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Selenium Assimilation and Volatilization from Dimethylselenoniopropionate by Indian Mustard[1]

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Earlier work from our laboratory on Indian mustard (*Brassica juncea* L.) identified the following rate-limiting steps for the assimilation and volatilization of selenate to dimethyl selenide (DMSe): (a) uptake of selenate, (b) activation of selenate by ATP sulfurylase, and (b) conversion of selenomethionine (SeMet) to DMSe. The present study showed that shoots of selenate-treated plants accumulated very low concentrations of dimethylselenoniopropionate (DMSeP). Selenonium compounds such as DMSeP are the most likely precursors of DMSe. DMSeP-supplied plants volatilized Se at a rate 113 times higher than that measured from plants supplied with selenate, 38 times higher than from selenite, and six times higher than from SeMet. The conversion of SeMet to selenonium compounds such as DMSeP is likely to be rate-limiting for DMSe production, but not the formation of DMSe from DMSeP because DMSeP was the rate of Se volatilization from faster than from SeMet and SeMet (but no DMSeP) accumulated in selenite- or SeMet-supplied wild-type plants and in selenate-supplied ATP-sulfurylase transgenic plants. DMSeP-supplied plants absorbed the most Se from the external medium compared with plants supplied with SeMet, selenate, or selenite; they also accumulated more Se in shoots than in roots as an unknown organic compound resembling a mixture of DMSeP and selenocysteine.

Selenium accumulation and volatilization by plants have been shown to be effective for the phytoremediation of Se-contaminated soil and water (Bañuelos et al., 1995; Hansen et al., 1998; Terry and Zayed, 1998). Se volatilization is particularly attractive for the phytoremediation of contaminated environments because inorganic Se is converted to the gas dimethylselenide (DMSe), which is approximately 600 times less toxic than inorganic Se (McConnell and Portman, 1952; Wilber, 1980). By understanding the physiology and biochemistry of Se assimilation and volatilization, it should be possible to enhance the efficiency of Se phytoremediation. Indian mustard (*Brassica juncea* L.) has been used as a model plant to study Se metabolism because field experiments have shown it to be an excellent candidate for the phytoremediation of Se-contaminated soil (Bañuelos and Meek, 1990; Bañuelos et al., 1995). This plant is not a Se hyperaccumulator but it takes up and volatilizes Se at high rates compared with other plants (Terry et al., 1992).

Se, a chemical analog of S, is thought to be assimilated and volatilized by plants using the same enzymes of the S assimilation pathway (Brown and Shrift, 1982; Terry and Zayed, 1994). Indeed, recent experiments with transgenic plants demonstrated that the proposed Se assimilation and volatilization pathway (Terry and Zayed, 1994) uses enzymes of the S assimilation and volatilization pathway. For example, selenate, the analog of sulfate, was shown to be transported by sulfate permease (S. Hwang and N. Terry, unpublished results) and then reduced via ATP sulfurylase (APS), the enzyme that activates sulfate (Pilon-Smits et al., 1999). Selenate and selenite (the inorganic species of Se) are incorporated into seleno-Cys (SeCys) and seleno-Met (SeMet) in a manner similar to the assimilation of sulfate into Cys and Met (Brown and Shrift, 1982; Anderson and Scarf, 1983; Anderson, 1993). Kinetic studies have demonstrated that selenate uptake and reduction were rate-limiting steps for Se assimilation and volatilization (de Souza et al., 1998). Rhizosphere bacteria facilitate the accumulation of selenate into plant tissues, thereby overcoming the first rate-limiting step of selenate uptake (de Souza et al., 1999). Subsequently, the enzyme APS was shown to be responsible for selenate reduction in vivo by overexpressing APS in Indian mustard, which resulted in increased reduction of selenate to organic Se forms such as SeMet (Pilon-Smits et al., 1999). X-ray absorption spectroscopy (XAS) showed that wild-type Indian mustard treated with selenate accumulated Se, mainly in the form of selenate, in root and shoot tissues, whereas selenite- or SeMet-treated plants accumulated an organoselenium compound similar to SeMet (de Souza et al., 1998; Zayed et al., 1998). SeMet is volatilized much more readily than selenate or selenite; the rate of Se volatilization measured separately from roots and shoots of Indian mustard supplied with SeMet was 48 to 100 times higher than plants supplied with selenate or selenite (Zayed et al., 1998).

In order for volatile DMSe to be produced from SeMet, a methyl group has to be added to SeMet, producing a selenonium compound such as Se-methylSeMet or dimethylselenoniopropionate (DMSeP). This view is derived by analogy to the pathway of dimethylsulfide (DMS) production, where the major precursors of DMS in higher plants are the sulfonium compounds *S*-methyl-Met and dimethylsulfoniopropionate (DMSP) (Dacey et al., 1987; Mudd

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and Datko, 1990; Rennenberg, 1991), which are also thought to serve as osmoprotectants (Hanson et al., 1997). *Spartina alterniflora*, an aquatic halophyte that accumulates very high concentrations of DMSP and also produces DMS at very high rates (Dacey et al., 1987), was recently shown to accumulate Se-methylSeMet and DMSeP (Ansede et al., 1999). These results show that plants can convert selenate to DMSeP. The conversion of selenate to SeMet is likely to occur by the enzymes of the S assimilation pathway (Terry and Zayed, 1994), while SeMet would be converted to DMSeP by the DMSP biosynthetic pathway from Met (Hanson et al., 1994, 1997; Kocsis et al., 1998). Thus, SeMet may be methylated to Se-methylSeMet, which would be the most likely stable precursor of DMSeP. Evidence supporting this view was obtained by Lewis et al. (1974), who showed that Se-methylSeMet was the source of DMSe production in cabbage leaves. DMSeP served as the substrate for DMSe in bacteria (Ansede and Yoch, 1997), which produced DMSe via the enzyme DMSP lyase (de Souza and Yoch, 1995).

That DMSeP is the precursor of DMSe has been established for the halophyte *S. alterniflora* under conditions of high Se concentrations (52–260 μM selenate) and high salinity (Ansede et al., 1999). To determine if DMSeP is the precursor of DMSe in Indian mustard under the physiological conditions used in our earlier studies on rate limitation (de Souza et al., 1998; Zayed et al., 1998; Pilon-Smits et al., 1999), we used HPLC, gas chromatography (GC), and XAS to detect DMSeP in plant tissues. Secondly, assuming that DMSeP is a precursor in Indian mustard, we wished to determine which step in DMSe production from SeMet is rate limiting, the conversion of SeMet to DMSeP or the conversion of DMSeP to DMSe. This was tested by supplying SeMet and DMSeP to plants and comparing the rates of volatilization and the amount and form of Se accumulated in plant tissues for each form supplied.

MATERIALS AND METHODS

To compare the assimilation of DMSeP with other chemical forms of Se by Indian mustard (*Brassica juncea* L.), selenate, selenite, SeMet, and DMSeP were supplied to plants. To show that plants can assimilate and volatilize DMSeP, several analytical tools were used to: (a) demonstrate that the salt that was synthesized was indeed DMSeP, (b) determine the amount and form of Se accumulating in plant tissues, and (c) measure the amount of Se volatilized.

DMSeP Synthesis and Mass Spectroscopy (MS) Analysis

DMSeP was synthesized by the method described for DMSP synthesis (Chambers et al., 1987), except that DMSe was used instead of DMS. Bubbling of HCl gas into the DMSe/acrylic acid mixture resulted in a hygroscopic precipitate, which was washed and recrystallized with ethanol to obtain white crystals. The DMSeP crystals were placed in a dessicator with silica gel at room temperature. Crystals of DMSeP were weighed and a 20 mM stock solution was prepared in water. When stock solutions of higher concentration were prepared and kept at 4°C, DMSeP precipitated in the form of spherical crystals. DMSP was obtained from Research Plus (Bayonne, NJ). For this study, freshly prepared 20 mM stock solutions were prepared before each experiment. Positive ion electrospray liquid chromatography/MS was used to determine the purity of the DMSeP preparation, and carried out on a triple quadrupole mass spectrometer (VG Trio 3, Analytica, Branford, CT) using an Atmospheric Pressure Ionization source. Samples were analyzed by directly injecting them into the spectrometer. A mobile phase of deionized water: acetonitrile with 0.1% (w/v) acetic acid was used (1:1, v/v).

Plant Growth, Se Treatment, and Analysis

Indian mustard seeds were obtained from the North Central Regional Plant Introduction Station in Ames, IA (accession no. 173847). Plants were grown in soil (UC mix: a mixture of peat moss, fertilizer, and coarse sand) for 6 weeks, after which time they were washed in water and placed in boxes containing one-half-strength Hoagland solution (Hoagland and Arnon, 1938). The solutions were aerated, and the boxes maintained in a greenhouse with a 9-h daylength and a controlled temperature of between 25°C and 30°C. The plants were allowed to grow under these conditions for 1 week, after which time Se (20 μM) was added as DMSeP, SeMet, sodium selenite, or sodium selenate. The plants were treated with Se for 1 week, after which time the hydroponic solution containing the appropriate Se form was replaced, and Se volatilization was measured in airtight chambers as described previously (Zayed and Terry, 1992; de Souza et al., 1998). After 24 h of collection of volatile Se in alkaline peroxide trap solution, the plants were washed thoroughly in running distilled water, and dried at 55°C for 3 d. Shoots and roots were separated from each other, weighed, and ground to a fine powder in a Wiley mill. The dry weights of all plants used in this study were not significantly different from each other ($P > 0.05$), with shoots and roots averaging 1.56 and 0.7 g, respectively. The Se content of the dried plant material was measured by vapor-generation atomic absorption spectroscopy of acid-digests of the dried plant material (Martin, 1975; Logan et al., 1987). Three replicates were used for all treatments. The total amount of Se in plant tissues was obtained by multiplying the Se concentration in the plant part by the dry weight of that part. The total uptake of Se by plants supplied with different chemical forms of Se was estimated by adding the total amount of Se accumulated per plant (root plus shoot) to the amount of Se volatilized over the 8-d exposure to Se. The amount of Se volatilized by plants over the entire 8-d exposure to Se was calculated from the rate of Se volatilization measured after 7 d of Se pretreatment, because this rate was shown to be linear over a 14-d period (de Souza et al., 1998).

HPLC Analysis

Analysis of DMSeP was carried out on a HPLC (LC-10A, Shimadzu, Columbia, MD) with a diode array detector (model SPD-M10, AVP, Mason Techology, Dublin, Ire-

land). Separations were performed by cation exchange chromatography at room temperature with a stainless steel column (250- × 4.6-mm i.d.) packed with Partisil 10 SCX (phenyl sulfonic acid groups bound to a silica support). The analyses were carried out under isocratic conditions. The mobile phase was water containing 5% methanol buffered with 0.05 M KH_2PO_4. The column and mobile phase were those used by Gorham (1984) for the separation of Gly betaine and DMSP, and more recently by Colmer et al. (1999), for the simultaneous separation and detection of Pro, Gly betaine, DMSP, Pro betaine, and Arg in plant leaf extracts. Dried plant samples (0.3 g) were ground using liquid nitrogen. Before the samples thawed, 1 mL of 10% HCl was added. A few drops of water were added to produce a homogenous paste. This paste was transferred to a centrifuge tube, and centrifuged for 15 min at 1,500*g*. The liquid phase was filtered through a 0.45-μm membrane filter, transferred to a volumetric flask, and made up to 10 mL, giving a clear solution for injection into the sample loop (20 μL) of the HPLC. DMSP and DMSeP in the plant extracts were detected by comparing residence times of peaks with those obtained from standard solutions of these onium compounds.

Analysis of DMSeP Content by GC

DMSeP in plant tissues was analyzed by measuring base-hydrolyzable DMSe, as described by Ansede et al. (1999). In this assay, plant tissues were digested with NaOH, which carries out the same elimination reaction as the enzyme DMSP lyase, cleaving DMSeP to an equimolar amount of DMSe. Samples (5–25 μL) of a 10 mM DMSeP stock solution or 0.3 g of plant tissue were treated with 5 mL of 4.5 M NaOH in 15-mL glass serum vials. The vials were immediately capped with Teflon-faced butyl rubber stoppers, and sealed with aluminum crimp caps. The base elimination reaction producing DMSe was allowed to proceed at room temperature for 24 h. DMSe was analyzed by injecting 0.25 mL of headspace gas into a gas chromatograph (model 3700, Varian, Palo Alto, CA) equipped with a flame ionization detector. The column contained 10% Carbowax 1000 on a Chromosorb W-AW support. The injector and detector temperatures were 150°C, and the oven temperature was 75°C. These conditions allowed for the separation of DMSe from its S analog, DMS, similar to the chromatographic data presented in Ansede et al. (1999).

XAS Analysis

Roots and shoots of plants supplied with 20 μM SeMet or DMSeP were frozen at −80°C, and analyzed by XAS to determine the chemical form of Se present in tissues without using chemical extraction methods. The XAS analysis was performed at the Stanford Synchotron Radiation Laboratory on beam line 4-3. The electron energy was 3.0 GeV, with a current of approximately 50 to 100 mA. A Si(220)-8 double-crystal spectrometer was used to monochromatize the x-rays, which were detuned 50% for harmonic rejection, and positioned with a 1-mm entrance slit that produced a beam of approximately 1-eV band width. Stock solutions of standard reference solutions of 10 mM DMSeP, SeMet, and SeCys were used. SeCys was synthesized from selenocystine and dithiothreitol as described previously (Esaki et al., 1981). Standard reference solutions or plant tissue samples were placed in a sample chamber at a 45° angle to the x-ray beam. Fluorescent x-ray spectra were obtained by a series of replicate scans dependent on trace element concentration. The energy positions of all spectra were calibrated against a Se reference foil.

RESULTS

Analysis of DMSeP by MS, HPLC, and GC

The white crystals of DMSeP that were synthesized from DMSe and acrylic acid were analyzed by MS (Fig. 1), and showed peaks which exactly matched the predicted pattern based on the formula $C_5H_{11}O_2Se$. Furthermore, the mass spectrum showed that no other major contaminants were present in the preparation. The DMSeP was also analyzed by HPLC, using a method developed to separate DMSeP (Fig. 2A) from its S analog, DMSP (Fig. 2B). DMSeP had a longer retention time compared with its chemical analog, DMSP. The finding that DMSeP could be separated by HPLC from its chemical analog is important, because many different plants produce DMSP (Paquet et al., 1995).

Indian mustard plants treated with 20 μM selenate contained trace levels of DMSeP in the shoots (Fig. 2C). The indirect GC assay for DMSeP (i.e. DMSe produced from selenate-treated plants digested with strong base) could not measure detectable levels of DMSeP (data not shown). Similar results were obtained from plants supplied with selenite and from selenate-treated transgenic plants overexpressing ATP sulfurylase (E.A.H. Pilon-Smits, M.P. de Souza, and N. Terry, unpublished data).

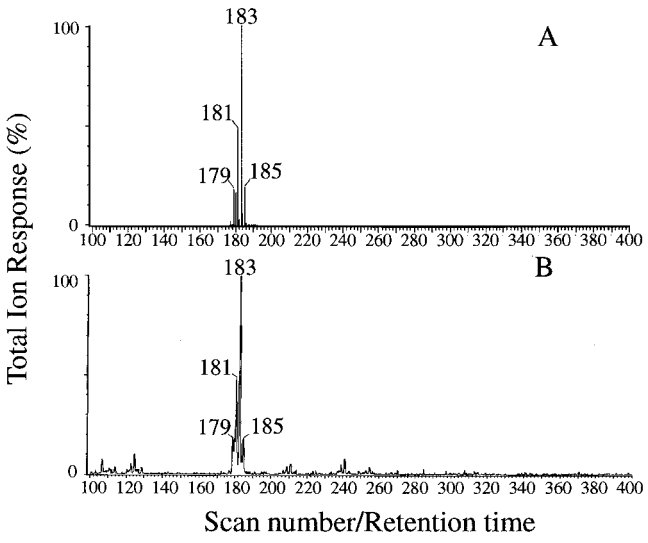

Figure 1. The predicted mass spectrum of DMSeP (A) from a computer model based on the M_r of the compound. The mass spectrum of DMSeP salt crystals (B) was very similar to the predicted spectrum.

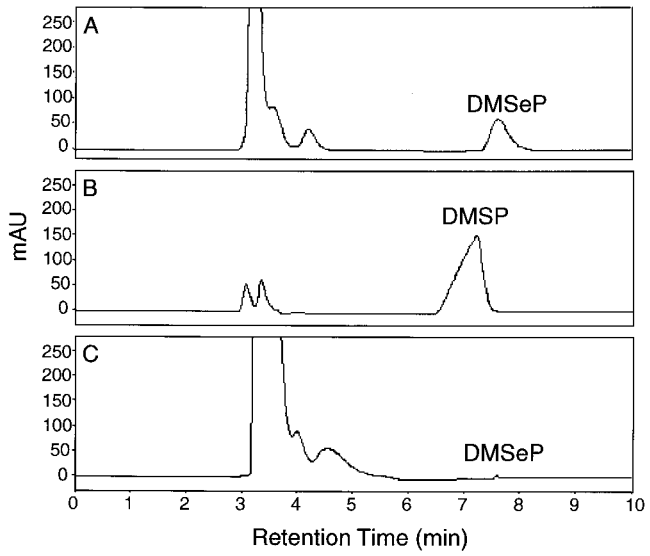

Figure 2. Analysis of DMSeP and DMSP by HPLC. DMSeP (A, 10 mM) had a longer retention time than its sulfonium analog, DMSP (B, 25 mM). An extract of an Indian mustard plant treated with 20 μM selenate showed only a trace amount of DMSeP (C). The peaks with earlier retention times that DMSeP and DMSP represent anionic compounds including acrylic acid, a by-product of the degradation of DMSeP or DMSP to DMSe or DMS, respectively.

Chemical Speciation of Se in Tissues of Plants Supplied with SeMet or DMSeP

DMSeP had a very distinct XAS spectrum from other organic Se forms to which it is related, SeCys and SeMet (Fig. 3A). The x-ray absorption near edge spectrum (XANES) of Se in roots and shoots of plants supplied with SeMet were similar to each other and to the SeMet reference (Fig. 3B). Thus, SeMet-supplied plants accumulate Se in tissues in the form of SeMet. XANES of DMSeP-treated Indian mustard leaf and root samples were similar to each other (Fig. 4A); however, there were differences in x-ray energy and post-edge characteristics in both leaf and root XANES of the unknown Se compound compared with the DMSeP reference (Fig. 4A), suggesting a change in valence or that the Se is bound to a different ligand. Plants supplied with DMSeP transformed it to an unidentified organic form of Se, most similar to a combination of DMSeP and another organic Se species such as SeCys (Fig. 4B). In comparison, plants supplied with selenite accumulated an organic Se form very similar to SeMet, whereas plants treated with selenate accumulated mainly selenate (Table I).

Se Volatilization and Accumulation from DMSeP-Supplied Plants

The highest rates of Se volatilization were obtained from DMSeP, followed by SeMet, selenite, and selenate (Fig. 5). Rates of Se volatilization were six times higher from plants supplied with DMSeP compared with SeMet, 38 times higher than selenite, and 113 times higher than selenate. DMSeP-treated plants accumulated significantly less Se in roots than plants supplied with other forms of Se (Fig. 6). DMSeP-treated plants and selenate-treated plants accumulated more Se in shoots than in roots, whereas plants treated with selenite or SeMet accumulated more Se in roots compared with shoots. Plants supplied with DMSeP, selenate, or SeMet accumulated more Se per plant (root plus shoot) than selenite-supplied plants. The concentrations of Se and the amount of Se in each plant part showed similar patterns for the different Se treatments, because all plants had very similar dry weights. In spite of the large shoot biomass (compared with roots), the distribution pattern of Se accumulated in shoots and roots was the same on a concentration basis or when expressed as the total amount of Se per plant part. The only exception was for the selenate-treated plants, which accumulated similar concentrations of Se in roots and shoots. The total amount of Se absorbed (amount volatilized plus amount accumulated) was highest for plants supplied with DMSeP, followed in descending order by SeMet, selenate, and selenite (Table I).

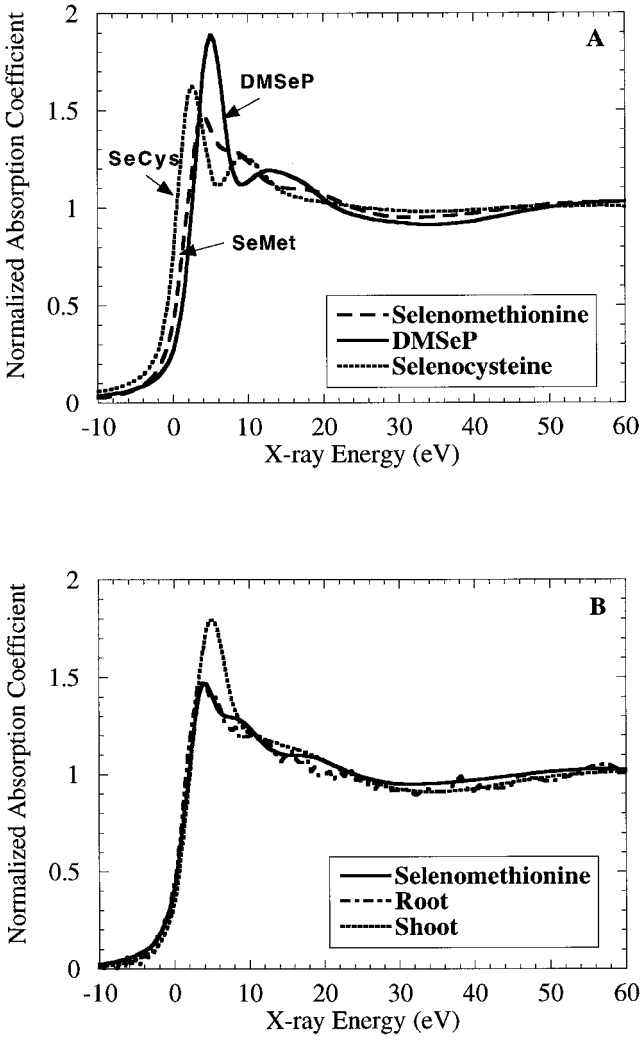

Figure 3. A, DMSeP shows a distinct XAS compared with SeMet and seleno-Cys. B, XAS analysis of an Indian mustard plant treated with 20 μM SeMet shows that its shoot and root accumulate an organic Se form with a spectrum very similar to that of an SeMet standard. All standards were analyzed at a concentration of 10 mM.

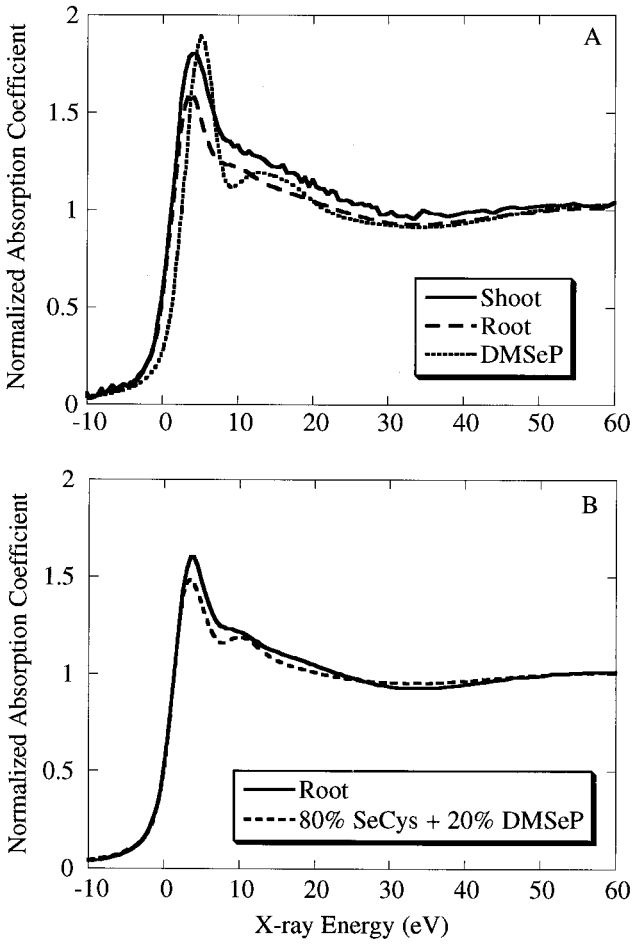

Figure 4. XAS analysis of the shoot and root of an Indian mustard plant treated with 20 μM DMSeP shows that plants can metabolize DMSeP to an organic Se form with a spectrum different from DMSeP (A). The spectral characteristics of the unknown form are most similar to a blend of 80% SeCys and 20% DMSeP (B).

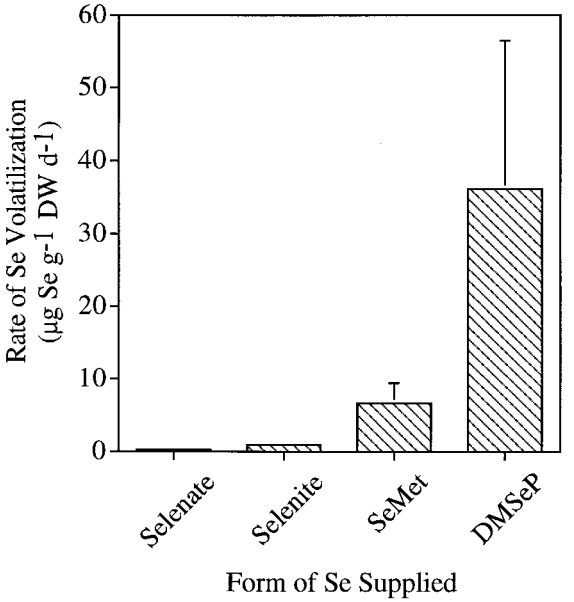

Figure 5. The rate of Se volatilization measured from DMSeP compared with other organic and inorganic Se forms supplied to Indian mustard plants at a concentration of 20 μM. The mean and SD of three replicates is shown.

DISCUSSION

Although DMSeP was detected by the HPLC assay in shoots of Indian mustard plants supplied with selenate under the same physiological conditions used in our earlier studies on rate-limitation, the concentrations measured were very low. Since volatile Se was produced from DMSeP at very high rates (113 times higher than selenate), it is perhaps not surprising that we were unable to detect high concentrations of DMSeP in plants supplied with selenate. In fact, almost 60% of the Se taken up by DMSeP-supplied plants was volatilized, compared with 21.5% in SeMet-supplied plants, 6.3% in selenite-supplied plants, and only 2% in selenate-supplied plants (Table I). The fact that DMSeP was so readily taken up, assimilated, and volatilized is consistent with the view that DMSeP may serve as a precursor of DMSe in Indian mustard. Furthermore, plants supplied with selenate are unlikely to accumulate DMSeP, in spite of the fact that they contain 100 $\mu g\ g^{-1}$ (1.25 mM) total Se in shoots and in roots, because of the rate limitations involved in the conversion of inorganic Se to SeMet and selenonium compounds such as DMSeP (see below). Indeed, XAS showed that selenate-supplied Indian mustard plants mostly accumulated Se in tissues in the form of selenate (de Souza et al., 1998).

Assuming that selenonium compounds such as DMSeP serve as precursors of DMSe, it would appear that the

Table I. *Use of XAS to determine the chemical form of Se accumulated in shoots and roots of Indian mustard and broccoli (both Brassicaceae) supplied with different forms of Se*

These data are summarized from Figs. 3B and 4 (de Souza et al., 1998; Zayed et al., 1998).

Form of Se Supplied	Type of Plant	Form of Se in Plant Tissue	Total Absorption[a]	% Volatilized (of Se Absorbed)
			μg Se	
Selenate	Broccoli or Indian mustard	Selenate; small amount of SeMet	382 ± 151	1.8
Selenite	Broccoli or Indian mustard	All SeMet	157 ± 61	6.3
SeMet	Broccoli or Indian mustard	All SeMet	529 ± 114	21.5
DMSeP	Indian mustard	Unknown organic form[b]	953 ± 375	59.6

[a] Total absorption is the sum of the amount of Se volatilized and the amount accumulated in plant tissues after 8 d. [b] The unknown organic Se form most closely resembles a mixture of 80% SeCys and 20% DMSeP.

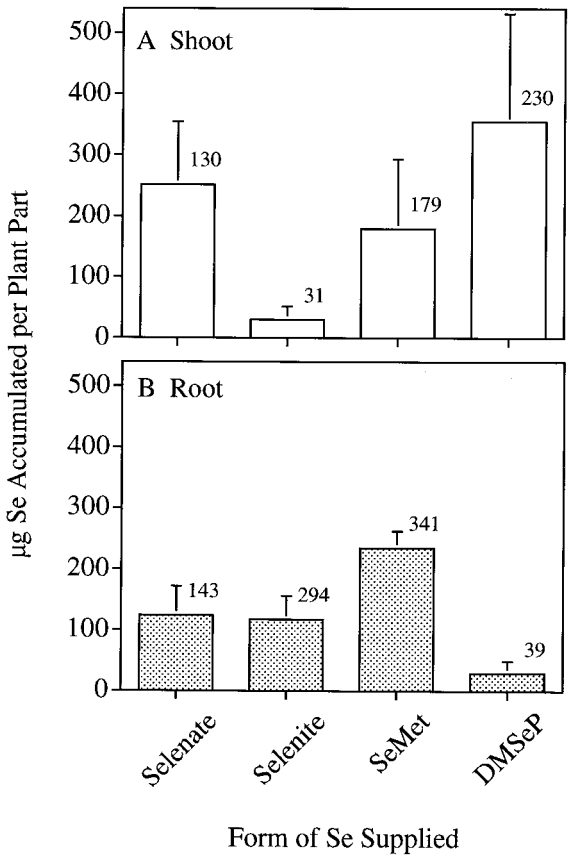

Figure 6. Total amount of Se accumulated in plant shoots (A) and roots (B) after 8 d of treatment with DMSeP and other forms of Se supplied to Indian mustard, all at 20 μM. The mean and SD of three replicates is shown. The values beside the bars represent μg g^{-1} Se in each plant part.

synthesis of selenonium compounds such as DMSeP from SeMet is rate-limiting for DMSe production rather than the conversion of DMSeP to DMSe. The evidence for this comes from the facts that: (a) DMSeP is volatilized six times faster than SeMet, and (b) selenite- or SeMet-supplied wild-type Indian mustard plants and ATP-sulfurylase transgenic plants supplied with selenate accumulate SeMet (Fig. 3B; Table I; de Souza et al., 1998; Pilon-Smits et al., 1999) but do not accumulate DMSeP (M.P. de Souza, E.A.H. Pilon-Smits, and N. Terry, unpublished GC data). Thus, we have developed the following model of the rate-limiting steps in selenate assimilation and volatilization: The first rate-limiting step in the pathway is the uptake of selenate (de Souza et al., 1998) via sulfate permease (S. Hwang and N. Terry, unpublished data). The second rate-limiting step is the activation of selenate by ATP sulfurylase (Pilon-Smits et al., 1999). From the present work it would appear that the third rate-limiting step is the conversion of SeMet to selenonium compounds such as DMSeP. Thus, we have identified at least three rate-limiting steps in the selenate volatilization pathway.

By analogy to the S volatilization pathway, in which the major precursors of DMS in higher plants are the sulfonium compounds *S*-methyl-Met and DMSP (Mudd and Datko, 1990; Rennenberg, 1991), the selenonium compounds Se-methylSeMet and DMSeP may serve as precursors for DMSe production in Indian mustard. The synthesis of Se-methylSeMet and DMSeP from SeMet may occur via the same biochemical pathway proposed for the synthesis of their S analogs, *S*-methyl-Met and DMSP, from Met in higher plants (Hanson et al., 1994, 1997; Kocsis et al., 1998). The first step in the DMSeP biosynthetic pathway would be the methylation of SeMet to Se-methylSeMet by the cytosolic enzyme Met methyltransferase (James et al., 1995; Bourgis et al., 1999). Se-methylSeMet is likely to be converted to DMSeP in the chloroplast by the enzymes of the DMSP biosynthetic pathway, and two of these have been identified so far: a transaminase (Rhodes et al., 1997) and a dehydrogenase (Vojtechova et al., 1997). The formation of DMSe from DMSeP (with the release of acrylic acid and a proton) takes place via an elimination reaction catalyzed by strong base or the enzyme DMSP lyase (Ansede et al., 1997), which has been purified from an *Alicaligenes* sp. (de Souza and Yoch, 1995) and is thought to exist in plants (Dacey et al., 1987). In the shoots, volatile DMSe can be directly produced from Se-methylSeMet (Lewis et al., 1974), presumably by the enzyme *S*-methyl-Met hydrolase, which produces DMS from *S*-methyl-Met in higher plants (Giovanelli et al., 1980; Mudd and Datko, 1990).

Plants supplied with DMSeP, selenate, or SeMet absorbed more Se per plant than selenite-supplied plants (Table I). The reason that SeMet and selenate were absorbed to a much greater extent is very likely because they are actively transported into the root (Leggett and Epstein, 1956; Sandholm et al., 1973; Abrams et al., 1990). Selenite uptake is thought to be passive (Bange, 1973; Arvy, 1993), which would explain its relatively low absorption. Since DMSeP was absorbed to the greatest extent compared with the other Se forms, it is possible that its uptake is active, and that it is taken up by the SeMet transport protein because of its chemical similarity to SeMet. The DMSeP-supplied plants accumulated Se in their tissues as an unknown organic compound most resembling a mixture of DMSeP and SeCys, which may have been produced by methyltransferase reactions.

The model we have developed above for selenate assimilation to DMSe includes several chloroplastic enzymes. Since the root is the major site of Se volatilization (roots volatilized Se at rates that were 26 times higher than shoots [Zayed and Terry, 1994]), it is evident that the enzymes of DMSe production (methyl-Met hydrolase and DMSP lyase) must be present in roots. Furthermore, the substrates for DMSe production (Se-methylSeMet and DMSeP) must be present in roots. By analogy to the S pathway, Se-methylSeMet could be synthesized in the roots by the cytosolic enzyme Met methyltransferase (James et al., 1995). On the other hand, DMSeP, which is likely to be synthesized in the chloroplast by the enzymes of DMSP synthesis (Hanson et al., 1997), would have to be transported to the root for conversion to DMSe. Consistent with the fact that the root is the major site of Se volatilization, roots of plants supplied with DMSeP contained much lower concentrations of Se than shoots (Fig. 6).

DMSe is the predominant form of Se produced by Se non-accumulators (Lewis, 1971) such as Indian mustard. The present work provides evidence that the selenonium compound DMSeP is converted to volatile Se much more efficiently than SeMet or inorganic Se compounds. Thus, the conversion of SeMet to selenonium compounds such as DMSeP is likely to be rate-limiting for Se volatilization in addition to the rate-limiting steps of selenate uptake and reduction.

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