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MAMMALIAN SELENIUM-CONTAINING PROTEINS

Dietrich Behne and Antonios Kyriakopoulos
Hahn-Meitner-Institut Berlin, Department Molecular Trace Element Research in the Life Sciences, Glienicker Strasse 100, D-14109 Berlin, Germany; e-mail: behne@hmi.de; kyriakopoulos@hmi.de

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■ **Abstract** Mammalian selenium-containing proteins can be divided into three groups: proteins containing nonspecifically incorporated selenium, specific selenium-binding proteins, and specific selenocysteine-containing selenoproteins. Selenoproteins with known functions identified so far include five glutathione peroxidases, two deiodinases, several thioredoxin reductases, and selenophosphate synthetase 2. Alternative splicing leads to a greater variety of selenoproteins, as was shown in the cases of a specific sperm nuclei glutathione peroxidase and some thioredoxin reductases. Selenoprotein P, selenoprotein W, a 15-kDa selenoprotein, an 18-kDa selenoprotein, and several selenoproteins identified in silico from nucleotide sequence databases were found to contain selenocysteine but their functions are not known. Gel electrophoretic separation of tissue samples from rats labeled in vivo with ^{75}Se showed the existence of further selenium-containing proteins.

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INTRODUCTION

As early as 1916, selenium was detected in normal human tissue samples, and it was suggested that "it may have a position in the organism which will without doubt be of the utmost significance in the study of the life processes" (49). However, this finding remained without any impact, as was emphasized in 1953 in the following statement (52): "[T]he data of T. Gassmann, who claimed to have found small amounts of selenium in normal human bone and tooth tissue and who attributes an essential importance to selenium, may be the result of an error." Four years later, Schwarz & Foltz showed that liver necrosis induced in rats by feeding them a purified vitamin E–deficient diet could be prevented by adding selenium (88). Subsequent studies of animals and humans proved that selenium is an essential element necessary for growth and fertility, and that selenium deficiency or a combined low selenium and low vitamin E status may lead to various disorders (37). Tissues that in animals were found to be affected include cardiac muscle, erythrocytes, eye, liver, kidney, pancreas, skeletal muscle, skin, smooth muscle, spermatozoa, and testis. In humans, selenium was shown to have a protective effect against Keshan disease, an endemic cardiomyopathy that occurred in selenium-deficient areas in China. Selenium deficiency also seems to be a pathogenic factor in Kaschin-Beck disease, an osteoarthropathy endemic in selenium-deficient regions in Northeast Asia, and cardiomyopathy and muscular disorders have been observed in patients on parenteral nutrition with a very low selenium intake.

Ever since the discovery of its essentiality, in 1957, the question of the chemical form in which selenium is biologically active has been of great interest. This question seemed to be settled in 1973, when glutathione peroxidase (GPx) was identified as a selenoenzyme (81). It catalyzes the reduction of peroxides and is thus part of the cellular antioxidant defense system.

The biological role of selenium in the form of GPx could explain several, but not all, effects of selenium deficiency. This indication that there might be further biologically active forms of selenium was supported by the finding that about two thirds of selenium present in the organism is not bound to this enzyme but is contained in other compounds (18). The discovery of a codon responsible for the incorporation of selenium in the form of selenocysteine into specific proteins in bacteria as well as in mammalian cells (30, 106) suggested that various selenoproteins are expressed in this way.

Information on the presence of a larger number of selenium-containing proteins was then obtained from experiments in which rats and mice were labeled in vivo by administration of ^{75}Se-selenite and the selenium-containing proteins identified from the tracer distribution after chromatographic or gel electrophoretic

separation (10, 29, 40, 45, 56). These compounds differed in their distribution among tissues (10) and subcellular fractions (17). This suggested that they are part of several metabolic pathways of the element and might be involved in different intracellular processes. The discovery of a hierarchy among the selenoproteins that, with insufficient selenium intake, results in the preferential supply of the element to certain selenium-containing proteins, with GPx being last in the ranking order (10), likewise indicated the existence of further biologically important selenoproteins.

Following these findings, numerous studies have been carried out that led to the identification of several selenoenzymes with key roles in the physiological processes. However, although with the advanced methods in molecular biology and protein biochemistry our knowledge in this field has rapidly increased, this work is still far from being completed. In this review, the state of research on the mammalian selenium-containing proteins is presented and discussed.

CLASSIFICATION OF THE SELENIUM-CONTAINING PROTEINS

The selenium-containing proteins known so far can be divided into three groups: proteins into which the element is incorporated nonspecifically, specific selenium-binding proteins, and specific proteins that contain selenium in the form of genetically encoded selenocysteine and that have been defined as selenoproteins. In addition there are proteins in which selenium has been detected but for which no information on its binding form is as yet available. The incorporation of dietary selenium into the different types of selenium-containing proteins is shown schematically in Figure 1.

In several distribution studies, of which only a few are mentioned here as examples, the retention of selenium in the tissues was found to be much higher when given as selenomethionine than when given as selenocystine, selenite, or selenate (42, 85, 104). In the investigation of the selenium-containing proteins in rat tissues after labeling with normal and large doses of selenite and selenomethionine, it was shown that the higher tissue selenium contents were due to nonspecific incorporation into a large number of proteins (14). The studies carried out in this field indicated that the distribution of the element among the different selenium-containing proteins depends to a certain extent on the chemical form and dosage of dietary selenium, as is summarized in the schematic diagram in Figure 1.

Specific Selenoproteins

After ingestion of normal amounts of selenite, selenate, or selenocysteine, nearly all of the element is transported via an intermediary pool into specific selenocysteine-containing selenoproteins, which are responsible for its biological

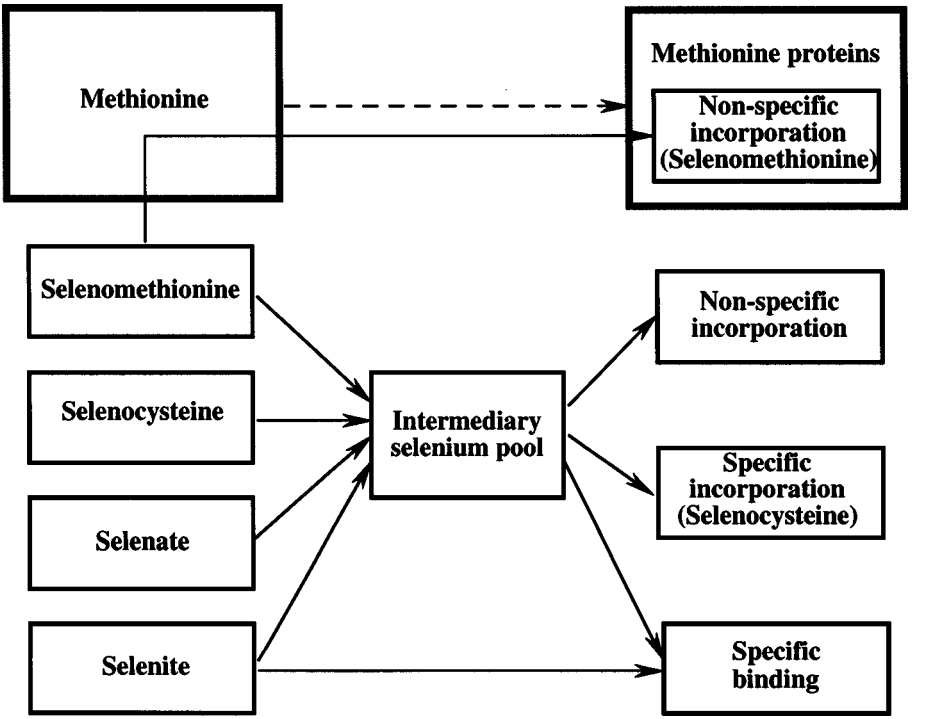

Figure 1 Types of selenium-containing proteins present in the mammalian organism and effects of the chemical forms of dietary selenium on the uptake of the element by these proteins. (For details see text.)

effects. Their levels are homeostatically controlled and cannot be increased by additional selenium supplementation.

Nonspecific Selenium-Containing Proteins

In the case of dietary selenomethionine, a part of the element is metabolized in the same way as the other selenium compounds. A certain percentage, however, is deposited directly nonspecifically into proteins in place of methionine and is therefore mainly found in methionine-rich proteins present in the organism in higher concentrations. This part that follows the metabolic pathways of methionine appears to be dependent only on the ratio of selenomethionine and methionine. It can therefore be influenced by changing the selenomethionine concentration as well as by changing the methionine concentration in the diet. This means that the amount of selenium incorporated nonspecifically increases with increased intake of selenomethionine (14, 42, 85, 104), but also with diets with low methionine levels (27, 102). In the latter case, however, with normal selenomethionine supply, the increase in nonspecific incorporation results in a decrease in the concentrations and effects of the specific selenoproteins (102).

Selenium-Binding Proteins

The third group of selenium-containing proteins comprises the specific proteins in which selenium is only attached to the molecules. So far they include two proteins of 14 kDa (5) and 56 kDa (6), which have been detected in mouse liver. The chemical form of selenium in these compounds is not known, but from the findings that the TGA codon responsible for selenocysteine incorporation is not present in the coding regions of the genes and that the levels of the two proteins are not dependent on dietary selenium supply, it could be concluded that the element is only firmly bound to these compounds. No information is available on the function of the 56-kDa protein, but it has been suggested that the 14-kDa protein may act as a growth regulatory molecule and that by modulating its function selenium may inhibit cell growth.

In mouse tissues, a protein of about 17 kDa has been found that specifically binds selenite administered either in vitro or in vivo (87). Its function is not known, but it has been suggested that it may be active in the intracellular transport of the element.

MAMMALIAN SELENOPROTEINS: General Points

The biological effects of selenium in mammals are due to certain proteins that contain the element in the form of covalently bound selenocysteine. For the specific uptake of selenium by these proteins, several factors are necessary. They prevent the substitution of selenium by sulfur, which has similar chemical and physical properties and is present in the biosphere in much higher concentrations. One of the unique features in the incorporation of selenocysteine is the use of the UGA codon, which normally serves as a termination signal and needs an mRNA stem-loop structure located in the 3′ untranslated region and specific translation factors to be recognized as the codon for selenocysteine insertion (70). Another one is the biosynthesis of selenocysteine, which takes place on its tRNA and is achieved by serine being first loaded onto this tRNA and then transformed into the selenoamino acid by reaction with selenophosphate. However, the knowledge of the different stages of selenoprotein expression in mammals (21, 70) is less complete than in the case of the bacterial systems (23, 24).

A further interesting aspect in the formation of mammalian selenoproteins is the existence of a hierarchy in selenoprotein expression, which ensures that in periods of insufficient dietary selenium intake, the selenium levels in certain tissues are maintained and within each tissue the levels of certain selenoproteins (10). Recent studies of rats showed that even after extreme experimental selenium depletion over six generations, which led to a drastic decrease in the selenium concentrations in liver, skeletal muscle, and blood below 1% of normal levels, the brain still contained 60% of the concentration found in control animals. In this hierarchy the brain is followed by spinal marrow, pituitary, thyroid, ovaries, and adrenals (16). Within most of the tissues, the phospholipid hydroperoxide GPx (PHGPx)

and an 18-kDa selenoprotein were most preferentially supplied with the element whereas the cellular GPx and the plasma GPx (pGPx) were supplied last (D Behne & A Kyriakopoulos, submitted for publication). The intracellular hierarchy in the expression of the selenoproteins may be regulated mainly by differences in the stability of their mRNAs in selenium deficiency (19, 32, 94). The ranking order among tissues and selenoproteins with regard to the incorporation of the element may supply information on the sites that are likely to be affected in selenium deficiency, and on the selenoproteins involved in selenium-related diseases. The preferential supply of selenium to certain high-priority selenoproteins may also explain the fact that the total disruption of selenoprotein synthesis achieved by knocking out the selenocysteyl-tRNA gene in mice resulted in early embryonal lethality (25), whereas in rats fed a selenium-deficient diet for 16 generations no increased mortality could be observed (D Behne, unpublished data).

SELENOPROTEINS WITH KNOWN FUNCTIONS

All the selenoproteins identified so far are enzymes, with the selenocysteine residue responsible for their catalytic functions. Their metabolic importance is based on the fact that in contrast to the thiol in the cysteine-containing enzymes, the selenol is fully ionized at normal physiological pH and that under comparable conditions it is of much higher reactivity than the thiol group (89). The selenoenzymes known so far are listed in Table 1. They include the GPxs, the iodothyronine deiodinases, the thioredoxin reductases, and a selenophosphate synthetase. With the exception of the latter, they are catalytically active in redox processes by using thiols as electron donors. Although enzymatic functions have been established, for most of them information on metabolic role and biological significance is far from complete.

Glutathione Peroxidases

GPxs catalyze the reduction of hydrogen peroxide and organic hydroperoxides and thus protect the cells from oxidative damage. As the name indicates, glutathione normally serves as the electron donor, but there are cases where other thiols are oxidized in order to fulfill a specific biological role. So far, five selenocysteine-containing GPxs have been detected: the cytosolic or classical GPx, a GPx found in the gastrointestinal tract, pGPx, PHGPx, and, as the most recent member of this family, another tissue-specific GPx, which is only present in the sperm nuclei.

Cytosolic or Classical Glutathione Peroxidase The cytosolic GPx (cGPx) was the first identified selenoprotein (46, 81). It is present in nearly all tissues but is unevenly distributed. In rats, the measurement of GPx activity in the tissue cytosols, which almost completely stems from cGPx, showed the highest values

TABLE 1 Mammalian selenoproteins with known functions

Selenoprotein	Abbreviations used	Significant studies
Glutathione peroxidases	GPxs	
Cytosolic or classical GPx	cGPx, GPx1	46, 81
Gastrointestinal GPX	GI-GPx, GPx-GI, GPx2	33
Plasma GPx	pGPx GPx3	95
Phospholipid hydroperoxide GPx	PHGPx, GPx4	99
Sperm nuclei GPx	snGPx	78
Iodothyronine deiodinases		
Type 1 deiodinase	D1, 5′DI	3, 13
Type 2 deiodinase?	D2, 5′DII	
Type 3 deiodinase	D3, 5′DIII	39
Thioredoxin reductases	TrxRs	
Thioredoxin reductase 1	TrxR1	96
Thioredoxin reductase 2	TrxR2	47, 67, 74, 103
Thioredoxin reductase 3	TrxR3	90
Selenophosphate synthetase 2	SPS2	55

in the liver and erythrocytes whereas at the other end of the scale the levels in the skeletal muscle and the brain were lower by about two orders of magnitude (18). The enzyme, which consists of four identical selenocysteine-containing subunits of about 22 kDa, catalyzes the reduction of hydrogen peroxide and various soluble organic peroxides. In this way it contributes to the antioxidant defense against reactive molecules and free radicals and complements the effects of vitamin E, which acts as a free radical scavenger. However, the losses in cGPx activity in selenium-deficient animals did not lead to pathological changes (94). Even after drastic selenium depletion in rats fed a selenium-deficient diet for 16 generations, with a decrease in liver cGPx activity below the detection limit, no lesions were observed that could be attributed to the loss in cGPx activity (D Behne, unpublished data). The same was true with cGPx knockout mice, which showed normal development (59). Effects were observed, however, after application of relatively high doses of paraquat in the knockout mice, which were affected to a much greater extent than the control animals (31, 43). The fact that the mutation of a benign strain of coxsackie virus into a virulent myocarditis-inducing genotype, previously observed in selenium-deficient mice (7), also occurred in the knockout mice indicated that this mutation was due to the lack in cGPx (8). These effects, together with the pathological changes observed in animals with combined selenium and vitamin E deficiency (37), suggest that under normal physiological conditions a low cGPx activity may be compensated for by other components of the antioxidative system, but that the protective effects of cGPx are of particular importance when the organism is exposed to additional stress factors.

Gastrointestinal Glutathione Peroxidase The gastrointestinal GPx (GI-GPx) is similar to cGPx in that it is a cytosolic selenoenzyme that consists of four identical selenocysteine-containing subunits slightly below 22 kDa and catalyzes the reduction of various peroxides (33). Unlike cGPx, however, it is a tissue-specific enzyme that was found in rats only in the GI tract and in humans only in the GI tract and the liver. In the epithelium of the rodent GI tract it contributes to about half the total GPx activity (44). Because of its tissue specificity, GI-GPx may be a major component in the defense system against ingested lipid hydroperoxides (44) and may be of importance in the prevention of colon cancer (35).

Plasma Glutathione Peroxidase Plasma GPx (pGPx) was identified as a tetrameric GPx with subunits of approximately 23 kDa. It differs from cGPx and GI-GPx in that it is a glycoprotein and is present in the extracellular fluids (95). It is expressed in various tissues, from where it is secreted into the extracellular fluids, but the kidney has the highest concentration of pGPx mRNA and is the main site of production for this enzyme (4, 34, 105). In vitro experiments showed that like the other tetrameric GPxs, it catalyzes the reduction of hydrogen peroxide and various organic peroxides when glutathione is used as a substrate. However, its specific enzymatic activity is only 10% that of cGPx (95). Although it was identified more than 10 years ago, its biological significance is still not clear. This is mainly due to the fact that the glutathione concentration in blood plasma is too low to serve as a suitable substrate for this enzyme. However, it has been shown that in this catalytic reaction, thioredoxin and glutaredoxin are better suited as electron donors than is glutathione (22), and this finding might stimulate research on the biological role of pGPx.

Phospholipid Hydroperoxide Glutathione Peroxidase Phospholipid hydroperoxide GPx (PHGPx) was the second mammalian selenoenzyme to be identified (99). Unlike the three GPxs described above, it is a monomer of 19.7 kDa. It is found in the tissues in both cytosolic and membrane-associated forms (83). In vitro translation of the full-length PHGPx mRNA showed the existence of a PHGPx precursor with an additional N-terminal leader sequence responsible for the specific import of the enzyme into the mitochondria (1, 79). Unlike the other tetrameric GPxs, PHGPx can directly reduce phospholipid and cholesterol hydroperoxides (97, 99) and was thus considered primarily as a factor in the protective system against the oxidative destruction of biomembranes. In the meantime, the results of numerous studies suggest that the enzyme may have important functions in the redox regulation of a variety of processes, such as inflammation and apoptosis, although in most of these cases it is not yet known to what extent the other GPxs may also be involved in these reactions. A significant role of PHGPx, only fulfilled by this enzyme, has, however, been found in spermatogenesis.

Earlier findings that selenium is highly enriched in the spermatozoa in the form of a selenoprotein located in the outer mitochondrial membrane (28), that the testicular selenium concentration rises sharply after the onset of puberty (9) and changes considerably by gonadotropin-related interruption and reconstitution

of spermatogenesis (11), and that a 19.7-kDa selenoprotein, present not only in the testis but also in all the other tissues, is by far the most prominent selenium compound in the spermatozoa (10) were explained when it was shown that the sperm mitochondrial membrane selenoprotein is in fact PHGPx (98) and that the increase in testis selenium during pubertal maturation is due to the abundant expression of this enzyme in the round spermatids (72). However, while in the rat testis PHGPx activity is very high (82), it is below the limit of detection in the epididymal spermatozoa (98). Here the enzyme constitutes at least 50% of the proteins present in the mitochondrial capsule, but it is present in an inactive, oxidatively cross-linked form. These findings indicate a change in the biological role of PHGPx from that of an enzyme protecting against peroxide-induced damage to that of an inactive, but still important, matrix component.

Sperm Nuclei Glutathione Peroxidase After labeling rats in vivo with ^{75}Se and separating the tissue homogenates by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), a 34-kDa selenoprotein was detected that was present only in testis and spermatozoa (10). It appeared after the onset of puberty and was localized in the nuclei of the late spermatids (12). During these stages of sperm development, the nuclei undergo considerable changes characterized by the replacement of the histones by the protamines and the reorganization and condensation of the DNA, which result in compact, very tightly packed nuclei stabilized by cross-linking of the protamine thiols. The protein was identified as a specific sperm nuclei GPx (snGPx), which differs from PHGPx in its N-terminal sequence (78). This sequence, which is encoded for by an alternative exon in the first intron of the PHGPx gene, is responsible for the specific biological role of snGPx. It contains a signal for the localization of the enzyme within the nuclei, where it is the only selenoprotein present, and a polyarginine-rich region by which it is attached to the DNA. In selenium-depleted rats where the concentration of snGPx had decreased to one third of normal, chromatin condensation was severely disturbed. We were able to show that the enzyme acts as a protamine thiol peroxidase responsible for disulfide cross-linking and thus is necessary for sperm maturation and male fertility.

Iodothyronine Deiodinases

The iodothyronine deiodinases have major physiological roles in that they catalyze the activation and inactivation of the thyroid hormones that regulate various metabolic processes and are indispensable for the normal development of fetal brain. The family of the deiodinases consists of three members that differ with regard to their tissue distribution and their role in the deiodination of thyroxine and its metabolites. With the numerous reviews available on this subject, only a few main findings are presented.

Type 1 Deiodinase A second important enzymatic function of selenium was detected in 1990, when two groups showed that a membrane-bound,

selenium-containing 27-kDa protein, which had been found in the thyroid, liver, and kidney of rats (10), was identical to the subunit of type 1 deiodinase (D1) and in this way identified this enzyme as a selenoenzyme (3, 13). It was also shown that it contains one covalently bound selenium atom per molecule in its active center (13). Cloning of rat D1 cDNA proved the existence of an in-frame UGA codon responsible for the incorporation of selenocysteine into this enzyme (20). D1 is located mainly in the thyroid, liver, kidney, and pituitary. It can catalyze monodeiodination of the iodothyronines at the 5′-position of the phenolic ring or at the 5-position of the tyrosyl ring. 5′-Deiodination of thyroxine (T_4) results in the formation of the biologically active hormone 3,3′,5-triiodothyronine (T_3), whereas by means of 5-deiodination, the inactive isomer 3,3′,5′-triiodothyronine (reverse T_3) is produced. 5-Deiodination of T_3 and 5′-deiodination of reverse T_3 then lead to the inactive 3,3′-diiodothyronine (T_2). The biological role of D1 is to provide T_3 to the plasma, to inactivate T_4 and T_3, and to eliminate reverse T_3 from the circulation. The decrease in T_3 production from T_4 found in the liver of selenium-deficient rats (3, 13) shows the importance of an adequate selenium supply with regard to thyroid hormone metabolism.

Type 2 Deiodinase Although cDNA cloning has shown that type 2 deiodinase (D2) in amphibian tissue is a selenoenzyme (41) and evidence has been provided that the same is true with the mammalian D2 (38, 86), final proof is still not available, as in the clones assumed to code for selenocysteine-containing D2 in mammals, the structures responsible for the incorporation of this amino acid have not been identified. A nonselenocysteine-containing, biologically active D2 subunit has recently been found (68). Further studies are therefore needed to solve the question of the role of selenium in the enzymatic D2 activity. D2 is membrane bound, has subunits of about 30 kDa, and is expressed predominantly in brain, brown adipose tissue, pituitary, and placenta. It only catalyzes 5′-monodeiodination and converts T_4 to T_3 and reverse T_3 to T_2. Its main biological role is the local intracellular production of T_3 from circulating plasma T_4 in the tissues that express this enzyme and is thus a major factor in tissue-specific regulation.

Type 3 Deiodinase Cloning of the type 3 deiodinase (D3) cDNA from rat established that D3 is a selenoenzyme (39). It is a 32-kDa selenoprotein mainly located in the central nervous system, placenta, and skin. D3 catalyzes the deiodination of the tyrosyl ring and is thus able to inactivate the thyroid hormones by producing reverse T_3 from T_4 and T_2 from T_3. In this way it protects the developing mammalian brain from exposure to excessive amounts of T_3 (62) and regulates the supply of T_4 and T_3 from the mother to the fetus (75).

Thioredoxin Reductases

Mammalian thioredoxin reductases are a family of homodimeric flavoenzymes present in various tissues. In addition to the flavin and the active site of the prokaryotic homologs with their redox-active disulfide, they also contain

selenocysteine as the penultimate C-terminal amino acid residue (50), which is indispensable for their enzymatic activity (53). Thioredoxin reductases are named for their ability to catalyze the NADPH-dependent reduction of oxidized thioredoxin. Reduced thioredoxin is a central factor in cellular redox regulation. It provides reducing equivalents for various redox-dependent systems, e.g. for ribonucleotide reductase essential for DNA synthesis and for the redox regulation of transcription factors, and has important functions in regulating cell growth and inhibiting apoptosis (77). The significance of thioredoxin for the mammalian organism was shown in an experiment in which the disruption of the thioredoxin gene resulted in early embryonic lethality (73). In addition to thioredoxin, mammalian thioredoxin reductases are able to use other substrates, including hydroperoxides, dehydroascorbate, and various enzymes and proteins (60). This broad substrate specificity has been attributed to the presence of selenocysteine situated in the flexible C-terminal extension (53).

Thioredoxin Reductase 1 A selenium-containing 56-kDa protein, purified from ^{75}Se-labeled human lung cancer cells, was the first thioredoxin reductase to be identified as a mammalian selenocysteine-containing thioredoxin reductase (96). This cytosolic enzyme, later named thioredoxin reductase 1 (TrxR1), is a dimer with two identical 56-kDa subunits. The sequence of the TrxR1 cDNA, obtained from a human placental library, was found to have 44% identity with that of the eukaryotic and prokaryotic glutathione reductases, but only 31% with that of the prokaryotic thioredoxin reductases (48).

Thioredoxin Reductase 2 A second selenocysteine-containing thioredoxin reductase, the mitochondrial thioredoxin reductase 2 (TrxR2), was described by four groups in 1999, when either its cDNA was cloned from human tissues (47), human adrenal (74), and rat liver (67), or its sequence was determined after purification of the protein from bovine adrenal cortex (103). They were around 56 kDa for the human and bovine proteins and about 53 kDa for the rat enzyme. The sequence identity between the mitochondrial TrxR2 and the cytosolic TrxR1 of the same species was found to be 54% (47), 56% (74), 54% (67), and 57% (103). TrxR2 differs from TrxR1 by an N-terminal extension identified as a mitochondrial leader sequence (74). The biological role of TrxR2 in the mitochondria is not known, but it may be mainly involved in the protection against mitochondria-mediated oxidative stress.

Further Thioredoxin Reductases A third selenocysteine-containing thioredoxin reductase, listed here as thioredoxin reductase 3 (TrxR3), was purified from ^{75}Se-labeled mouse testis, where it is preferentially expressed (90). The deduced sequence of the human enzyme shows 70% identity to that of TrxR1. It contains a long N-terminal extension and, at about 65 kDa, has a higher molecular mass than the other two isozymes.

It has recently been observed that TrxR1 isolated from mouse liver, mouse liver tumor, and a human T-cell line exhibited considerable heterogeneity, which,

as with the production of snGPx (78), is due to alternative splicing of the first exons of the TrxR1 gene. By means of homology analyses, three isoforms of mouse and rat TrxR1 mRNA could be distinguished. Expression of multiple mRNA forms was also observed for human TrxR2 (91). By means of an algorithm that scans nucleotide sequence databases for mammalian selenocysteine insertion elements, two selenoproteins, SelZf1 and SelZf2, were identified that share a common domain with TrxR2 and probably are produced by alternative splicing (69). The findings of the two studies suggest the existence of further thioredoxin reductase species, which may differ with regard to their distribution among tissues and subcellular compartments and may have specific biological roles.

Selenophosphate Synthetase 2

Selenophosphate synthetase catalyzes the reaction of selenide with AMP. The product, selenophosphate, acts as the selenium donor for the biosynthesis of selenocysteine. In addition to selenophosphate synthetase 1, which contains threonine in its active center (71), a selenocysteine-containing homolog of about 50 kDa has been identified in various human and mouse tissues (55). Information on the differences in the functions of the two enzymes in the biosynthesis of the mammalian selenoproteins is not yet available. The detection of a selenoenzyme that is involved in the production of the selenoproteins is of special interest with regard to the regulation of the mammalian selenium metabolism.

SELENOPROTEINS WITH UNKNOWN FUNCTIONS

Selenoprotein P

A selenium-containing protein not related to GPx was found in rat plasma in 1977 (57) and was then shown to contain selenium in the form of selenocysteine (76). Selenoprotein P (SelP) (see Table 2 for a list of all selenoproteins with unknown functions) is a glycoprotein of 43 kDa and constitutes more than 60% of the plasma selenium (80). Cloning of rat liver cDNA showed that SelP contains 10 selenocysteines (58) and thus is different from all other selenoproteins so far identified, which have only one selenocysteine residue per molecule or subunit. A cDNA obtained from bovine brain suggested the existence of a second SelP with 12 selenocysteine residues (84). SelP is mainly expressed in liver but is also present in other tissues. Although it was the second selenoprotein to be detected, its function is still unknown. Because of its extracellular location and its high selenium content, it was thought to act as a selenium transport protein (76). On the other hand, the fact that protection against diquat-induced liver lesions found after administration of selenium to selenium-deficient rats coincided with the appearance of SelP, suggested that it may act as an antioxidant (26). This hypothesis is supported by the finding that in human plasma, it contributes to the destruction

TABLE 2 Mammalian selenoproteins with unknown functions

Selenoprotein	Abbreviations used	Significant studies
Selenoprotein P	SelP	58, 76
Selenoprotein W	SelW	100, 101
15-kDa selenoprotein	Sel15	51, 61
18-kDa selenoprotein	Sel18	65
Selenoprotein R	SelR	63
Selenoprotein T	SelT	63
Selenoprotein N	SelN	69
Selenoprotein X	SelX	69
Selenoprotein Zf1	SelZf1	69
Selenoprotein Zf2	SelZf2	69

of peroxynitrite, thought to be an important factor in inflammatory toxicity (2). Further studies are needed to find out more about its significance and biological role.

Selenoprotein W

Selenoprotein W (SelW) was first purified from rat skeletal muscle and was shown to be a cytosolic selenoprotein of slightly less than 10 kDa (100). Cloning of its cDNA indicated that it contains one selenocysteine residue per molecule (101). It is enriched in skeletal and heart muscle, brain, testis, and spleen but was also found in a large number of other tissues (93). Its biochemical and physiological role is not known. However, the protein isolated from muscle tissue was found to contain glutathione, which may have been bound as a reactant in an enzymatic redox cycle (54). The finding that glial cells with overexpressed levels of SelW were more resistant to peroxidation than normal cells (92) could likewise suggest a redox function.

15-kDa Selenoprotein

By labeling rats with ^{75}Se, isolating a 15-kDa selenium compound, and analyzing its amino acids, a selenocysteine-containing protein was identified. It is an acid protein with a pI value of 4.5 and stems from a cytosolic selenoprotein of 240 kDa. It is present in various tissues but is highly expressed in the epithelial cells of the prostate gland (61). A 15-kDa selenoprotein was then found in ^{75}Se-labeled human T cells and was shown to contain a selenocysteine residue encoded by TGA. It, too, has a native form of 200–240 kDa and is likewise highly expressed in the prostatic tissue (51). We have recently been able to show that the 15-kDa selenoprotein

detected in rats and the human 15-kDa selenoprotein are the same protein in two different mammalian species (D Röthlein, A Kyriakopoulos, D Behne, submitted for publication), and therefore we refer to both as the 15-kDa selenoprotein (Sel15). Its function and biological significance are not known; however, further investigation of it is of special interest because of the decreased incidence of prostatic cancer with selenium supplementation (36). The recent finding that the gene for Sel15 is located on a chromosome often affected in cancer (64) supports the hypothesis that this protein might play a role in the observed relationship between selenium and this disease.

18-kDa Selenoprotein

An 18-kDa selenium-containing protein was detected in various tissues of rats (10). It was identified as a selenocysteine-containing selenoprotein with a pI of about 4.6–4.8, which is mainly present in the mitochondrial membranes (65). Its biological function is still unknown. However, an interesting characteristic is the fact that in the hierarchy of selenium distribution, it was found to be one of the most preferentially supplied proteins (D Behne & A Kyriakopoulos, submitted for publication), which can be taken as an indication of its biological significance.

Further Selenoproteins Identified in Silico

Two computer programs have been developed that allow the identification of mammalian selenoprotein genes by scanning the nucleotide sequence databases for the selenocysteine insertion sequence elements necessary for decoding UGA as selenocysteine (63, 69). Nucleotide sequences corresponding to two novel selenoproteins, SelR and SelT, have been found in one of the studies (63). Calculated from their cDNA sequences, the human proteins are 12.6 kDa and 18.8 kDa for SelR and SelT, respectively. The other study describes four novel selenoproteins, SelN, SelX, SelZf1, and SelZf2, the latter two of which, both TrxR2 homologs, were mentioned above (69). SelN, SelX, and SelZ are calculated to be 58, 16, and 48 kDa, respectively. By means of Northern blot hybridization of human tissues, SelN mRNA was found to be ubiquitously expressed, but was enriched in pancreas, ovary, prostate and spleen. SelX mRNA was mainly present in liver and leukocytes and was low in lung, placenta, and brain. SelZ mRNA was enriched in kidney, liver, testis, and prostate and was low in thymus. With the exception of the TrxR2 homologs, no information on the functions of these selenoproteins is available.

SELENIUM-CONTAINING PROTEINS NOT YET IDENTIFIED

Labeling experiments with ^{75}Se in combination with electrophoretic and chromatographic methods of protein separation (10, 29, 40, 45, 56) have been valuable tools in selenium research, which, with most of the mammalian selenoproteins

identified so far, gave a first indication of their existence and their distribution in the organism. After further improvement of these labeling techniques, mainly by using severely selenium-depleted rats and ^{75}Se-selenite with a very high specific activity, we were able to determine selenium compounds present in the organism at very low concentrations. After separation by SDS-PAGE and autoradiography of the labeled compounds, 28 selenium-containing bands ranging from 116 to 8 kDa could be distinguished (15). This range was extended by applying a modified tricine-SDS-PAGE, which allows the determination of smaller proteins. Using this method, four additional selenium-containing proteins of approximately 7, 5, 4, and 3 kDa were detected (66). By applying two-dimensional SDS-PAGE/isoelectric focusing, some of the selenium-containing bands could be further resolved into several spots with different isoelectric points. Although a few of these compounds may be precursors or metabolic products of the same selenoprotein, and selenium-binding proteins also have to be taken into account, these findings suggest that in addition to the selenocysteine-containing proteins detected so far, there are further mammalian selenoproteins awaiting identification.

OUTLOOK

Considerable progress has recently been made in selenium research and particularly in the identification of novel selenocysteine-containing proteins. The findings that selenoproteins are produced by alternative splicing (78, 91) and that there are several additional selenium-containing proteins present in the tissues (15, 66) suggest that further mammalian selenoproteins exist. With the advanced methods now available in molecular biology, protein biochemistry, and protein analysis, it may be assumed that most of these compounds will be identified in the near future.

The more difficult task, however, is the functional characterization of novel selenoproteins that do not belong to one of the selenoenzyme families already known. Selenoprotein P, which was detected more than 20 years ago, is an example of this difficulty.

An even greater challenge is presented by studies carried out to clarify the biological roles of the mammalian selenoproteins. With the exception either of specific tasks, such as the regulation of thyroid hormone metabolism by the deiodinases and the production of selenophosphate by selenophosphate synthetase 2 (55), or of tasks restricted to a certain site of action, as with PHGPx in the sperm mitochondrial membrane (98) and snGPx in the sperm nuclei (78), relatively little evidence is available concerning the physiological roles of the selenoproteins. This is mainly due to the fact that because of the similarities in the enzymatic functions of the different members of one selenoenzyme family and the metabolic links between these families, it is often difficult to allocate a certain selenium-mediated effect to a single selenoprotein among all the others present in the tissues.

Another difficulty arises from the hierarchy in the selenium distribution between tissues and selenoproteins (10, 16). It is not possible to study pathological changes

in relation to the concentration of a preferentially supplied selenoprotein in a high-priority tissue, as its level cannot be decreased sufficiently, even in severe selenium depletion.

In these cases gene knockout studies of experimental animals will therefore be valuable tools to investigate the selenoproteins to clarify their roles in reproduction, development, stress management and the maintenance of various metabolic processes.

Another important task for the near future is the investigation of the regulatory mechanisms responsible for selenium distribution and homeostasis and the study of the protective effects of additional selenium supplementation. In this way it will be possible to answer the question regarding the optimal selenium intake for achieving the most beneficial effects.

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