Annu. Rev. Nutr. 1990. 10:451–74

MOLECULAR BIOLOGY OF SELENOPROTEINS[1]

Roger A. Sunde

Department of Human Nutrition & Foods and Department of Biochemistry, University of Missouri-Columbia, Columbia, Missouri 65211

KEY WORDS: selenium, glutathione peroxidase, molecular biology, selenocysteine, UGA codon, proteins, selenium-containing

---

CONTENTS

[1]Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 11,174.

0199-9885/90/0715-0451$02.00

INTRODUCTION

Essential trace elements are required in the diet to maintain the health, growth, and function of an animal. The discovery in 1973 (63) that glutathione peroxidase (GPX) contains an integral, stoichiometric quantity of selenium (Se) was important because it demonstrated a biochemical role for the essential trace element, and because it provided a most useful biochemical marker for monitoring the Se status of both humans and animals. Subsequently, a lack of GPX seemed to explain logically the effects of Se deficiency observed in animals, and GPX activity was used successfully by numerous researchers to identify Se deficiency and to monitor restoration of Se status. Unfortunately, the lack of any characterized selenoenzymes other than GPX in higher animals also may have slowed further progress in understanding the complete role of Se in cells of higher animals. In the last four years, however, progress has been rapid and exciting as new techniques have been used to study the biochemistry of Se. The result is that there is a new vitality in selenium nutritional biochemistry.

The objectives of this review are to discuss recent developments in the molecular biology of selenoproteins, with an emphasis on higher animals, and to integrate these developments into what we know about Se metabolism. I (i) review Se metabolism leading to the Se precursors used for selenoprotein synthesis in order to justify the classification of selenoproteins into four different classes, and then I discuss new developments in our understanding of (ii) classical GPX, (iii) other glutathione peroxidases, (iv) other selenoproteins, and (v) the regulation of GPX expression by Se status. Several comprehensive reviews describe previous developments in Se biochemistry (2a, 15, 70, 74, 79, 90).

The term "molecular biology" is used in this review expansively—that is, dealing with any aspect of Se biochemistry at a molecular level, including research using metabolic techniques as well as techniques of recombinant DNA and of protein chemistry. While the full extent of the roles of Se in an organism is not known, we are getting a much better picture of the underlying mechanisms involved in the nutritional biochemistry of Se, and of why Se is essential in humans and in animals.

SE METABOLISM AND FOUR CLASSES OF SELENOPROTEINS

Selenoamino Acids

Se metabolism was initially assumed to follow the pathways of sulfur metabolism, and this remains an important concept. The hypothesis that these pathways were the same or similar was primarily based on the chemical similarity of S and Se; they have similar covalent radii and the ability to use $d\pi$-$p\pi$ multiple bonding. Substrates that contain Se bound between two carbons were thus presumed to be readily metabolized by the enzymes that metabolize the analogous S compounds (67). This assumption was strengthened by the demonstration that plants and bacteria metabolize inorganic forms of Se into the selenoamino acids, selenomethionine ([Se]Met) and selenocysteine ([Se]Cys) (10). Some plants that accumulate Se can synthesize additional unique selenoamino acids, such as selenocystathionine or methylselenocysteine, to sequester the Se (14). The synthetic enzymes for these selenoamino acids, however, do not appear to be confined just to accumulator plants (56), so the sequestration of Se would appear to be directed by regulation of gene expression and/or enzyme activity.

[Se]Met is a ready substrate for the enzymes that use methionine. [Se]Met is metabolized to [Se]adenosyl methionine (SeAM) by the adenosylmethionine synthetases in bacteria and yeast, and this reaction appears to be more favorable for [Se]Met than for methionine (Met) (48). SeAM is also reported to be a better methyl donor than SAM in mammalian systems (7). Soda and coworkers (24) have demonstrated that [Se]Met is a better substrate than Met for the bacterial L-methionine-γ-lyase-catalyzed α,γ-elimination which releases methane selenol in a manner similar to the Met transamination pathway in animals reported by Benevenga and coworkers (72). Thus, Se analogs of the sulfur amino acids clearly can follow the pathways of sulfur metabolism in plants and bacteria.

Animals are unable to synthesize selenomethionine ([Se]Met) directly from inorganic Se (19). [Se]Cys in tissues of animals fed inorganic forms of Se may arise solely from the synthesis of GPX and other selenoproteins. Soda and coworkers (23) have shown that [Se]Cys can be synthesized from [Se]Met by cystathionase in rat liver; they could, however, find no evidence for formation of [Se]Cys from selenide. Hawkes et al (33), however, have reported that up to 80% of the body's Se may be present as [Se]Cys. Recent discoveries (see below) indicate that this [Se]Cys has been synthesized in conjunction with the synthesis of selenoproteins using inorganic Se in a tRNA-mediated process.

Selenoamino Acid tRNA

[Se]Cys and [Se]Met are readily esterified to the corresponding sulfur amino acid tRNA's ($tRNA^{Met}$ and $tRNA^{Cys}$) in bacteria (10), and thus [Se]Met is readily incorporated into protein in *E. coli* in place of Met (49); *E. coli* grown on selenate or [Se]Met can replace over 50% of the Met residues with [Se]Met without loss of catalytic activity (38). In higher animals [Se]Met-$tRNA^{Met}$ formation by the methionyl-tRNA synthetases is only slightly less favorable than Met-$tRNA^{Met}$ formation (Km's: 11 μM [Se]Met vrs. 7 μM Met) (36), and thus [Se]Met is readily incorporated into rat liver protein (50). This explains why supplementation of humans in New Zealand (with Se intake about 50% of that in the US) with 100 μg Se/day as [Se]Met resulted in a dramatic rise in erythrocyte (RBC) Se but only a moderate rise in plasma or RBC GPX activity, whereas 90 μg Se/day as selenite produced moderate parallel rises in both blood Se and GPX activity (62).

Inorganic Selenium

On the inorganic side of Se metabolism, little is known about the selenate-selenite interconversion, but it is thought to involve APS and/or PAPS analogues (cf 2a, 74). Although the covalent size and bond energies of Se and S are similar, the greater reduction potential of Se as compared to S results in a tendency for Se metabolism to go towards reduction in most biological systems. The reduction of selenite to selenide and the subsequent methylation is the only well characterized pathway of Se metabolism in animals (37). Erythrocytes rapidly take up and metabolize selenite and then transport the Se back into the plasma (66). The Se that leaves the erythrocyte has been shown to be selenide (28). Thus, selenide appears to be the common and perhaps important intermediate in the metabolism of both inorganic and amino acid forms of Se (74, 80).

Diagram of Se Metabolism

The metabolic pathways of sulfur metabolism that evidently are in common with Se metabolism, the reductive pathway of inorganic Se compounds, and the Se-specific reactions including Se incorporation into GPX can be combined into the hypothetical scheme of Se metabolism shown in Figure 1 (see 74, 75, 80, 83, for earlier versions of this diagram). Briefly, [Se]Met degradation is shown to follow the Met transamination pathway described by Steele & Benevenga (72); an enzyme like the L-methionine-γ-lyase described by Soda and coworkers (24) might in fact release methane selenol in animals. [Se]Cys metabolism does not follow cysteine metabolism (with oxidative release of selenite); instead the [Se]Cys-specific enzyme selenocysteine lyase directly releases elemental Se which is reduced to selenide (22). Selenite is shown following Ganther's (37) selenite reduction pathway leading to sele-

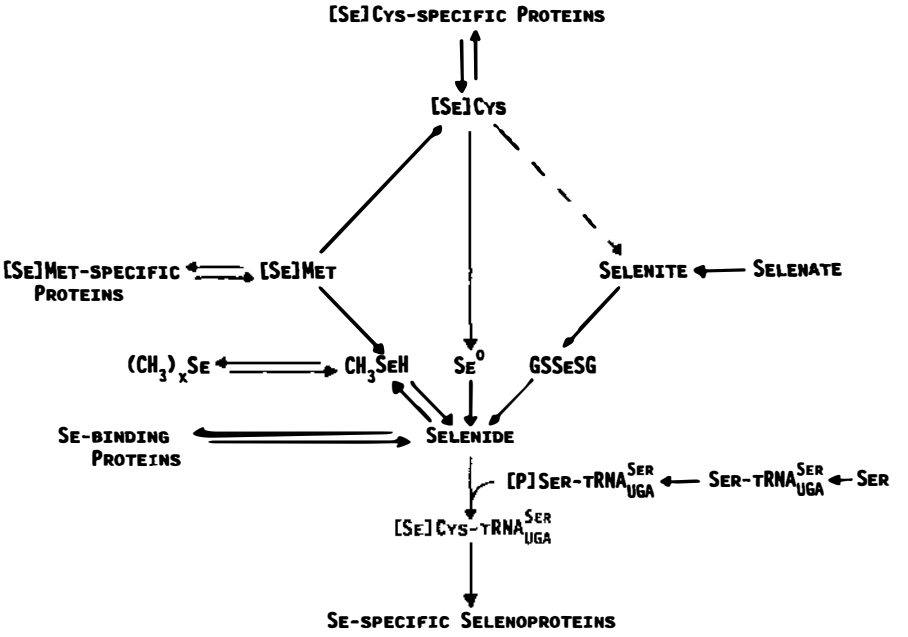

Figure 1 Selenium metabolism and the four classes of selenoproteins. The diagram shows the various pathways of selenium metabolism and the Se precursors that lead to the synthesis of each class of selenoproteins.

nide formation. Methylation of selenide is shown leading to formation of the methylated selenium species that are excreted in urine and breath. This hypothetical scheme is very useful as an aid in understanding Se metabolism and selenoprotein synthesis, but it also shows that much of Se metabolism in animals remains to be investigated in detail.

Nomenclature for Selenoproteins

The metabolism of Se thus leads to several possible precursor forms of Se that can be used for the synthesis of selenoproteins. The model permits classification of selenoproteins into four different classes depending on Se precursor and mechanism used for Se incorporation (see Table 1). One class of selenoproteins is the *[Se]Met-specific proteins* which have [Se]Met as the Se moiety which is incorporated translationally into a growing peptide chain via [Se]Met-tRNA^{Met} at positions specified by the Met codon. The Se stoichiometry will depend on the ratio of [Se]Met versus Met esterified to tRNA^{Met}. Specific examples include thiolase (68) and β-galactosidase (38), as well as muscle in [Se]Met-fed animals (6a). A similar class of selenopro-

Table 1 Classification of Selenoproteins

I.	*[Se]-Specific Selenoproteins*
	glutathione peroxidase (13)
	formate dehydrogenase (101)
	[NiFeSe]hydrogenase (87a)
	selenoprotein-P (34)
II.	*[Se]Met-Specific Proteins*
	thiolase (68)
	β-galactosidase (38)
	muscle proteins (6a)
III.	*[Se]Cys-Specific Proteins*
	β-globin (92)
	yeast proteins (5)
IV.	*Se-binding Proteins*
	[Se]-fatty acid binding protein (3)
	130-kD plasma Se-binding protein (28)
	77-kD mitochondrial Se-binding protein (8)

teins is the *[Se]Cys-specific proteins* which have preformed [Se]Cys as the Se moiety which is incorporated translationally at positions specified by Cys codons using [Se]Cys-$tRNA^{Cys}$. Examples of this class would include the competitive incorporation of [Se]Cys and Cys into protein in a cell-free translation system using globin mRNA (92), and apparent [Se]Cys-specific proteins in yeast (5). A most important class of selenoproteins is the *Se-specific selenoproteins*. The discovery that UGA codes for the [Se]Cys in GPX and in formate dehydrogenase (discussed in depth below) provides the definition for this class of selenoproteins. [Se]Cys is the Se-moiety, but the Se is incorporated cotranslationally, using selenide (HSe^-) and serine as the precursors, in a $tRNA^{Ser}_{UGA}$-mediated process at the position specified by a UGA codon. GPX, formate dehydrogenase, Se-dependent hydrogenase, and selenoprotein-P are the only enzymes in this class that have known nucleotide sequences which confirm that they belong to this class. Glycine reductase is almost certainly an additional member of this class. The last class of selenoproteins are the *Se-binding proteins*. This operational class contains the selenoproteins with Se bound tightly enough so that the Se remains attached during standard protein purification procedures that produce discrete ^{75}Se-labeled species. This class includes proteins that bind Se tightly, perhaps attached posttranslationally to an amino acid residue in the peptide backbone, or perhaps in an unknown arrangement such as in bacterial xanthine dehydrogenase. This class also includes selenoproteins which have not been characterized sufficiently to be classified into one of the other classes, such as

fatty acid binding protein ([Se]FABP). It also includes proteins labeled in vitro with Se, such as the 130 kd plasma protein (28).

CLASSICAL SE-DEPENDENT GLUTATHIONE PEROXIDASE (GPX)

GPX was the first enzyme shown to contain a stoichiometric quantity of Se and to require Se for catalytic activity. The classical GPX is a tetrameric protein containing four identical subunits with an M_r of about 23 kd. The pioneering work by Epp and coworkers (21) showed that the four spherical subunits are arranged in an almost square planar configuration, with one Se per subunit located in a depression at the active site.

[Se]Cys Is the Se Moiety

More than a decade ago, Cone, Stadtman and coworkers discovered that the Se in clostridial glycine reductase was present as [Se]Cys (17). Following this demonstration, Tappel and coworkers (27) and Wendel and coworkers (91) showed that [Se]Cys was also the Se moiety in reduced GPX. Ganther and coworkers (42) used mass spectroscopy of a nitrophenyl derivative to show elegantly that the Se moiety from ovine GPX was [Se]Cys. The [Se]Cys was reported to be the 35th residue from the N-terminal end of crystallized bovine erythrocyte GPX (21) and the 41st residue of the N-terminal polypeptide fragment of rat liver GPX (16). Amino acid sequence analysis of bovine GPX found the [Se]Cys at the 45th residue from the N-terminal end (29). The amino acid sequence suggests (to the author) that cleavage of 9 hydrophobic residues from the N-terminus (plus omission of one other residue) may account for the discrepancy between the two versions of the bovine GPX amino acid sequence. We have observed similar changes in the apparent M_r of rat liver GPX during purification that are apparent proteolytic modifications of GPX that do not affect enzyme activity (81).

The reports that Se was present in GPX as [Se]Cys were soon followed by a preliminary report that [Se]Cys incorporation into GPX was mediated by a [Se]Cys-tRNA (31). This apparent translational process for incorporating Se into GPX was in conflict with the process implicated by isotope dilution experiments (80) that showed that inorganic forms of Se, such as selenite or selenide, were more readily incorporated into GPX than was preformed [Se]Cys. Selenite thus appeared to be more readily metabolized than [Se]Cys to the immediate precursor, perhaps selenide, used for GPX synthesis. Thus, in 1980 a posttranslational (or cotranslational) modification of an amino acid residue already present in the peptide backbone appeared to be the mechanism used for formation of the [Se]Cys moiety of GPX (80).

Serine is the [Se]Cys Precursor

To further study the mechanism used to incorporate Se into GPX, Sunde & Evenson (75) perfused isolated rat liver with ^{14}C- or ^{3}H-labeled amino acids, purified the GPX to homogeneity, derivatized the [Se]Cys to carboxymethyl-[Se]Cys ([Se]Cys(Cm)), hydrolyzed the protein, and then subjected the hydrolysate to amino acid chromatography. The specific activity of the individual amino acids was measured to determine the origin of the carbon skeleton of [Se]Cys in GPX. When a liver was perfused with 20 or 60 μCi [U$-^{14}$C]cystine, cysteine was labeled without discernible labeling of [Se]Cys(Cm). Because there are three cysteine residues per subunit, a [Se]Cys(Cm) peak one third the size of the cysteine peak should have been observed if cysteine was the precursor of [Se]Cys. Perfusion with 100 μCi [U$-^{14}$C]serine substantially labeled serine and glycine (the folate-linked metabolite of serine) and significantly labeled [Se]Cys(Cm) with nearly the same cpm/μmole as serine. Perfusions with 250 μCi [3$-^{3}$H]serine also yielded serine and [Se]Cys(Cm) peaks with equal cpm/μmole, but glycine was not labeled. This lack of ^{3}H labeling eliminated glycine as the precursor. The specific labeling of [Se]Cys with ^{14}C or ^{3}H from serine demonstrated that serine is the carbon source for [Se]Cys. The nearly equal specific activity of serine and [Se]Cys(Cm) indicated that the serine pool used to acylate Ser-tRNA and to synthesize [Se]Cys was the same, and it further suggested that the [Se]Cys moiety of GPX is formed cotranslationally (75).

UGA Codon Specifies [Se]Cys

At the Beatson Cancer Institute in Glasgow, P. R. Harrison has been studying erythroid cell differentiations as a model for regulation of gene expression. In 1986, this group (13) reported that they had selected, cloned, and sequenced a 19-kd protein from murine fibroblasts because it was expressed at higher levels in mouse erythroblasts than in erythroid stem cells. Comparison of the amino acid sequence, implied by the nucleotide sequence, with amino acid sequences in a computerized protein sequence data base revealed that they had serendipitously cloned GPX without knowing its identity. The mouse GPX gene coded for a 201 amino acid polypeptide that had different residues at only 27 positions throughout the protein, when compared to the published bovine GPX sequence. Surprisingly, a UGA codon[2], normally one of three termination codons (the others are UAA and UAG), specified the position of the [Se]Cys at residue 47 in the middle of an open reading frame in the first of two exons in the gene. UGA is a well-used termination codon in higher animals, so this discovery implies that there must be a unique aspect of the

[2]Throughout the text, the three-nucleotide triplet that specifies [Se]Cys will be called UGA whether referring to the UGA codon in mRNA or to the TGA in DNA.

UGA in the mRNA for GPX such that it specifies [Se]Cys incorporation rather than polypeptide termination as in most mRNA species.

Confirmation that [Se]Cys in selenoproteins can be specified by UGA was rapid. Bock et al (58) had previously shown that mutants that produced truncated formate dehydrogenase polypeptides still incorporate Se into the polypeptide if a deletion occurs on the 3' side of the Se moiety. This shows that a full polypeptide was not required for Se incorporation. In June, 1986, Bock, Stadtman and coworkers (101) also reported that the [Se]Cys in formate dehydrogenase (formate:hydrogen lyase) was specified by a UGA in the open reading frame of the *E. coli* gene. Experiments with fusion genes (100) further indicated that the polypeptide chain was not synthesized beyond the UGA codon if Se was missing from the media. This strongly suggests that Se must be present for elongation of the peptide beyond the position of [Se]Cys. By analogy with GPX and formate dehydrogenase, this work also implies that a UGA codon specifies the location of [Se]Cys in glycine reductase. Recently, Voordouw et al (87a) have shown that the [NiFeSe]hydrogenase from *Desulfovibrio* species is a Se-specific selenoprotein with a UGA codon near the 3' end of the gene.

GPX Sequences

The nucleotide sequence for a human GPX has now been determined by Mullenbach and coworkers (54) who sequenced a GPX cDNA from human kidney. The human gene encodes a 201 amino acid polypeptide with [Se]Cys located at residue 47 and specified by a UGA codon. The amino acid and nucleotide sequence homologies with the mouse GPX sequences were 85% and 90%, and the intron locations were identical, indicating that the sequence of GPX is well conserved in higher animals. Nucleotide sequences for the GPX for bovine pituitary (55), mouse placenta (55), rat liver (35, 60, 96), and human liver (73) have all now been published, and these illustrate that the GPX gene is highly conserved, especially surrounding the UGA codon and the rest of the 5'-half of the sequence.

Cotranslational Se Incorporation

The discovery that the [Se]Cys moiety in selenoproteins is encoded by the UGA codon has exciting implications for Se biochemistry as well. The reports that Se incorporation into GPX was mediated by a [Se]Cys-tRNA (31, 32) were in apparent disagreement with our demonstration that inorganic Se was more readily metabolized to the form inserted into GPX than was selenocystine (80). We had also shown, however, that cycloheximide inhibition of protein synthesis would block Se incorporation into GPX (78), suggesting that incorporation of Se into GPX occurred translationally or cotranslationally. Lastly we demonstrated that serine provides the carbon skeleton of the

[Se]Cys in GPX (75). These conflicting observations were hypothetically resolved with the help of certain seryl-tRNAs in higher animals that are specific for the UGA codon (30). Serine while esterified to these tRNAs is further phosphorylated by a specific kinase to form [P]Ser-tRNA$^{Ser}_{UGA}$ (51). We proposed (75) that a metabolic exchange of Se for the phosphate would thus result in a [Se]Cys-tRNA$^{Ser}_{UGA}$ that would incorporate [Se]Cys into GPX at the position specified by UGA[3]. This three-step pathway would certainly be a novel means of synthesis and incorporation of an essential moiety into an enzyme, and such a process was shown in 1988 to occur at least in prokaryotes (46).

[Se]Cys-tRNA

Leinfelder and coworkers (46) identified four genes whose products are required for [Se]Cys incorporation into formate dehydrogenase in *E. coli*. Screening of a cosmid bank resulted in identification of a 223-bp clone which would complement one of the mutant genes (selC, formerly called fdhC). Sequencing revealed that this clone encoded a tRNA molecule with a number of unique features including (i) an anticodon sequence UCA to hybridize with the UGA codon, (ii) a length of 95 bases versus the usual 76 ± 5, (iii) a D-loop with 22 versus the usual 10 bases, (iv) an amino acid acceptor stem of 8 rather than 7 base-pairs, and (v) several deviations from the consensus nucleotides usually found in *E. Coli* tRNAs. This tRNA was shown to be acylated by serine and to incorporate [Se]Cys into formate dehydrogenase, thus supporting the identification of serine as the precursor of the [Se]Cys moiety in GPX (75). This is apparently the prokaryotic equivalent to the putative species used to incorporate Se into GPX. Two other genes (selA, selD) necessary for [Se]Cys incorporation encoded 50-kd and 37-kd protein gene products, respectively, which might be enzymes necessary for the conversion of serine to [Se]Cys while bound to the tRNA.

The UGA codon is one of three termination codons, and it is well used in higher animals [e.g. rabbit β-globin (20) and human P-450 microsomal hydroxylase (86)], so there must be a unique aspect of the UGA in GPX and in formate dehydrogenase so that it will specify [Se]Cys incorporation rather than polypeptide termination. Examination of the base sequence adjacent to the UGAs in the GPX and in formate dehydrogenase does not readily reveal a "consensus sequence" of bases that would differentiate a [Se]Cys-UGA from a termination UGA. To allow UGA to specify [Se]Cys rather than chain termination, Mizutani & Hitaka (52) proposed that a stem motif at the UGA was important. Mullenbach et al (55) have proposed that a lack of an adjacent stem motif may be critical. It now appears likely that an additional factor is

[3]In this text, this tRNA species with an anticodon (UCA) that hybridizes to the UGA codon in the mRNA is referred to as tRNA$^{Ser}_{UGA}$.

necessary to discriminate between [Se]Cys incorporation and polypeptide termination (45). In *E. coli,* four genes are required for the full metabolism of Se leading to selenoprotein synthesis: *sel*C codes for the $tRNA^{Ser}_{UGA}$, and *sel*A and *sel*D appear to code for 50 and 37 kd proteins that hypothetically may be enzymes in the [Se]Cys-$tRNA^{Ser}_{UGA}$ synthesis pathway. Leinfelder et al (45) have suggested that the 70 kd protein encoded by *sel*B may be the necessary factor.

Little is known about the analogous eukaryotic proteins and genes, but Hatfield and coworkers (44) have recently shown that a similar tRNA in a rat mammary tumor cell line (TMT-081-MS) will facilitate synthesis of [Se]Cys from selenite and serine. However, this fact does not conclusively establish that Se incorporation is a cotranslational event. Mizutani & Hitaka (52) have shown that traces of [P]Ser-$tRNA^{Ser}_{UGA}$ will be converted to [Se]Cys-$tRNA^{Ser}_{UGA}$ in vitro using selenide and crude mouse liver enzyme preparations. These workers, however, also reported that the same preparations would convert [P]Ser residues in casein to [Se]Cys, suggesting that the ability to modify [P]Ser residues posttranslationally is also present in cells. This experiment may have been complicated by the use of a reducing cocktail (NADPH, DTT, GSH, GSH reductase) to generate selenide. Thus the exact pathway for synthesis of the [Se]Cys moiety in selenoproteins is not yet conclusively established.

Selenide As the Se Precursor

The numerous Se supplementation studies using selenite, selenate, and [Se]Met provided the first indication that a common intermediate rather than one of these species was used for incorporation into GPX. Sunde & Hoekstra proposed in 1980, based on isotope dilution experiments (80), that selenide or a metabolically close species was the precursor used for selenoprotein synthesis. Subsequent work with the [Se]Cys-$tRNA^{Ser}_{UGA}$ pathway continues to support this view. Our work using the SDS/PAGE analysis has shown that two species on the inorganic side of Se metabolism, [^{75}Se]selenite (25) and [^{75}Se]selenodiglutathione (76), and two species on the organic side of Se metabolism, [^{75}Se]Met (88) and [^{75}Se]betaine (dimethylselenoacetate) (26), all result in the same labeling pattern of selenoproteins. This strongly suggests that selenide or a closely related species is the sole precursor species used to synthesize the selenoproteins detected using the SDS/PAGE procedure.

OTHER GLUTATHIONE PEROXIDASES

Phospholipid Hydroperoxide GPX (GPX-II)

An apparently new form of GPX was reported in 1985 by Ursini et al (87) to be a second Se-dependent GPX. This species, called GPX-II, has been purified from porcine heart and liver, and shown to be a monomeric species of

22 kd with 1 g-atom Se/mole. GPX-II will degrade phospholipid hydroperoxides, such as phosphatidylcholine hydroperoxide, which are not substrates for classical GPX. The relationship of this monomeric peroxidase to tetrameric GPX is not known, but it appears to have a different amino acid composition, and thus there may be a separate gene for GPX-II. This species may be the minor 20-kd selenoprotein that is detected by SDS/PAGE analysis of ^{75}Se-labeled rat liver (25); a higher ratio of ^{75}Se incorporation into GPX-II/GPX in Se-deficient versus Se-adequate liver suggests that Se is preferentially incorporated into GPX-II in Se deficiency.

Plasma GPX (GPX-P)

Cohen and coworkers (84) have purified GPX from human plasma and shown that it is immunochemically distinct from erythrocyte GPX. GPX-P is also a tetrameric protein with subunit M_r of 23 kd and with 4 g-atom Se/mole enzyme. As expected for a plasma species, GPX-P was shown to be a glycoprotein. Interestingly, GPX-P has a specific activity that is only 10% of that found for erythrocyte GPX; this fact leads to confusing calculations in the literature concerning the percentage of plasma Se accounted for by GPX. Both classical GPX and GPX-P are synthesized by hepatocytes in culture, indicating that GPX-P originates in the liver. Amino acid sequence analysis of several tryptic peptides of GPX-P has indicated recently that the amino acid sequence for GPX-P and classical GPX are different, suggesting that GPX-P may be encoded by a separate gene (2).

Non Se-Dependent GPX

A non-Se-dependent glutathione peroxidase activity in the liver of Se-deficient animals was discovered in 1976 (43). This activity was shown to be due to the GSH-S-transferases (59), which are one of the most abundant liver proteins. The enzyme, which normally catalyzes GSH conjugation and does not contain Se, cannot catalyze the reduction of H_2O_2, so enzyme assays using H_2O_2 will only measure classical GPX (if properly conducted to avoid catalase and hemoglobin-mediated peroxidase activity). This non-Se-dependent GPX will reduce hydroperoxides, but with a much higher Km than GPX (59). The importance of the peroxidase activity of GSH-S-transferases in animals is not known.

OTHER MAMMALIAN SELENOPROTEINS

At least eight selenoenzymes have been discovered in bacteria (cf 2a, 74), but the nature of other selenoproteins in animals besides GPX has not been studied in detail until recently. Sunde & Hoekstra (78) determined the distribution of different size classes of Se-binding proteins in liver, using Seph-

adex G-150 chromatography of liver cytosol from rats fed Se-adequate (0.2 μg Se/g diet as selenite) or Se-deficient diets, when the rats were injected i.v. with a tracer dose of [^{75}Se]selenite. They found that each class size of protein was generally present in both Se-adequate and Se-deficient rats, but the distribution of ^{75}Se differed with Se status. Hawkes et al (33) used column chromatography to determine the size and charge classes of selenoprotein in rats labeled by long-term administration of [^{75}Se]selenite. Notably, these workers reported that 92% of the muscle Se was protein-bound, as compared to 70% for liver and 54% for testes and epididymides, that [Se]Cys accounted for over 100% of the Se in liver but only 56% in kidney, and that 80% of the whole body Se in a selenite-fed rat was present as [Se]Cys. Thus, a number of potential selenoproteins are present in minor amounts that may be important for normal cellular metabolism.

SDS/PAGE Analysis

These chromatographic procedures, however, had several major problems that limited further advances in the study of mammalian selenoproteins. For instance, the procedures were unable to completely resolve various selenoproteins one from another, and thus Burk & Gregory (9) showed that an apparent 80-kd species observed with gel filtration chromatography was both GPX and a second selenoprotein. Additionally, some of these apparent selenoproteins may only be proteins with weak affinity for Se, rather than true selenoproteins, and the low-molecular-weight peaks might in fact be inorganic or low-molecular-weight Se not attached to any particular protein. Thus, new procedures were needed in order to evaluate more critically the distribution of Se between various selenoproteins. Evenson & Sunde (25) developed a procedure using SDS slab gel gradient electrophoresis (SDS/PAGE) which was able to separate the various Se-containing protein subunits. This procedure electrophoretically strips the Se from proteins that bind the Se only weakly; the SDS/PAGE thus evaluates more critically how much of the Se is actually bound to proteins. To compare the SDS/PAGE procedure to standard chromatographic analysis, Evenson & Sunde (25) determined the ^{75}Se labeling patterns of selenoproteins in Se-deficient rat liver cytosol 3 hr after [^{75}Se] selenite administration. Sephadex G-150 chromatography revealed four ^{75}Se-labeled peaks, as reported previously (78). Analysis of the same liver cytosol, using the SDS/PAGE procedure, showed that the Se-deficient liver cytosol contained two major ^{75}Se-labeled proteins of 65 and 23 kd, as well as minor ^{75}Se-labeled proteins of 77, 58, 20, 14, and 10 kd. SDS/PAGE analysis of the GPX peak from the Sephadex G-150 chromatography showed that the 80-kd peak actually contained both the 23-kd GPX subunit and the 65-kd selenoprotein as well as the minor 77- and 58-kd selenoproteins. The apparent G-150 20–30-kd selenoprotein peak actually contained the 20-, 14-, and 10-kd

selenoprotein species. Thus, techniques more specific than gel filtration chromatography are necessary to completely resolve these variously labeled selenoproteins.

The SDS/PAGE procedure is currently the best technique that I know to study selenoprotein metabolism, because of its sensitivity and quantitative ability. Because we can load up to 1500 μg protein per lane, and because the counting efficiency for ^{75}Se is >60%, we can detect incorporation of ^{75}Se that is not detected using autoradiography. In fact, the sensitivity is such that we can detect significant ^{75}Se-labeling of GPX after ^{75}Se administration to Se-deficient rats when our antibody procedures (ELISA or immunoblotting) or enzyme activity assays do not detect any GPX.

Dietrich Behne (6) has recently reported that Se-deficient rats (0.002 μg Se/g diet) and Se-adequate rats (0.3 μg Se/g diet), injected with 30 μCi [^{75}Se]selenite (0.13 μg Se) and killed 36 to 45 days later, had 13 selenoproteins in various tissues with molecular weights of 12.1, 15.6, 18.0, 19.7, 22.2, 23.7, 27.8, 33.3, 55.5, 59.9, 64,9, 70.1, and 75.4 kd. Thus both short-term and long-term labeling show a number of selenoproteins that await assignment of a function.

57-kd Plasma Selenoprotein P ([Se]P)

One of the most prominent "new" selenoproteins is the 57-kd plasma selenoprotein (called plasma selenoprotein P ([Se]P)). This protein confounded researchers because it lost its Se during purification, and because the weight of the native protein, determined by gel filtration chromatography, was 80 kd (9), whereas the SDS/PAGE ^{75}Se-labeled protein weighed 57 kd. Thus, it appeared that the native protein also had a 30-kd non-Se-containing subunit in addition to the 57-kd portion (53). Yang et al (94) purified [Se]P by preparing monoclonal antibodies against partially purified [Se]P, and then used these antibodies in an affinity chromatography step to purify the native species. They discovered that [Se]P was a single 57-kd polypeptide; periodic acid-Shift reagent staining indicated that the protein is a glycoprotein, as was expected for a plasma protein. These researchers have reported that the Se in Se-P is present as [Se]Cys, that there are 10 g-atom Se per mole, and UGA is used to specify at least some of the [Se]Cys moieties (34).

17-kd Sperm Selenoprotein ([Se]MCP)

A 17-kd sperm selenoprotein has been isolated from both rat (11) and bovine spermatozoa (57). It is 15–17-kd protein found in the mitochondrial capsule in the mid-piece region of the sperm in association with the mitochondrial helix. Calvin et al (12) gave this species the name *mitochondrial capsule protein ([Se]MCP)*. [Se]MCP is a cysteine-rich protein, so the high Se content may be due to [Se]Cys substitution for cysteine rather than specific Se incorpora-

tion. The Se in [Se]MCP is stabilized during purification by carboxymethylation, but the Se stoichiometry of [Se]MCP has not been established, and the Se has not yet been shown to be [Se]Cys. Thus, [Se]MCP has been listed as a Se-binding protein, although further research may discover that it is a [Se]Cys-specific or a Se-specific selenoprotein.

14-kd Se-Fatty Acid Binding Protein ([Se]FABP)

One of the minor selenoproteins has recently been shown to be a most interesting selenoprotein. Bansal et al (3) purified a 14-kd selenoprotein from mouse liver and determined the amino acid sequence of 75% of the polypeptide. Comparison of the resulting sequence with other sequences revealed a 92% amino acid sequence homology with rat liver fatty acid binding protein (FABP). Thus, FABP appears to be a selenoprotein. The genes for rat and human FABP have been cloned and sequenced and do not contain an open reading frame UGA to specify [Se]Cys. This suggests that Se is specified and incorporated into FABP by a mechanism unlike that used for GPX. The exact nature of the Se binding and the Se stoichiometry remain to be determined, and thus [Se]FABP is presently listed as a Se-binding selenoprotein. The sequenced portion of [Se]FABP contains Cys as well as Ser and Met residues, so translational incorporation of [Se]Cys or [Se]Met, or posttranslational modification of Ser to [Se]Cys, could account for the Se in FABP.

Other Selenoproteins

Behne (6) has reported that a 77-kd selenoprotein is found in high amounts in thyroid, and Arthur (1) has reported that severe Se deficiency alters the ratio of T3 to T4 in rats; together these facts suggest that a selenoprotein may have a role in thyroid function. A 130-kd Se-binding selenoprotein in plasma remains uncharacterized (28), as does a 77-kd Se-binding protein in liver mitochondria (8). The status of the 10-kd selenoprotein observed in muscle also remains unclear (cf 74). Other selenoproteins detected by SDS/PAGE analysis now are only ^{75}Se hot spots, but the advent of amino acid and nucleotide sequence data banks may make identification of the species rather straightforward in the future.

REGULATION OF GPX EXPRESSION BY SE

The common forms of Se used for dietary supplementation of humans and animals are selenite, selenate, and [Se]Met, and these forms are generally equally available from the diet for maintenance of GPX activity (cf 74). Marked differences in availability can occur, however, under certain circumstances (77, 89). When animals are fed a Se-deficient diet (typically <0.1

μg Se/g diet), growing animals rapidly exhibit a drop in GPX activity, which indicates that body Se stores are being depleted. A dietary Se level of 0.05 μg/g diet will maintain growth in most young animals whereas a dietary level of 0.1 μg Se/g diet is necessary to maintain GPX activity levels. When dietary Se level is increased above 0.1 μg Se/g diet, GPX enzyme activity in most tissues tends to plateau, which suggests that the level of GPX is regulated homeostatically. This plateau has been used to determine the Se requirement for animals and humans (cf. 47). Other methods, such as measuring tissue Se concentration and, more recently, the level of a plasma selenoprotein have been proposed, but GPX activity remains the best estimate of biochemical Se status. The underlying mechanism used to regulate GPX expression may be the reason why GPX activity has proved to be such a reliable parameter of Se status.

Eukaryotic Gene Regulation

Regulation of gene expression by nutritional factors (e.g. metals, lactose, amino acids, etc) in prokaryotes occurs principally at the level of transcription, but regulation in eukaryotes occurs at a variety of levels. For instance, zinc administration increases the level of metallothionein by increasing the synthesis of metallothionein mRNA (18). This example alone would imply that regulation in eukaryotes of gene expression by a metal also occurs at the level of transcription, but this is not universal. Iron loading of rats leads to an increase in ferritin levels, but the increase is due to increased translatability of existing mRNA rather than de novo transcription of ferritin mRNA (98). This regulation has recently been shown to involve an upstream "iron-responsive element" (64). Similarly, copper administration to copper-deficient rats results in increased ceruloplasmin levels, but copper administration is only necessary for the increase in enzymatic function (oxidase activity), not for the increase in immunoprecipitable material stimulated by interleukin-1 (4). Thus, regulation of expression by metals appears to use several distinct mechanisms in higher animals.

Effect of Se Status on GPX Protein

In 1982, Yosida and coworkers (95) used immunoprecipitation to show that the loss of GPX activity in Se-deficient rat liver is also accompanied by a similar loss of GPX protein. In humans, the low level of GPX activity in erythrocytes in a Se-deficient individual was also shown by Cohen and coworkers (85) to be accompanied by a low level of GPX protein, using polyclonal antibodies to human erythrocyte GPX. During Se repletion, the increase in GPX activity paralleled the increase in GPX protein, further demonstrating that Se status can control the level of GPX protein as well as activity. GPX activity and GPX protein also increased in parallel during Se repletion of HL-60 cells.

To study more carefully the effect of dietary Se deficiency on the regulation of the protein portion of GPX, Knight & Sunde (40) fed weanling rats a Se-deficient (< 0.02 μg/g Se) or a Se-supplemented (0.2 μg/g Se as Na_2SeO_3) diet and killed the rats at intervals up to 28 days later. GPX activity was assayed using H_2O_2 so only the Se-dependent GPX was measured. Anti-GPX antibodies, produced in a rabbit by three injections of purified rat liver GPX, were used in an ELISA for GPX protein. Immunoblotting showed that the antibodies were highly specific for GPX. In Se-supplemented rats, liver GPX activity increased 66% and GPX protein increased 50% over the 28 days. In Se-deficient rats, liver GPX activity decreased exponentially to 0 with a half-life of 2.8 days. Liver GPX protein also decreased exponentially, but with a longer half-life of 5.2 days ($p<0.001$) (40), and the anti-GPX antibody-reactive protein did not decrease to 0. This experiment shows that both GSH-Px activity and GPX protein decrease exponentially during progressive dietary Se deficiency.

Knight & Sunde (41) then further examined how Se status controls the level of GPX during Se repletion. Se-deficient rats were fasted overnight and then fed a diet supplemented with 0.1 or 0.5 μg/g Se as selenite. The rats were sacrificed at intervals up to 14 days after the start of Se repletion, and the changes in liver GPX protein and activity were determined. A significant increase in GPX protein and activity was observed 24 hr after the rats were supplemented with 0.5 μg/g Se, but rats supplemented with 0.1 μg/g Se did not show a significant increase until 14 days after the start of Se repletion. GPX protein and GPX activity increased in a coordinate manner for both treatment groups. This experiment further demonstrates that Se status regulates the level of GPX. A short-term Se repletion experiment (41) indicated that both GPX protein and activity increases do not begin immediately after an increase in cellular Se, which suggests that other processes besides Se incorporation may be required before the rate of GPX synthesis increases in a repleted Se-deficient rat.

Effect of Se Status on GPX mRNA

The cloning and sequencing of GPX by Chambers et al (13) opened the door to study the regulation of GPX mRNA. Saedi and coworkers (65) obtained the cloned murine GPX gene from P. R. Harrison (13) and used RNA blot hybridization analysis to determine the effect of long-term Se status on the level of mRNA for GPX. Long-term Se-deficient (8–12 weeks) and Se-adequate rats were killed, and total liver RNA and poly A^+ RNA were isolated and subjected to analysis using the 0.7 kb EcoR1 restriction fragment of the cloned murine GPX gene. Total RNA from Se-adequate rats contained a single band that hybridized with the ^{32}P-labeled GPX probe. This rat RNA species was the same size (13S) as reported for murine GPX (13). Total RNA from the Se-deficient rats contained 7% and 10% of the level of GPX mRNA

found in the Se-adequate rats, and the poly A^+ RNA contained 17% of the level of RNA. RNA blot-hybridization analysis of the same samples using a 400 bp chicken β-actin probe showed that rat liver β-actin levels were relatively unchanged as compared to the 83–93% reduction in GPX mRNA in Se deficiency. These facts indicate that the effect of Se status was not a general effect of Se on all RNA species. This experiment thus demonstrated that long-term Se-deficient rat liver has greatly reduced levels (83–93% reductions) of mRNA for GPX as compared to Se-adequate rat liver.

To determine the effect of progressive Se deficiency on mRNA levels for GPX (82), we used RNA blot-hybridization analysis of total liver RNA from weanling rats fed either a Se-deficient (<0.02 μg Se/g) or a Se-adequate (0.2 μg Se/g) diet and killed at intervals up to 28 days after the start of the experiment. GPX activity was assayed using H_2O_2; GPX protein was assayed using anti-GPX antibodies in an ELISA (40); and GPX mRNA was measured using RNA blot-hybridization analysis. In the initial week of deficiency, GPX activity, GPX protein, and GPX mRNA all fell exponentially with half-lives of 3.3, 5.0, and 3.2 days, demonstrating a rapid effect of decreased cellular Se on mRNA levels.

To characterize the effect of an increase in liver Se on GPX mRNA in Se-deficient rats, Smith et al (69) injected Se-deficient rats with 11 of 44 μg Se as selenite (equivalent to the daily Se ingestion of a rat fed a diet with 0.5 or 2.0 μg Se/g and equivalent to the dose of Se used previously to characterize the response of GPX protein to short-term Se repletion), and sacrificed at periods up to 24 hr after Se injection. Neither GPX activity nor GPX mRNA increased significantly until 12 hr after Se injection in spite of increased ^{75}Se in the liver within 1 hr after injection. With 11 μg Se, GPX mRNA levels remained constant from 12 to 18 hr, and then fell at 24 hr, and GPX activity remained at the 12 hr level. With 44 μg Se, mRNA increased from 12 to 18 hr before decreasing at 24 hr, and GPX activity continued to increase from 12 to 24 hr. At the peak levels, mRNA was 60% of that detected in Se-adequate controls, and GPX activity was 9% of that in Se-adequate controls. Thus, a single injection of Se resulted in a substantial but transient rise in GPX mRNA, and the increase in GPX mRNA was delayed for at least 6 hr, in a way similar to the delayed increase in GPX protein levels.

Considerable disagreement exists in the recent published literature concerning the effect of Se deficiency on mRNA levels for GPX and for β-actin. Imura (39) also reported that GPX mRNA decreased dramatically as Se deficiency progressed in the mouse. In contrast, Reddy et al (61) first reported that levels of Se-GPX mRNA were increased in the deficient state. Later they reevaluated the blots and reported that GPX I mRNA levels in Se-adequate and Se-deficient rats were nearly the same (60). Yoshimura et al (97) reported recently that the mRNA for GPX was markedly diminished in Se deficiency,

but that β-actin mRNA was outstandingly increased with the selenium deficiency. Thus, this apparent disagreement concerning the effect of Se deficiency on the level of GPX mRNA and other mRNA species is an important research question that we have attempted to answer.

To evaluate more completely the effect of Se deficiency on GPX mRNA versus other mRNA species, Smith in our lab (unpublished) fed 3 male weanling rats the Se-deficient diet and 3 rats a Se-adequate diet for 33 days. Liver GPX activity in the Se-deficient rats was 1.6% of that in the Se-adequate animals. Liver Se was 12.5% of that found in the Se-adequate animals, and liver RNA for GPX fell 88% in the Se-deficient animals, as compared to a nonsignificant 15% increase for β-actin, and as compared to no significant change for carbonic anhydrase. Thus, this experiment with three replicates demonstrates that the dramatic decrease in GPX mRNA with Se deficiency is specific for GPX mRNA relative to mRNA for β-actin and carbonic anhydrase. Furthermore, in these Se-deficient rats (supplemented with vitamin E and methionine and still growing when killed), β-actin was not significantly affected by Se deficiency.

Mechanism of Regulation

The mechanism that makes the level of GPX mRNA sensitive to Se status is unknown. The finding that mRNA levels were almost completely reduced by Se deficiency demonstrates that the regulation does not occur at the translational level, but the modulation of GPX mRNA levels could occur either transcriptionally or posttranscriptionally. It would be most exciting to find that a "selenium responsive element" regulates transcription of the GPX gene. Alternatively, the level of GPX mRNA could be controlled by the rate of degradation. Perhaps the GPX mRNA is a much better target for ribonuclease when Se and [Se]Cys-tRNA$^{Ser}_{UGA}$ are not present, leading to the low observed levels of mRNA. We have recently found, using an in vitro nuclear run-off transcription assay, that liver nuclei from long-term Se-deficient rats are able to synthesize GPX mRNA at rates equal to that found in Se-adequate nuclei (99). Thus, the use of the novel [Se]Cys-tRNA$^{Ser}_{UGA}$ may be a regulatory alternative to a selenium-responsive element. Whichever the case, the ability of cellular Se concentration to regulate GPX expression means that, when we monitor Se status using GPX levels, we are tapping into the Se-sensing regulatory system of the cell. It also means that in times of low Se status, the cell may be able to divert Se away from GPX and into other selenoproteins with more critical roles.

ACKNOWLEDGMENT

This work was supported in part by the NIH (CA 45164 and AM 32942) and by the USDA (85CRCR11583).

Literature Cited

1. Arthur, J. R., Beckett, G. J. 1989. Selenium deficiency and thyroid hormone metabolism. See Ref. 90, pp. 90–95
2. Avissar, N., Slemmon, R., Cohen, H. J. 1990. Partial sequencing of plasma glutathione peroxidase. *FASEB J.* 4:A1061

2a. Axley, M. J., Stadtman, T. C. 1989. Selenium metabolism and selenium-dependent enzymes in microorganisms. *Annu. Rev. Nutr.* 9:127–37

3. Bansal, M. P., Cook, R. C., Danielson, K. G., Medina, D. 1989. A 14-kilodalton selenium-binding protein in mouse liver is fatty acid-binding protein. *J. Biol. Chem.* 264:13780–84
4. Barber, E. F., Cousins, R. J. 1988. Interleukin-1-stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats. *J. Nutr.* 118: 375–81
5. Batley, B. L., Sunde, R. A. 1989. Selenium incorporation into proteins follows sulfur amino acid incorporation in yeast. *FASEB J.* 3:A781
6. Behne, D., Hilmert, H., Scheid, S., Gessner, H., Elger, W. 1988. Evidence for specific selenium target tissues and new biologically important selenoproteins. *Biochim. Biophys. Acta* 966:12–21

6a. Beilstein, M. A., Whanger, P. D. 1988. Glutathione peroxidase activity and chemical forms of selenium in tissues of rats given selenite and selenomethionine. *J. Inorg. Biochem.* 33: 31–46

7. Bremner, J., Natori, Y. 1960. Behaviour of some selenium compounds in transmethylation. *Biochim. Biophys. Acta* 44:367–76
8. Brian, W. R., Sunde, R. A., Hoekstra, W. G. 1985. A previously unreported ^{75}Se-binding protein in rat liver mitochondria. *Fed. Proc.* 44:1669
9. Burk, R. F., Gregory, P. E. 1982. Characteristics of ^{75}Se-P, a selenoprotein found in rat liver and plasma, and comparison of it with selenoglutathione peroxidase. *Arch. Biochem. Biophys.* 213:73–80
10. Burnell, J. N., Whatley, F. R. 1977. Sulfur metabolism in *Paracoccus denitrificans:* Purification, properties and regulation of cysteinyl- and methionyl-tRNA synthetase. *Biochim. Biophys. Acta* 481:266–78
11. Calvin, H. I. 1978. Selective incorporation of selenium-75 into a polypeptide of the rat sperm tail. *J. Exp. Zool.* 204:445–52
12. Calvin, H. I., Cooper, G. W., Wallace, E. 1981. Evidence that selenium in rat sperm is associated with a cysteine-rich structural protein of the mitochondrial capsules. *Gamete Res.* 4:139–49
13. Chambers, I., Frampton, J., Goldfarb, P., Affara, N., McBain, W., Harrison, P. R. 1986. The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. *EMBO J.* 5:1221–27
14. Chen, D. M., Nigam, S. N., McConnell, W. B. 1970. Biosynthesis of Se-methylselenocysteine and S-methylcysteine in astragalus-bisulcatus. *Can. J. Biochem.* 48:1278–83
15. Combs, G. F. Jr., Spallholz, J. E., Levander, O. A., Oldfield, J. E. 1987. *Selenium in Biology and Medicine*. New York: AVI
16. Condell, R. A., Tappel, A. L. 1982. Amino acid sequence around the active-site selenocysteine of rat liver glutathione peroxidase. *Biochim. Biophys. Acta* 709:304–9
17. Cone, J. E., del Rio, R. M., Davis, J. M., Stadtman, T. C. 1976. Chemical characterization of the selenoprotein component of clostridial glycine reductase: identification of selenocysteine as the organoselenium moiety. *Proc. Natl. Acad. Sci. USA* 73:2659–63
18. Cousins, R. J., Dunn, M. A., Leinart, A. S., Yedinak, K. C., DiSilvestro, R. A. 1986. Coordinate regulation of zinc metabolism and metallothionein gene-expression in rats. *Am. J. Physiol.* 251:E688–94
19. Cummins, L. M., Martin, J. L. 1967. Are selenocystine and selenomethionine synthesized in vivo from sodium selenite in mammals? *Biochemistry* 6:3162–68
20. Efstratiadis, A., Maniatis, T., Kafatos, F. C. 1977. The primary structure of rabbit beta-globin messenger-RNA as determined from cloned DNA. *Cell* 10:571–85
21. Epp, O., Ladenstein, R., Wendel, A. 1983. The refined structure of the selenoenzyme glutathione peroxidase at 0.2 nm resolution. *Eur. J. Biochem.* 133: 51–69
22. Esaki, N., Nakamura, T., Tanaka, H., Soda, K. 1982. Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. *J. Biol. Chem.* 257: 4386–91
23. Esaki, N., Nakamura, T., Tanaka, H.,

Suzuki, T., Morino, Y., Soda, K. 1981. Enzymatic synthesis of selenocysteine in rat liver. *Biochemistry* 20:4492–96
24. Esaki, N., Tanaka, H., Uemura, S., Suzuki, T., Soda, K. 1979. Catalytic action of L-methionine-γ-lyase on selenomethionine and selenols. *Biochemistry* 18:407–10
25. Evenson, J. K., Sunde, R. A. 1988. Selenium incorporation into selenoproteins in the Se-adequate and Se-deficient rat. *Proc. Soc. Exp. Biol. Med.* 187: 169–80
26. Evenson, J. K., Sunde, R. A. 1990. Metabolism of ^{75}Se from [^{75}Se] selenobetaine into selenoproteins in rats. *FASEB J.* 4:A371
27. Forstrom, J. W., Zakowski, J. J., Tappel, A. L. 1978. Identification of the catalytic site of rat liver GSH-Px as selenocysteine. *Biochemistry* 17:2639–44
28. Gasiewicz, T. A., Smith, J. C. 1978. Properties of the cadmium and selenium complex formed in rat plasma *in vivo* and *in vitro*. *Chem. Biol. Interact.* 23:171–83
29. Günzler, W. A., Steffens, G. J., Grossman, A., Kim, S-M. A., Ötting, F., et al. 1984. The amino-acid sequence of bovine glutathione peroxidase. *Hoppe-Seyler's Z. Physiol. Chem.* 365:195–212
30. Hatfield, D., Diamond, A., Dudock, B. 1982. Opal suppressor serine transfer-RNAS from bovine liver form phosphoseryl-transfer RNA. *Proc. Natl. Acad. Sci. USA* 79:6215–19
31. Hawkes, W. C., Lyons, D. E., Tappel, A. L. 1979. Identification and purification of a rat liver selenocysteine-specific transfer RNA. *Fed. Proc.* 38:820 (Abstr.)
32. Hawkes, W. C., Lyons, D. E., Tappel, A. L. 1982. Identification of a selenocysteine-specific aminoacyl transfer RNA from rat liver. *Biochim. Biophys. Acta* 699:183–91
33. Hawkes, W. C., Wilhelmsen, E. C., Tappel, A. L. 1985. Abundance and tissue distribution of selenocysteine-containing proteins in the rat. *J. Inorg. Biochem.* 23:77–92
34. Hill, K. E., Read, R. W., Yang, J.-G., Lloyd, R. S., Burk, R. F. 1990. Cloning and expression of a cDNA probe for rat plasma selenoprotein P. *FASEB J.* 4:A371
35. Ho, Y.-S., Howard, A. J., Crapo, J. D. 1988. Nucleotide sequence of a rat glutathione-peroxidase cDNA. *Nucleic Acids Res.* 16:5207
36. Hoffman, J. L., McConnell, K. P., Carpenter, D. R. 1970. Aminoacylation of *Escherichia coli* methionine tRNA by selenomethionine. *Biochim. Biophys. Acta* 199:531–34
37. Hsieh, H. S., Ganther, H. E. 1977. Biosynthesis of dimethyl selenide from sodium selenite in rat liver and kidney cell-free systems. *Biochim. Biophys. Acta* 497:205–17
38. Huber, R. E., Criddle, R. S. 1967. The isolation and properties of β-galactosidase from *Escherichia coli* grown on sodium selenate. *Biochim. Biophys. Acta* 141:587–99
39. Toyoda, H., Himeno, S., Imura, N. 1989. The regulation of glutathione peroxidase gene expression: implication for species difference and the effect of dietary selenium manipulation. See Ref. 90, pp. 3–7
40. Knight, S. A. B., Sunde, R. A. 1987. The effect of progressive selenium deficiency on anti-glutathione peroxidase antibody reactive protein in rat liver. *J. Nutr.* 117:732–38
41. Knight, S. A. B., Sunde, R. A. 1988. Effect of selenium repletion on glutathione peroxidase protein in rat liver. *J. Nutr.* 118:853–58
42. Kraus, R. J., Foster, S. J., Ganther, H. E. 1983. Identification of selenocysteine in glutathione-peroxidase by mass spectroscopy. *Biochemistry* 22:5853–58
43. Lawrence, R. A., Burk, R. F. 1976. Glutathione peroxidase activity in selenium deficient rat liver. *Biochem. Biophys. Res. Commun.* 71:952–58
44. Lee, B. J., Worland, P. J., Davis, J. N., Stadtman, T. C., Hatfield, D. L. 1989. Identification of a selenocysteyl-$tRNA^{Ser}$ in mammalian cells that recognizes the nonsense codon, UGA. *J. Biol. Chem.* 264:9724–27
45. Leinfelder, W., Stadtman, T. C., Böck, A. J. 1989. Occurrence in vivo of selnocysteyl-transfer RNA (UCA)ser in *Escherichia coli*. *J. Biol. Chem.* 264: 9720–23
46. Leinfelder, W., Zehelein, E., Mandrand-Berthelot, M.-A., Böck, A. 1988. Gene for a novel transfer-RNA species that accepts L-serine and cotranslationally inserts selenocysteine. *Nature* 331: 723–25
47. Levander, O. A. 1983. Considerations in the design of selenium bioavailability studies. *Fed. Proc.* 42:1721–25
48. Markham, G. D., Hafner, E. W., Tabor, C. W., Tabor, H. 1980. S-Adenosylmethionine synthetase from *Escherichia coli*. *J. Biol. Chem.* 255: 9082–92

49. McConnell, K. P., Hoffman, J. L. 1972. Methionine-selenomethionine parallels in *E. coli* polypeptide chain initiation and synthesis. *Proc. Soc. Exp. Biol. Med.* 140:638–41
50. McConnell, K. P., Hoffman, J. L. 1972. Methionine-selenomethionine parallels in rat liver polypeptide chain synthesis. *FEBS Lett.* 24:60–62
51. Mizutani, T., Hashimoto, A. 1984. Purification and properties of suppressor seryl-transfer RNA: ATP phosphotransferase from bovine liver. *FEBS Lett.* 169:319–22
52. Mizutani, T., Hitaka, T. 1988. The conversion of phosphoserine residues to selenocysteine residues on an opal suppressor transfer-RNA and casein. *FEBS Lett.* 232:243–48
53. Motsenbocker, M. A., Tappel, A. L. 1982. A selenocysteine-containing selenium-transport protein in rat plasma. *Biochim. Biophys. Acta* 719:147–53
54. Mullenbach, G. T., Tabrizi, A., Irvine, B. D., Bell, G. I., Hallewell, R. A. 1987. Sequence of a cDNA coding for human glutathione peroxidase confirms TGA encodes active site selenocysteine. *Nucleic Acids Res.* 15:5484
55. Mullenbach, G. T., Tabrizi, A., Irvine, B. D., Bell, G. I., Tainer, J. A., Hallewell, R. A. 1988. Selenocysteine's mechanism of incorporation and evolution revealed in cDNAs of 3 glutathione peroxidases. *Protein Eng.* 2:239–46
56. Ng, B. H., Anderson, J. W. 1978. Synthesis of selenocysteine by cysteine synthases from selenium accumulator and non-accumulator plants. *Phytochemistry* 17:2069–74
57. Pallini, V., Bacci, E. 1979. Bull sperm selenium is bound to a structural protein of mitochondria. *J. Submicrosc. Cytol.* 11:165–70
58. Pecher, A., Zinoni, F., Bock, A. 1985. The seleno-polypeptide of formic dehydrogenase (formate hydrogen-lyase linked) from *Escherichia coli:* genetic analysis. *Arch. Microbiol.* 141:359–63
59. Prohaska, J. R., Ganther, H. E. 1977. Glutathione peroxidase activity of glutathione-S-transferases purified from rat liver. *Biochem. Biophys. Res. Commun.* 76:437–45
60. Reddy, A. P., Hsu, B. L., Reddy, P. S., Li, N.-Q., Thyagaraju, K., Reddy, C., et al. 1988. Expression of glutathione peroxidase I gene in selenium-deficient rats. *Nucleic Acids Res.* 16:5557–68
61. Reddy, C. C., Li, N. Q., Reddy, P. S., Reddy, A. P., Hsu, B., et al. 1988. Evidence for cotranslational insertion of selenium into glutathione-peroxidase from rat liver. *FASEB J.* 2:A765
62. Robinson, M. F., Thomson, C. D. 1983. The role of selenium in the diet. *Nutr. Abstr. Rev.* 53:3–26
63. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., Hoekstra, W. G. 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179:588–90
64. Rouault, T. A., Hentze, M. W., Caughman, S. W., Harford, J. B., Klausner, R. D. 1988. Binding of a cytosolic protein to the iron-responsive element of human ferritin messenger RNA. *Science* 241:1207–10
65. Saedi, M. S., Smith, C. G., Frampton, J., Chambers, I., Harrison, P. R., Sunde, R. A. 1988. Effect of selenium status on mRNA levels for glutathione peroxidase in rat liver. *Biochem. Biophys. Res. Commun.* 153:855–61
66. Sandholm, M. 1973. The initial fate of a trace amount of I.V. administered selenite. *Acta Pharmacol. Toxicol.* 33:1–5
67. Schwarz, K. 1965. Role of vitamin E, selenium and related factors in experimental nutritional liver disease. *Fed. Proc.* 24:58–67
68. Sliwkowski, M. X., Stadtman, T. C. 1985. Incorporation and distribution of selenium into thiolase from *Clostridium kluyveri. J. Biol. Chem.* 260:3140–44
69. Smith, C. G., Saedi, M. S., Sunde, R. A. 1989. The effect of selenium repletion on glutathione peroxidase mRNA and activity in rats. *FASEB J.* 3:A451
70. Stadtman, T. C. 1987. Specific occurrence of selenium in enzymes and amino acid transfer-RNAs. *FASEB J.* 1:375–79
71. Deleted in proof
72. Steele, R. D., Benevenga, N. J. 1979. The metabolism of 3-methylthiopropionate in rat liver homogenates. *J. Biol. Chem.* 254:8885–90
73. Sukenaga, Y., Ishida, K., Takeda, T., Takagi, K. 1987. cDNA Sequence coding for human glutathione-peroxidase. *Nucleic Acids Res.* 15:7178
74. Sunde, R. A. 1984. The biochemistry of selenoproteins. *J. Am. Oil Chem. Soc.* 61:1891–1900
75. Sunde, R. A., Evenson, J. K. 1987. Serine incorporation into the selenocysteine moiety of glutathione peroxidase. *J. Biol. Chem.* 262:933–37
76. Sunde, R. A., Evenson, J. K. 1990. Metabolism of ^{75}Se from [^{75}Se] selenodiglutathione into selenoproteins in rats. *FASEB J.* 4:A1061

77. Sunde, R. A., Gutzke, G. E., Hoekstra, W. G. 1981. Effect of dietary methionine on the biopotency of selenite and selenomethionine in the rat. *J. Nutr.* 111:76–86
78. Sunde, R. A., Hoekstra, W. G. 1980. Incorporation of selenium into liver glutathione peroxidase in the Se-adequate and Se-deficient rat. *Proc. Soc. Exp. Biol. Med.* 165:291–97
79. Sunde, R. A., Hoekstra, W. G. 1980. Structure, synthesis and function of glutathione peroxidase. *Nutr. Rev.* 38:265–73
80. Sunde, R. A., Hoekstra, W. G. 1980. Incorporation of selenium from selenite and selenocystine into glutathione peroxidase in the isolated perfused rat liver. *Biochem. Biophys. Res. Commun.* 93: 1181–88
81. Sunde, R. A., Knight, S. A. B., Geyman, T. W., Evenson, J. K. 1988. Apparent proteolytic modification of glutathione peroxidase. In *Trace Element Metabolism in Man and Animals—TEMA 6*, ed. L. S. Hurley, C. L. Keen, B. Lonnerdal, pp. 21–22. New York: Plenum
82. Sunde, R. A., Saedi, M. S., Knight, S. A. B., Smith, C. G., Evenson, J. K. 1989. Regulation of expression of glutathione peroxidase by selenium. See Ref. 90, pp. 8–13
83. Sunde, R. A., Sonnenburg, W. K., Gutzke, G. E., Hoekstra, W. G. 1981. Biopotency of selenium for glutathione peroxidase synthesis. In *Trace Element Metabolism in Man and Animals-4*, ed. J. M. Howell, J. M. Gawthorne, C. L. White, pp. 165–67. Canberra, Aust: Aust. Acad. Sci.
84. Takahashi, K., Avissar, N., Whitin, J., Cohen, H. 1987. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. *Arch. Biochem. Biophys.* 256: 677–86
85. Takahashi, K., Newburger, P. E., Cohen, H. J. 1986. Glutathione peroxidase protein: Absence in selenium deficiency states and correlation with enzymatic activity. *J. Clin. Invest.* 77: 1402–4
86. Umbenhaur, D. R., Martin, M. V., Lloyd, R. S., Guengerich, F. P. 1987. Cloning and sequence determination of a complementary DNA related to human liver microsomal cytochrome p-450 S-mephenytoin 4-hydroxylase. *Biochemistry* 26:1094–99
87. Ursini, F., Maiorino, M., Gregolin, C. 1985. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim. Biophys. Acta* 839:62–70
87a. Voordouw, G., Menon, N. K., LeGall, J., Choi, E. S., Peck, H. D. Jr., Przybyla, A. E. 1989. Analysis and comparison of nucleotide sequences encoding the genes for [NiFe] and [NiFeSe] hydrogenases from *Desulfovibrio gigas* and *Desulfovibrio baculatus*. *J. Bacteriol.* 171:2894–99
88. Waschulewski, I. H., Batley, B. L., Sunde, R. A. 1988. The effect of dietary methionine on ^{75}Se incorporation from [^{75}Se]selenomethionine into selenoproteins. *FASEB J.* 2:A1089
89. Waschulewski, I. H., Sunde, R. A. 1988. Effect of dietary methionine on tissue selenium and glutathione peroxidase activity in rats fed selenomethionine. *Br. J. Nutr.* 60:57–68
90. Wendel, A. 1989. *4th International Symposium on Selenium in Biology and Medicine*. Heidelberg: Springer-Verlag
91. Wendel, A., Kerner, B., Graupe, K. 1978. The selenium moiety of glutathione peroxidase. In *Functions of Glutathione in Liver and Kidney*, ed. H. Sies, A. Wendel, pp. 107–13. Berlin: Springer-Verlag
92. Wilhelmsen, E. C., Hawkes, W. C., Tappel, A. L. 1983. Investigations of specificity of selenocysteine incorporation into selenoproteins via a rabbit reticulocyte lysate synthesis system. *Fed. Proc.* 42:2152
93. Deleted in proof
94. Yang, J.-G., Morrison-Plummer, J., Burk, R. F. 1987. Purification and quantitation of a rat plasma selenoprotein distinct from glutathione peroxidase using monoclonal antibodies. *J. Biol. Chem.* 262:13372–75
95. Yoshida, M., Iwami, K., Yasumoto, K. 1982. Purification and immunochemical analysis of rat liver glutathione peroxidase. *Agric. Biol. Chem.* 46:41–46
96. Yoshimura, S., Komatsu, N., Watanabe, K. 1980. Purification and immunohistochemical localization of rat liver glutathione peroxidase. *Biochim. Biophys. Acta* 621:130–37
97. Yoshimura, S., Takekoshi, S., Watanabe, K., Fujii-Kuriyama, Y. 1988. Determination of nucleotide sequence of cDNA coding rat glutathione peroxidase and diminished expression of the messenger-RNA in selenium deficient rat liver. *Biochem. Biophys. Res. Commun.* 154:1024–28

98. Zähringer, J., Baliga, B. S., Munro, H. N. 1976. Novel mechanism for translational control in regulation of ferritin synthesis by iron. *Proc. Natl. Acad. Sci. USA* 73:857–61
99. Zhou, X., Sunde, R. A. 1990. Effect of selenium status on glutathione peroxidase gene transcription in isolated rat liver nuclei. *FASEB J.* 4:A1061
100. Zinoni, F., Birkmann, A., Leinfelder, W., Böck, A. 1987. Cotranslational insertion of selenocysteine into formate dehydrogenase from *Escherichia coli* directed by a UGA codon. *Proc. Natl. Acad. Sci. USA* 84:3156–3260
101. Zinoni, F., Birkmann, A., Stadtman, T. C., Böck, A. 1986. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 83:4650–54.