Plants, selenium and human health

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Selenium is an essential nutrient for animals, microorganisms and some other eukaryotes. Although selenium has not been demonstrated to be essential in vascular plants, the ability of some plants to accumulate and transform selenium into bioactive compounds has important implications for human nutrition and health, and for the environment. Selenium-accumulating plants provide unique tools to help us understand selenium metabolism. They are also a source of genetic material that can be used to alter selenium metabolism and tolerance to help develop food crops that have enhanced levels of anticarcinogenic selenium compounds, as well as plants that are ideally suited for the phytoremediation of selenium-contaminated soils.

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Abbreviations

APSe	adenosine 5′-phosphoselenate
DMDSe	dimethyldiselenide
DMS	dimethylsulfide
DMSe	dimethylselenide
DMSeP	dimethylselenoiopropionate
DMSP	dimethylsulfoniopropionate
GMCys	glutamyl-methylcysteine
MCys	methylcysteine
MCysSO	methylcysteine sulfoxide
MSeC	methylselenocysteine
SECIS	selenocysteine insertion sequence
SeMM	Se-methylmethionine
SMM	*S*-methylmethionine
SMT	selenomethyltransferase

Introduction

Selenium (Se) is an essential nutrient for animals, humans, and microorganisms. Interest in Se has historically focused on its toxicity, and this is especially important in the southwest United States where soils that contain naturally high concentrations of Se soils. Poisoning results when plants that accumulate high concentrations of Se are eaten by livestock and other animals [1]. In fact, it is thought that General Custer might have survived his trip to the Little Bighorn if reinforcements had not been delayed by pack-animals that were apparently suffering from Se-induced lameness. The potential health benefits of some Se compounds and an interest in the phytoremediation of sites that are contaminated by Se have resulted in the increased study of Se biochemistry in plants [2,3•]. The existence of naturally occurring Se-accumulating plants has been known in the scientific literature for at least 70 years. Only recently, however, has an interest developed in using these unusual plants as tools to improve our basic understanding of Se biochemistry in plants. Such Se-accumulating plants also provide a rich genetic resource that we are beginning to exploit to develop food crops that are enriched in anticarcinogenic Se compounds for improved public health. These genetic resources are also being used to develop plants that are ideally suited for the phytoremediation of Se-contaminated soil and water. In this review, we discuss plant selenium biochemistry, selenium hyperaccumulators and the potential to alter selenium metabolism in economically important plants.

Selenium biochemistry

Se is primarily taken up from the soil by plants as selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}). Selenate directly competes with sulfate for uptake by plants, and *Arabidopsis thaliana* mutants that lack a functional sulfate transporter are resistant to selenate [4•,5•,6,7]. After uptake, it has been proposed that selenate is primarily transported into the chloroplasts, where it is processed by the sulfur assimilation pathway (see [8••,9] for reviews). Selenate is thought to be activated by ATP sulfurylase, forming adenosine 5′-phosphoselenate (APSe) [10,11]. Overexpression of ATP sulfurylase in Indian mustard (*Brassica juncea*) has confirmed that the activation of selenate to APSe by ATP sulfurylase is one of the rate-limiting steps for selenate assimilation in plants [12]. This finding agrees with previous studies which showed that the reduction of SeO_4^{2-} to SeO_3^{2-} in Indian mustard is the rate-limiting step in the biosynthesis of organic Se compounds [13]. In the presence of glutathione *in vitro*, bound APSe is non-enzymatically reduced to selenite [14]. In *Escherichia coli*, however, the reduction of APSe clearly requires 3′-phosphoadenosine 5′-phosphosulfate (PAPS) reductase [15]. It would therefore appear likely that *in planta* selenate is activated to APSe by ATP sulfurylase and then reduced to selenite by adenosine 5′-phosphosulfate (APS) reductase. Once selenate is reduced to selenite, there is strong evidence to suggest that it is non-enzymatically reduced to selenide by glutathione [16]. Ng and Anderson [16] observed the

synthesis of selenocysteine from *O*-acetyl serine (OAS) and selenite only in the presence of purified cysteine synthase, glutathione reductase, glutathione and NADPH. Selenocysteine was synthesized from OAS and selenide by cysteine synthase, leading Ng and Anderson to conclude that the only source of selenide in this assay was the non-enzymatic reduction of selenite by glutathione [16]. The existence of this non-enzymatic pathway for the reduction of selenite to selenide explains why selenite is more readily assimilated by plants than selenate [13]. The reduction of selenite results in the production of selenoamino acids, such as selenocysteine and selenomethionine. The non-specific incorporation of selenoamino acids into proteins contributes to Se toxicity [17].

Once synthesized by the methionine biosynthetic pathway, selenomethionine can be methylated and converted to dimethylselenide (DMSe) and then volatilized [18••]. The first step in the biosynthesis of dimethylselenide is the methylation of selenomethionine to form methylselenomethionine. This step is catalyzed by *S*-adenosyl-*L*-methionine:*L*-methionine *S*-methyltransferase [18••]. When the expression of this enzyme is eliminated in *A. thaliana*, Se volatilization is greatly reduced [18••]. The addition of methylselenomethionine to the growth medium restores the volatilization of DMSe. The conversion of Se-methylmethionine (SeMM) to DMSe is probably carried out by *S*-methylmethionine hydrolase, which normally converts *S*-methylmethionine (SMM) to dimethylsulfide (DMS) [19]. Alternatively, DMSe can be produced by the conversion of SeMM to dimethylselenoiopropionate (DMSeP) in the chloroplast [20]. Just two enzymes, a transaminase/decarboxylase and a dehydrogenase, have been identified in the biosynthesis of dimethylsulfoniopropionate (DMSP) from SMM. It is likely that these enzymes are also responsible for the conversion of SeMM to DMSeP [21]. It has been suggested that DMSP lyase is responsible for the conversion of DMSP and DMSeP to DMS and DMSe, respectively [20]. DMSP lyase, however, has not yet been identified in plants. Volatilization of DMSe from Indian mustard plants that were supplied with DMSeP was significantly higher than that of plants supplied with selenomethionine, suggesting that DMSeP can be converted to DMSe in plant shoots [20]. Both pathways for the production of DMS/DMSe may exist in plants. Recently, Se volatilization by plants has received attention as a possible method for the phytoremediation of Se-contaminated soils. Phytovolatilization would have the advantage over phytoaccumulation of not producing highly Se-enriched plant material that would require special disposal [2].

Essentiality of selenium

Organisms that require Se for normal cellular function contain essential selenoproteins, such as glutathione peroxidase, formate dehydrogenase, and selenophosphate synthase. Interestingly, the incorporation of selenocysteine into these selenoproteins is directed by a specific tRNA that recognizes a UGA-opal codon. Normally, the UGA codon acts to terminate translation. In combination with a selenocysteine insertion sequence (SECIS), however, the UGA codon is recognized by the selenocysteine tRNA, which directs the insertion of selenocysteine [22,23]. There is no direct evidence for the specific incorporation of selenocysteine in vascular plants. Several selenoproteins that include a glutathione peroxidase homologue and selenocysteine tRNA have, however, been identified in the model plant system *Chlamydomonas reinhardtii* [24••,25••]. Genes that encode selenoproteins in *C. reinhardtii* contain UGA codons at a position in their sequence that corresponds to the occurrence of selenocysteine in the protein sequence. These genes also contain SECIS elements, but these elements differ from those of animals. Interestingly, the *C. reinhardtii* SECIS elements are capable of directing the correct insertion of selenocysteine into selenoproteins in mammalian cells. This suggests that the plant and animal selenocysteine-insertion systems may share a common origin [24••].

Evidence for the specific insertion of selenocysteine in vascular plants is less definitive. Genes that have homology to those encoding animal selenoproteins have been identified in some plants, but they contain the codon for cysteine rather than that for selenocysteine [26]. A selenocysteine tRNA has, however, been identified in sugar beet [27]. Recently, two laboratories isolated a chloroplastic cysteine desulfurase gene from *Arabidopsis* [28••,29••]. This gene is a member of the cysteine desulfurase family, which includes enzymes that convert cysteine to alanine and elemental sulfur or selenocysteine to alanine and elemental Se. Members of this family are widespread in both eukaryotes and bacteria, and function in the biosynthesis of Fe–S clusters, thiamine, molybdopterin and elemental Se, as well as in iron homeostasis. The elemental Se that is produced in these reactions is transferred to selenophosphate synthase for the biosynthesis of monoselenophosphate [30•]. Monoselenophosphate is an intermediate in the production of selenocysteine tRNA, which is used to specifically incorporate SeCys into protein. The cysteine desulfurase from *A. thaliana* shows activity with both cysteine and selenocysteine as substrates, but its activity is much greater when selenocysteine is the substrate [28••]. It is unclear if this enzyme functions in S or Se metabolism or both. A possible specific role for Se in higher plants remains to be identified.

Selenium and human health

The importance of Se to human health has become a focus in recent years. Although Se deficiency is rare in the US, it does occur in several parts of the world, such as China, where concentrations of Se in the soil are low. Se deficiency can lead to heart disease, hypothyroidism and a weakened immune system [31]. The toxic effects of

excess Se have been known for some time but, in the past decade, it has become more evident that Se has many potential health benefits beyond meeting basic nutritional requirements. Numerous studies have demonstrated the anticarcinogenic activities of some organic forms of Se against certain types of cancer [3•,32,33]. In a long-term double-blind study, supplemental Se was associated with significant reductions in lung, colorectal and prostate cancers [34]. There is a great deal of variation, however, in the efficacy of Se compounds against cancer [3•,34]. One of the most effective anticarcinogenic Se compounds is methylselenocysteine (MSeC), which is found in some members of the *Brassica* and *Allium* families, as well as in Se-accumulating *Astragalus* species. MSeC can comprise upwards of 0.6% of the shoot dry weight in *Astragalus* species.

Selenium hyperaccumulators

Most plants contain only low foliar concentrations of Se, of less than 25 μg/g dry weight, and rarely exceed 100 μg/g dry weight even when grown on High-SE soils. They are termed non-accumulators [7]. However, a limited number of specialized plants, which are often found growing on soils that are naturally enriched in Se, can accumulate high concentrations of Se in their foliage. These accumulating plants can be divided into two groups: primary accumulators (hyperaccumulators) and secondary accumulators (indicator species). Primary accumulators have discrimination coefficients ($DC_i = [Se/S]_{plant}/[Se/S]_{solution}$) of more than one in solution culture, and have concentrations of Se in the range of thousands of mg/kg [35]. Examples of primary accumulators include various *Astragalus* species, which are members of the Fabaceae, as well as *Stanleya pinnata*, a member of the Brassicaceae [36]. Secondary accumulators take up Se in proportion to the amount of Se available in the soil, they have a DC_I of less than one, and tissue concentrations of Se in the hundreds of mg/kg [7]. Members of this group include species of *Aster*, *Atriplex* and *Melilotus*, as well as *Brassica juncea* (Indian mustard) [37,38].

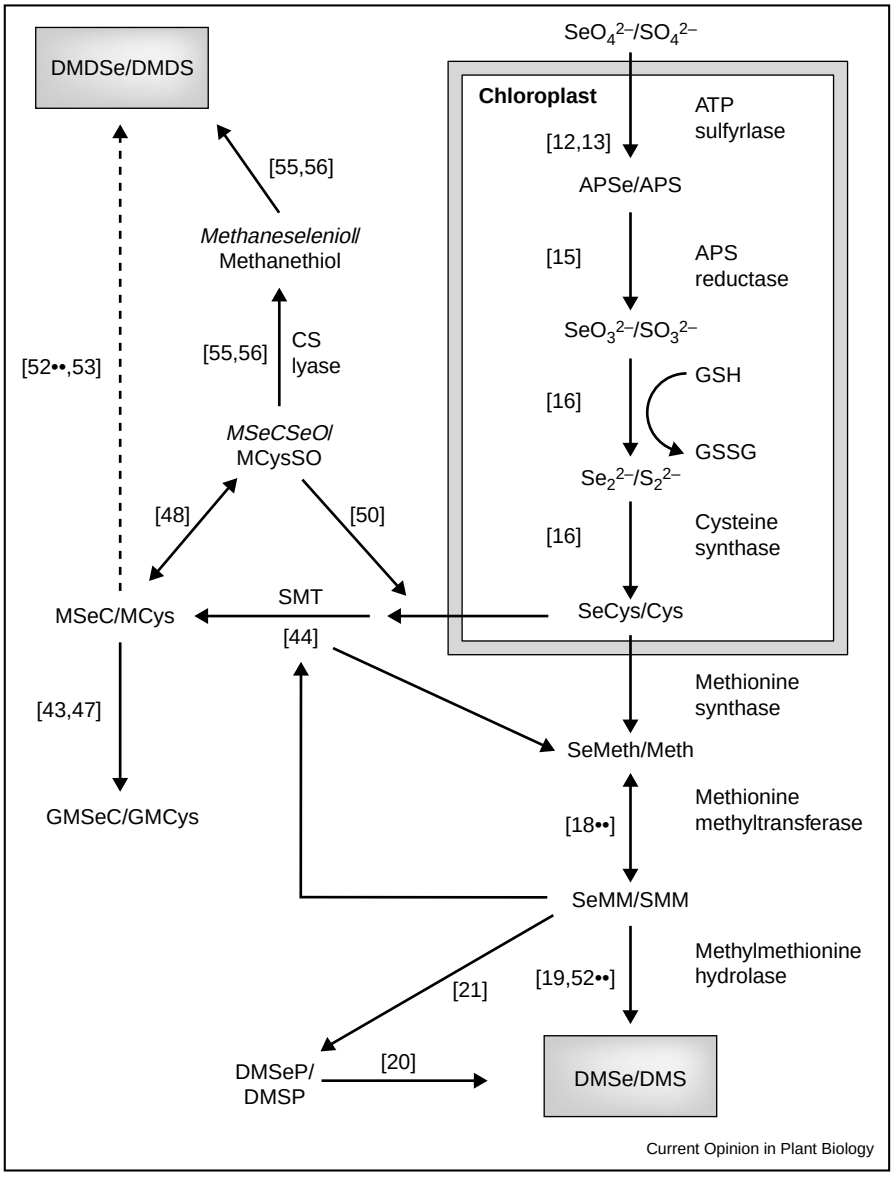
The best-characterized primary Se accumulator is *Astragalus bisulcatus*. This species grows on soils that have naturally high Se concentrations in the southwestern US. It can accumulate up to 0.6% of its shoot dry weight as MSeC [39]. When these plants are grown hydroponically in the presence of selenate, the Se in the older leaves is predominantly inorganic, but young leaves and roots contain 90–95% of their Se as organic Se [40,41••,42]. The non-protein amino-acid methylselenocysteine is the predominate organic Se constituent in these plants [43]. The amino acids MSeC and methylcysteine constitute 5–10% and 20–30%, respectively, of the total free-amino-acid pool in the young shoots of hydroponically grown *A. bisulcatus* (D Salt, unpublished data). MSeC and methylcysteine (MCys) are produced from the methylation of selenocysteine and cysteine, respectively, by the enzyme selenomethyltransferase (SMT) using *S*-methymethionine as the methyl donor [44]. Methylation of selenocysteine prevents its non-specific incorporation into proteins and its conversion into selenomethionine, helping to confer Se tolerance in *A. bisulcatus* [45]. Selenocysteine methyl transferase is expressed equally in the old and young leaves of *A. bisulcatus*, so changes in the expression of this enzyme do not account for the decrease in MSeC observed in older leaves [41••]. The seeds of *A. bisulcatus* also accumulate a conjugated form of MSeC, gamma-glutamyl-methylselenocysteine (GMSeC).

Numerous Fabaceae species, including members of the *Phaseolus* and *Vigna* genera as well as *Meliltius indicus*, produce sulfur analogues of the Se compounds that are observed in *A. bisulcatus*, including MCys in their tissues and gamma-glutamyl-methylcysteine (GMCys) in their seeds. It has been suggested that these genera use MCys as a transportable form of reduced sulfur and GMCys as a storage form in their seeds, as they contain low concentrations of the sulfur amino acids (cysteine and methionine) [46,47]. MCys is also produced by members of the *Brassica*, *Raphanous*, and *Allium* genera [48]. Members of the *Brassica* and *Raphanous* have very little free MCys as it is converted to and primarily accumulated as methylcysteine sulfoxide (MCysSO), which can be converted back to free cysteine [49,50]. The methyl group of the MCys originates from methionine, and it has been assumed that the methyl donor is *S*-adenosyl-methionine. Recent evidence suggests, however, that the methyl donor may be *S*-methylmethionine. The enzyme(s) responsible for producing MCys in these genera have not been identified, and may or may not resemble the selenocysteine methyl transferase from *A. bisulcatus*. It seems likely, however, that members of the Fabaceae produce MCys using an enzyme that is related to *A. bisulcatus* selenocysteine methyl transferase. The presence of methylselenocysteine seleno oxide (MSCSeO) has not been confirmed in plants that produce MCysSO, but it seems likely that MSCSeO is produced in these species.

Indian mustard plants that were putatively transformed with SMT from *A. bisulcatus* (AbSMT) produced approximately 80% of their organic Se as MSeC, and had a reduced concentration of selenomethionine compared to wildtype plants [51••]. These plants volatilize less DMSe than wildtype plants when treated with SeO_4^{2-} or SeO_3^{2-} [52••]. Overexpression of SMT would be expected to lead to increased methylation of selenocysteine, resulting in decreased production of selenomethionine. The resulting reduction in selenomethionine concentration would then cause the observed reduction in the volatilization of DMSe, supporting the proposed pathway for DMSe. The increase in DMSe volatilization in these plants when treated with selenomethionine may result from methylation of selenomethionine by SMT. Alternatively, elevation of selenomethionine

concentrations in the presence of SMT may indirectly impact DMSe production by altering the pool size of other metabolites, including the SMT methyl donor methylmethionine. One of the two putatively SMT-transformed *B. juncea* lines also showed greater volatilization of dimethyldiselenide (DMDSe) than that of wildtype plants, supporting Meija *et al.*'s suggestion that DMDSe is produced from MSeC [52••]. DMDSe production has been reported in the Se hyperaccumulator *Astragalus racemosus*, which is also known to hyperaccumulate MSeC, further supporting the hypothesis that DMDSe is produced from MSeC [53]. Recently, a thiopurine methyltransferase that is capable of producing DMSe and DMDSe when expressed in bacteria grown with SeO_3^{2-}, SeO_4^{2-} and MSeC but not with selenomethionine has been isolated from *Pseudomonas syringae* [54•].

Figure 1



Pathway of sulfate assimilation and proposed pathway of selenate assimilation in higher plants. Subcellular localization of reactions from SMT onward has not been confirmed. Volatile compounds are within shaded boxes. Specific references are given in parenthesis for individual reactions. Compounds that have been suggested to be involved in these pathways but not yet identified are in italics. Dotted arrows indicate reactions that have been suggested but lack sufficient supporting data. The basic pathway for sulfate assimilation was compiled from [8••,9]. The production of MCysSO has only been identified in members of the *Brassica* and *Raphanous* genera. The presence of members of the cysteine sulfoxide lyase family has only been identified in members of the *Allium* and *Brassica* genera. The enzymes that convert SMM and SeMM to DMSeP and DMSP are located in the chloroplast. APS, adenosine 5′-phosphosulfate; Cys, cysteine; DMDS, dimethyldisulfide; GMSeC, glutamyl-methylselenocysteine; GSH, oxidized glutathione; GSSG, reduced glutathione; Meth, methionine; MSeCSeO, methylselenocysteine selenooxide.

Ranjard *et al*. [54•] suggest that methaneselenol may be a key intermediate between these Se compounds and the production of DMSe and DMDSe. This enzyme belongs to a family of thiol methyltransferases that are found in many different animals. It is possible that a similar enzyme or reaction might effect the conversion of MSeC to DMDSe in plants. Members of the *Brassica* and *Allium* genus contain cysteine sulfoxide lyases that (depending on the specific enzyme) can breakdown conjugated cysteine sulfoxides, methylcysteine sulfoxide, and cysteine, leading to the production of volatile compounds such as dimethyldisulfide (DMDS) [55,56]. These reactions occur primarily after tissue injury, suggesting that they play a role in plant defense. It has also been suggested that these reactions function in sulfur remobilization, and it seems likely that these same processes could also result in the production of DMDSe.

Conclusions

Although the recent increase in research on Se biology in plants has expanded our understanding of Se biochemistry, there is still much that is not known. A summary of the proposed pathway for Se assimilation is given in Figure 1. Data to demonstrate whether or not Se is essential in higher plants are still not definitive. The SMT gene from *Astragalus bisulcatus* is currently the only gene involved in Se tolerance that has been isolated from a primary Se accumulator. Primary Se accumulators present an opportunity to further elucidate Se biochemistry. These species are a potential source of genetic material that could be used to alter the Se metabolism of economically important plants. The close relationship between Se and sulfur means that insights gained in studies of Se biology also have the potential to expand our understanding of sulfur metabolism.

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