoprotein was occurring that protected the liver against the reactive oxygen intermediates produced by diquat cycling. Further experiments, in which selenium doses were varied, associated selenoprotein P with that protection (13, 34).

Ultrastructural characterization of the diquat liver injury revealed that centrilobular sinusoidal endothelial cells were injured within minutes of diquat

administration to selenium-deficient rats (4). The endothelial cells became detached within an hour, leaving the hepatocytes exposed to the blood. At two hours, the hepatocytes were necrotic. Based on these results and the report that selenoprotein P associates with endothelial cells, it was postulated that selenoprotein P protects the sinusoidal endothelial cells against oxidative molecules resulting from diquat cycling (4).

Similar studies were attempted in mice in anticipation of using *Sepp*$^{-/-}$ mice to test the hypothesis. However, because the severe and rapid liver necrosis observed in selenium-deficient rats given diquat did not occur in selenium-deficient mice (R.F. Burk, unpublished observations); mice cannot be used to evaluate this proposed function of selenoprotein P.

STUDIES IN HUMAN BEINGS

Human and rat selenoprotein P amino acid sequences are 72% identical and each contains ten selenocysteine residues (32). The first selenocysteine in each is residue 40 and it is paired with a cysteine at residue 43. However, the second selenocysteine, which delimits the selenium-rich *C*-terminal domain from the *N*-terminal domain, is residue 286 in the human sequence and residue 245 in the rat one. The *N*-terminal domain is thus longer in the human protein than in the rat protein and the selenium-rich *C*-terminal domain is only 22% of the human sequence compared with 33% of the rat sequence.

Human selenoprotein P has been purified by several groups, but only rudimentary characterization of it has been carried out. Two groups have found two protein bands on SDS-PAGE, which suggests the presence of isoforms (1, 55). Another group carried out purification in the presence of diisopropylfluorophosphate and found only one band (64). That group proposes that proteolysis generated the second band seen by the other groups and that isoforms are not present. Resolution of this point will require further work.

Early methods to quantitate selenoprotein P in plasma were cumbersome. One method relied on chromatographic separation of selenoprotein P, glutathione peroxidase, and albumin with subsequent determination of the selenium in each fraction (77). Although it provided some useful information, this method lacked specificity and was superseded by antibody-based assays. The first specific method employed was a radioimmunoassay that used a polyclonal antibody preparation (36). It relied on competition between the selenoprotein P in the plasma sample and ^{75}Se-labeled selenoprotein P. Production of the labeled protein required culturing human hepatoblastoma cells in the presence of ^{75}Se-labeled selenite. Because ^{75}Se has a relatively short half-life, this method could not be widely used.

ELISA assays that have the advantage of not requiring labeled selenoprotein P are now being used. Takahashi and colleagues have produced monoclonal antibodies suitable for accurate determination of human selenoprotein P (67). They reported a mean value ±SD in the plasma of healthy Japanese subjects of

5.3 ± 1.1 mg per liter, $n = 73$. Using different monoclonal antibodies, our group has determined that our reference U.S. plasma contains 5.8 mg selenoprotein P per liter (78). Application of the ELISA should facilitate studies of selenoprotein P in human subjects.

Selenoprotein P in Populations

Åkesson's group has investigated selenoprotein P in Europe and Scandinavia (58b, 59). Both these regions have selenium intakes that are marginal with respect to maintenance of plasma glutathione peroxidase activity. Åkesson's studies showed that selenoprotein P concentration correlated with plasma glutathione peroxidase activity and selenium concentration (59).

A selenium supplementation trial was carried out in Finland before selenium was added to fertilizer there (49). Åkesson's group assayed stored plasma samples from that study for selenoprotein P (59). Pre-supplementation values were approximately 60% of the maximal values of selenoprotein P after supplementation (59). A study carried out in New Zealand, another low-selenium country, gave similar results (25). Thus, New Zealand and many countries in Scandinavia and Europe have plasma levels of selenoprotein P that are below the optimal value. This suggests that residents of those countries are mildly selenium deficient.

More severe selenium deficiency occurs in certain areas of China. A study in Sichuan Province revealed selenoprotein P levels in healthy men that were 13% of the U.S. standard (36). Plasma selenium concentration and glutathione peroxidase were similarly depressed. These determinations of selenoprotein P, and of other selenium biomarkers, demonstrate that large areas of the world have selenium intakes that are not adequate to optimize selenoproteins in the body. This implies that these subjects are selenium deficient.

Effect of Disease

Although other plasma biomarkers of selenium have been measured in a number of diseases, selenoprotein P has been assessed in detail only in cirrhosis (7). Selenoprotein P was depressed in cirrhotics in proportion to clinical severity of disease. Patients with severe disease had levels as low as 50% of the levels in healthy subjects. In the same study, plasma glutathione peroxidase activity was increased in proportion to clinical severity of disease. Thus, the two plasma selenoproteins responded to cirrhosis differently. Plasma selenoprotein P, much of which originates in the liver, was depressed, most likely because of impaired liver function. The rise in plasma glutathione peroxidase activity, however, may be a compensatory response to physiological factors in the kidney caused by the disease. In any case, it seems unlikely that patients with cirrhosis have nutritional selenium deficiency. It is likely, however, that cirrhosis alters selenium disposition in the body because of the central role of the liver in selenium homeostasis.

Depletion of selenoprotein P by apheresis treatment of hypercholesterolemia has been noted (60). Blood was passed through columns containing dextran sulfate

cellulose and returned to the subjects. Selenoprotein P presumably bound to that substance because its plasma concentration was halved. Such treatment might affect biological functions dependent on selenoprotein P until normal levels are restored, but it is unlikely to deplete the body of selenium because only 1% of whole-body selenium was removed by a treatment (60).

Effect of Selenium Supplementation

In addition to the above-mentioned studies in Finland and New Zealand (25, 49), a recent trial in a low-selenium area of China assessed the response of selenoprotein P to 20 weeks of selenium supplementation (78). Plasma glutathione peroxidase activity increased to the level found in selenium-replete subjects with supplementation of 37 μg selenium daily, but selenoprotein P reached only 75% at the highest supplementation level, 61 μg (Figure 2). This indicates that optimization of selenium in all the tissues secreting selenoprotein P requires a greater selenium intake than does optimization of selenium in kidney, the organ that produces plasma glutathione peroxidase (5).

The major implication of this study is that selenoprotein P concentrations are a better biomarker than glutathione peroxidase for selenium in the whole body. Plasma glutathione peroxidase likely is a biomarker principally for selenium in the kidney. Thus, it can be recommended that selenoprotein P replace glutathione peroxidase as the indicator of selenium nutritional status in the healthy person.

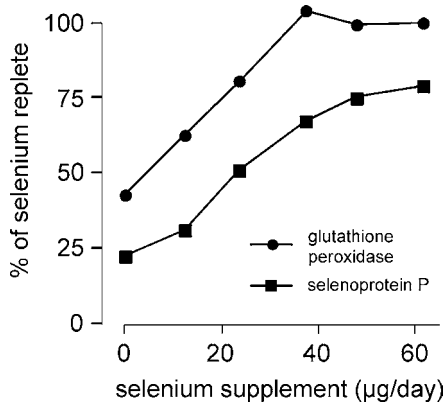

Figure 2 Response of plasma selenoproteins to selenium supplementation of selenium-deficient human subjects (78). Average dietary selenium intake was 10 μg per day. Each symbol represents the mean of ten subjects receiving selenium daily as selenomethionine for 16–20 weeks. Glutathione peroxidase reached its highest activity at a supplementation level of 37 μg. Selenoprotein P reached only 75% of the U.S. standard value at the highest supplementation level of 61 μg.

CONCLUSIONS

Selenoprotein P stands out, even among selenoproteins, for its unusual physical and metabolic characteristics. The *N*-terminal domain of its primary structure has a selenium-containing redox center, a pH-sensitive heparin-binding site, and two histidine-rich stretches. Thus, this part of the molecule has both enzymatic and binding capabilities as well as the capacity to localize the molecule to areas of low pH. The smaller *C*-terminal domain is selenium rich and contains some selenenylsulfide bonds. Thus, this part of the molecule provides selenium to cells and might be redox active.

Four isoforms that range from just the *N*-terminal domain to the full-length protein are present in rat plasma. Their presence implies that the *N*-terminal domain can function independently of the rest of the molecule but that the *C*-terminal domain needs the *N*-terminal domain for it to function.

The flux of selenium through selenoprotein P is very high. This indicates that the protein is turning over at a rapid rate. Reasons for this rapid turnover might be that the protein serves to transport selenium to all parts of the body and/or that it serves as an antioxidant molecule that cannot be reduced back to an active form. Mice with selenoprotein P deleted demonstrate that it is involved in the provision of selenium to the brain, testis, and fetus, but that it does not appear to be necessary for supply of selenium to most other tissues. The high turnover cannot be accounted for by supply of selenium to brain and testis. Thus, the purpose of this high turnover of selenium, and presumably of the entire protein, needs to be determined. It should provide important clues to selenoprotein P function.

Human selenoprotein P is an accessible index of selenium nutritional status that appears to be more useful than plasma glutathione peroxidase activity or selenium concentration. There are indications that selenium metabolism is altered in diseases such as cirrhosis, so study of selenoprotein P in these diseases can be expected to yield health benefits, as can studies of mutations affecting the protein. The investigation of selenoprotein P in humans is at an early stage, but work already done indicates that selenium and selenoprotein P are important to human health.

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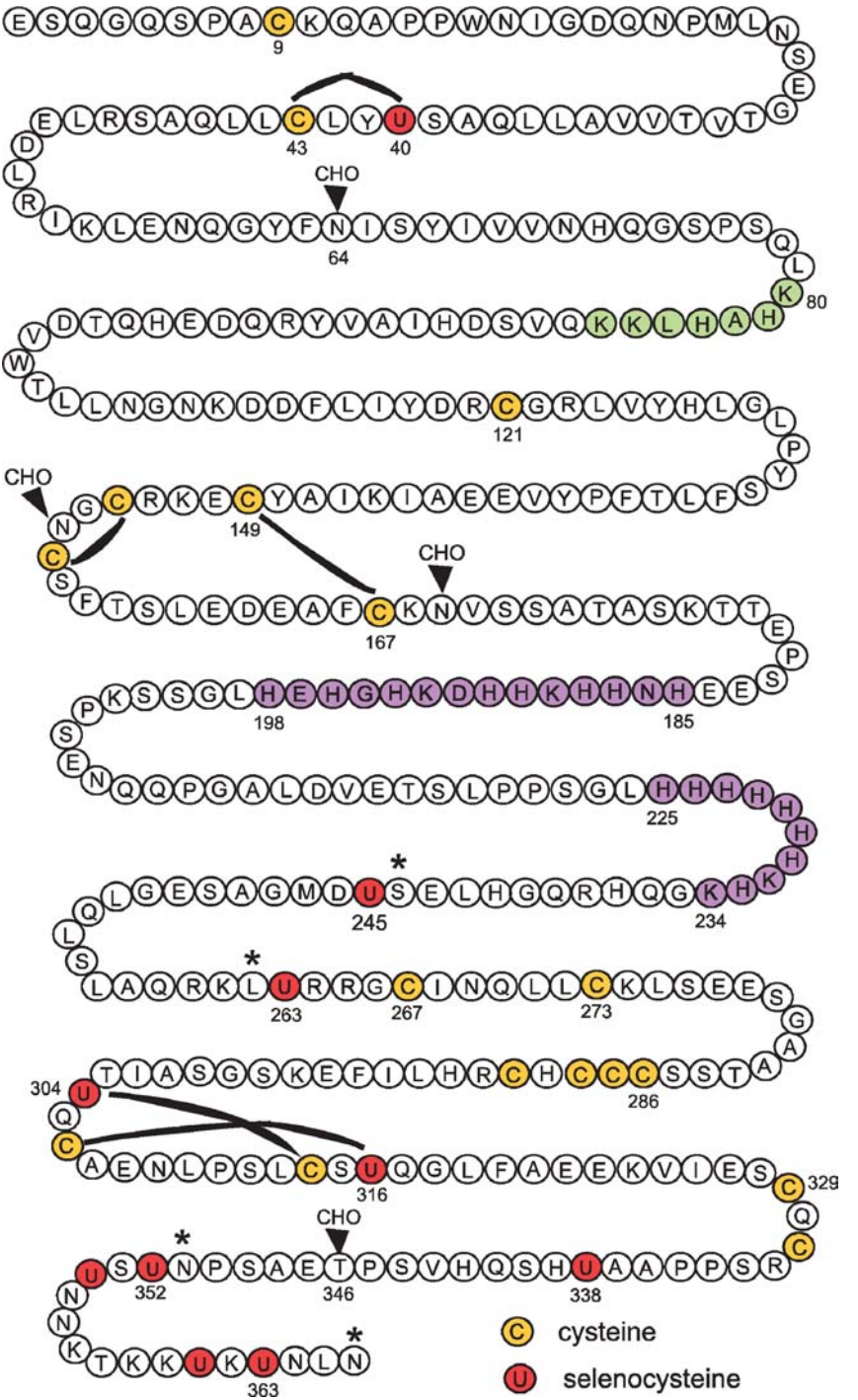

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Figure 1 Rat selenoprotein P. The amino acid sequence of the mature protein is presented. Asterisks denote the *C*-terminal residues of the four isoforms. CHO indicates the three occupied *N*-glycosylation sites (at residues 64, 155, and 169) and the *O*-glycosylation site (at residue 346). The heparin-binding site is shown in green and the two histidine-rich sequences are shown in purple. Selenenylsulfide and disulfide bridges are represented by dark lines that connect amino acid residues.

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