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Selenium Biotransformation by the Salt Marsh Cordgrass *Spartina alterniflora*: Evidence for Dimethylselenoniopropionate Formation

JOHN H. ANSEDE,[†] PERRY J. PELLECHIA,[‡] AND DUANE C. YOCH*,[†]

Department of Biological Sciences and Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208

Phytoremediation of toxic inorganic selenium compounds by accumulation, assimilation, and volatilization is an ideal way to rid contaminated soils and sediments of these molecules. In this context, salt marsh cordgrass (*Spartina alterniflora)* was investigated for its potential to produce dimethylselenoniopropionate (DMSeP), which as we have shown can serve as a precursor for the enzymatic volatilization of the relatively nontoxic gas, dimethylselenide (DMSe). Plants grown in sand culture, under varying saline conditions amended with the environmentally toxic form of selenium (selenate) were analyzed for organoselenonium compounds. DMSeP was positively identified in plant tissue and partially purified plant extracts by alkaline degradation to DMSe, ^{1}H and ^{77}Se NMR, and by enzymatic cleavage by DMSP lyase to DMSe (and acrylate). DMSeP levels were highest in plants grown in high salt (full-strength seawater) and high selenium. Preliminary evidence suggests that cordgrass may also produce *Se*-methyl selenomethionine, the putative precursor of DMSeP. This appears to be the first report for the biological assimilation of selenate into DMSeP by a plant species. These findings suggest a possible mechanism for the volatilization of selenium, as DMSe, analogous to that of dimethylsulfide (DMS) production by the salt tolerant cordgrass, *Spartina alterniflora*.

Introduction

The biogenic emission of dimethylselenide (DMSe) from soil (*1*−*3*), plants (*4*−*6*), and algae (*7*, *8*) is recognized as an important process affecting the toxicity and mobility of selenooxyanions. Furthermore, volatilization is seen as a potential mechanism for the bioremediation of selenium-contaminated environments, which is a serious problem in many areas of the Western United States (*9*). In several well-studied areas, agricultural irrigation leaches the water-soluble selenate and selenite salts from soils derived from Cretaceous rock. The irrigation water eventually ends up in reservoirs and evaporation ponds where the selenooxyanions accumulate to toxic levels (*9*, *10*). These selenium-contaminated areas are also associated with elevated levels of salinity (mainly chloride and sulfate salts) (*11*, *12*). Selenium toxicity has been the cause of death and deformity of waterfowl and fish populations inhabiting these environments (*13*), which heightens the need for new, cost-efficient solutions to this problem. Phytoremediation results from plant and algal species that take up and reduce selenate and selenite to volatile forms including DMSe and dimethyldiselenide (DMDSe) (*5*, *7*, *8*), which are 500−700 times less toxic than the selenium oxyanions (*14*). Little is known about the mechanism of selenium volatilization. It has been suggested that the putative precursors of DMSe in terrestrial plants and DMDSe in selenium accumulator plants are *Se*-methyl selenomethionine and methylselenocysteine, respectively (*5*, *8*, *15*). However, the mechanism, metabolic intermediates, and enzymology involved in selenium assimilation in plant and algal species remain a matter of speculation.

The structural similarity between DMSe and DMS suggests that their chemistry and biochemistry may also be similar, and therefore some of what is known about DMS production may be applicable to DMSe. DMS is widespread in nature as a volatile degradation product of dimethylsulfoniopropionate (DMSP), an internal compatible solute involved in osmoregulation by phytoplankton and other marine organisms (*16*). DMSP lyase, the enzyme responsible for DMSP degradation and DMS production, has been identified in marine bacteria, marine algae, and phytoplankton (*16*). DMS is also volatilized from DMSP by the salt marsh halophyte, *Spartina alterniflora*, commonly known as cordgrass (*17*, *18*). We postulate that the biochemical mechanism of DMSe production by some plant and algal species growing in selenate-contaminated soil and water may be similar to that which produces DMS from DMSP in marine organisms. For this premise to have validity, it is essential to demonstrate production of the DMSe precursor, DMSeP, in phytoplankton and plants grown in selenium-contaminated environments. In this study, we have shown that the cordgrass, *S. alterniflora*, which produces high concentrations of DMSP (*18*), is also capable of producing DMSeP when grown in selenate with varying levels of salinity.

Experimental Design

Plant Collection and Cultivation. Young *S. alterniflora* Loisel plants (6−12 in.) with their roots washed free of sediment were potted in filter sand in 13 × 13 cm plastic pots lined with plastic bags. Plants were divided into three groups and watered the first time with either distilled water (dH_2O), half-strength or full-strength natural seawater (i.e., 0, 50, or 100% (35‰ salinity) seawater, respectively). Plants were grown outside, received ca. 9−11 h of sunlight day^{-1} and were watered daily with dH_2O to maintain the original seawater concentration. Air temperatures ranged from ca. 21 °C at night to as high as 41 °C during midday. Quarter-strength Miracle-Gro was added to the pots on days 2, 17, 30, 54, 67, and 88. After 25 days of growth all plants were detopped, and new shoots were allowed to grow. On day 31, six plants each growing in the three seawater concentrations received sodium selenate at 0, 0.5, 10, or 50 mg L^{-1} from a 1 g L^{-1} sodium selenate stock solution diluted to the appropriate concentration in 200 mL of dH_2O. The corresponding molar values of selenate in the plant pore water was 0, 6.3, 126.6, and 633 μM, respectively. The selenate amendments were repeated on days 57 and 78. Plants were harvested on day 105, washed, and frozen at −70 °C until needed for analyses.

Analysis of Plant Tissue for Dimethylselenonium Compounds by Alkaline Hydroelimination. Alkaline hydro-

* Corresponding author tel: (803)777-2322; fax: (803)777-4002; e-mail: yoch@biol.sc.edu.
[†] Department of Biological Sciences.
[‡] Department of Chemistry and Biochemistry.

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elimination is a method used to determine the presence of sulfonium compounds in algal or plant tissue by the emission of DMS (*19*). Because of the similarity between DMSP and DMSeP, this method has also been used to analyze for the latter by identifying DMSe (*15, 20*). As applied to *Spartina*, 2 g wet weight of shoots (leaves plus stems) or 3 g of root material, cut into ca. 1 cm pieces, were placed in 14.5-mL siliconized glass serum bottles along with 2 mL of 4.24 M NaOH and immediately capped with Teflon-lined butyl rubber stoppers. Headspace gases were analyzed for DMSe after incubating the bottles 16 h at ambient temperature or 15 min at 115 °C (*19*), respectively. The difference between the quantity of DMSe released at 115 °C and ambient temperature was that assumed to be released from *Se*-methyl selenomethionine (*19*).

Identification and Quantitation of DMSe. The headspace gases (250 μL) were analyzed on a Shimadzu GC-8A gas chromatograph equipped with a flame ionization detector and a Haysep R column (Supelco). Injector and column temperatures were maintained at 190 and 165 °C, respectively; N_2 carrier gas flow rate was 36 mL min^{-1}. Peaks were integrated on a HP 3390A integrator. Standards of DMS and DMSe for GC and mass spectroscopy analysis were prepared by subjecting known amounts of pure DMSP or DMSeP to NaOH (as above). DMSP and DMSeP were synthesized from DMS (or DMSe) and acrylate as described previously (*20, 21*). The liquid stocks of DMS and DMSe were obtained from Aldrich and Alfa AESAR (Ward Hill, MA), respectively.

GC−MS analysis of headspace gases produced by NaOH treatment of cordgrass tissue and fractions from the DMSeP purification process was carried out on a Finnigan 4500 gas chromatograph−mass spectrophotometer equipped with a 0.25 mm i.d. × 3 m × 0.25 μm Rtx-5 column (Restek). He was used as the carrier gas at 62 kPa, and it was operated at ambient temperature. No other peaks were eluted as the column temperature was increased 10 °C min^{-1} up to 150 °C. MS acquisition parameters (analyzed on a Teknivent Vector 2 data system) were as follows: electron energy = 70 eV, full scan acquisition from 35 to 300 *m/z*.

Isolation of DMSeP from *S. alterniflora*. Due to the structural similarity of DMSP and DMSeP, a procedure used for the purification of DMSP from a *Limonium* species (*22*) was also employed for the isolation of DMSeP from *Spartina*. Plant tissue yielding the highest levels of DMSe g wet $weight^{-1}$ following alkaline treatment was frozen to −70 °C, lyophilized, and passed through a Wiley Mill with a 40-gauge mesh screen. Samples were macerated in sea sand and extracted with −70 °C MCW (methanol:chloroform:water at a ratio of 12:5:1) and chromatographed on a cation exchange column (AG 50W-X8) as described previously (*22*). Fractions (1.5 mL) were collected, and 100-μL aliquots were analyzed for dimethylselenonium compounds by the alkaline treatment described above. Fractions yielding the most DMSe were pooled (ca. 4.5 mL total), loaded onto a Sephadex G-10 column (1.5 × 47 cm) that was equilibrated and eluted with dH_2O. The G-10 column separated the HCl from the selenonium and sulfonium compounds which were pooled, lyophilized, and then resuspended in 1.5 mL of dH_2O and used for NMR analysis and enzyme assays.

The synthetic DMSeP (*20*) was also passed through an AG 50W-X8 column (1.5 mL) and then eluted with 3 M HCl to remove some of the breakdown products that might have accumulated in the time since it was synthesized. A trimethylselenonium ion impurity in the DMSeP preparation was not removed by this procedure; however, it did serve as a useful internal reference in comparing these results with other NMR data.

NMR Analysis. NMR analysis was used to confirm that the selenonium molecule isolated from the *Spartina* extract was in fact DMSeP. All NMR data were collected on a Varian Unity Inova 500 spectrometer. The ^1H (500.16 MHz) spectra of the organically synthesized DMSeP sample required presaturation of the dominant water resonance. Data were collected with a 7-kHz window centered on the water resonance (4.63 ppm); 240 transients were collected using a 45° pulse angle, 3 s acquisition time, and 4.5 s interpulse delay. ^{77}Se NMR (95.40 MHz) chemical shifts were acquired through indirect detection using the standard gradient-enhanced Heteronuclear Multiple Quantum Coherence sequence (gHMQC). The ^1H dimension was collected with 2048 complex points over a 5-kHz window that was centered at the resonance frequency of H_2O (4.63 ppm). The ^{77}Se dimension was collected with 512 fids (free induction decays) over a 400 ppm window that was centered at 150 ppm. The ^{77}Se chemical shifts were externally referenced to dimethylselenide (0 ppm). The equilibrium delay was 2 s, and a 50-ms delay was used for the magnetization transfer ($^2J_{H-Se} \approx 10$ Hz). The synthetic DMSeP was run with 16 scans/fid, while the *Spartina* sample required 112 scans for each fid. All data were collected at ambient temperatures.

Results

Preliminary Identification and Quantitation of DMSeP and *Se*-methyl selenomethionine in *Spartina*. To determine if *Spartina* produces DMSeP in the presence of added selenium, plants grown in selenate (50 mg L^{-1}) in 100% seawater were initially tested for DMSe following strong alkali treatment. Although this assay is not conclusive evidence for the presence of DMSeP, it is based on the fact that authentic DMSeP (*20*) and DMSP (*19*), when subjected to sodium hydroxide treatment, release DMSe and DMS, respectively. When the headspace from bottles that contained the alkali-treated plant tissue was analyzed by gas chromatography, two major components were identified. These components had GC retention times of 5.75 and 9.10 min (Figure 1), which matched those of DMS and DMSe generated by alkaline hydroelimination from DMSP and DMSeP standards, respectively (data not shown). Mass fragmentation patterns of 62 and 110 *m/z* from GC−MS analyses of the gases generated from alkali treatment of the plant tissue confirmed their identity as DMS and DMSe, respectively (Figure 1, inset).

The effect of various combinations of salinity and selenate was tested on the production of NaOH hydrolyzable organoselenonium compounds synthesized by *Spartina*. The amount of DMSe released by NaOH hydroelimination from the cordgrass shoot (stem/leaf) tissue ranged from 0 to 0.39 μmoles DMSe • g wet weight $tissue^{-1}$ (Figure 2). Plants yielding the highest level of DMSe were grown in 100% seawater and 50 mg L^{-1} selenate. At lower salinities (0 and 50% seawater), cordgrass grown in 50 mg L^{-1} selenate produced less DMSeP but was highly stressed as evidenced by the production of lower biomass and yellow dried-up leaves and shoots. The roots from each of the varying saline and selenium conditions produced about one-third the amount of DMSe as shoots (data not shown). This observation is consistent with the fact that *Spartina* produces only about one-third the amount of DMSP in roots as it does in shoots and leaves (*23*).

When plant tissue was autoclaved in the presence of strong alkali (i.e., increasing the heat from 23 to 115 °C), there was a substantial additional release of DMSe (Figure 3). These data suggest that in addition to DMSeP other molecules presumably *Se*-methyl selenomethionine may be present in *Spartina*.

Identification of Plant DMSeP. Proof of DMSeP biosynthesis by *Spartina* required its isolation and identification from plant tissue. The high degree of similarity between the DMSP and the putative DMSeP resulted in their co-purification when chromatographed on a cation exchange column as seen by the alkaline release of both DMSe and DMS (Figure 4). The single peak eluting from the column indicates that

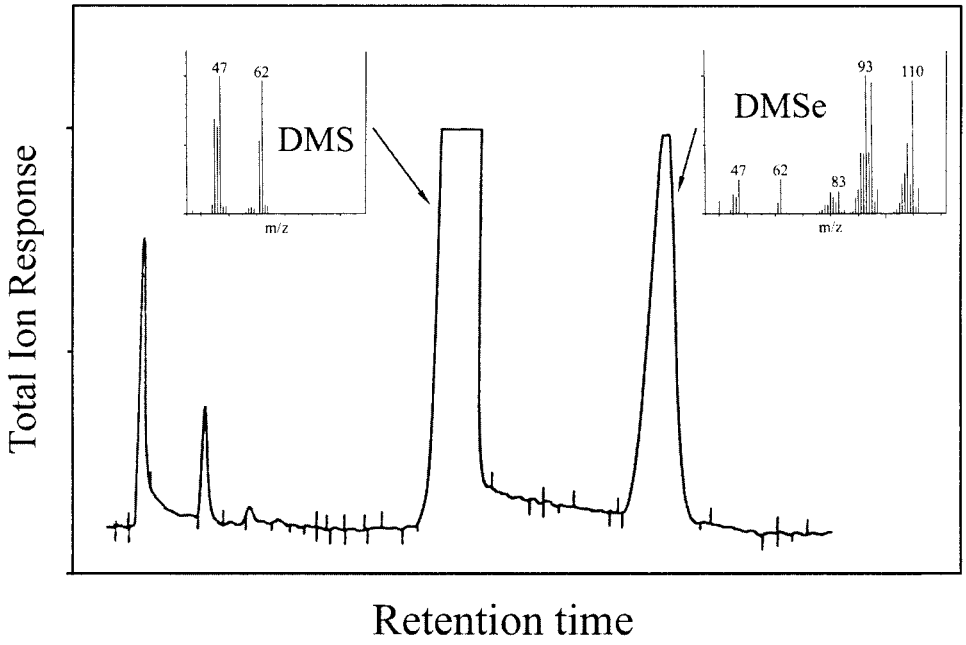

FIGURE 1. Gas chromatographic separation and identification of headspace gases (DMS and DMSe) generated from alkaline treatment of shoots (leaves and stems) from *Spartina*. Identities of these gases were confirmed by comparing retention times of products generated from alkaline hydroelimination of authentic DMSP and DMSeP, which were 5.75 and 9.10 min for DMS and DMSe, respectively. (Insets) The identification of these gases was confirmed by their GC−MS mass fragmentation patterns.

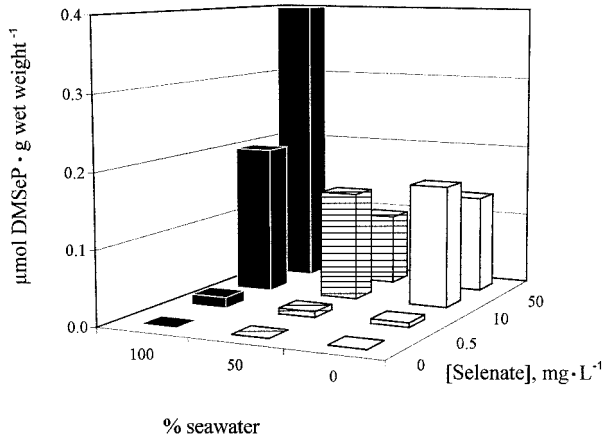

FIGURE 2. Effect of increasing salinity and selenate levels on the production of DMSeP by *Spartina*. DMSeP was measured as DMSe following alkaline treatment of plant shoots. Plants were grown under conditions of increasing salinity (0, 50, and 100% seawater); at each salinity level plants were amended with 0, 0.5, 10, or 50 mg of sodium selenate L^{-1}. Values are the mean from six plants. Variation in DMSe between plants receiving the same treatment was <25%.

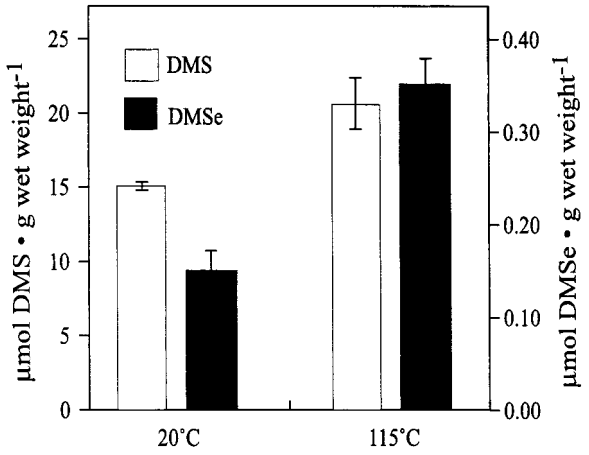

FIGURE 3. Effect of temperature (20 vs 115 °C) on the alkali-dependent release of DMS and DMSe from shoots of *Spartina*. The higher temperature releases DMS from *S*-methyl methionine and by inference DMSe from *Se*-methyl selenomethionine. Plants for this assay were grown in 100% seawater amended with 10 mg L^{-1} (126.6 μM) selenate. The headspace gas (DMS and DMSe) was analyzed by gas chromatography. Values are the mean of three replicas; the bars represent standard errors.

no other organoselenium compounds were present in the preparation at this stage of purification. The DMSeP extracted from this tissue was ca. 2.25% of the DMSP concentration. NMR and enzymatic analyses were performed to obtain conclusive evidence of the structural identity of the putative DMSeP molecule.

To have a basis to compare the dimethylselenonium compound found in cordgrass tissue, synthetic DMSeP was analyzed by electrospray ionization mass spectroscopy and ^{1}H and ^{77}Se NMR. Since it was possible that some of the DMSeP standard degraded over time, it was essential to determine what contaminants, if any, were present in the sample. Electrospray ionization mass spectroscopy of the synthetic DMSeP standard carried out ca. 20 months after it was prepared, showed patterns of two major selenonium species. One molecule with *m/z* of 183 was identified as DMSeP, and the other at 125 was identified as trimethylselenonium $(CH_3)_3Se^+$, presumably a breakdown product of DMSeP (Figure 5). The $(CH_3)_3Se^+$ produced the same *m/z* pattern as did the authentic standard synthesized by Fan et al. (*7*). It did not interfere with DMSeP analysis and, in fact, was used as an internal standard for NMR analysis (see below).

^{1}H and ^{77}Se NMR spectroscopy were used to confirm the identity of synthetic DMSeP and its selenium ion impurity [$(CH_3)_3Se^+$]. The ^{1}H NMR spectra of this mixture gave four assignable resonances (Figure 6). The largest singlet at 2.09 ppm was assigned to the protons of $(CH_3)_3Se^+$. A second lower intensity singlet at 2.14 ppm was assigned to the methyl groups of DMSeP. Both resonances have 10 Hz satellites at their base, which are indicative of methyl groups coupled to an adjacent selenium atom ($^{2}J_{H-Se}$) (*24*). Two coupled triplets ($^{3}J_{H-H}$ = 7 Hz) were assigned to the methylene protons of DMSeP at 2.38 and 2.81 ppm.

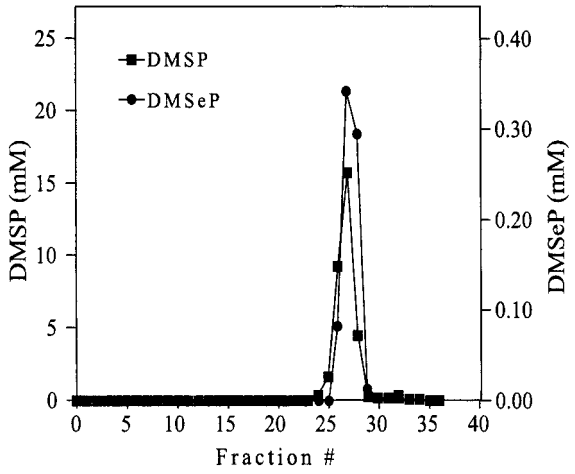

FIGURE 4. DMSP and DMSeP extracted from *Spartina* and chromatographed on an AG 50W-X8 cation exchange column. DMSP and DMSeP were analyzed by subjecting 100 μL to alkaline hydrolysis and quantitating the resulting DMS and DMSe by gas chromatography as shown in Figure 1. The plants were grown on 100% seawater and 50 mg L^{-1} selenate.

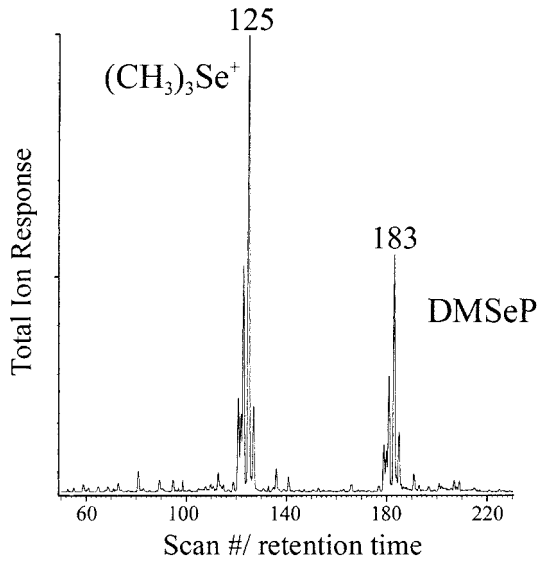

FIGURE 5. Positive ion electrospray liquid chromatography−mass spectroscopy of organically synthesized DMSeP standard containing a selenonium impurity identified as trimethylselenonium, $(CH_3)_3Se$. The analysis was performed using an Analytica API source on a VG Trio 3 triple quadrupole mass spectrometer. The mobile phase consisted of 50% dH_2O:50% acetonitrile with 0.1% acetic acid. Samples were analyzed by direct injection.

The gradient-enhanced $^1H\{^{77}Se\}$ HMQC spectrum of the synthetic DMSeP mixture (Figure 7A) showed two correlations. The ^1H resonance at 2.09 ppm has a ^{77}Se correlation at 256 ppm, with reference to DMSe. This chemical shift is comparable to that of Fan et al. (*7*), who reported a value of 258 ppm for synthetically prepared trimethylselenonium $[(CH_3)_3Se^+]$. These NMR analyses provided conclusive evidence that the 125 *m/z* electrospray component in our synthetic DMSeP standard was in fact $(CH_3)_3Se^+$. The other cross peak in this gHMQC spectrum was assigned to DMSeP, with chemical shifts of the methyl groups' ^1H, 2.14 ppm; ^{77}Se, 297 ppm. Neither methylene resonance showed a correlation in the gHMQC spectrum due to insufficient sensitivity, but their chemical shifts and intensity are consistent for DMSeP.

The partially purified selenonium sample from *Spartina* tissue was studied using the same NMR methods. The ^1H NMR spectrum (not shown) gave resonances of 2.17 (singlet), 2.42, and 2.85 ppm (triplets). These resonances, which are much less intense than the predominant DMSP in the sample, almost exactly matched those assigned to the synthetically prepared DMSeP. The proton frequencies differed by ca. +0.04 ppm for each resonance and were most likely due to the samples having a slightly different pH. The synthetic DMSeP was run under acidic conditions (3 M HCl) while the *Spartina* extract was run at pH 3. Both samples were referenced using the H_2O resonance (assigned 4.63 ppm), which is known to be dependent on pH, but this was disregarded in this study. The $^1H\{^{77}Se\}$ gHMQC spectrum of the *Spartina* extract gave a single resonance at 297 ppm in the selenium dimension that correlated to the singlet at 2.17. This supported the identification of the selenonium species in the *Spartina* shoot sample as DMSeP. There was no other

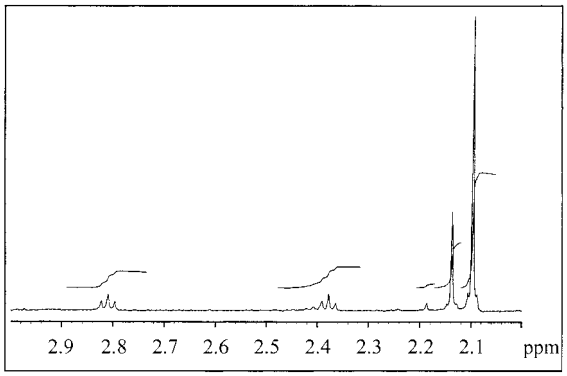

FIGURE 6. One-dimensional ^1H NMR analysis of DMSeP and $(CH_3)_3Se^+$.

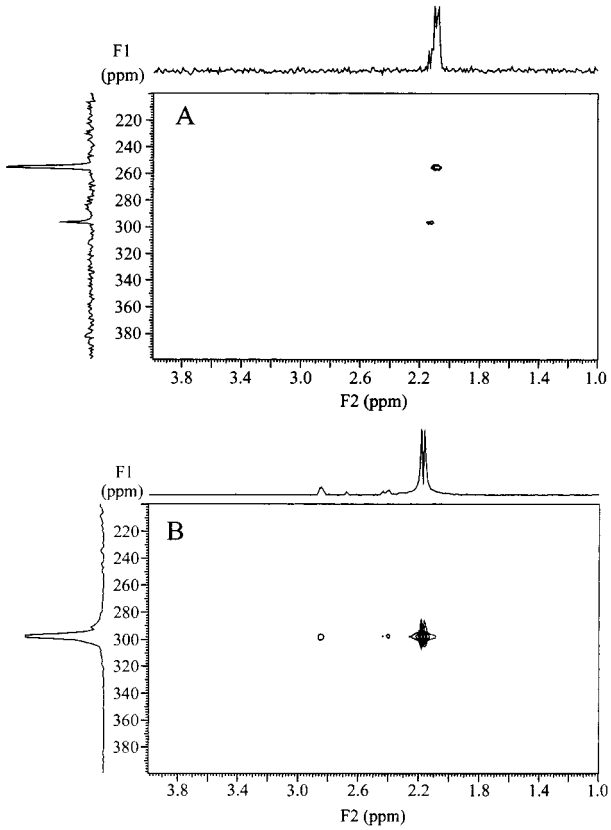

FIGURE 7. $^1H\{^{77}Se\}$gHMQC 2-D NMR analysis of (A) authentic DMSeP (with the trimethylselenonium impurity) and (B) the partially purified selenonium molecule from *Spartina*. A comparison indicates that the latter is identical to authentic DMSeP.

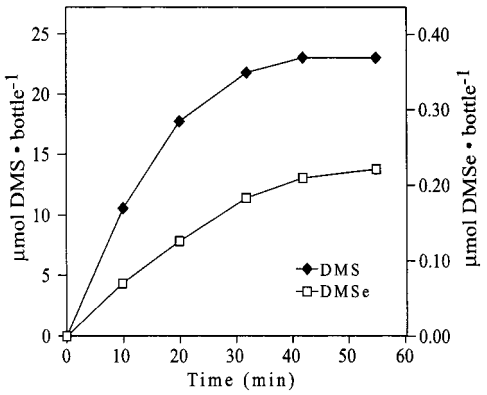

FIGURE 8. DMS and DMSe emissions by DMSP lyase from DMSP/DMSeP-rich partially purified fraction from *Spartina*. The reaction mixture contained the following in 1 mL: DMSP lyase partially purified from *Alcaligenes* strain M3A (200 µL) (*20*), the DMSP/DMSeP sample (200 µL), and 50 mM phosphate buffer, pH 7.4 (800 µL). Headspace gases were analyzed by gas chromatography and identified as DMS and DMSe consistent with the fact that the unknown selenium-containing molecule produced by cordgrass is DMSeP.

selenium species that could be identified by ^{77}Se NMR in the sample.

Plant DMSeP as a Substrate for DMSP Lyase. The final line of evidence that the dimethylselenenonium ion isolated from *Spartina* was DMSeP was indicated by the fact that bacterial DMSP lyase (*25*) recognizes this molecule as a substrate and degrades it to DMSe (Figure 8). This is consistent with our previous report that organically synthesized DMSeP serves as a substrate for DMSP lyase from two strains of marine bacteria (*Alcaligenes* sp. strain M3A and *Pseudomonas doudoroffii*), which resulted in the release of DMSe (*20*). The rate of both DMS and DMSe emissions were linear until the substrate was depleted. Controls in which DMSP lyase was not added or heat inactivated produced no DMS or DMSe (data not shown).

Discussion

Numerous pathways and precursors have been proposed to explain the volatilization of DMSe from soils and various plant species (*2*, *5*, *8*, *11*, *15*, *26*, *27*). In this paper, we have definitively identified DMSeP in the salt marsh cordgrass, *S. alterniflora*, as a molecule that has not yet been considered as a precursor of DMSe. The identification of DMSeP in cordgrass included the following: (i) alkaline hydroelimination yielded DMSe (which was identified by GC retention time and GC−MS) from both plant tissue and partially purified plant fractions; (ii) ^{1}H and ^{77}Se NMR analyses of the putative DMSeP in the partially purified fractions showed identical chemical shifts to that of the authentic standard; and (iii) the putative DMSeP served as a substrate for the bacterial DMSP lyase, an enzyme that does not use other dimethylsulfonium or presumably dimethylselenonium molecules as substrates (*28*).

The release of additional DMS and DMSe after alkaline treatment of plant tissue at temperatures >100 °C (Figure 3) indicated that *Spartina* also produced *S*-methyl methionine and by analogy *Se*-methyl selenomethionine when grown in selenate. In similar experiments with marine algae, White (*19*), after conducting preliminary experiments with pure sulfonium compounds (i.e., DMSP and *S*-methyl methionine), concluded that the additional DMS released from algae at higher temperatures "could conceivably" have been coming from *S*-methyl methionine. Therefore our data only provide circumstantial evidence for the presence of *Se*-methyl selenomethioine in cordgrass. It seems logical, however, that *Spartina* would synthesize DMSP and DMSeP by the same pathway, in which case *Se*-methyl selenomethionine would serve as a key intermediate in DMSeP biosynthesis, just as *S*-methyl methionine does in the production of DMSP (*29*).

The question arises as to whether molecules other than DMSeP could be converted to DMSe by alkaline hydroelimination, which would confound this supposition. Intermediates in the synthesis of DMSP by *Spartina*, DMSP aldehyde, and DMSP amine (*29*) are no doubt also susceptible to alkaline hydrolysis. This is especially true of DMSP aldehyde, which is spontaneously degraded to release DMS (*30*). The Se analogues of these intermediates would be expected to act similarly. Therefore in measuring the total amount of DMSeP in plant tissue by alkaline hydroelimination at ambient temperatures, it may be that some of the DMSe produced was in fact coming from these intermediates.

Since selenium and sulfur often compete for the same cellular uptake sites (*5*, *9*), it was assumed that as the selenium concentration was increased from 0 to 50 mg L^{-1} at each of the seawater concentrations, the amount of DMSeP produced might have increased as the salinity (i.e. seawater) decreased. The highest concentration of DMSeP was however found in plants that received 100% seawater and 50 mg L^{-1} selenate (Figure 2), suggesting instead a lack of competition between sulfur and selenium. Furthermore, the low concentration of DMSeP in plant tissue at lower seawater concentrations was also unexpected in that plants that received zero or half-strength seawater and 50 mg L^{-1} selenate had only ca. one-fourth the DMSeP concentrations of that found in the 100% seawater amendments. One possible explanation for this lower DMSeP production may be that the plants grown in the lower seawater concentrations (and therefore lower sulfate) were highly stressed by the high selenium additions, as they did not grow very well under these conditions. The stress on these plants was evidenced by low biomass production as compared to that of other plants; the leaves and shoots were yellow, shriveled, and looked almost dead at the time of harvest. It may be that the high concentration of sulfate in the 100% seawater amendments provide protection to the plants at these higher selenium concentrations (50 mg L^{-1}). As the sulfate concentrations were decreased (50 and 0% seawater), the higher selenate concentrations may have had a chance to compete for sulfate uptake sites and biochemical reactions involving sulfur, and it produced an inhibitory effect on plant metabolism and growth. The reason for the lower levels of DMSeP found in plants grown in lower levels of selenate (0.5 and 10 mg L^{-1}), where there was a more favorable selenium to sulfur ratio, is not immediately obvious. One possibility is that, over the 105-day growth period, the plants depleted the selenium in these pots by volatilizing it to DMSe.

Many sites requiring selenium remediation are associated with high salinity (chloride or sulfate salts) (*9*), and the need to identify salt-tolerant plants for phytoremediation of selenium-contaminated soils has been recognized (*12*). Selenium accumulation in cultivars, such as canola, kenaf, tall fescue, and birdsfoot trefoil, was shown to decrease in each of these plants with increasing salinity (*12*), while selenium accumulation and volatilization decreased progressively in brocolli as sulfate was increased in the plant growth solution beyond 0.25 mM (*31*). Similar sulfate inhibitory effects were seen in selenium accumulation by alfalfa and *Astragalus bisulcatus* (*32*). Cordgrass is not only salt-tolerant (sulfate in seawater is ca. 30 mM), but its sequestration of selenate into aboveground tissue as DMSeP was enhanced in plants growing in these higher levels of seawater (Figure 2). The increased DMSeP in tissue may mean that less selenium was incorporated into plant proteins and therefore diverted to the free Se-amino acid pool, which may be more readily converted to DMSeP. However such an

explanation is speculative and will require additional experiments to resolve.

Spartina accumulated ca. 0.4 μmol of DMSeP g wet weight^{-1} (ca. 1.3 μmol of DMSeP g dry weight^{-1}) of shoot tissue under optimal conditions of full-strength seawater and 50 mg L^{-1} selenate. At this time we have not yet determined the total amount of Se accumulation in *Spartina*, so we do not know what percentage of Se is found as DMSeP relative to the total Se pool. *Spartina* is known to release substantial amounts of DMS (800−18 000 nmol m^{-2} h^{-1}) from DMSP in salt marshes on the U.S. Atlantic Coast (*17*, *33*). Therefore, we hypothesize that *Spartina* would also produce DMSeP in both natural and constructed wetlands contaminated with salts and selenium oxyanions and volatilize the Se as DMSe. It has been suggested that microbes may play a role in this process; in this regard, aerobic bacteria, decomposing fungi, and microbes in anoxic sediments have already been demonstrated to volatilize DMSe from added DMSeP (*20*, *34*). Studies that determine the total amount of selenium accumulated by *Spartina* and its ability to volatize selenium as dimethylselenide will be needed to validate this hypothesis.

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