

SELENIUM IN HIGHER PLANTS

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■ **Abstract** Plants vary considerably in their physiological response to selenium (Se). Some plant species growing on seleniferous soils are Se tolerant and accumulate very high concentrations of Se (Se accumulators), but most plants are Se nonaccumulators and are Se-sensitive. This review summarizes knowledge of the physiology and biochemistry of both types of plants, particularly with regard to Se uptake and transport, biochemical pathways of assimilation, volatilization and incorporation into proteins, and mechanisms of toxicity and tolerance. Molecular approaches are providing new insights into the role of sulfate transporters and sulfur assimilation enzymes in selenate uptake and metabolism, as well as the question of Se essentiality in plants. Recent advances in our understanding of the plant's ability to metabolize Se into volatile Se forms (phyto volatilization) are discussed, along with the application of phyto remediation for the cleanup of Se contaminated environments.

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INTRODUCTION

Interest in selenium (Se) has escalated in the past two decades. In trace amounts, Se is an essential micronutrient and has important benefits for animal and human nutrition. At high dosages, however, it may be toxic to animals (96, 114, 165) and to humans (162). The concentration range from trace element requirement to lethality is quite narrow; the minimal nutritional level for animals is about 0.05 to 0.10 mg Se kg⁻¹ dry forage feed, while exposure to levels of 2 to 5 mg Se kg⁻¹ dry forage causes toxicity (165, 168). The first report of the nutritional benefit of Se was published in 1957 (138). In 1973, Se was shown to form part of the important antioxidant enzyme, glutathione (GSH) peroxidase (133). Other health benefits include carcinoma suppression (87) and the relief of certain symptoms associated with AIDS (82).

The toxicity of Se has been known for many years (54). However, it was not until the Kesterson Reservoir controversy in the 1980s that scientists, regulators, politicians, and the general public of the United States were made acutely aware of the importance of Se as an environmental contaminant. Selenium present in the waters at the natural wildlife refuge at Kesterson Reservoir, California, was shown to be the agent responsible for mortality, developmental defects, and reproductive failure in migratory aquatic birds (116) and in fish (135). The contamination of the Kesterson Reservoir arose from Se-laden agricultural drainage water that had been allowed to flow into it from neighboring farms. Selenium toxicity is encountered in arid and semiarid regions of the world that have seleniferous, alkaline soils derived from the weathering of seleniferous rocks and shales. In the western United States, the leaching of soluble, oxidized forms of Se, especially selenate, from these seleniferous soils is accelerated by intensive irrigation, with selenate being accumulated in high concentrations in drainage water. Selenium is also released into the environment by various industrial activities; for example, oil refineries and electric utilities generate Se-contaminated aqueous discharges.

The narrow margin between the beneficial and harmful levels of Se has important implications for human health. Plants can play a pivotal role in this respect: For example, plants that accumulate Se may be useful as a "Se-delivery system" (in forage or crops) to supplement the mammalian diet in many areas that are deficient in Se. On the other hand, the abilities of plants to absorb and sequester Se can also be harnessed to manage environmental Se contamination by phytoremediation, whereby green plants are used to remove pollutants from contaminated soil or water (153). One special attribute of plants that has potential benefit for Se phytoremediation is their ability to convert inorganic Se to volatile forms, predominantly dimethylselenide (DMS_e), a process called phytovolatilization.

The chemistry of Se has been reviewed extensively by several authors (e.g. 88, 91). It is of interest to note the following. Selenium was discovered by the Swedish chemist Jakob Berzelius in 1817. Its name comes from the Greek word for moon, selene. It is a group VIA metalloid with an atomic weight of 78.96. Selenium shares many similar chemical properties with sulfur (S), although the

Se atom is slightly larger; the radius of Se^{2+} is 0.5 Å whereas the radius of the S^{2+} is 0.37 Å. Like S, Se can exist in five valence states, selenide (2^-), elemental Se (0), thioselenate (2^+), selenite (4^+), and selenate (6^+) (91). The speciation of Se depends on redox conditions and pH: Selenate tends to be the major species in aerobic and neutral to alkaline environments, whereas selenide and elemental Se dominate in anaerobic environments. Selenium also exists in volatile forms other than DMSe, e.g. dimethyldiselenide (DMDSe), and probably dimethyl selenone, dimethyl selenylsulfide, and methaneselenol (65, 129).

In this review we have focused on the main conceptual developments in Se physiology and biochemistry in plants, including Se uptake and transport, the question of Se essentiality in plants, the assimilation and volatilization of Se, as well as mechanisms of toxicity and tolerance. We have also included sections on phytoremediation and phytovolatilization because of the important implications of these strategies in dealing with environmental Se contamination.

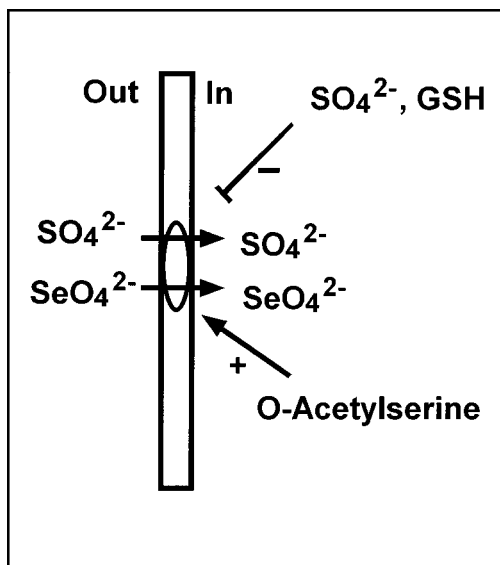
ACCUMULATORS AND NONACCUMULATORS

Plants differ in their ability to accumulate Se in their tissues. Certain native plants are able to hyperaccumulate Se in their shoots when they grow on seleniferous soils. These species are called Se accumulators and include a number of species of *Astragalus*, *Stanleya*, *Morinda*, *Neptunia*, *Oenopsis*, and *Xylorhiza* (29, 84, 132, 156). They can accumulate from hundreds to several thousand milligrams of Se kg^{-1} dry weight in their tissues. On the other hand, most forage and crop plants, as well as grasses, contain less than 25 mg Se kg^{-1} dry weight and do not accumulate Se much above a ceiling of 100 mg Se kg^{-1} dry weight when grown on seleniferous soils. These plants are referred to as Se nonaccumulators (29). Crop plants grown on nonseleniferous soils typically have Se concentrations ranging from 0.01 to 1.0 mg kg^{-1} dry weight (102). On soils containing moderate concentrations of Se, Bureau et al (33) found that tissue Se levels in 17 different crops rarely exceeded 1 mg Se kg^{-1} dry weight, although salt-tolerant species of genera such as *Distichlis* and *Atriplex*, grown at similar soil Se levels, exhibited concentrations of 10 to 20 mg Se kg^{-1} dry weight (83, 120).

Although Se accumulators grow on seleniferous soils, not all plant species on seleniferous soils are Se accumulators: Some plants accumulate only a few milligrams of Se kg^{-1} dry weight. For example, the genus *Astragalus* contains both Se-accumulating species and nonaccumulating species and these can grow next to each other on the same soil (58). Trelease & Beath (156) observed that several species of plants growing on seleniferous soil of the Niobrara Formation had markedly different tissue Se concentrations, e.g. *Astragalus bisulcatus* contained 5530, *Stanleya pinnata* 1190, *Atriplex nuttallii* 300, and grasses 23, mg Se kg^{-1} dry weight.

A third category of plants, known as secondary Se accumulators (29), grow on soils of low-to-medium Se content and accumulate up to 1000 mg Se kg^{-1} dry

Figure 1 Selenate uptake across the root plasma membrane is mediated by the high-affinity sulfate transporter. The expression of the high-affinity sulfate transporter is regulated positively by O-acetylserine, and negatively by sulfate and glutathione. SO_4^{2-} = sulfate; SeO_4^{2-} = selenate; GSH = reduced glutathione.



weight. Examples of plants in this group are species of *Aster*, *Astragalus*, *Atriplex*, *Castilleja*, *Comandra*, *Grayia*, *Grindelia*, *Gutierrezia*, and *Machaeranthera* (120). Most recently, research by Bañuelos and his colleagues has identified the fast-growing *Brassica* species, Indian mustard (*Brassica juncea*) and canola (*B. napus*), as new secondary Se-accumulator plant species with a typical Se concentration of several hundred milligrams of Se kg^{-1} dry weight in their shoot tissues when grown on soils contaminated with moderate levels of Se (11).

UPTAKE AND TRANSPORT

Uptake of Selenate, Selenite, and Organic Se

Selenate is accumulated in plant cells against its likely electrochemical potential gradient through a process of active transport (29). Selenate readily competes with the uptake of sulfate and it has been proposed that both anions are taken up via a sulfate transporter in the root plasma membrane (Figure 1) (1, 5, 27, 39, 94). Selenate uptake in other organisms, including *Escherichia coli* (100) and yeast (38), is also mediated by a sulfate transporter.

Research with yeast enabled the first sulfate transporter genes to be cloned from plants. The approach was to select for resistance to high concentrations of selenate as a means of isolating yeast mutants defective in sulfate transport (27, 38, 146). Using functional complementation, three genes (*SHST1*, *SHST2*, and *SHST3*) encoding the sulfate transporter were isolated from the tropical legume

Stylosanthes hamata (146) and one gene (*HVST1*) was isolated from barley (*Hordeum vulgare*) (145). Sequence analysis predicts that the transporter contains 12 membrane-spanning domains. Furthermore, the transporter genes exhibit a high degree of sequence conservation with other sulfate transporters cloned from animals and microorganisms (44, 146). By using the sequence of these highly conserved regions, homologs of the sulfate transporter gene have now been cloned from many plants including *Arabidopsis* (149, 169), Indian mustard (80), and corn (23). Kinetic and expression studies have indicated that the sulfate transporters belong to two main classes, i.e. transporters that are either high affinity or low affinity for sulfate. The high-affinity transporter, which is likely to be the primary transporter involved in sulfate uptake, has a K_m for sulfate of $\sim 7\text{--}10\ \mu\text{M}$ and is expressed primarily in the roots (145, 146). The low-affinity transporter has a higher K_m for sulfate ($100\ \mu\text{M}$) and is expressed in both shoots and roots; this transporter may be involved in the internal intercellular transport of sulfate (146).

The expression of the transporter genes is regulated by the S status of the plant, as well as by the regulators, glutathione (GSH) and O-acetylserine (44; see Figure 1). While high levels of sulfate and GSH decrease transcription, high levels of O-acetylserine increase transcription of the high-affinity transporter genes as well as sulfate uptake (44). Thus, increasing O-acetylserine levels can potentially increase selenate uptake: Our results show that application of O-acetylserine increased selenate accumulation in Indian mustard almost twofold compared to untreated plants (MP de Souza & N Terry, unpublished data). O-acetylserine, a precursor of cysteine (Cys) and a product of the nitrate assimilation pathway, may be of pivotal importance as a coregulator of the S and nitrogen metabolic pathways (44).

Because of the role of the sulfate transporter in selenate uptake, we have undertaken studies to determine whether overexpression of either the high-affinity (*SHST1*) or low-affinity (*SHST3*) transporter genes from *S. hamata* increases the uptake of Se in Indian mustard. Overexpression of *SHST1* increased selenate accumulation up to twofold in transgenic plants compared to wild type; on the other hand, transgenic plants overexpressing *SHST3* did not differ significantly from wild type in their accumulation of selenate (S Huang & N Terry, unpublished data). These data support the view that the high-affinity sulfate transporter is involved in selenate uptake.

Unlike selenate, there is no evidence that the uptake of selenite is mediated by membrane transporters (1, 5, 6, 143). Selenite uptake was inhibited by only 20% by the addition of a respiratory inhibitor, hydroxylamine, to nutrient solution; selenate uptake, however, was inhibited by 80% (5). Asher et al (6) showed that, although the Se concentration in the xylem exudate of selenate-supplied detopped roots exceeded that of the external medium by 6 to 13 times, Se concentration was always lower in the xylem exudate than outside when selenite was supplied. Plants can also take up organic forms of Se such as selenomethionine (SeMet) actively. Abrams et al (1) showed that SeMet uptake by wheat seedlings followed Michaelis-Menten kinetics and that uptake was coupled to metabolism, as indicated by

evidence of inhibition by metabolic inhibitors (e.g. dinitrophenol) and by anaerobic conditions (i.e. nitrogen bubbling).

Interaction with Salinity

As indicated above, sulfate competes with selenate for uptake by the sulfate transporter. It is hardly surprising therefore that sulfate salinity drastically inhibits plant uptake of selenate (19, 107, 141, 166, 171). Not all plant species are affected to the same extent by sulfate salinity. In Se-accumulators, selenate is taken up preferentially over sulfate. Calculation of the Se/S discrimination coefficient (the ratio of the plant Se/S ratio to the solution Se/S ratio) indicated that *A. bisulcatus*, rice, and Indian mustard are able to take up Se preferentially in the presence of a high sulfate supply (19). Other species (e.g. alfalfa, wheat, ryegrass, barley, broccoli) had discrimination coefficient values lower than 1, and selenate uptake was significantly inhibited by the increases in sulfate supply. Chloride salinity had much less effect on selenate uptake than sulfate salinity (107, 141, 166). Generally, there is a small decrease in shoot accumulation of Se with increasing salt levels (15).

Transport and Distribution

The translocation of Se from root to shoot is dependent on the form of Se supplied. Selenate is transported much more easily than selenite, or organic Se, such as SeMet. Zayed et al (171) showed that the shoot Se/root Se ratio ranged from 1.4 to 17.2 when selenate was supplied but was only 0.6 to 1 for plants supplied with SeMet and less than 0.5 for plants supplied with selenite. Arvy (5) demonstrated that within 3 h, 50% of the selenate taken up by bean plant roots moved to shoots, whereas in the case of selenite, most of the Se remained in the root and only a small fraction was found in the shoot. Time-dependent kinetics of Se uptake by Indian mustard showed that only 10% of the selenite taken up was transported from root to shoot, whereas selenate (which was taken up twofold faster than selenite) was rapidly transported into shoots (49). Thus, plants transport and accumulate substantial amounts of selenate in leaves but much less selenite or SeMet. The reason why selenite is poorly translocated to shoots may be because it is rapidly converted to organic forms of Se such as SeMet (171), which are retained in the roots.

The distribution of Se in various parts of the plant differs according to species, its phase of development, and its physiological condition (156). In Se accumulators, Se is accumulated in young leaves during the early vegetative stage of growth; during the reproductive stage, high levels of Se are found in seeds while the Se content in leaves is drastically reduced (58). Nonaccumulating cereal crop plants, when mature, often show about the same Se content in grain and in roots, with smaller amounts in the stems and leaves (17). Distribution of Se in plants also depends on the form and concentration of Se supplied to the roots and on the nature and concentration of other substances, especially sulfates, accompanying the Se (49, 171).

Plants can absorb volatile Se from the atmosphere via the leaf surface. Zieve & Peterson (174) demonstrated that *Agrostis tenuis*, *H. vulgare*, *Lycopersicon esculentum*, and *Raphanus sativus* accumulate ^{75}Se when fumigated with (^{75}Se)-DMSe in a sealed system. The Se absorbed by the leaves was accumulated in roots as inorganic selenite, selenogluthathione (SeGSH), SeMet, and protein-bound SeMet. Using an isotope dilution method, Haygarth et al (79) assessed the importance of soil and atmospheric Se inputs to ryegrass; with soil pH of 6, 47% of the ^{75}Se in ryegrass leaves was derived from the soil, whereas at pH 7, it was 70%. It was assumed that the remainder of the Se came from the atmosphere.

BIOCHEMISTRY OF Se

The Question of Se Essentiality in Plants

Although there is mounting evidence that Se is required for the growth of algae (126, 170), the question of the essentiality of Se as a micronutrient in higher plants is unresolved and remains controversial. There are indications that Se may be required for Se-accumulating plants, which are endemic to seleniferous soils. Trelease & Trelease (157) observed an increase in biomass production when the Se accumulator, *Astragalus pectinatus*, was treated with 0.38 mM Se. These results were challenged subsequently by Broyer et al (31), who attributed the growth stimulation in the *Astragalus* plants to the ability of Se in the nutrient solution to counteract phosphate toxicity; at low phosphate concentrations, growth was not stimulated by Se treatment. There is no evidence for a Se requirement in nonaccumulators (142). When alfalfa and clover plants were grown in hydroponic culture with highly purified salts, addition of selenite did not result in an enhancement of growth (30). However, this type of experiment does not prove that there is no requirement for Se; it merely shows that, if there is a requirement, it must be satisfied at an Se concentration lower than that obtained in the plant tissue under the conditions of the experiment (since there are always trace amounts of Se in plants coming from impurities in the nutrient salts or from the atmosphere).

In order to investigate the essentiality of Se in higher plants, attempts have been made to establish whether plants contain essential selenoproteins, such as those discovered for bacteria and animals. For example, several Se-dependent enzymes have been identified in which an integral selenocysteine (SeCys) residue is inserted in the catalytic site (67, 147). Selenoenzymes, such as formate dehydrogenase from bacteria and Archaea, and the GSH peroxidase (GPX) and type 1 iodothyronine deiodinase family of enzymes from animals, are involved in oxidation-reduction reactions in which the Se (present in the reduced selenol form) functions as a redox center (148). Mutagenesis studies have shown that this SeCys residue plays a critical role because replacement of the active site SeCys by a Cys residue greatly reduced catalytic activity (9, 20).

The incorporation of the active site-SeCys into these essential selenoproteins is a cotranslational process directed by a UGA codon (22). UGA normally functions as a universal termination codon; in order for UGA to function as a SeCys codon, both specific secondary structural elements in the mRNA and a unique SeCys-charged tRNA^{ser/sec} that contains the UGA anticodon are required (148). The biosynthesis of SeCys is itself a unique process because it occurs in a tRNA-bound state distinct from the S/Se assimilation pathway (see next section). A key reaction in this biosynthesis is the activation of selenide to form selenophosphate by the enzyme selenophosphate synthetase (148). Selenophosphate is the Se donor for the conversion of the serine-bound tRNA^{ser} to the SeCys-bound tRNA^{sec} (148).

Attempts have been made to establish whether plants also contain these essential selenoproteins. Radioactive labeling experiments have so far failed to detect essential selenoproteins in plants. For example, Anderson (2), using radioactively labeled [⁷⁵Se]selenite and [³⁵S]sulfate, was unable to detect proteins with significantly higher ⁷⁵Se/³⁵S ratios than other proteins. A second approach to detecting selenoproteins in plants is to test for the presence of a family of selenoproteins, the GSH peroxidases (GPX), which catalyze the reduction of hydrogen peroxide by reduced GSH in order to protect cells against oxidative damage (see 60). The presence of GPX is determined by GSH-dependent reduction of hydrogen peroxide. Based on this enzymatic assay, plant extracts with GPX activity and partially purified preparations of GPX were reported recently from various plants (reviewed in 60). Sabeh et al (134) found a 16-kd tetrameric protein in *Aloe vera*, which they conclude is a GPX selenoprotein similar to that found in mammals.

Molecular evidence has been obtained that suggests that, although there are GPX-like enzymes in higher plants, they may not be selenoproteins. For example, genes with significant sequence homology to animal GPX genes were recently isolated from a number of plants including *Nicotiana sylvestris* (42), *Citrus sinensis* (81), and *Arabidopsis* (Genbank accession no. X89866). Analysis of these sequences indicates that the plant genes showed homology to only one subgroup of animal GPX genes, the phospholipid hydroxypoxide GSH peroxidases (60). However, in contrast to animal GPX genes that contain a SeCys codon, UGA, in the active site, the plant genes contain the Cys codon, UGU (60). The gene isolated from *Citrus sinensis* was expressed into protein and assayed; it exhibited only 0.2 to 0.8% activity of the animal GPX (18, 81). Thus, it would appear to have another function in plants (60). Furthermore, peptide sequencing of the purified protein has confirmed that Cys, and not SeCys, is at the active site of the enzyme (64). On the basis of all the available information published, we conclude that no essential higher plant selenoprotein has been clearly identified by either protein or DNA sequence analysis to date.

Although there is no evidence for an essential selenoprotein in higher plants, there is evidence that part of the machinery for synthesizing selenoproteins may be present, i.e. a UGA decoding-tRNA^{sec} was demonstrated to be present in *Beta vulgaris* (sugar beet) (76). This tRNA^{sec} was observed in bacterial (95) and mammalian cells (93) and has also been identified in the diatom *Thalassiosira pseudonana* (77),

and in the Ascomycete *Gliocladium virens* (76). Hence, UGA appears to have the dual function of termination codon and codon for SeCys in all groups of organisms.

Se Assimilation, Volatilization, and Incorporation into Proteins

In addition to the possible incorporation of trace amounts of Se into specific or essential selenoproteins, higher plants metabolize Se via the S-assimilation pathway (172). This involves the nonspecific incorporation of Se into selenoamino acids and their proteins, as well as volatilization, which occurs when Se is supplied to plants in excess of any potential Se requirement. Most of the available information on Se assimilation and volatilization is for Se nonaccumulators (Figures 2–5); the pathway for Se accumulators is presented separately (Figure 6).

Role of ATP Sulfurylase After selenate is absorbed into the root via the sulfate transporter, it is translocated without chemical modification (49, 171) through the xylem to the leaves. Once inside the leaf, selenate enters chloroplasts where it is metabolized by the enzymes of sulfate assimilation. The first step in the reduction of selenate is its activation by ATP sulfurylase to adenosine phosphoselenate (APSe), an activated form of selenate (Figure 2). ATP sulfurylase has been shown to activate selenate, as well as sulfate, *in vitro* (35, 53, 140). Molecular studies in our laboratory provided the first *in vivo* evidence that ATP sulfurylase is responsible for selenate reduction, and that this enzyme is rate limiting for both selenate reduction and Se accumulation (125). Transgenic Indian mustard plants that overexpressed ATP sulfurylase were developed using a gene construct containing the *Arabidopsis thaliana* *aps1* gene (97), with its own chloroplast transit sequence, fused to the Cauliflower Mosaic Virus 35S promoter. X-ray absorption spectroscopy (XAS) analysis of wild-type Indian mustard plants supplied with selenate showed that selenate was accumulated in both roots and shoots, but when selenite was supplied, an organo-Se compound (similar to SeMet) accumulated (49). We concluded therefore that the reduction of selenate was rate limiting to selenate assimilation. This rate-limiting step was overcome in transgenic plants overexpressing ATP sulfurylase because these plants accumulated a SeMet-like compound when supplied with selenate (125). Furthermore, when the shoots of the transgenic plants were removed, and the detopped roots supplied with selenate, plants were unable to reduce the selenate, which remained as the principal form of Se in roots. This supports the view that the chloroplasts are the site for selenate reduction (125).

Reduction of APSe to Selenide There is evidence that APSe can be reduced nonenzymatically to GSH-conjugated selenite (GS-selenite) following the pathway outlined in Figure 2A (3, 53). The GS-selenite is then reduced via GSH to produce the intermediate selenodiglutathione (GS-Se-SG). When selenite is taken up by plants (instead of selenate), it reacts nonenzymatically with GSH to form

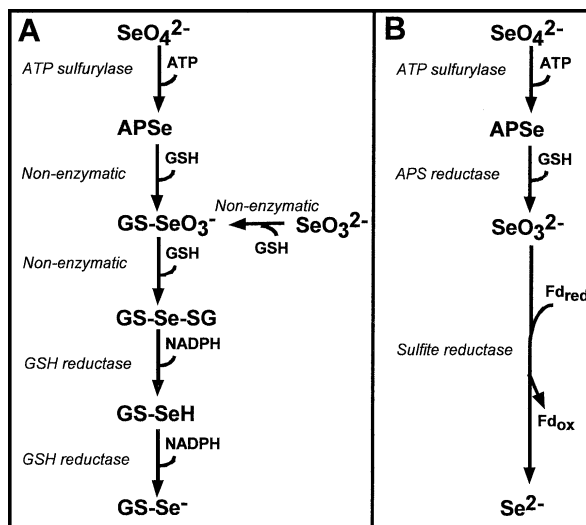


Figure 2 The activation of selenate by ATP sulfurylase, which is followed by (A) reduction to selenite (Se^{2-}) via nonenzymatic reactions and glutathione reductase, (B) reduction to selenide via APS reductase and sulfite reductase. Selenite (SeO_3^{2-}) can enter the pathway via a nonenzymatic reaction to selenodiglutathione (GS-Se-SG), which is reduced to the selenol, GS-SeH . GS-Se^- is glutathione-conjugated selenide.

GS-Se-SG (2, 3). GS-Se-SG is reduced with NADPH to the corresponding selenol (GS-SeH), and subsequently to GSH-conjugated selenide (GS-Se^-) by the enzyme GSH reductase (113). Later, Anderson & Scarf (3) proposed that the reduction of GS-Se-SG to GS-SeH may also proceed nonenzymatically with GSH as a reductant.

In the S-assimilation pathway, the S analog of APSe, 5'-adenylylsulfate (APS), is at a branchpoint where it can be converted to either sulfite (72, 136, 139) or 3'-phosphoadenosine 5'-phosphosulfate (PAPS), which is used to form sulfated compounds (e.g. sulfolipids) (21). By analogy to the S-assimilation pathway, APSe could be reduced to selenite with GSH by APS reductase (21), and then to selenide by the enzyme, sulfite reductase (using ferredoxin as a reductant) (Figure 2B). Genes encoding the chloroplast enzymes, APS reductase and sulfite reductase, have been cloned from *A. thaliana* (32, 72, 139). However, there is no evidence that APS reductase and sulfite reductase are involved in selenate reduction *in vivo*, or that there is a Se analog for PAPS or selenolipids in plants (35).

Although GSH reductase is clearly an important enzyme in the reduction of selenate (Figure 2), we were unable to obtain molecular evidence that it is a rate-limiting enzyme. We transformed Indian mustard plants to overexpress a bacterial GSH reductase in the cytoplasm (cytGR) and in the chloroplast (cpGR) (EAH Pilon-Smits & N Terry, unpublished data). The GSH reductase gene was cloned

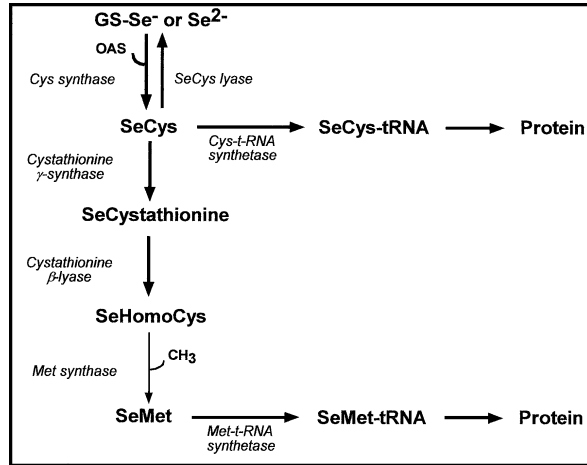


Figure 3 Incorporation of selenide into SeCys, SeMet, and proteins. SeCys lyase is a Se-specific enzyme, whereas all the other enzymes shown recognize both S and Se. The only exception is Met synthase, which is involved in Met synthesis, and is very likely to be involved in SeMet synthesis although there is no evidence supporting this (represented by the thin arrow).

from *E. coli* and adapted for expression in plants by Foyer et al (69). However, transgenic cpGR and cytGR plants treated with selenate or selenite did not show any differences from the wild type with respect to the form of Se accumulated, Se uptake, or Se volatilization rate.

Selenite can undergo other transformations besides its assimilation into selenide; for example, plants supplied with selenite have been shown to oxidize it to selenate (6; D Hansen, CM Lytle & N Terry, unpublished data). Although common in bacteria, the reduction of selenate or selenite to elemental Se (52, 101, 117, 118) has not been reported in plants.

Amino Acid and Protein Synthesis Plants are thought to assimilate SeCys in a manner similar to bacteria (2), where SeCys is metabolized to SeMet, both of which are nonspecifically incorporated into proteins. In plants, the formation of SeCys very likely takes place within the chloroplasts (113). SeCys is formed by the action of Cys synthase, which couples selenide with O-acetylserine (112) (Figure 3). GS-Se⁻ may be the physiological substrate of Cys synthase rather than free Se²⁻ (159). The activity of Cys synthase may be influenced by the ratio of sulfide to selenide; selenide inhibits the synthesis of Cys while excess sulfide inhibits the synthesis of SeCys (112). When the Cys synthase gene (cloned from spinach; 137) was overexpressed in Indian mustard, the transgenic plants treated with selenate or selenite did not show any differences from the wild type with respect to Se uptake, Se species accumulated, or Se volatilization (EAH Pilon-Smits, CM Lytle &

N Terry, unpublished data). The results from this overexpression study suggest that Cys synthase is not rate limiting for selenate or selenite assimilation to SeMet.

In mammals, SeCys can be reconverted to selenide by the Se-specific enzyme SeCys lyase, with the concomitant release of L-alanine (Figure 3). This enzyme, which was purified from pig liver, is specific for SeCys because it discriminates against Cys as a substrate (59). The same enzyme may also exist in plants, since an *Arabidopsis thaliana* homolog of the mammalian SeCys lyase gene was recently cloned (Genbank accession no. CAA16686).

By analogy to the methionine (Met) biosynthetic pathway (55, 128), SeMet may be produced from SeCys via SeCystathionine and SeHomoCys (Figure 3). There is some evidence for this from kinetic studies of *in vitro* enzymes. Cystathionine- γ -synthase, which catalyzes the condensation of phosphohomoserine with SeCys to form SeCystathionine, exhibited a preference for SeCys: It had a higher affinity for SeCys ($K_m = 70 \mu\text{M}$) than for Cys ($K_m = 240 \mu\text{M}$) (45). Cystathionine- β -lyase, which most likely cleaves selenocystathionine to selenohomoCys, did not differentiate between the Se and S forms of cystathionine, since the enzyme had a similar affinity for cystathionine ($K_m = 0.31 \text{ mM}$) and selenocystathionine ($K_m = 0.35 \text{ mM}$) (105).

The most likely enzyme for the synthesis of SeMet from SeHomoCys is the cytosolic enzyme, Met synthase (Figure 3), which has been cloned from *Arabidopsis*, *Catharanthus roseus*, and *Coleus blumei* (57, 122, 128). In plants, Met synthase uses methyl-tetrahydrofolate as a methyl donor (40). It is possible that SeMet is subject to the recycling pathways that have been described for Met (55).

Selenium is readily incorporated into proteins in nonaccumulator plants treated with Se (28). The incorporation into proteins occurs through the nonspecific substitution of SeCys and SeMet in place of Cys and Met, respectively (29) (Figure 3). With regard to Cys versus SeCys incorporation, Burnell & Shrift (36) showed that the mung bean cysteinyl-tRNA synthetase, the enzyme catalyzing the first step in the incorporation of Cys into protein, supported Cys- and SeCys-dependent PPI-ATP exchange at rates that were approximately equal. Similar studies showed that both Met and SeMet are substrates for the methionyl t-RNA synthetase (34, 62).

Selenomethionine to DMSe Lewis et al (99) showed that SeMet may be methylated to Se-methylSeMet, which was cleaved to DMSe by an enzyme in a crude extract of cabbage leaves (Figure 4). The most likely enzyme responsible for this reaction is S-methylMet hydrolase, which produces DMS from S-methylMet in higher plants (70, 71, 78). Another possible pathway for DMSe production is via the intermediate, dimethylselenoniopropionate (DMSeP) (Figure 4). Evidence for this was obtained by Ansele et al (4), who showed that selenate-supplied *Spartina alterniflora* plants accumulated the selenium compounds, Se-methylSeMet and DMSeP, especially at high salinity and at Se concentrations greater than $50 \mu\text{M}$. The production of DMSeP very likely will occur via the same biochemical pathway proposed for the synthesis of its S analog, dimethylsulfoniopropionate (DMSP), in higher plants (74, 75, 90). If so, the first step would be the methylation of SeMet by the cytosolic enzyme, Met methyltransferase (MMT) (25, 85). The enzymes

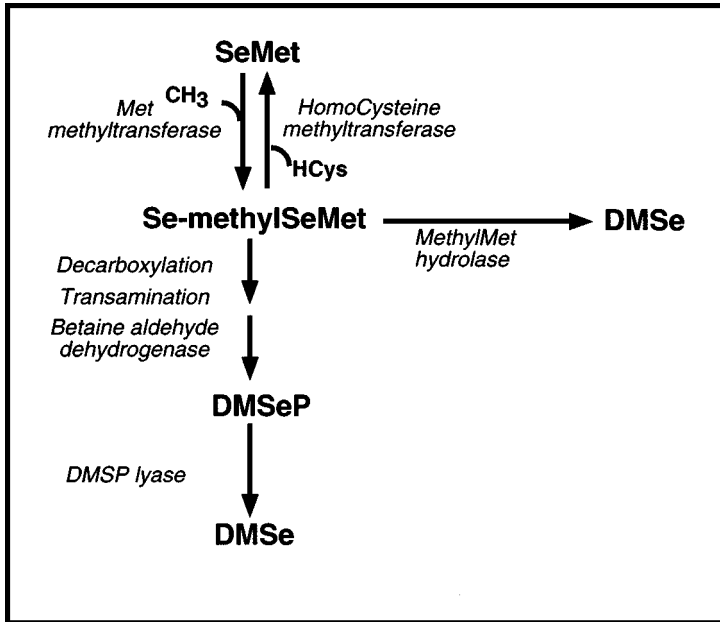


Figure 4 Production of DMS from SeMet can take place directly from Se-methylSeMet, which is produced by the methylation of SeMet, or it can be produced after the conversion of SeMet to DMSeP.

for the formation of DMSP from S-methylMet are not known although DMSP-aldehyde and DMSP-amine have been identified as intermediates, depending on the plant species (86, 90). The biochemical steps involved are a decarboxylation, a transamination involving the conversion of methylMet to DMSP-aldehyde, and the chloroplastic enzyme betaine aldehyde dehydrogenase (BADH), which oxidizes DMSP-aldehyde to DMSP (131, 161). The formation of DMS from DMSeP may proceed in plants as it does in bacteria, i.e. DMSeP may be volatilized to DMS by the enzyme DMSP lyase (4), which is thought to exist in plants (43) (Figure 4).

A futile S-methylMet cycle occurs in plants. In this cycle, homoCys methyltransferase catalyzes the conversion of S-methylMet to Met by donating the methyl group to homoCys (71, 109). A similar reaction could exist to form SeMet from Se-methylSeMet.

Localization of the Pathway The movement of Se through the plant and the localization of enzymes involved in Se assimilation and volatilization may be summarized as follows. Selenate is transported to the chloroplasts where it is reduced. All the enzymes involved in this reduction, ATP sulfurylase, APS reductase, GSH reductase, sulfite reductase, Cys synthase, cystathionine γ -synthase, and cystathionine β -lyase, are chloroplastic (32, 68, 72, 89, 92, 112, 128, 139, 160, 163).

The production of SeMet from SeHomoCys and the methylation of SeMet to Se-methylSeMet by Met synthase and Met methyltransferase, respectively, most likely takes place in the cytosol because both of these enzymes are cytosolic (57, 85, 163). In shoots, Se could be volatilized through the action of methylMet hydrolase producing DMSe directly from methyl-SeMet (70, 71, 99). By analogy with dimethyl sulfide (DMS) production from DMSP that occurs in leaves (43), DMSeP synthesis from Se-methylSeMet could take place in the chloroplast (158), with DMSe being produced by DMSP lyase. However, since roots volatilize Se as much as 26 times more than shoots, it is clear that most of the DMSe is produced from the roots (173). For this to occur, the DMSe precursors, Se-methylSeMet and/or DMSeP, would have to be transported to roots in order to form DMSe. This presumes that methyl-Met hydrolase and DMSP lyase are also present in roots.

Other Possible Rate-Limiting Steps In addition to the rate limitation imposed by ATP sulfurylase, there is evidence of other possible rate-limiting steps for Se assimilation and volatilization. Since SeCys may move readily into other pathways (e.g. protein synthesis), one might speculate that the availability of SeCys might be rate limiting for Se volatilization. To test this hypothesis, de Souza et al (50) supplied Indian mustard plants with a crude preparation of SeCys (which contained impurities of selenocystine and dithiothreitol). The SeCys-supplied plants accumulated high concentrations of Se in their roots but did not volatilize Se significantly faster than selenate or selenite; thus, there was no evidence that SeCys was rate limiting (Figure 5). Plants supplied with SeMet on the other hand, volatilized Se at rates that were almost fivefold higher than the rates measured from SeCys, even though the plants accumulated slightly lower amounts of Se in roots. Thus, the synthesis of SeMet would appear to be rate limiting to Se volatilization. Furthermore, the rate of Se volatilization from DMSeP-supplied plants was fivefold higher than that measured from SeMet-supplied plants, even though the roots of plants supplied with SeMet accumulated eightfold more Se than DMSeP-supplied plants (Figure 5). This suggests that the conversion of SeMet to DMSeP is also rate limiting. In support of this view, XAS and HPLC data showed that a SeMet-like compound accumulates in selenite- and SeMet-supplied plants, but only relatively low levels of selenonium compounds (MP de Souza, CM Lyttle, MM Mulholland, ML Otte, N Terry, unpublished data). That DMSeP is volatilized at very high rates but does not accumulate in significant amounts in Indian mustard provides evidence that the production of DMSe from DMSeP is not likely to be rate limiting.

Pathway in Se Accumulators The pathway for the assimilation of inorganic forms of Se to SeCys in Se accumulators is believed to be the same as for nonaccumulators (29, 154) (Figure 6). However, Se accumulators differ from nonaccumulators in that they metabolize the SeCys primarily into various nonprotein selenoamino acids. The synthesis of these nonprotein selenoamino acids probably occurs along pathways associated with S metabolism (29). Three possible pathways are shown in Figure 6 for the conversion of SeCys to (I) Se-methylSeCys, which has been found in many Se-accumulators (29); (II) Se-cystathionine, which

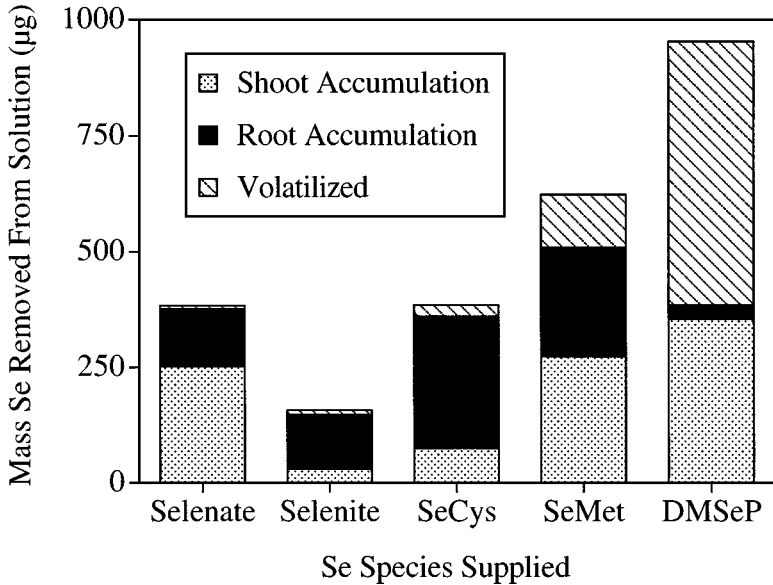


Figure 5 Total amounts of Se removed from hydroponic media per plant when Indian mustard plants were supplied with different forms of Se at 20 μM . The total amount of Se shown includes the amount of Se volatilized and the amounts accumulated in roots and shoots over an 8-day period under hydroponic culture conditions.

has been observed to accumulate in *Neptunia amplexicaulis* and *Morinda reticulata* (123); and (III) the dipeptide, γ -glutamyl-Se-methylSeCys, which has been observed in two *Astragalus* Se-accumulator species (114). Se-methylSeCys is the product of the enzyme SeCys methyltransferase, which has recently been purified and cloned from *Astragalus bisulcatus* (110, 111). SeCys methyltransferase methylates SeCys specifically (i.e. not Cys) into methylSeCys (110). It may be further methylated to produce DMDSe, which is volatilized (Figure 6) (63, 98). In (II), Se-cystathionine accumulates because the enzyme cystathionine β -lyase (which cleaves cystathionine (Figures 3, 6), is unable to cleave the Se analog (123).

Toxicity and Tolerance

When plants are exposed to high concentrations of Se in their root medium, they may exhibit symptoms of injury including stunting of growth, chlorosis, withering and drying of leaves, decreased protein synthesis, and premature death of the plant (106, 156). There are striking differences between the Se-accumulating plants and the nonaccumulators in the amount of Se they may absorb without showing symptoms of toxicity. In nonaccumulators, the threshold Se concentration in shoot tissue resulting in a 10% reduction in yield varied from 2 mg Se kg^{-1} in rice to 330 mg Se kg^{-1} in white clover (108). Se accumulators, on the other hand, may contain Se concentrations in excess of 4000 mg Se kg^{-1} without exhibiting any negative

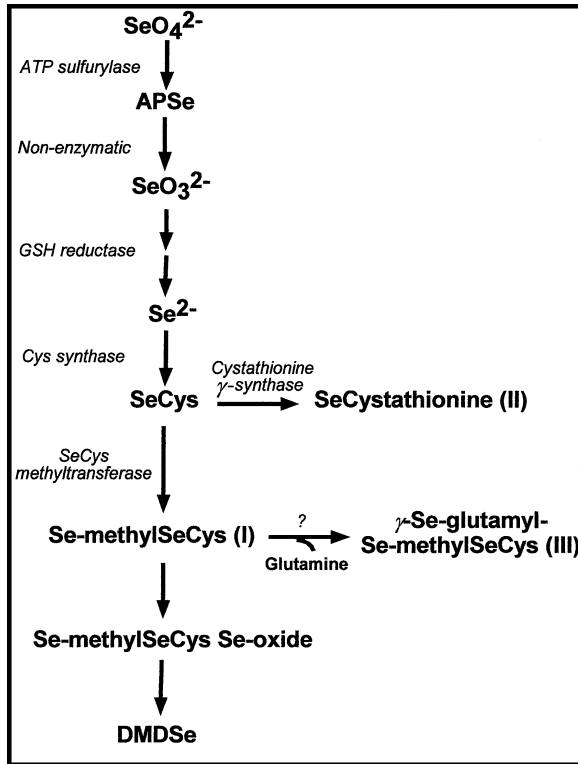


Figure 6 The assimilation of selenate by Se accumulators. The incorporation of selenate to SeCys takes place in a manner similar to Se nonaccumulators (Figures 1–3). In Se-accumulating *Astragalus* species, SeCys is methylated to Se-methylSeCys(I), which can be volatilized to DMDSe, and/or conjugated to glutamine to the dipeptide, γ -glutamyl-Se-methyl-SeCys(III). In other Se-accumulators SeCys may be converted to SeCystathionine, which is accumulated (II).

effects on growth (142). The threshold Se concentration may also vary with plant age and with sulfate supply. For example, Rosenfeld & Beath (132) observed that in the nonaccumulators, wheat and corn, younger plants were more susceptible and growth inhibition was greater than in mature plants. Tolerance to Se toxicity may increase with increasing sulfate supply so that threshold Se concentrations may not be the same at different sulfate concentrations in the root environment (108).

In nonaccumulators, the threshold toxicity concentration is also dependent on the form of Se accumulated. Selenate and selenite are the major forms that are toxic to plants because both are readily absorbed by the plant and assimilated to organic Se compounds. Some studies indicate that selenite is more toxic than selenate (e.g. 132). This may be due to the faster conversion of selenite to selenoamino acids (171), which may then be incorporated into plant proteins in replacement of S and cause toxicity to the plant (see below). Other studies have shown that

selenate is more toxic than selenite: Wu et al (167) observed that selenate caused a greater growth inhibition than selenite with respect to the growth of tall fescue.

The major mechanism whereby high Se accumulation in plant tissues induces Se toxicity is almost certainly associated with the incorporation of SeCys and SeMet into proteins in place of Cys and Met, respectively (28, 61). The differences in size and ionization properties of S and Se may result in significant alterations in protein structure. The bond between two Se atoms is approximately one seventh longer and one fifth weaker than the disulfide bond (29). Therefore, the incorporation of SeCys in place of Cys into protein could interfere with the formation of disulfide bridges, resulting in a slightly altered tertiary structure of S-proteins and a negative effect on their catalytic activity (3, 29). Furthermore, Se may diminish the rate of protein synthesis; this is because the substitution of SeMet for Met into proteins may be less effective as a substrate for peptide bond formation during translation (62).

There are other ways by which Se can induce toxicity in plants. Se induces chlorosis, possibly through an adverse effect on the production of porphobilinogen synthetase, an enzyme required for chlorophyll biosynthesis (119). Selenate and selenite interfere with the *in vivo* reduction of nitrate in leaves (7). Selenate may interfere with the synthesis of GSH. The addition of selenate strongly reduced sulfate-induced GSH accumulation in spinach leaf disks (46). Also, incubation of spruce needles with selenate led to a substantial decrease in GSH content (24). Thus, interference of GSH synthesis in plants by selenate or other Se compounds may diminish plant defense against hydroxyl radicals and oxidative stress.

Tolerance Mechanisms Given the toxic effects of Se on plant function, how are Se-accumulators able to tolerate such high concentrations of Se in their cells? The main mechanism appears to be the reduction of the intracellular concentration of SeCys and SeMet, which otherwise would be incorporated into proteins with damaging effects on plant function. This may be achieved by accumulating Se in nonprotein seleno-amino acids, e.g. Se-methylSeCys and SeCystathionine, or as the dipeptide, γ -glutamyl-Se-methylSeCys, which accumulates in some species of *Astragalus* (114) (see discussion above). Since SeCys methyltransferase has threefold less activity with Cys compared to SeCys in the methylation reaction, Se detoxification by Se-methylSeCys accumulation can proceed without depleting the cell of Cys (110). Neuhierl et al (111) demonstrated that expression of the SeCys methyltransferase gene in *E. coli* could confer tolerance to high concentrations of Se and reduce the nonspecific incorporation of Se into proteins. The role of this enzyme in Se tolerance is further reinforced by the finding that moderate tolerance of the nonaccumulator *Astragalus cicer* to selenite coincided with the expression of an enzyme with SeCys methyltransferase activity (164).

There is evidence that a similar enzyme to SeCys methyltransferase exists in bacteria and in nonaccumulators but the enzyme has a different function. This was shown by the work of Neuhierl et al (111) who found genes that had sequence similarity to the *A. bisulcatus* SeCys methyltransferase gene in *E. coli* (*yagD*), *Arabidopsis*, and rice. Biochemical analysis of the enzyme encoded by *yagD* showed that it was involved in the catabolism of S-methylMet, i.e. it was a

methyltransferase gene. These researchers postulated that the SeCys methyltransferase in Se accumulators may have developed under selective pressure from a S-methylMet-dependent thiol/selenol methyltransferase found in all plants.

An alternative mechanism for excluding Se from proteins may be through the ability of Se accumulators to discriminate against the incorporation of selenoamino acids into proteins. For example, in the Se accumulator, *A. bisulcatus*, cysteinyl-tRNA synthetase is unable to attach SeCys to the Cys tRNA, thereby excluding SeCys from cellular incorporation into proteins (37). Selenium tolerance may also be achieved by compartmentation into the vacuole as selenate and, in the case of Se accumulators, as nonprotein seleno-amino acids.

PHYTOVOLATILIZATION

Variation Among Plant Species

The rate of Se volatilization varies substantially among plant species (56, 124, 151). Terry et al (151) measured the rate of Se volatilization from 15 crop species grown in solution culture in the presence of 20 μM selenate. Rice, broccoli, and cabbage volatilized Se at the highest rates, i.e. 200–350 $\mu\text{g Se m}^{-2}$ leaf area day^{-1} , and sugar beet, bean, lettuce, and onion exhibited the lowest rates, $>15 \mu\text{g Se m}^{-2}$ leaf area day^{-1} . Duckart et al (56) found that *Astragalus bisulcatus* and broccoli showed the highest rates of Se volatilization, followed by tomato, tall fescue, and alfalfa, respectively. For wetland plant species cultured hydroponically, there was a 50-fold variation in Se volatilization rates; the highest Se volatilization rate from selenate was attained by mare's tail, 1.0 mg Se kg^{-1} dry weight d^{-1} , and from selenite, 4.0 mg Se kg^{-1} dry weight d^{-1} (attained by *Azolla*) (124). Terry & Lin (152) compared the rate of volatilization of 11 different plant species, including pickleweed, saltgrass, cordgrass, Eucalyptus, cotton, and canola, under field conditions. They found that pickleweed (*Salicornia bigelovii*) had the highest average volatilization rate, 420 $\mu\text{g Se m}^{-2}$ soil surface d^{-1} , a rate that was 10 to 100 times greater than rates measured for the other species.

Plant/Microbe Interactions

Bacteria, fungi, and algae can volatilize Se at high rates independently of plants. Pure cultures of bacteria, fungi, and algae tested under laboratory conditions volatilized Se at rates as high as or higher than plants when expressed per gram dry weight of tissue (26, 51, 65, 66, 127, 155). A complicating factor in the assessment of the contribution of Se volatilization from plants is the volatilization of Se by soil microorganisms (154). An important question to resolve is: To what extent is Se volatilization by plants dependent on the presence and activity of microbes in the rhizosphere?

One of the first indications that bacteria are involved in plant Se volatilization was obtained when detopped roots of broccoli were treated with penicillin-G and chlortetracycline (173). These antibiotics inhibited 95% of the Se volatilization

activity from selenate-supplied detopped roots, supporting the view that rhizosphere bacteria may be essential for plant Se volatilization. Several attempts have been made to determine whether plants can volatilize Se independently of microorganisms. One approach to resolving this problem was to culture plants axenically, i.e. from seeds washed with ethanol and hypochlorite to remove microorganisms. When sterile and nonsterile plants were supplied with selenate, selenite, or SeMet, sterile plants volatilized SeMet at slightly lower rates than nonsterile plants but with selenate or selenite, sterile plants produced very little if any volatile Se (AM Zayed, N Terry, unpublished data). Thus, these data suggest that bacteria in the rhizosphere of Indian mustard are required for Se volatilization from selenate and selenite but not from SeMet.

What role do rhizosphere bacteria play in the phytovolatilization of selenate or selenite? De Souza et al (47, 48) obtained evidence that bacteria facilitate the uptake of selenate into root tissues. Two different approaches were used to inhibit microbial activity in roots: antibiotics and axenic plants. Using these approaches, rhizosphere bacteria were shown to increase selenate uptake in different plant species two- to threefold faster than in plants with inhibited rhizosphere activity (47, 48). De Souza et al (47) also obtained evidence that the role of bacteria in selenate uptake may be the production of a heat-labile compound(s) that was proteinaceous in nature. One possibility is that bacteria may stimulate selenate uptake in plants by producing O-acetylserine and serine in the rhizosphere (sterile plants inoculated with bacteria had twofold more O-acetylserine and tenfold more serine in their rhizosphere than sterile plants had). In support of this view, O-acetylserine and serine supplied to axenic plants were shown to stimulate selenate uptake two- and 1.5-fold, respectively (MP de Souza & N Terry, unpublished data).

The effect of rhizosphere microorganisms on Se uptake appeared to be specific for selenate; there was no such effect on selenite uptake. There is some evidence that the role of bacteria in selenite volatilization by plants may be in the production of organo-Se compounds such as SeMet, which is more readily volatilizable than selenite or selenate (171): XAS speciation of selenite-supplied plants showed that plants treated with ampicillin to inhibit rhizosphere bacteria accumulated SeCys, whereas plants not treated with ampicillin accumulated Se in the form of SeMet (MP de Souza, CM Lytle & N Terry, unpublished data).

Environmental Factors

Several studies indicate that the ability of plants to volatilize Se is influenced by the concentration of Se in the root medium and by the chemical form of Se supplied. The rate of Se volatilization was shown to be correlated linearly with the external Se concentration and with the internal plant tissue Se concentration for Indian mustard plants supplied with selenate or selenite over an increasing concentration range (49). Terry et al (151) showed the rate of Se volatilization by different plant species was strongly correlated with the plant tissue Se concentration in selenate-supplied plants. The dependence of Se volatilization rate on the form of Se supplied was demonstrated by Lewis et al (99), who showed that cabbage

leaves from plants supplied with selenite released 10 to 16 times more volatile Se than those taken from plants supplied with selenate. Our research showed that plants of broccoli, Indian mustard, sugar beet, and rice volatilized SeMet at the highest rate, followed by selenite, then selenate (171), whereas Indian mustard plants supplied with DMSeP volatilized Se at a rate that was 6 times higher than that measured from plants supplied with SeMet, and 26 times higher than from SeCys (50). Under field conditions, Zijian et al (175) showed that when soils from a low-Se region in China were amended with SeMet, the rate of Se volatilization was enhanced 14-fold, while the addition of less reduced forms of Se resulted in only a fourfold increase. In experiments where SeMet and SeCys were added to soil, Martens & Suarez (103) showed that 50% to 80% of the added SeMet was volatilized, whereas little or no volatile Se was measured from SeCys-amended soil because it was converted mainly to selenite and selenide.

A very important factor influencing phytovolatilization is the concentration of sulfate compared to selenate in the substrate. Selenium volatilization by broccoli was inhibited strongly by increased sulfate supply: Zayed & Terry (173) showed that with increase in the sulfate concentration in nutrient solution from 0.25 to 10 mM, the rate of Se volatilization by broccoli plants decreased from 97 to 14 $\mu\text{g Se m}^{-2}$ leaf area day⁻¹. The inhibition in volatilization with sulfate increased progressively with the increase in the ratio of S/Se in plant tissue. These results suggested that sulfate competed with selenate for uptake and that other S compounds out-competed their Se analogs for the active sites of enzymes responsible for the conversion of inorganic Se to volatile forms. The inhibitory effects of sulfate on Se volatilization by plants were less detectable when Se was supplied as selenite or SeMet (171).

Selenium volatilization rates vary enormously in the field, with very high rates occurring at certain times of the year, especially in spring and early summer (73, 152). Lin & Terry (unpublished data) examined the potential effects of 15 different environmental and plant factors on Se volatilization in a constructed wetland, including the Se concentrations in water, sediment, and plants, the microbial biomass in sediments, and the pH, salinity (EC), dissolved oxygen, standing depth, and temperature of water. Multiple-regression analysis indicated that water temperature, Se concentration in sediment (or in roots), and the level of microbial biomass (especially in the rhizosphere) were among the most important environmental factors influencing phytovolatilization.

PHYTOREMEDIATION

Plants have been shown to be highly effective in the phytoremediation of Se-contaminated soils. Bañuelos and his colleagues reduced the total Se inventory in the top 75 cm of soil from California's Central Valley by almost 50% over a period of 3 years (14). With their copious root systems, plants can scavenge large areas and volumes of soils, removing Se as selenate, selenite, and organic

forms of selenium such as SeMet. Once absorbed by plant roots, Se is translocated to the shoot, where it may be harvested and removed from the site, a process called phytoextraction. One difficulty with phytoextraction is that Se is accumulated in plant tissues where it may become available to wildlife, especially birds.

Phytovolatilization, on the other hand, circumvents this problem because it removes Se completely from the local ecosystem into the atmosphere. This minimizes the entry of Se into the food chain, particularly as Se is volatilized by roots (173). Studies have shown that volatile Se entering the atmosphere is dispersed and diluted by air currents directly away from the contaminated areas, with deposition possibly occurring in Se-deficient areas (8).

Many species have been evaluated for their efficacy in phytoremediation (13, 19, 104, 121, 130, 132, 167). Certain species of *Astragalus* were found to accumulate the most Se (e.g. *A. bisulcatus*), which led some researchers to suggest the use of the Se-accumulators, *A. bisulcatus* and *A. racemosus* (121). However, these are slow-growing plants and Se accumulated in *Astragalus* shoots is mostly soluble and can easily be leached from plant tissues back to the soil by rainfall (41).

The ideal plant species for phytoremediation of Se is one that can accumulate and volatilize large amounts of Se, grow rapidly and produce a large biomass on Se-contaminated soil, tolerate salinity and other toxic conditions, and provide a safe source of forage for Se-deficient livestock. Indian mustard (*Brassica juncea*), which typically contains 350 mg Se kg⁻¹ dry weight, has most of the desired attributes (10, 13, 172). It is also easily genetically engineered (16, 172). EAH Pilon-Smits et al (125) transformed Indian mustard by overexpressing the gene encoding ATP sulfurylase; the transgenic plants exhibit much promise in the cleanup of Se from contaminated soils in that they accumulate two- to threefold more Se per plant than the wild-type plants.

Another major environmental problem is how to cleanup Se from contaminated water. One effective solution to this problem is to use constructed wetlands: up to 90% of the Se from oil refinery effluents was shown to be removed by this means (73). Although much of the Se is retained in the sediments, a substantial portion is taken up and immobilized into plant tissue, and a further portion (possibly as much as 10% to 30%) may be volatilized to the atmosphere (73). The choice of suitable wetland species is important in the cleanup of Se-contaminated wastewater by constructed wetlands because different species vary substantially in their ability to absorb, accumulate, and volatilize Se. Ten wetland cells were planted with different plant species in mono- and mixed cultures to evaluate the best plant species composition for maximum removal of Se from irrigation drainage water (150). This study, located at the Tulare Lake Drainage District at Corcoran, California, is now entering its third year; early results indicate that Se removal is greatly enhanced by the planting of wetland vegetation and that cattail (high biomass) and widgeon grass (high bioaccumulation) removed the greatest amount of Se per unit area of wetland (150).

One problem most often raised in connection with Se phytoremediation is how to dispose of Se-containing vegetation. Because Se is an essential trace element for adequate nutrition and health in humans and animals, one solution is to use seleniferous plant materials as a forage blend in Se-deficient regions to improve the Se status of animals. Another option is to add Se as a source of organic Se fertilizer to soils supporting forage crops (e.g. alfalfa; 12). Plants used for the phytoremediation of Se may generate other useful byproducts such as fibers for the production of paper and building materials, energy for heat production by the combustion of dried plant material or by fermentation to methane or ethanol. In addition, a large variety of chemical compounds (e.g. oil, sugars, fatty acids, proteins, pharmacological substances, vitamins, and detergents) are naturally produced by plants and may be useful byproducts of the phytoremediation process (115).

CONCLUSIONS AND FUTURE DIRECTIONS

Despite the fact that Se has been shown to be an essential micronutrient for animals, bacteria, and probably algae, the question of whether Se is required for the growth of higher plants is still controversial and unresolved. Clearly, one of the most important goals for future Se research is to continue to search for the presence of essential selenoproteins in higher plants using protein or DNA sequence analysis. Advances in the sequencing of genomes of higher plants such as *Arabidopsis* and rice may facilitate the discovery of genes containing in-frame UGA codons or homologs of the genes encoding SeCys incorporation enzymes (e.g. selenophosphate synthetase). This would provide strong (though indirect) evidence that SeCys-containing selenoproteins are present in plants and thus support the view of Se essentiality in plant growth.

Molecular studies have enabled significant strides to be made in other areas of Se physiology and biochemistry. Overexpression of genes encoding transporters and enzymes involved in the uptake, assimilation, and volatilization of sulfate has been highly successful in elucidating the role of these proteins in Se metabolism and in identifying rate-limiting steps in the assimilation of selenate to volatile Se. For example, it is now well established that selenate is taken up actively via a sulfate transporter in the root plasma membrane, that the S enzyme, ATP sulfurylase, mediates the reduction of selenate in plants, and that the reduction of selenate and the conversion of organic Se to volatile Se is rate limiting to Se uptake and assimilation.

One important area in need of more research is the role of rhizosphere bacteria in Se volatilization by plants. Plants volatilize relatively low amounts of selenate or selenite in the absence of bacteria, and it is not clear why this is so. Part of the reason may be that rhizosphere bacteria are required to facilitate the uptake of selenate into root tissues, possibly by providing key proteinaceous compounds. Furthermore, bacteria promote the conversion of SeCys to SeMet, thereby facilitating selenite volatilization.

The ability of plants to absorb, sequester, and volatilize Se has important implications in the management of environmental Se contamination by phytoremediation. Indian mustard has been identified as a plant species that has a superior ability to accumulate and volatilize Se, grow rapidly on Se-contaminated soil, tolerate salinity and other toxic conditions, and provide a safe source of forage for Se-deficient livestock. By overexpressing genes encoding key enzymes in the S assimilation pathway, it has been possible to genetically engineer plants to enhance their ability for Se phytoremediation.

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