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SOME SELENIUM-DEPENDENT BIOCHEMICAL PROCESSES

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I. Introduction

Selenium is located between sulfur and tellurium in the Periodic Table and has been classified both as a metal and a nonmetal. It resembles sulfur in many of its chemical properties and exists in the same valency states, namely, −2, 0, +2, +4, and +6. In contrast to the sulfur oxides the most stable oxide of selenium is SeO_2 rather than SeO_3. Selenium dioxide is a solid that readily reacts with water to form H_2SeO_3 and is easily reduced to red elemental selenium (atomic weight 78.96). There are a number of naturally occurring stable isotopes and one of these, ^{77}Se, which has a nuclear spin of $\frac{1}{2}$, is suitable for nuclear magnetic resonance spectrometry. Although the natural abundance of this isotope is low (about 7.5%) ^{77}Se enriched to about 95% abundance is available, which should prove to be useful for labeling selenoproteins, especially those synthesized by microorganisms. A radioactive isotope of selenium, ^{75}Se, which is a

gamma emitter with a half-life of 122 days, is employed extensively in studies on selenium metabolism in bacteria and animals.

Selenium is used in industry for the hardening of steel, in the production of rectifiers for conversion of alternating to direct current, as a catalyst in many photochemical processes (e.g., as a component of the photosensitive plate of the Xerox machine) and recently as an additive to animal feeds. Copper refinery slimes and dust left after extraction of copper from ore are major sources of selenium. In soil and water selenium occurs both as selenites and selenates.

II. Some General Aspects of Selenium Nutrition

Selenium, the element once considered to be of biological importance solely because of its toxic properties, is now generally recognized as an essential micronutrient for mammals, birds, and several bacteria. It is believed that fish and many other animal species probably also depend on selenium. Whether it is an essential element for higher plants is debatable at present.

A variety of disease syndromes of domestic and laboratory animals were eventually proved to be deficiency syndromes that could be prevented, or sometimes cured, by the administration of small amounts of selenium. Among these diseases are a nutritional type of muscular dystrophy known as white muscle disease that frequently afflicts young lambs and calves (1–3), exudative diathesis of chicks and turkeys (4,5), and necrotic liver degeneration in rats (6,7) and swine (1). The responsiveness of the last two syndromes to dietary selenium provided the first experimental evidence in support of a nutritional requirement for this element (4,6). The high incidence of selenium deficiency diseases among domestic animals in parts of New Zealand (1), Oregon (2), and in many areas of high rainfall where the available selenium in the soil is low is correlated with a concomitant low content of selenium in the forage crops. In contrast, there appear to be no well-documented cases of selenium deficiency in humans, presumably because very few people at present subsist exclusively on foods produced in a single geographical area. For example, cereal grains and meat products from the western interior regions of the United States and Canada generally contribute much more selenium than do the corresponding products from eastern United States. Also, consumption of fish and other marine products

can compensate for the low selenium content of water and local foods in many deficient areas. In spite of this lack of a directly demonstrable human dietary requirement for selenium the fact that we, in common with other mammals, possess at least one important selenium-dependent enzyme, glutathione peroxidase, is indicative of our need of this trace element. Based on analyses of the selenium content of foodstuffs and drinking water, the average daily intake for humans in many parts of the world has been calculated. From this range of values and, also, by extrapolation from experimentally determined animal requirements, a daily dietary requirement of about 0.75 μequivalent (60 μg Se) for the adult human is indicated (8). Clearly this is only an approximation, because in all cases the total selenium content is measured and at present neither the chemical form nor the relative availability of this element in most natural products is known. The average North American diet supplies a total of 1–3 μequivalents of selenium per day (8). This amount appears to be adequate to prevent any deficiency syndromes and is significantly below the estimated toxic range of 10–30 μequivalents/day (0.8–2.4 mg/day) for adult humans.

A report (9) that selenium could partially replace fetal calf serum in the medium used for culture of erythroid cells and granulocyte/macrophage cells from mouse bone marrow is interesting both from the standpoint of mammalian cell nutrition and for economic considerations.

The selenium requirement of several bacteria that synthesize selenium-dependent enzymes, for example, formate dehydrogenase or glycine reductase, is in the micromolar range. *Clostridium sticklandii*, for example, is maximally stimulated to synthesize both of these enzymes when the standard culture medium is supplemented with 0.5–1 μM selenite. The rich basal medium, which contains formate plus complex mixtures of amino acids and peptides, supports luxuriant growth of the organism, but its selenium content is adequate for the production of only very low levels of formate dehydrogenase and glycine reductase. At present it is not known whether these are the only selenoenzymes produced by *C. sticklandii* or if the traces of selenium normally supplied in the medium are used preferentially for the synthesis of one or more indispensable selenoenzymes. Data from a number of cultures grown in the presence of $Na_2{}^{75}SeO_3$ (1 μM) show that 30–40% of the selenium of the medium is incorpo-

rated into the cell mass, which averages about 600 mg (dry weight) from a liter of medium. Of this selenium that is fixed in the cells, about 30% is present in a single protein, the 12,000-dalton glycine reductase selenoprotein. The extent to which formate dehydrogenase accounts for the remaining 60–70% is not yet known.

For *Escherichia coli* and various anaerobic bacteria that synthesize selenium-dependent formate dehydrogenases selenium supplements of 0.1–10 μM are commonly employed. Although the higher levels are seldom inhibitory to growth, they are greatly in excess of the actual requirement unless the levels of other nutrients in the medium are adequate for very high cell yields.

The ability of many bacteria to reduce tellurite is well-known, but selenium cannot be replaced by tellurium, its next higher homolog, for the synthesis of glycine reductase or formate dehydrogenase by *C. sticklandii* or for formate dehydrogenase by *Methanococcus vannielii*. Growth of these bacteria was not noticeably affected by levels of tellurite up to 10 μM. Selenate, as well as selenite, is utilized by *C. sticklandii* and *M. vannielii* as a source of selenium.

Both the early observations on the nutritional importance of selenium and many recent studies point to an intimate biochemical relationship between this element and α-tocopherol or vitamin E. Although the mode of action of α-tocopherol is still unknown, like other vitamins it is required in only minute amounts and therefore clearly must play a specific catalytic role in some type of biochemical process. A cofactor role in a reaction sequence leading to the synthesis of an essential selenoenzyme or coenzyme is an attractive possibility.

III. Selenium Toxicity

The marked toxicity of selenium to animals and plants was recognized long before there was any indication that it might also be an essential trace element. In fact, the pronounced toxic effects of selenium at relatively low concentrations served as a deterrent to its acceptance as a required nutrient and it is only recently that its use as an animal food additive has been authorized by the United States Food and Drug Administration.

"Alkali disease" and "blind staggers" two commonly known diseases of grazing animals were shown to be due to selenium poisoning (10). Animals afflicted with alkali disease show loss of hair from manes

and tails, grossly misshapen hoofs, stiffness of joints, and lack of vitality. These symptoms are caused by the intake of high, but sublethal, amounts of selenium that accumulate in the hair and hoof keratins and in liver, kidney, and various other organs of the body. Organic forms of selenium present in the proteins of cereal grains, hay, and grasses grown in soils high in selenium are considered to be the predominant cause of this type of selenium poisoning. In contrast, blind staggers, a syndrome of acute selenium poisoning, is often exhibited by grazing animals that ingest lethal amounts of selenium contained in certain species of plants known as selenium-accumulator plants. The animals, after eating such plants, walk around aimlessly in circles, froth at the mouth, clearly are in great pain, and may die of respiratory failure within 24 hours. Among the selenium compounds that have been identified in accumulator plants of the genus *Astragalus* and *Stanleya* are Se-methylselenocysteine, γ-glutamyl peptides of Se-methylselenocysteine (11,12), selenocystathionine (13), and volatile compounds such as dimethylselenide and dimethyldiselenide (14). These low-molecular-weight, soluble, organoselenium compounds appear to be detoxification products synthesized by the accumulator plants as a means of preventing the indiscriminate incorporation of selenium instead of sulfur into their proteins and other essential cell constituents. This device enables the selenium-accumulator plants to grow and thrive in seleniferous soils that are toxic to ordinary plants. The total selenium content of some accumulator plants may be as high as 2 or occasionally even 15 g per kilogram of dry plant tissue (11). Although the above-mentioned compounds accumulated by these plants appear to be a more common cause of acute selenium poisoning in range animals and thus may be more toxic per se, the considerable variation in the relative toxicity values reported for several organic and inorganic forms of selenium indicates the additional involvement of many other factors.

Numerous studies have been performed (10,15,16) on the ameliorative effects of arsenite on selenium poisoning and of selenium on arsenic, mercury, lead, cadmium and silver toxicity, but it is not at all clear how these effects are exerted. The greater affinity of selenols as compared to sulfhydryl compounds for mercury and methyl mercury may account for the protective effect of selenium against mercury toxicity that is observed in some instances (17).

For some laboratory animals a "minimal fatal dose" of selenium

has been defined as the amount, administered in the form of sodium selenite, that would cause death, usually within 24 hr. For rats, this dose is 3.5 mg per kilogram of body weight (18) and for mice 6 mg per kilogram of body weight (19). For 60–70 adult humans these values of a lethal dose extrapolate to 200–400 mg as selenium or approximately 0.5–1 g as sodium selenite.

IV. Biological Reactions that Occur with Both Sulfur and Selenium Isologs

A. ENZYME-CATALYZED INCORPORATION OF SELENIUM INSTEAD OF SULFUR IN NORMAL CELL CONSTITUENTS

In an earlier review on selenium biochemistry (20) it was pointed out that a number of enzymes that normally catalyze reactions resulting in the transformation of sulfur compounds also react with the corresponding selenium analogs. Since then additional examples of nonspecific enzymes of this type have been documented. In some instances a particular enzyme is known to be equally reactive with the selenium analog and with the normal substrate provided the two are compared at equivalent concentrations, but such an enzyme should not be capable of producing appreciable amounts of the selenium metabolite *in vivo* unless high or toxic levels of selenium are present. Otherwise, at normal sulfur to selenium ratios, the relative number of selenium substrate molecules transformed would be very small. Hence, although the lack of specificity of enzymes involved in sulfur metabolism may play a key role in selenium toxicity, different enzymes that selectively react with selenium compounds at extremely low concentrations must serve as the catalysts in normal essential selenium metabolism. In so far as selenium toxicity is concerned, neither the biological effects of the selenium product of a particular nonspecific enzyme nor the combined effects of several such substitution reactions are known in detail. However, it is reasonable to suppose that the indiscriminate substitution of selenium for sulfur in biopolymers such as proteins, nucleic acids, and derivatives of complex carbohydrates could have marked deleterious effects on the organism in which this occurs.

Table I is a partial list of enzyme-catalyzed reactions in which a selenium compound can substitute to some degree for the normal

TABLE I
Some Enzymes that Utilize Both S and Se Compounds as Substrates

Enzyme	Reaction catalyzed (or reaction product)
ATP sulfurylase	ATP + SO_4^{2-} ⇆ adenosine-5′-sulfatophosphate + PP_i
Cysteinyl-tRNA synthetase	Cysteine + ATP + $tRNA^{cys}$ → cys-$tRNA^{cys}$ + AMP + PP_i
tRNA sulfur transferase	Cysteine + ATP + tRNA → (4-thiouracil)-tRNA
Methionyl-tRNA synthetase	Methionine + ATP + $tRNA^{met}$ → methionyl-tRNA + AMP + PP_i
Amino acid polymerase	Methionyl-tRNA + protein synthesis system → polypeptide-methionine
S-Adenosylmethionine synthetase	ATP + methionine → S-adenosylmethionine + PPP_i
S-Adenosylmethionine methyl transferase	S-Adenosylmethionine + acceptor → S-adenosyl-homocysteine + methylated product

substrate. These include enzymes that react with inorganic forms of sulfur and those concerned with the metabolism of sulfur amino acids. Additional examples of selenium substitution for sulfur by plants and microorganisms can be found in a chapter by Shrift in *Organic Selenium Compounds: Their Chemistry and Biology* edited by Klayman and Günther (21).

Studies on the ATP sulfurylases from yeast (22,23), spinach (24), and both selenium-accumulator and nonaccumulator species of *Astragalus* (25) indicate that the forms of the enzyme from these sources are similar. All catalyze both a sulfate- and a selenate-dependent reversible release of inorganic pyrophosphate from ATP, from which it is concluded that the enzyme is capable of forming adenosine-5′-selenophosphate, as well as adenosine-5′-sulfatophosphate. Moreover, with purified *Saccharomyces cerevisiae* ATP sulfurylase it has been possible to demonstrate directly the formation of an acid-labile, ^{75}Se-labeled product from ATP and $^{75}SeO_4^{2-}$ (23). Although the instability of the compound precluded extensive characterization, its properties were consistent with those expected of adenosine-5′-selenophosphate. Release of elemental selenium from this product in the presence of reduced glutathione presumably involved thiolytic cleavage of the selenium anhydride to form a thioselenic acid and subsequent reduction of the latter to elemental selenium. A scheme pos-

tulated (23) to account for this transformation involves the intermediate formation of a "selenotrisulfide," diglutathione selenide (GSSeSG), which in the presence of excess reduced glutathione yields H_2Se. In the absence of strictly anaerobic conditions the latter is reoxidized to Se^0. Alternatively, in the absence of oxygen, GSSeSG decomposes in alkali to red elemental selenium and GSSG. Either the nonenzymic reactions or a coupled enzyme-catalyzed process in which GSSeSG is reduced by NADPH and glutathione reductase (26,27) to H_2Se an account for the overall reduction of selenate after its activation by ATP sulfurylase. Whether these reactions do in fact account for the entry of selenate and selenite into the normal metabolic pathways of plants and animals is debatable.

In recent studies with cultured tobacco cells (28) it has been shown that susceptibility to selenate toxicity is greater under conditions that cause the apparent derepression of ATP sulfurylase than under conditions where the *in vivo* activity of the enzyme is low. This result supports the hypothesis that the sulfur pathway is used for selenate activation under toxic conditions. Reuveny observed that repression of the sulfurylase by sulfate was overcome by subtoxic levels of selenate added at only one-tenth the concentration of sulfate and suggested that this might be due to the formation of a selenium analog of the repressor sulfur compound and the mutual antagonism of their effects (28). These studies provide an example of how interference of the normal regulation of the level or activity of a sulfur pathway enzyme by selenium could be an important factor in selenium toxicity.

Little is known at present regarding the specific effects of the replacement of sulfur amino acids in proteins by their selenium analogs. Clearly, substitution of a selenocysteine or a selenomethionine residue for its sulfur analog at the catalytic site of a key enzyme or at positions important for the maintenance of the tertiary structure of a protein could be of much greater significance than if such replacements occurred in less-critical regions.

The β-galactosidase of *E. coli* provides an interesting example of an enzyme that can tolerate extensive replacement of methionine by selenomethionine (29,30). Replacement of 70–75% of the methionine residues by the selenium analog, when synthesis was induced in the presence of high levels of selenomethionine, did not result in significant alteration of the catalytic activity of the enzyme. Such indiscriminate insertion of selenomethionine into proteins of *E. coli* and also

presence of high levels of selenomethionine, did not result in significant alteration of the catalytic activity of the enzyme. Such indiscriminate insertion of selenomethionine into proteins of *E. coli* and also in the rat can be explained partially by the lack of specificity both of the methionyl-tRNA synthetase and the amino acid polymerase from these sources (Table I). On the other hand, evidence for the nonspecific occurrence of selenocysteine instead of cysteine in proteins, although reported both in plants and animals, is not convincingly documented chemically. It has been shown that $tRNA^{cys}$ can be charged with selenocysteine by *E. coli* cysteinyl-tRNA synthetase (31), although the sulfur amino acid is the preferred substrate. Also, selenocysteine was used as substrate by a 200-fold purified L-cysteinyl-tRNA synthetase from *Phaseolus aureus* (32). However, direct demonstration of the utilization of the selenocysteine-charged $tRNA^{cys}$ by an amino acid polymerase with resultant incorporation of the analog into a polypeptide appears to be lacking.

Selenium can also be incorporated enzymically into the aminoacyl formed by modification of a uracil residue after the polymer is synthesized (33,34). The sulfur is derived from cysteine and is transferred in an ATP-dependent reaction by tRNA sulfur transferase. Selenocysteine can replace cysteine in this reaction with the result that a tRNA containing a 4-selenouracil is formed (35). Since activity of the enzyme with the two amino acid substrates is similar, appreciable amounts of 4-selenouracil should be introduced into the tRNA when selenocysteine and cysteine levels are comparable. *In vivo* labeling of tRNA with ^{75}Se occurred when *E. coli* was grown in a medium containing magnesium sulfate (0.8 m*M*) as the sulfur source and 0.04 μM [^{75}Se]selenite (36). The extent to which selenium was incorporated into tRNA under these conditions was not reported, but one of the ^{75}Se-labeled nucleosides isolated from the enzymic digests of the radioactive tRNA cochromatographed with authentic 4-selenouridine.

The selenium analog of the biologically important methyl donor, S-adenosylmethionine, has been prepared enzymically and, in this case, selenomethionine is a slightly better substrate than methionine for S-adenosylmethionine synthetase (37). Also, the methyl group from Se-adenosylselenomethionine was transferred to guanidinoacetic acid by hog liver S-adenosylmethionine methyl transferase and creatine was formed. However, at present there is little information

concerning possible deleterious effects *in vivo* of significant levels of Se-adenosylmethionine and its demethylated product, Se-adenosylhomocysteine.

B. BIOLOGICAL ACTIVITY OF CHEMICALLY SYNTHESIZED SELENIUM ANALOGS OF SOME SULFUR-CONTAINING COENZYMES, POLYPEPTIDES, AND NATURAL PRODUCTS

Stimulated by a growing interest in the biochemical aspects of selenium chemistry, organic chemists have prepared selenium analogs of several naturally occurring sulfur compounds and have examined their biological effects. A few of these analogs are given in Table II and are discussed below. Numerous other selenium analogs of naturally occurring compounds in which oxygen or methylene groups are replaced by selenium also have been prepared and studied (20,-21,38,39).

Substitution of selenium for sulfur in the iron–sulfur centers of the 2Fe–2S ferredoxins from parsley, adrenal cortex (adrenodoxin), and *Pseudomonas putida* (putidaredoxin) has been reported (40–42). Homologs of the naturally occurring proteins in which all or part of the sulfur was replaced with ^{77}Se or ^{80}Se were biologically active. Information concerning the geometry of the Fe_2Se_2 and Fe_2SSe centers was provided by electron paramagnetic resonance spectroscopy of the proteins derivatized with ^{77}Se. The peptide hormone oxytocin, which contains two half cystine residues in a disulfide bridge, has been synthesized both in its normal and selenium analog forms (38,43). Derivatives in which selenocysteine replaced one or both of

TABLE II
Synthetic Selenium Analogs of Some Natural Products

Protein, coenzyme, or natural product	Substitution
Ferredoxins	Se for S in Fe_2S_2 centers
Oxytocin	Selenocysteine for one or both cysteine residues
tRNA	Se for S in 4-thiouridine moiety
Coenzyme A	Selenocysteamine for cysteamine moiety
Biotin	Se for S in ring
Nicotinamide-adenine dinucleotide phosphate (NADP)	Se or S for O in amide group of micotinamide

the cysteine residues exhibited biological activities characteristic of oxytocin. Conformational changes induced by these substitutions were extensively investigated by a number of techniques, including optical rotatory dispersion spectroscopy, circular dichroism spectroscopy, and nuclear magnetic resonance spectroscopy (38). A chemical procedure developed for elimination of sulfur from the 4-thiouridine residues of transfer ribonucleic acids (tRNA) by treatment with cyanogen bromide and its replacement with selenium has proved to be a practical method for the production of significant amounts of seleno-tRNA (44). It will be of particular interest to evaluate the biological effects of this selenium analog of tRNA.

Selenium analogs of two sulfur-containing coenzymes, coenzyme A (45) and biotin (46), also have been prepared. Selenocoenzyme A, if maintained in its reduced form by treatment with dithiothreitol, can be acetylated enzymically (47), and the selenium analog of its pantetheine moiety effectively replaces pantetheine as a growth factor for *Lactobacillus helveticus* (48). The biotin analog, (+)selenobiotin, functions as a growth factor for several biotin-requiring microorganisms and is used by an *E. coli* biotin auxotroph for synthesis of holo acetylCoA carboxylase (49). The activity of the selenobiotin-containing carboxylase was about 62% that of the normal biotin-containing carboxylase.

One particularly interesting example of a selenocoenzyme analog in which the selenium is substituted for oxygen occurs in the series NADP, S-NADP, Se-NADP (50). In this instance the oxygen of the carbamoyl group of nicotinamide in nicotinamide-adenine dinucleotide phosphate was replaced either with sulfur or with selenium. The coenzyme activity of the three compounds towards a number of type A and type B NADP-linked dehydrogenases decreased in the order NADP > S-NADP > Se-NADP. Unlike NADP(H) and S-NADP(H), the reduced Se-NADP reacted only with the type B NADP-linked dehydrogenases, which suggests that the selenium analog might be a very useful substrate for investigations of the stereospecificity of hydrogen transfer (50).

V. Enzymes and Proteins in Which Selenium is Essential

In contrast to the above-mentioned examples of selenoorganic compounds, which are either toxic or have no marked effect on biological

systems, there are at least three, and possibly four, proteins that are now known to be biologically active only when they *do contain* selenium. Since only trace amounts of selenium are required for optimal synthesis of these selenoproteins, the mechanism by which this essential element is introduced must be highly specific and noncompetitive with its more abundant analog, sulfur. For example, synthesis of the clostridial glycine reductase selenoprotein, which contains a single essential selenocysteine residue in the same polypeptide chain as two sulfur cysteine and three methionine residues, occurs even though the bacteria are cultured in the presence of a several-thousand-fold molar excess of sulfur over selenium (51,52). The mammalian enzyme glutathione peroxidase is comprised of four subunits and contains 4 gram atoms of selenium, presumably one per subunit (53–55). Perhaps it is purely coincidental that, as in the case of the bacterial protein, there are also two cysteine and three methionine residues per subunit of this enzyme (54), but at least this composition further emphasizes that a highly efficient discrimination between selenium and sulfur is obligatory.

A. MAMMALIAN AND AVIAN GLUTATHIONE PEROXIDASE

Several nutritionally related disorders of mammals and birds can now be attributed to their inability to synthesize a specific selenoprotein, glutathione peroxidase. This protein catalyzes the decomposition of peroxides by reaction with reduced glutathione (eq. 1) and is thought to be primarily responsible for the protection of red cell membranes and other tissues from damage due to organic peroxides (56,57).

$$2GSH + H_2O_2 \text{ (or R-OOH)} \rightarrow GSSG + 2H_2O \text{ (or } H_2O + ROH) \qquad (1)$$

It was isolated in crystalline form (58) and many of its catalytic and physical properties were investigated (59,60) before it was known to depend on selenium for its activity. Identification of glutathione peroxidase as a selenoenzyme grew out of investigations by a University of Wisconsin research group on the biochemical basis of the greater sensitivity of membranes of erythrocytes from selenium-deficient rats and sheep to oxidative damage as compared to those from normal animals (61). It was observed that the erythrocytes from normal animals were protected from hemolysis *in vitro* by glucose, whereas those from selenium-deficient animals were not. The deficiency did