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THE NUTRITIONAL BIOCHEMISTRY OF SELENIUM

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INTRODUCTION

Selenium (Se) was first recognized to be important in normal metabolism in 1957 by Schwarz & Foltz (150), who found it prevented necrotic degeneration of the liver in the vitamin E–deficient rat. This finding was immediately confirmed by work that demonstrated the prevention of the vitamin E deficiency disease of the chick, exudative diathesis, by low dietary levels (approximately 0.1 ppm) of Se (129, 149). Thus, the recognition of Se as nutritionally important came with the discovery of its ability to prevent certain vitamin E deficiency diseases in laboratory animals fed partially purified diets. Because vitamin E was thought to function as a biologically specific antioxidant in the prevention of in vivo lipid peroxidation, hypotheses for the mode of action of Se in animals held that it, too, functioned as an antioxidant. These hypotheses were borne out in 1973 when Rotruck et al (140) discovered that Se is an integral component of glutathione peroxidase (EC 1.11.1.9), an enzyme that

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metabolizes hydroperoxides. Recent studies therefore have been concerned with the utilization of dietary Se for the synthesis and function of Se-dependent glutathione peroxidase in protection from oxidative stress.

SELENIUM IN FOODS AND FEEDS

Selenium occurs naturally in foods and feedstuffs almost exclusively in organic compounds, the primary forms of which are selenomethionine, Se-methyl-selenomethionine, selenocystine, and selenocysteine (120, 147). The amounts of Se contained in most feed and food plants vary according to the concentration and biological availability of Se in the soil. Thus, while many regions of the world produce feed and food plants containing adequate Se to meet the nutritional needs of animals and people, some areas deficient in Se produce plants with insufficient concentrations of the mineral (i.e. less than 0.10 ppm) to prevent Se deficiency diseases. Examples of regions deficient in Se are the northeastern, Pacific northwestern, and extreme southeastern United States; north-central and eastern Canada; eastern Finland; the North Island of New Zealand; parts of Australia; and much of the People's Republic of China, especially the northeastern, southwestern, and east-central areas. In parts of the world where the Se levels in natural feedstuffs are low, animal feeds are generally supplemented with Na_2SeO_3, so selenite is an important dietary form of Se.

Human foods also vary in Se content, so there are substantial differences in Se intake between people in different parts of the world. Reports indicate very low average dietary Se intakes (less than 60 μg/person/day) in New Zealand, Finland, and parts of Sweden and China; and relatively high intakes of Se (at least 200 μg/person/day) in Venezuela and parts of Sweden, Canada, and China (167). China has the greatest known variation in human Se intakes, with vast areas of endemic Se deficiency occurring where residents consume an average of 11 μg/person/day (19, 20) and also a few pockets of high-Se exposure where the average Se intake has been estimated to be as much as 4990 μg/person/day (S. Yang, personal communication, 1981).

ENTERIC ABSORPTION OF SELENIUM

The major dietary forms of Se (i.e. selenocystine, selenocysteine, selenomethionine and Se-methyl-selenomethionine from natural sources, and selenite from nutritional supplements) appear to be well absorbed by monogastrics. Selenite can be indistinguishable from sulfite in biological systems, as indicated by competition for uptake by microorganisms and higher animals. Studies with *Chlorella vulgaris* (156) and *Pencillium chrysogenum* (179) demonstrated that selenate and sulfate compete for uptake from the medium. The authors of the latter study felt that the two species competed at the level of binding by sulfate permease, i.e. that the enzyme could transport either compound indiscriminately. Methionine and its Se-containing analog, selenomethionine, are also transported via the same mechanisms and hence compete for transport. This was demonstrated in *Chlorella vulgaris* (157) and in the hamster (99). The latter investigators measured the transmucosal flux of L-selenomethionine and L-methionine in everted duodenal sacs in vitro and found that both amino acids are actively transported by the same system. In contrast, the transport of selenocystine or selenite by the hamster intestine did not proceed against concentration gradients and was not inhibited by cysteine or sulfite.

Selenite may be better utilized by monogastrics than by ruminants. Wright & Bell (177) found that the retention of Se orally administered as selenite was approximately 77% in the pig but only 29% in the sheep. Their studies indicated that the lower small intestine, cecum, and colon were primary sites of Se absorption in both species, and that little if any absorption occurred in the rumen and abomasum. Probably as the result of the active transport of Se as a methionine analog, abosorption of Se as selenomethionine by monogastrics is generally high, while the absorption of Se as selenite is generally much lower and more variable. The enteric absorption of selenomethionine has been found to be 91% in rabbits (167) and 75% in humans (138). However, the absorption of Se as selenite has been reported to be as low as 45% in humans (138) and as high as 93% in rats (166). Studies by Combs (25, 26) demonstrated that the enteric absorption of selenite by the chick is affected by the dietary concentrations of vitamin E, vitamin A, and ascorbic acid, each of these being capable of promoting Se absorption when added to the diet at levels higher than those normally considered to be required. Thus, it is likely that these and other dietary factors may markedly affect the absorption and hence the nutritional value of forms of Se such as selenite, which do not have active transmucosal uptake.

Absorbed Se is transported through the body primarily bound with plasma proteins. The protein binding of Se has been shown to require an initial uptake and metabolism by erythrocytes that reduce Se, probably to H_2Se, using reduced glutathione (58, 77, 99). The subsequent release of the reduced Se product is associated with its rapid binding to plasma proteins (77, 91, 133, 141, 142). Almost all of the Se present in plasma occurs in protein-bound form. It is mainly in the albumin fraction in the mouse (141); in the human it is mainly in the very low-density lipoprotein fraction (9, 141). The transport function of these proteins was demonstrated by Porter et al (133), who found that they transfer Se readily to lymphocytes in vitro. Only a small percentage of the total plasma Se, approximately 1.5%, is present as Se-dependent glutathione peroxidase (4). Although this enzyme accounts for only 10% of the total Se in human erythrocytes, it is the dominant form of Se in erythrocytes of the sheep, accounting for about 80% of the total Se (117), and it accounts for 100% of the total Se in rat erythrocytes (4).

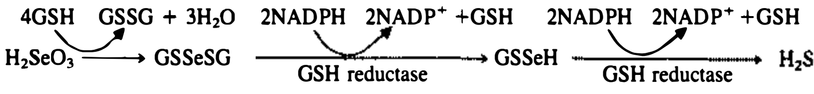
Concentrations of Se in tissues depend on the level and chemical form of ingested Se (16). When animals consume Se at rates near their nutritional requirements (i.e. dietary levels of 0.05–0.20 ppm), the mineral is found at highest concentrations (0.2–2 ppm, fresh weight basis) in kidney, liver, and other glandular tissues. The Se contents of heart muscle (0.15–0.20 ppm) are usually greater than those of skeletal muscle (0.05–0.20 ppm); the Se concentrations of brain and nervous tissue tend to be low (less than 0.10 ppm). At very low levels of intake, i.e. less than 0.05 ppm in the diet, the Se concentrations of blood and liver are markedly reduced; those of kidney are less affected. At high levels of intake, i.e. dietary levels in excess of 1 ppm, the concentrations of Se in liver and kidney may reach several parts per million. Tissue Se concentrations are generally greater when the mineral has been consumed in organic form. Thus, Se naturally occurring in foods and feeds as well as Se in selenomethionine produce greater levels of Se in animal tissues than equivalent amounts of the mineral fed as selenite. A notable exception to this is the Se present in fish meal and tuna; it has very poor biologic availability, perhaps because of heavy metal complexing.

METABOLISM OF SELENIUM

Although the chemistry of Se resembles that of sulfur (S) in several respects, these elements have important biochemical differences and are therefore not completely interchangeable in animal systems. The first difference is with respect to reduction of their oxyanions. The quadrivalent Se in selenite tends to undergo reduction, while the quadrivalent S in sulfite tends to undergo oxidation. Thus, Se compounds tend to be metabolized in animals to more reduced states; S compounds tend to be oxidized. The second difference is in the relative acid strengths of their hydrides. Although the analogous oxyacids of Se and S have comparable strengths, the hydride H_2Se (pK_a 3.8) is a much stronger acid than H_2S (pK_a 7.0). This difference is reflected in the dissociation behaviors of the selenohydryl group in selenocysteine (pK_a 5.24) and the sulfhydryl group of cysteine (pK_a 8.25) (71). Thus, whereas thiols such as cysteine are mainly protonated at physiological pH, the selenohydryl groups of selenols such as selenocysteine are largely dissociated. This behavior appears to be important in the catalytic roles of Se in selenoenzymes.

When Se is consumed as selenomethionine and other organic forms that occur naturally in foods, it is released as selenite by postabsorptive catabolism (120). Given this and the fact that Se supplements to animal diets consist largely of selenite, it is clear that the metabolism of Se by animals is intimately related to the metabolism of selenite. Ganther (46) first showed that Se is reductively metabolized by animals when he demonstrated the formation of dimethyl selenide from selenite by cell-free preparations from mouse liver. This process requires reduced glutathione (GSH) and is stimulated by NADPH and anaerobic conditions. The reductive metabolism of selenite occurs via a sequence of reactions commencing with the nonenzymic reaction of selenite by GSH to form an intermediate consisting of two GSH molecules joined by a S–Se–S linkage. This product is one of several compounds characterized by Ganther (47) as having the general formula RSSeSR, which are thus referred to as "selenotrisulfides" (i.e. bis(alkylthio)selenides). Selenotrisulfides are formed by the reaction of selenite with thiols (e.g. cysteinyl residues, 2-mercaptoethanol, CoA, GSH) (48, 50). The work of Ganther (47) indicates that the usual stoichiometry of this reaction is as follows: $4RSH + H_2SeO_3 \rightarrow RS\text{–}Se\text{–}SR + RSSR + 3H_2O$. Kice (80) has pointed out the complexity of this reaction. It is thought to proceed first via a rapid initial stage involving the reversible reaction of thiol with selenite to yield an alkylthioselenic acid intermediate ($RSSeO_2H$); second, by the reaction of $RSSeO_2H$ with a second thiol molecule to yield RSSe(O)SR; third, by the reaction of RSSe(O)SR and a third thiol molecule to yield RSSeSR and sulfenic acid, RSOH; fourth, by the conversion of the RSOH either to RSO_2SR or to RSSR by reaction with a fourth thiol molecule. The ratio of the final products appears to depend on the nature of the R group in the reacting thiol. When GSH is the reacting thiol, the selenotrisulfide reaction produces selenodiglutathione (GSSeSG) and oxidized glutathione (GSSG) quantiatively as follows: $4GSH + H_2SeO_3 \rightarrow GSSeSG + GSSG + 3H_2O$.

Selenium in the form of selenodiglutathione is enzymically reduced by glutathione reductase (EC 1.6.4.2) and NADPH to yield H_2Se. This reduction proceeds via two steps, the first of which results in the production of selenopersulfide intermediate, GSSeH (48). The further NADPH-linked reduction of the selenopersulfide to yield an acid-volatile selenide thought to be H_2Se is also catalyzed by glutathione reductase and is most active under anaerobic conditions (48, 69). This step is thought to involve the direct reduction of the Se–Se bond. Hydrogen selenide can also be formed by nonenzymic reduction with thiols (69, 137). Ganther (50) has offered this as an explanation for the reduction of selenite to dimethyl selenide in the absence of NADPH by washed microsomal preparations. The following sequence summarizes the reactions in the glutathione reductase pathway for the reduction of selenite to H_2Se in the cytosol:



$$H_2SeO_3 \xrightarrow{4GSH \;\curvearrowright\; GSSG + 3H_2O} GSSeSG \xrightarrow[\text{GSH reductase}]{2NADPH \;\curvearrowright\; 2NADP^+ + GSH} GSSeH \xrightarrow[\text{GSH reductase}]{2NADPH \;\curvearrowright\; 2NADP^+ + GSH} H_2Se$$

Gasiewicz & Smith (57, 58) showed that the form of Se released by erythrocytes into plasma is H_2Se, produced as the result of GSH-dependent metabolism in the blood cells. The glutathione reductase pathway of selenite reduction therefore appears to be important in the vascular transport of Se by converting selenite to the readily transportable H_2Se. Studies by Diplock and coworkers (17, 36) have suggested that selenide may have a specific role in tissues. They found that as much as 43% of the total Se in liver microsomal preparations of rats labeled in vivo was released in acid-volatile form, presumably as selenide. The amount of label recovered in this fraction was greatest when the homogenizing medium contained either α-tocopherol or mercaptoethanol or when the rats had been fed vitamin E. Diplock (34) suggested that selenide may be a component, replacing sulfide, in the active site of microsomal nonheme iron protein in the microsomal electron transport system. However, subsequent tests of this hypothesis have not provided conclusive evidence for such a role of Se in metabolism (35).

Selenodiglutathione, the substrate for the glutathione reductase pathway, can also react with other thiols. Vernie et al (170) found that GSSeSG reacted very rapidly with a component, i.e. an elongation factor, of an in vitro polyribosomal amino acid incorporating system, which thus inhibited amino acid incorporation from [^{14}C]leucyl-tRNA. This inhibition was not reversible by addition of GSH to the system, but it was blocked by the prior addition of GSH. Also, the inactive elongation factor could be activated by treatment with NADPH in the presence of glutathione reductase. Those workers concluded that the inhibition of in vitro protein synthesis by selenite occurred as a result of the reaction of GSSeSG with critical sulfhydryl groups in functional proteins in these systems. This reaction may have physiological significance in circumstances of high Se exposure and may play a role in the toxicity of Se.

Biological systems reduce selenate to selenite. Wilson & Bandurski (175) proposed that selenate was activated with ATP and ATP sulfurylase, followed by hydrolysis of the pyrophosphate thus formed. Their evidence included formation of a Se compound presumed to be adenosine-5'-selenophosphate on the basis of its electrophoretic mobility, which was similar to that of its S analog. Subsequent work (33) confirmed the formation of an adenosine-5'-selenophosphate–type compound by electrophoresis, but such a product could not be isolated by column chromatography on DEAE-Sephadex, perhaps because of the instability of the Se anhydride. Shaw & Anderson (154) failed to demonstrate the formation of an adenosine-5'-selenophosphate–type product using a plant ATP sulfurylase, but their use of [^{75}Se]selenate of relatively low specific activity may have precluded the detection of this product. The observation that thiols such as GSH caused the deposition of elemental Se from a sulfurylase incubation medium led Dilworth & Bandurski (33) to suggest that the nonenzymic thiolytic cleavage of the phosphoselenate anhydride resulted in the formation first of a thioselenic acid, e.g. $GSSeO_3^-$) and then of selenite, which was subsequently reduced by GSH, glutathione reductase, and NADPH as described above. Therefore, two reactions appear to be important in the reduction of selenate to selenite. The first step is the enzymic activation of selenate of adenosine 5′-selenophosphate,

$$SeO_4^{-2} + ATP \xrightarrow[\text{sulfurylase}]{Mg^{+2}} \text{adenosine-5}'\text{-selenophosphate} + PPi;$$

the second step is the nonenzymic thiolytic cleavage of that product,

$$\text{adenosine-5}'\text{-selenophosphate} \xrightarrow{GSH} GSSeO_3^- \longrightarrow SeO_3^{-2} + GSH\ .$$

These reactions can explain the occurrence of selenate reduction in animals that lack the sulfate reductase systems of plants and microorganisms.

While the glutathione reductase pathway for reduction of selenite to H_2Se occurs in the cytosolic fraction of the cell, the pathways for methylation of the H_2Se so produced occur in both the cytosolic and microsomal fractions (54). Ganther (46) demonstrated that the methylation of Se added as selenite to liver homogenate systems utilized S-adenosylmethionine as the methyl donor and required both microsomal and cytosolic components for maximal activity. The major product of this system was identified as $(CH_3)_2Se$. Hsieh & Ganther (70) indicated that the methylation process occurred via methyltransferases acting upon the H_2Se formed from the reduction of selenite, either nonenzymically by GSH or other reducing agents in microsomes, or enzymically by the NADPH-linked glutathione reductase system in the cytosol. The microsomal methylation system can be induced by changing the type of diet or by exposure to xenobiotics (37, 56, 70) and is inhibited in vitro by arsenite (51, 52, 70). The cytosolic Se-methyltransferase involved in the methylation of H_2Se has been purified from rat liver and kidney (70); however, it is not known whether this enzyme is specific for H_2Se or whether it is one of several methyltransferases known to be active in methylating compounds containing sulfur and oxygen.

Hydrogen selenide produced by the reduction of selenite can thus become bound to proteins in an acid-labile form or it can undergo methylation. Because H_2Se is one of the most toxic Se compounds (50), the methylation pathways are important means of its detoxification. Gaseous H_2Se at levels as low as 1 μg per liter of air have been shown to cause olfactory fatigue in humans; higher levels can irritate mucous membranes and ultimately lead to pulmonary edema (31). The toxicities of dimethyl selenide and trimethyl selenonium, in contrast, are several orders of magnitude less (102, 103, 116, 127, 128). That methylation is important in the detoxification of Se is also indicated by the observations that intoxicating doses of Se produced depletions of S-adenosylmethionine in tissues (67) and that supplemental dietary methionine decreases selenite toxicity (176).

The methylated compounds of Se are the major forms in which the mineral is excreted. The volatile dimethyl selenide is lost across the lungs and is exhaled (37, 51, 56, 104). The very soluble trimethylselenonium cation is excreted in the urine and is the major excretory form of the mineral (10, 12, 14, 81, 125, 126). This metabolite accounts for 20–50% of total urinary Se in the rat, and the only study of humans available to date indicates that the trimethylselenonium ion comprises a similar percentage of human urinary Se (10). In addition to trimethyl-selenonium, a second form, which Burk (10) called the U-2 fraction, accounts for one third of urinary Se. In conditions of physiologic intakes of Se, i.e. dietary levels less than 0.5 ppm, Se homeostasis is regulated by urinary excretion (10, 12) with little pulmonary excretion as dimethyl selenide. The pulmonary excretion of dimethyl selenide is of quantitative significance only under conditions of excessive Se intake. McConnell & Roth (104) showed that rats injected with 5 μg of Se as selenite per kg body weight excreted as much as 50% in the form of dimethyl selenide within the first 24 hours.

Evidence of the reductive metabolism of selenate and selenite in animals suggests the possibility that amino acids containing Se are synthesized from inorganic forms of the mineral in the diet. Microorganisms can synthesize the Se analogs of the sulfur amino acids methionine and cysteine (6, 73, 168), but it is not apparent that mammals have this ability. Godwin et al (60) isolated [^{75}Se]selenomethionine from the milk of ewes previously treated intraruminally with $Na_2{}^{75}SeO_3$, but it is possible that the rumen microflora, and not the host, produced selenomethionine that was in turn absorbed and assimilated into milk proteins. Several researchers have reported the apparent synthesis of amino acids containing ^{75}Se in animals given $Na_2{}^{75}SeO_3$(59, 105, 121, 151, 155). However, the presence of ^{75}Se as both selenomethionine and selenocysteine in hydrolysates of tissue proteins from animals pretreated with $Na_2{}^{75}SeO_3$ is open to question because it is now recognized that both selenoamino acids are unstable under the hydrolysis conditions employed in that study (71, 155) and because selenite has the ability to react in vitro with methionine and cysteine to form adducts with chromatographic properties similar to those of the parent sulfur amino acids (151). Cummins & Martin (32) used exhaustive dialysis for two weeks to remove noncovalently bound ^{75}Se from tissue proteins prepared from rabbits previously fed $Na_2{}^{75}SeO_3$ for 35 days. After hydrolysis of the dialyzed protein was performed, ion exchange chromatography revealed no ^{75}Se in those fractions with elution volumes of the selenoamino acids. Those authors concluded that selenite is not utilized for the synthesis of methionine and/or cysteine; however, the strongly alkaline conditions employed in that study may have led to degradation of any selenocysteinyl residues.

Olson & Palmer (121) detected several radiolabeled metabolites in enzymic digests of liver and kidney from rats treated with $Na_2{}^{75}SeO_3$. Selenocysteine, identified by ion exchange co-chromatography, was found in only small amounts, and a major metabolite co-eluted with 2,7-diamino-4-thio-5-selenooctanedioic acid. The authors concluded that selenocysteine is synthesized from selenite by the rat and is subsequently converted to the sulfoselenide compound. They suggested that the synthesis may first involve the reduction of selenite to selenide and then the condensation of the latter with serine to yield selenocysteine in a reaction analogous to the condensation of homocysteine with serine, which is catalyzed by serine hydrolase and yields cystathionine. An alternative hypothesis for the synthesis of selenocysteine in tissue proteins was suggested by Ganther (49), who proposed that H_2Se may react with peptide serinyl residues post-translationally to yield selenocysteinyl residues.

Selenocysteine is the form in which Se occurs in the active sites of several, if not all, of the known Se-dependent enzymes. Studies of protein A of clostridial glycine reductase (29, 30), bacterial formate dehydrogenase (78), and mammalian glutathione peroxidase (44) have indicated that the chemical form of Se in each enzyme is selenocysteine. The procedures used in these studies involved alkylation of the reduced ^{75}Se-labeled enzymes using iodoacetamide and identification of the ^{75}Se-carboxymethyl derivative after acid hydrolysis or of the ^{75}Se-carboxyamidomethyl derivative after enzymatic hydrolysis. The chemical identity of the radiolabeled Se derivatives was deduced by comparing elution volumes from ion-exchange columns or by comparing R_f values in one- or two-dimensional thin-layer chromatography. Ganther & Kraus (55) have cautioned that because these studies have relied upon co-chromatography for the chemical identification of the Se moieties in these enzymes, conclusive proof of the identity of each must await the successful production of mass spectra of the Se-containing derivatives. They have also pointed out that even if such data prove that the alkylated hydrolysis products are selenocysteine derivatives, it must be determined whether that product is related to the form of Se in the native enzyme or to a breakdown product of an unstable initial alkylated compound, e.g. a selenonium species.

Hawkes et al (65) presented evidence of a transfer RNA specific for selenocysteine in rat liver. This observation (reviewed by Tappel, 163) implies either the existence of a pathway for the de novo biosynthesis of selenocysteine or an adaptation for the utilization of preformed selenocysteine of dietary origin. It also suggests that selenocysteine may be incorporated into at least some selenoproteins, e.g. glutathione peroxidase, during translation. Another equally plausible possibility for the mode of incorporation of Se into selenocysteine in glutathione peroxidase is via post-translational modification(s) as described above. The likelihood of the latter possibility is supported by the studies of Sunde & Hoekstra (161), which demonstrated that the incorporation of radioactivity from L-[^{75}Se]selenocysteine into glutathione peroxidase by the isolated perfused rat liver was decreased by addition of a 9-fold excess of nonlabelled selenite or selenide to the perfusing medium, while addition of a 100-fold excess of unlabelled selenocysteine was relatively ineffective in reducing the incorporation of radioselenium from [^{75}Se]selenite.

Selenomethionine, an important naturally occurring form of Se in foods and feeds, follows the metabolic pathways of methionine. Huber & Criddle (72) found that *E. coli* grown on a medium containing selenate in place of sulfate produced β-galactosidase that had 75% of its methioninyl residues replaced by selenomethioninyl residues without affecting its catalytic properties. Studies have also shown that selenomethionine can replace methionine in the aminoacylation of Met-tRNA (68) and in polypeptide chain synthesis in rat liver and *E. coli* (100, 101). Selenomethionine can be readily metabolized as a mimic of its S-containing analog; therefore, when methionine is the first-limiting amino acid in the diet, it can be expected that selenomethionine may be well utilized for general protein synthesis rather than being degraded and releasing its Se. The studies of Sunde et al (160), which showed that the apparent biological availability of Se from selenomethionine for the rat was improved by the addition of methionine to a methionine-limiting diet, support this hypothesis.

Selenomethionine originating both from the diet and from degraded tissue proteins is apparently metabolized to yield selenocysteine (148) and can be degraded to release selenide. Selenide may be released from the metabolism of selenomethionine via the methionine transamination pathway (158) proceeding through a methylselenol intermediate; however, as the equilibrium of the methionine-α-keto-γ-methiolbutyrate transamination step strongly favors the amino acid, it is more likely that selenide release occurs as the result of the metabolism of selenomethionine via the pathway for methionine transsulfuration to cysteine. Selenocysteine is produced directly by this route and indirectly by the reduction of selenocysteine; it is presumed that this can occur in a fashion analogous to the reduction of cystine, both nonenzymically via reduction by GSH and enzymically via a thioltransferase and GSH (3). Schwarz (148) suggested that selenocysteine is metabolized by cystathionase, which has cysteine desulfhydrase activity, and that this results in the release of selenite, which ultimately is reductively metabolized to selenide.

The gaps in the present understanding of the metabolism of the selenoamino acids have been bridged, perhaps naively, by assuming that they are metabolized by the pathways for their S-containing analogues. Research is needed to determine the pathways of degrading selenoamino acids in order to determine the extent to which these assumptions have been valid. A better knowledge of these metabolc processes will increase our understanding of the factors that affect the utilization of dietary Se, particularly the organic Se components normally found in foods and feedstuffs.

ROLE OF SELENIUM IN GLUTATHIONE PEROXIDASE

The only known biochemical function of Se in animals is as an essential constituent of glutathione peroxidase (GSHpx) (glutathione: H_2O_2 oxidoreductase, EC 1.11.1.9). This enzyme, present in the cytosol and mitochondrial matrix space, was described in 1957 by Mills (109), who found that it protects erythrocytes from hemoglobin oxidation and hemolysis induced in vitro by H_2O_2 or ascorbic acid. Subsequent work showed that GSHpx utilized reducing equivalents from GSH in the reduction of H_2O_2 (110), lipid hydroperoxides (95), or sterol hydroperoxides (94) according to the following general reaction:

$$\text{ROOH} + 2\,\text{GSH} \xrightarrow{\text{GSHpx}} \text{ROH} + H_2O + \text{GSSG}.$$

In 1972, studies by Rotruck et al (139) of the effects of dietary Se on oxidative damage to rat erythrocytes led to the discovery of erythrocyte GSHpx Se-dependent, which contains Se as an essential constituent (140).

Glutathione peroxidase has been purified from several tissues of a number of species. The molecular weight of the enzyme, determined by sedimentation equilibrium, has been found to be 83,800 ± 1,200 for bovine erthrocytes (39), 76,000 ± 1,000 for rat liver (113), and 95,000 ± 3,000 for human erythrocytes (1). Estimates of molecular weight of the enzyme made by gel filtration range from 77,000 to 96,600. The isoelectric points of erythrocyte GSHpx from cow, sheep, and human have been found to be 5.6–6.0 (41), 6.0–6.5 (159), and 4.9 (1) respectively. The pH optimum of the enzyme has been reported as 8.8 from bovine erthyrocytes (41), 8.5 from human erythrocytes (1), and 8.8–9.1 from rat lung (21). Studies indicate that regardless of source, GSHpx is an homologous tetramer. Oh et al (117) found that the enzyme purified from ovine erythrocytes contained 3.8 g-atoms Se/mol. This finding was confirmed by Flohe et al (41), who determined a stoichiometry of 4.04 for the enzyme from that tissue. Subsequent studies have found stoichiometries near 4 for GSHpx from rat liver (113), human erythrocytes (1), and human placenta (2). Sunde et al (159) reported much lower values for GSHpx preparations from sheep erythrocytes and liver; however, they suggested that this may have been due to the known lability of Se in aqueous preparations of the enzyme.

Purified GSHpx does not contain heme or flavin moieties (43, 113) or metals other than Se (41). The amino acid composition of the rat liver enzyme was determined by Nakamura et al (113), who found it contained 153 amino acid residues per subunit, including 2 cysteinyl, 3 methionyl, and 13 aromatic amino acid residues. The amino acid composition of the slightly larger (182 amino acid residues per monomer) enzyme from bovine erythrocytes has been found to be very similar (83).

Bovine erythrocyte GSHpx has been crystallized; Ladenstein and colleagues (82–85) have determined its three-dimensional structure at 2.8 Å resolution. Their studies show the native enzyme as a tetramer composed of a nearly planar arrangement of two dimers measuring 90.4 Å × 109.5 Å × 58.6 Å. Each dimer is composed of two nearly spherical and apparently identical subunits of 18.7 Å radius, each of which contains one Se atom. The four Se atoms in the tetramer are located on its surface, with a separation of 21 Å between adjacent Se atoms within a dimer. This separation suggests that only one Se atom is present per active site. Each subunit appears to consist of a central core of two parallel and two antiparallel chains in pleated sheet configurations, surrounded by four α-helixes. Each Se atom is located at the end of a long α-helix in a region surrounded by aromatic amino acids. The region around the active site forms a flat depression on the surface of the subunit, which makes the active site accessible to substrates. Electron density mapping indicates that Se is present in the enzyme as selenocysteine or a derivative, which confirms the results of chemical analyses of the enzyme.

The Se-dependent GSHpx has a marked specificity for GSH as the donor substrate (39, 110) but has much less specificity for acceptor substrates. The enzyme will reduce several free fatty acid hydroperoxides, t-butyl hydroperoxide and cumene hydroperoxide, at rates (i.e. V_{max} values) comparable to that of H_2O_2 (23, 63, 95); however, it will not reduce esterified fatty acid hydroperoxides (97), which would be expected to be present in biological membranes. In addition, GSHpx will reduce hydroperoxides of some steroids (94), of thymine and nucleic acids (23), and of prostaglandins (8, 17, 115, 169). Because GSHpx inhibits vitamin K–mediated carboxylation of prothrombin precursors, it has been suggested that it can also reduce vitamin K–hydroperoxide (86).

Understanding the mechanism of action of GSHpx has been complicated because various forms of the enzyme may exist depending upon the conditions of isolation and study. Ganther & Kraus (55) have provided evidence for at least three forms of Se-dependent GSHpx that are distinguished on the basis of their sensitivities to inhibitors and their binding of GSH. One form, R, is fully reduced and is prepared by treatment of the freshly purified enzyme with an excess of GSH. The R form is inhibited by iodoacetate, which apparently involves the formation of a carboxymethyl derivative of the selenocysteine at the active site and is insensitive to inhibition by cyanide. A second form, A, is fully oxidized. It is produced by oxidation of the R form with a large excess of H_2O_2. In contrast to R, the A form is insensitive to iodoacetamide and is inhibited only very slowly by cyanide. A third form, C, is produced by partial reduction by GSH under conditions that permit the spontaneous oxidation of the enzyme, e.g. dialysis in the presence of air. The C form binds GSH and is insensitive to iodoacetamide, but it is extremely sensitive to cyanide.

The existence of various forms of Se-dependent GSHpx gives an indication of its catalytic mechanism. Ganther (49) proposed a model of its mechanism that involves the enzyme cycling between the three forms during catalysis. The first cycle involves the cycling between the most reduced form, R; a selenol form, RSeH; and a selenenic acid form, RSeOH. This part of the model is consistent with x-ray photoelectron spectroscopic studies (21, 172) and x-ray crystallographic studies (85). The model includes a second cycle of the enzyme between the selenenic acid (RSeOH) form and a seleninic acid form, RSeOOH, the latter form being the fully oxidized form A. Sunde & Hoekstra (162) extended this two-cycle model to include four successive reductions by GSH in the conversion of the most oxidized form of the enzyme (the seleninic acid form A) to the iodoacetamide-sensitive selenol form (form R) that proceed through two intermediate forms with cyanide-sensitive Se–S bonds. Although their model proposes the reaction of the enzyme with 2 mol of hydroperoxide acceptor substrate per 4 mol of GSH, the relatively high physiologic concentrations of GSH are expected to maintain the enzyme in the more reduced forms. Therefore, under physiological conditions, the enzyme is thought to function via selenol-selenenic acid cycling as proposed by Ganther et al (53). This mechanism is consistent with the studies of Flohe and colleagues (38, 42, 63) of the kinetics of GSHpx. They describe the reaction as a ter uni ping pong mechanism in which the enzyme is not saturable by either substrate and shows similar V_{max} values for most hydroperoxide acceptor substrates.

Glutathione peroxidase can be assayed either by direct measurement of the decrease in GSH (110) or by the use of exogenous glutathione reductase to couple the redox cycling of GSH and GSSG to the oxidation of NADPH (123). Because the former method is somewhat cumbersome and lacks the sensitivity of a kinetic type of assay, the latter method has been used more frequently in recent years. The assay is conducted with a defined initial concentration of GSH and a concentration of peroxide substrate well in excess of the apparent K_m for the latter. (The apparent K_m values for each substrate are independent of the concentration of the other substrate.) In samples that may contain catalase (e.g. tissue extracts), that activity is inhibited by the use of sodium azide rather than cyanide, as the latter inhibits GSHpx (134). Stable hydroperoxides (e.g. H_2O_2, *t*-butyl hydroperoxide, cumene hydroperoxide) can be used as acceptor substrates. Because the nonenzymic reaction of H_2O_2 with GSH is faster than that of the other hydroperoxides listed, use of *t*-butyl hydroperoxide or cumene hydroperoxide generally results in lower blank values and has been found preferable for that reason. However, the glutathione-S-transferases also have GSHpx activity when assayed using these substrates (87, 135). Although the peroxidase activity of these enzymes apparently is not physiologically important, there is a 10- to 30-fold difference in the apparent K_m values (63, 89, 135). However, assays using the organic hydroperoxide substrates may give positively biased results when samples contain significant amounts of these transfereses.

Because hemoglobin also catalyzes the oxidation of GSH by H_2O_2 (123), samples such as whole blood or erythrocyte lysates can also give spurious results. This problem may be averted by converting hemoglobin to methemoglobin before assaying for GSHpx; however, this technique does not eliminate the problem, which appears to be particularly acute in the case of human hemoglobin. It is therefore recommended that erythrocytes and whole blood be avoided as means of assessing nutritional Se status by GSHpx activity. Recent work by Levander et al (93) indicates the platelets may be a useful biopsy material; they are rich in GSHpx, do not contain GSH-S-transferases, and respond to changes in Se nutriture in a time frame corresponding to that of liver. Plasma is another material of choice for assessing Se status; because it appears to be of hepatic origin and changes rapidly with changes in dietary Se level, its value is greatest in assessing short-term (hours to days) responses to dietary Se in living animals.

The tissue distribution and individual tissue levels of Se-dependent GSHpx vary between animal species and in response to the Se nutriture of the individual. For example, under normal conditions of adequate Se intake, the greatest specific activities of this enzyme are found in the liver in the rat (88, 90) and chick (122); the lamb (118, 119) and guinea pig (13) have very low hepatic activities. Dietary deficiencies of Se (i.e. concentrations of less than approximately 0.10 ppm for most species) are associated with rapid decreases in GSHpx activity in plasma (114) followed by slower decreases in liver and other tissues (93, 114). Numerous investigators have demonstrated that Se supplementation of deficient animals is effective in restoring GSHpx activities to normal levels.

In general, GSHpx activity correlates well with total Se in whole blood or plasma of individuals with marginal or deficient rates of Se intake (107, 165). However, these two parameters do not correlate in individuals with adequate or better levels of Se intake (144). Although tissue activities of Se-dependent GSHpx vary directly with the level of Se added at low levels to low-Se diets, the activities in most tissues plateau at dietary concentrations of about 0.10 ppm of Se even though tissue Se content continues to rise at dietary levels above that level (64, 118, 119). The disparity between the activity of Se-dependent GSHpx and the content of Se in tissues in animals fed diets containing adequate amounts of Se appears to be the result of nonspecific incorporation of selenoamino acids into tissue proteins, which becomes more quantitatively significant with increasing Se intake.

METABOLIC FUNCTION OF GLUTATHIONE PEROXIDASE

Selenium-dependent GSHpx functions as part of a multicomponent antioxidant defense system within the cell. This system serves to protect unsaturated phospholipids and/or critical proteins from the adverse effects of reactive oxygen and free radical initiators of the oxygen (22). The deleterious reactions against which this system protects commence with the production of superoxide ($O_2^{\cdot -}$) by the univalent reduction of O_2 both enzymically and nonenzymically during metabolism. The disproportionation of $O_2^{\cdot -}$ is catalyzed by cytosolic and mitochondrial superoxide dismutases which return half of the O_2 and reduce the balance to H_2O_2 (45). Both $O_2^{\cdot -}$ and H_2O_2 can promote the peroxidation of the polyunsaturated phospholipids that make up biological membranes; however, a more important initiator of lipid peroxidation in the cell may be the hydroxyl radical ($\dot{O}H$), produced by the reaction of $O_2^{\cdot -}$ and H_2O_2 in the presence of trivalent iron according to the Haber-Weiss reaction (106). The formation of $\dot{O}H$ is prevented by reducing the concentrations of the reacting species $O_2^{\cdot -}$ (via superoxide dismutases) and H_2O_2 (via GSHpx). Another enzyme, catalase, can also reduce H_2O_2, but in most cells (the mammalian erythrocyte being a notable exception), catalase and GSHpx are separated by subcellular compartmentalization. Catalase is confined almost exclusively to the peroxisomes, while GSHpx is found in the cytosol and mitochondrial matrix space, so there is little competition of these enzymes for metabolically produced H_2O_2 (40). Therefore, glutathione peroxidase has central importance in protecting against the pro-oxidative consequences of metabolic activation of oxygen via the reduction of H_2O_2 in the cell. This funcion is thought to be the basis of the protective effect of Se against the toxicity of oxidant compounds (e.g. nitrofurantoin, paraquat), the metabolism of which yields $O_2^{\cdot -}$ (27, 131, 132).

The function of GSHpx is complementary to that of vitamin E. Vitamin E (i.e. α-tocopherol) is lipophilic and is located strictly within membranes; it is a good free radical scavenger and serves to remove reactive oxygen species as well as free radicals produced by lipid peroxidation. GSHpx can also reduce fatty acid hydroperoxides and might therefore be expected to interrupt the autocatalytic propagation of lipid peroxidation. However, the fact that the enzyme cannot use esterified fatty acid hydroperoxides (97) as substrate suggests that this may not be a normal metabolic function of GSHpx unless peroxidation is accompanied by de-esterification of fatty acids from membrane phospholipids. The normal function of GSHpx seems to be the reduction of H_2O_2 for the prevention of $\dot{O}H$ generation. Thus, Se-dependent GSHpx and vitamin E act in complementary fashion to block free radical formation and subsequent attack of membrane phospholipids and critical proteins, while interruption of lipid peroxidation autocatalysis is effected by vitamin E alone.

NUTRITIONAL ASPECTS OF SELENIUM

Impairment of cellular antioxidant protection caused by dietary deficiencies of Se and/or vitamin E can result in a multitude of disorders in animals. These include dysfunctions of the brain, cardiovascular system, liver, muscles, and fetus (28). In most Se deficiency diseases, the amount of the mineral required for normal function is inversely related to the dietary level of vitamin E; the two nutrients have mutually sparing effects upon the dietary requirements for both. The single exception to this phenomenon is the rather specific need by the chick for very low dietary levels of Se (less than 0.05 ppm) to prevent the atrophy and subsequent periacinar fibrosis of the exocrine pancreas (164). This disorder, until recently thought to be the only pathological condition resulting from Se deficiency uncomplicated by deficiencies of vitamin E or other nutrients, is now known to be prevented by dietary levels of vitamin E or synthetic antioxidants in excess of 300–500 ppm (174). Thus, all of the known Se deficiency diseases of animals also respond to vitamin E at some level. This close nutritional interrelationship of Se and vitamin E appears to be explained adequately on the basis of their known roles in antioxidant protection of cells of different organ systems in the body.

The roles of Se in certain diseases of humans are less clear than those established in experimental and agricultural animals. Epidemiological evidence has been cited that links certain types of cancers with low Se status (76, 143, 145, 152), and a growing body of experimental evidence indicates that Se, at least at supranutritional levels, can inhibit carcinogenesis in some experimental animal models (24, 61, 62, 74, 75, 96, 108, 111, 146, 171), which suggests that Se may play some role in the etiology of some human cancers. Ecological comparisons have also suggested a link between low Se status and increased incidence of cardiovascular disease (153). Levander (108) has proposed that Se-dependent GSHpx may be important in the regulation in platelets of balance of two arachidonic acid metabolites—the proaggregatory and vasoconstrictive thromboxane, and the antiaggregatory and vasodilatory prostacyclin—which implicates Se as a factor in cardiovascular diseases. According to this hypothesis, GSHpx would act by metabolizing arachidonic acid hydroperoxide (produced by the enzyme lipoxygenase), which can otherwise inhibit prostacyclin synthetase. This hypothesis is supported by the findings that platelets, otherwise rich sources of GSHpx, show severely diminished activities of the enzyme in Se-deficiency and by the findings of altered metabolism of arachidonic acid metabolites in Se-deficient rats (7) and in Se-depleted humans (8).

Studies over the past decade in the People's Republic of China have identified an endemic cardiomyopathy associated with severe Se deficiency (19, 20, 79). This disease, named for Keshan county in northeastern China where it was first described, affects post-weaning infants, children, and women of childbearing age among the rural population in a large area of endemic Se deficiency that stretches in a long belt from northeast to south central China. There are significant annual and seasonal variations in incidence of the disease. Occasionally, it affects as much as 11% of the population in the target age groups and the case-fatality rate is as high as 80%. Other potentiating factors have been proposed in the etiology of Keshan Disease, but the endemic nature of the disease appears to be because of severe nutritional Se deficiency. Keshan Disease patients have been found to be of extremely low Se status (e.g. blood Se, 0.017 ± 0.002 μg/ml) as compared to healthy persons from unaffected areas of China (e.g. blood Se, 0.136 ± 0.048 μg/ml). Residents of these Se-deficient areas have blood Se levels lower than those of people anywhere else in the world—approximately one half of the levels found in New Zealand and Finland, and one fifth or less of the level found in North America. In several large-scale intervention studies (79), prophylaxis with orally administered Na_2SeO_3 has been found to be extremely effective in reducing, but not completely eliminating, Keshan Disease. Although the exact role of Se in the etiology of Keshan Disease is not established, examination of diseased heart muscles have suggested a lesion in mitochondrial function. It is not known whether such lesions are a manifestation of chronic GSHpx deficiency of the heart muscle.

Burk (11) recently reviewed the effects of Se that appear to result from metabolic activities unrelated to GSHpx. These include alterations in the metabolism of glutathione and of certain xenobiotic agents. The metabolic bases for these effects are presently unknown.

Two selenoproteins of unknown function have been reported in animals. The first is a small (10,000-dalton) protein that appears in muscle tissue from Se-adequate lambs but is absent from muscle of Se-deficient lambs with nutritional muscular dystrophy (5, 130, 173). The second is a selenoprotein of mol wt 15,000–20,000 found in bovine and rat sperm (15, 98, 124, 178). These and other Se-binding proteins that have been observed but not characterized (18, 66, 112, 136) offer researchers the possibility of elucidating additional biochemical functions of this essential element.

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