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Studies of the naturally occurring biomethylation of selenium and the determination of the products

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A systematic study of the biomethylation of selenium and the determination of the methylated species indicates preliminarily that selenium is susceptible to natural biomethylation under certain environmental conditions. Detectable levels of methylated selenium species, including dimethyl selenide [$(CH_3)_2Se$], dimethyl diselenide [$(CH_3)_2Se_2$] and dimethylselenone [$(CH_3)_2SeO_2$] have been detected by gas chromatography–graphite furnace atomic absorption spectrophotometry (GC–GF AA) from a variety of environmental samples. Findings of naturally methylated selenium species from both soil samples and related air samples suggest that there may exist a localized cycle of selenium between ground soil and the ambient air.

Factors that influence the sensitivity and accuracy for the determination of alkyl selenide compounds by GC–GF AA have also been investigated. Flash-like injection mode and addition of about 10% of hydrogen gas to the argon carrier gas provide for highly sensitive detection. Reproducible determination can be obtained with a precision of about 6% and the detection limits are 0.3 ng Se m^{-3}.

Keywords: Natural environment, methylated selenium species, environmental samples, ground soil, smelter, murine exhalation, selenium metabolite, flash-like injection

INTRODUCTION

Biomethylation of inorganic selenium to alkyl selenide species in the environment has been investigated by several authors.[1–5] However, little information on the naturally occurring biomethylation of selenium and the quantitative determination of selenium-containing metabolites is available in the literature. In this paper, we report the results of a number of determinations of organoselenium compounds in various environmental samples. These include air samples collected at the water's edge of a lake, at the outlet of a sealed sewage digestion tower, in the vicinity of a heavy-metal smelter, and in the breath of mice administered with different selenium compounds, as well as from soil samples obtained from the ground surface of the above smelter.

Detectable levels of methylated selenium species including dimethyl selenide, dimethyl diselenide and dimethylselenone have been determined by a GC–GF AA system indicating that natural biomethylation of selenium is relatively widespread in certain environments, and that anaerobic conditions sometimes favour this process. GC–GF AA together with a cryogenic trapping system has also been applied to the study of selenium metabolism in mice. Animals, especially rats, given large doses of different selenium compounds exhale volatile selenides which have been identified primarily as dimethyl selenide.[6–17] To discover if dimethyl selenide was the only selenium-containing metabolite which could be detected by their method in the air exhaled by experimental animals treated with inorganic selenium compounds, Prochazkova and co-workers[18] used a synthetic adsorbent for trapping the volatile selenium species and separated them by gas chromatography. Dimethyl selenide was then proved to be the major compound whilst a smaller amount of another selenium-containing species was also detected without any identification. Quantitative data, however, for exhaled selenium are very scarce. At least two selenium-containing species other than dimethyl selenide are observed in our experiments. One of these has been identified as dimethyl diselenide and the other,

accounting for a large fraction of the metabolite, still remains unidentified up to now in this work.

Factors that influence the sensitivity and accuracy for the determination of alkyl selenide compounds have been investigated in the present work. The adoption of a flash-like injection mode and the addition of *ca* 10% hydrogen to the argon carrier gas enhance the sensitivity by about two-fold. Parameters reported here are also significant for the determination of other volatile organometallic species.

EXPERIMENTAL

Apparatus and reagents

A gas chromatography–graphite furnace atomic absorption spectrophotometry (GC–GF AA) system which was reported previously[19–22] was used for all the experiments. The spectrophotometer was equipped with a Perkin–Elmer HGA-74 graphite furnace. A selenium electrodeless discharge lamp (PE-1474), a deuterium background corrector and a PE-Hitachi 56 strip chart recorder were the main accessories. Argon was used as the carrier gas at a flow rate of about 125 cm^3 min^{-1}. The transfer line used was a 1 m length of nickel tubing (0.5 mm i.d.). At the beginning of the line, hydrogen at a flow rate of *ca* 12 cm^3 min^{-1} was doped onto the argon.

All chemicals used were of analytical-reagent grade. Alkyl selenides were obtained as standard materials from Strem Chemicals (Newburyport, MA, USA). Stock solutions were prepared by dissolution in pentane of analytical-reagent grade. Working solutions at nanogram levels were prepared daily prior to use. The packing materials used for cryogenic trapping included various kinds of beads of 4 mm diameter [glass beads, beads of poly(vinyl chloride) and polypropylene] and pyrex glass wool.

Procedure

Sampling of the alkyl selenide species in the air was based on cryogenic trapping.[20] Air was sucked by a DT/VT 1.5 Becker pump into the trap filled with glass wool at *ca* −140°C at a flow rate of about 3 dm^3 min^{-1} for at least 4 h without blocking of the trap due to ice deposition. A Nuclepore membrane filter of 0.4 μm pore size was used to collect the particulate matter. The volatile selenium compounds were determined with GC–GF AA, and the particulate selenium on the Nuclepore filter was determined by tube excited energy–dispersive X-ray fluorescence using the procedure of Van Espen and Adams.[23]

Brown–black loamy soil collected at random at a profile 1 cm under the ground surface inside a selenium-smelter grounds was used for the analysis of volatile selenium evolved from the soil samples. Water content of the soil samples was about 16.5% by weight. No addition of any form of selenium nor any nutrient was made to the experimental soil samples in this work. In addition, resort was not made to any incubation with selenium in the laboratory. The corresponding air samples were collected just outside the factory at a height of about 30 cm above the ground. Reference may be made to previous work of the authors for further details.[24]

Quantitative data on exhaled selenium are scarce. We also report in this and other[25] work a quantitative investigation of exhaled organoselenium compounds by mice treated with different selenium compounds by different routes. Three-week-old Swiss–Webster male mice with an average body weight of 11 g were used in all experiments. For breath sampling, the mice were temporarily transferred from the metabolic cages to a clean plastic box which was sealed into a polyethylene bag and the air inside the bag was sampled by a pump at a flow rate of 3 dm^3 min^{-1} into a cryogenic trap filled with glass wool followed by determination with the GC–GF AA system.

For measurement of the collection efficiency and recovery from the cryogenic trap at different temperatures, a sampling system was built in which the cryogenic trap was followed by a small U-shaped tube filled with the same materials as were used in the cryogenic trap, and the small tube was held in a liquid nitrogen bath to capture any alkyl selenides escaping from the trap. For each analysis the contents of the trap and the small tube were analysed for alkyl selenides to evaluate the collection efficiency of, and the recovery from, the trap. Reportedly,[21] the temperature of −120°C is sufficient for quantitative collection of tetra-alkyllead compounds. For alkyl selenides, however, we find the trap temperature must be maintained at −140°C.

Table 1 Organoselenium concentrations (ng m^{-3}) in air samples collected at various aquatic environments in Belgium

Site	Temperature, weather conditions	$(CH_3)_2Se$	$(CH_3)_2Se_2$	$(CH_3)_2SeO_2$
Campus lake	8°C, cloudy	0.47 ± 0.03	0.35 ± 0.05	<0.20
Smelter	17°C	1.41 ± 0.07	0.63 ± 0.1	0.3 ± 0.01
Fishing pond				
'Broek'	18°C, cloudy	<0.15	<0.30	<0.20
'Breeven'	8°C, raining	<0.15	<0.30	<0.20
Sewage treatment plant				
No. 1	16°C, raining	<0.15	<0.30	<0.20
No. 2	15°C	2.40 ± 0.04	<0.30	18.8 ± 0.70

RESULTS AND DISCUSSION

Table 1 summarizes results of a number of triplicate determinations. The air samples collected at the water's edge of a campus lake at the University of Antwerp contain detectable concentrations of dimethyl selenide, dimethyl diselenide and on one occasion of dimethylselenone. The concentration appears to increase with temperature, and the lake appears to be responsible for this emission, as the concentration drops below detection limits at a distance of 150 m. A microbial production process can be assumed but it is surprising that no organoselenium compounds were detected at two other nearby lakes. As appears from Table 2, the campus lake water contains a somewhat higher concentration of dissolved selenium than the other two lakes. This is probably connected with the emissions of a selenium-producing smelter at about 3 km in the dominating wind direction. We compared the organoselenium concentrations in air samples at various aquatic environments and the concentrations of inorganic selenium in the different environmental waters collected at the corresponding air sampling sites, but no correlation could be found.

Two locations on the Scheldt river near Antwerp were investigated. One about 1.5 km downstream and downwind of a coal-fired power plant shows a significant concentration of dimethyl selenide and dimethylselenone, while the other at about 3 km upstream of the same plant shows no detectable organoselenium compounds. Two different sewage treatment plants were investigated also. At the first plant neither at the aeration tanks, nor at the sedimentation tanks, could any volatile selenium compounds be detected. At the second plant, however, high concentrations of dimethylselenone (18.8 ng m^{-3}) and dimethyl selenide (2.40 ng m^{-3}) were detected at the outlet of a sealed sewage digestion tower. It is reasonable to conclude that anaerobic micro-organisms are responsible for methylation.

Results (Table 3) obtained for ground soil samples

Table 2 Inorganic selenium concentrations (μg dm^{-3}) in the different environmental waters at the air sampling sites

Site	Dissolved Se		Suspended Se
	Se(IV)	Total Se	
Campus lake (Antwerp)	0.15	0.23	<0.03
Fishing pond			
'Broek'	0.09	0.14	<0.03
'Breeven'	<0.04	<0.06	<0.03
Sewage treatment plant			
No. 1			
Influent	0.05	0.36	0.16
Effluent	<0.04	0.10	<0.03
No. 2			
Influent	0.13	0.47	0.27
Effluent	0.18	0.20	<0.03

Table 3 Methylated selenium species from soil and air samples

Chemical form	Evolution of soil samples (ng Se day^{-1})	Concentrations in air (ng Se m^{-3})	$\frac{Se_{soil}}{Se_{air}}$
$(CH_3)_2Se$	8.2	1.4	5.8
$(CH_3)_2Se_2$	4.3	0.6	7.2
$(CH_3)_2SeO_2$	1.9	0.3	6.4

Weight of soil in each experiment = 30 g

Table 4 Amount of selenium exhaled [ng (100 g body weight)$^{-1}$] for different selenium compounds

Time after administration (days)	10^{-5} mol dm^{-3} Se in drinking water in the form of: Selenite		Selenocystine		Selenomethionine			0.2 cm^3 intraperitoneally injected selenocystine (2×10^{-3} mol dm^{-3})	
	Exhaled compound: $(CH_3)_2Se$	$(CH_3)_2Se_2$	Exhaled compound: $(CH_3)_2Se$	$(CH_3)_2Se_2$	Exhaled compound: $(CH_3)_2Se$	$(CH_3)_2Se_2$	Unknown	$(CH_3)_2Se$	$(CH_3)_2Se_2$
2[a]	—	—	—	—	—	—	—	7.7	1.6
4[a]	—	—	—	—	—	—	—	9.9	2.6
7	<0.2	<0.3	<0.2	<0.3	<0.2	1.37	0.5	D[b]	D[b]
10	0.44	<0.3	0.53	<0.3	0.62	1.73	0.5	—	—
14	0.52	<0.3	0.65	<0.3	0.65	2.74	7.26	—	—
18	—	—	1.39	<0.3	—	—	—	—	—
22	—	—	3.69	0.57	—	—	—	—	—

[a]Hours after the injection; [b]D, animals dead.

indicate the evolution of dimethyl selenide, dimethyl diselenide and another selenium-containing compound which is tentatively identified as dimethylselenone. The higher ratio of Se_{soil} to Se_{air} for dimethyl diselenide is due to its lower stability than that of the other two species. These results suggest that natural biomethylation of selenium in soils produces at least three species. They support Chau's work[5] where he reported occasional detection of volatile selenium compounds in some specific lake sediments without addition of selenium.

In a further set of experiments 10^{-5} mol dm^{-3} Se as selenite, selenomethionine [$CH_3SeCH_2CH(NH_2)COOH$] or selenocystine [$(SeCH_2CH(NH_2)COOH)_2$] was added to the drinking water of mice. At selected periods after starting the administration of selenium, the animals were taken out of the metabolic cages and their breath was sampled by the trap. One set of control mice was also sampled. The results obtained are summarized in Table 4. Addition of selenite to drinking water results in very low selenide production, since only the dimethyl selenide metabolite is observed after 14 days of selenite administration. Selenium added to the drinking water in the organic form resulted in a higher production of alkyl selenides. It is interesting to mention that, for selenocystine, only after 18 days of administration did another species, dimethyl diselenide, appear on the breath. For selenomethionine, both selenide compounds were observed, with the diselenide compound being the predominant form, and also a third unidentified selenium species which contributes to a large fraction of the metabolites was observed. No methyl species above the 0.3 ng detection limit was observed in the breath of the control mice. All animals remained healthy during the entire experiment. In another experiment 31.6 μg selenium, as selenocystine, was injected intraperitoneally into each mouse and the breath was sampled two hours and four days after the injection. The results of the analyses are also included in Table 4. Very soon after injection both methyl selenides were observed in the breath with dimethylselenide as the predominant form. The injection dose, 2.9 mg Se (kg body weight)$^{-1}$ is very close to the published LD_{50}-value of 4 mg Se (kg body weight)$^{-1}$. Indeed, the animals died five days after injection. Selenocystine as well as selenite metabolism results in dimethylselenide as the predominant species in the breath regardless of the method of administration. Selenomethionine, however, metabolizes differently from selenocystine (although seleno-amino acids assimilate more readily) and results in the observation of selenium compounds soon after addition to drinking water. Not only dimethyl selenide but also dimethyl diselenide and a third species were detected in the metabolites. It appears that selenocystine behaves more similarly to selenite than to selenomethionine. Corresponding behaviour of selenite and seleocystine was previously demonstrated by Thomson and coworkers,[26,27] who intubated different selenium compounds into rats. Also, Greeder and Milner[28] proved

Table 5 Effect of cryogenic trap temperature on collection efficiency

Trap[a] temperature (°C)	Collection efficiency (%)		
	$(CH_3)_2Se$	$(C_2H_5)_2Se$	$(CH_3)_2Se_2$
−140	96	100	95
−120	90	99	98
−100	56	90	98
−78	15	20	93

[a]The trap used here was filled with pyrex glass wool.

that the effectiveness in limiting tumour growth in mice of selenite, selenate and selenocystine differed considerably from that of selenomethionine.

The collection efficiency of the cryogenic trap for alkyl selenide compounds is summarized in Table 5. Several absorbents used in the traps were investigated at a temperature of −140°C. Results are indicated in Table 6. Quantitative collection of the most thermally labile species, dimethyl diselenide, can be achieved only when the absorbent is deactivated by acid-washing or silanizing and kept at a temperature of −140°C. This suggests that the surface condition of the absorbent is critical, as partial decomposition of the labile compounds may not be negligible if adsorption is too strong.

It is well established[29] that for on-column injection, a sufficiently high temperature is required to ensure instantaneous vapourization of the sample on injection for good reproducibility and sensitivity. Studies, however, on the thermal stability of dimethyl diselenide prompted us to explore a new, flash-like, injection mode. As is shown in Table 7, flash-like injection[30] enhances the sensitivity for dimethyl diselenide significantly.

Deuterium background correction does not effectively correct for non-specific molecular absorption by various organic impurities at 196.1 nm. Chau *et al.*[31] circumvented this difficulty by burning off the organic matrix in a hydrogen flame using a precombustion section just in the front of a silica AA furnace. In the GC−AA system, Radziuk and Van Loon[32] used a hydrogen diffusion flame. We, in the present work, add about 10% hydrogen to the argon carrier gas and let the gas flow directly into the graphite furnace to overcome this problem and, moreover, increase the sensitivity for alkyl selenides by a factor of about 2 (Table 8).

Table 6 Collection efficiency of absorbents for alkyl selenides

Species	Glass beads	Poly(vinyl chloride)		Polypropylene		Pyrex glass wool	
		Untreated	Washed[a]	Untreated	Washed[a]	Untreated	Silanized
$(CH_3)_2Se$	98	73	100	73	84	93	96
$(CH_3)_2Se_2$	84	60	96	41	80	99	95
$(C_2H_5)_2Se$	89	79	97	81	80	96	100

[a]Washed with 1 mol dm^{-3} hydrochloric acid before use.

Table 7 Influence of injection mode and temperature on relative sensitivity for alkyl selenides

Injection temperature (°C)	Relative sensitivity (%)					
	$(CH_3)_2Se$		$(C_2H_5)_2Se$		$(CH_3)_2Se_2$	
	On-column	Flash	On-column	Flash	On-column	Flash
100	1.00	0.83	1.00	1.03	2.30	3.08
200	1.24	0.93	1.07	1.11	2.00	2.97
260	1.30	1.07	1.09	1.04	1.00	2.27

Table 8 Effect of hydrogen addition to the argon carrier gas on sensitivity

Species	Relative sensitivity	
	Without H_2 addition	10% H_2 addition
$(CH_3)_2Se$	1.0	1.9
$(C_2H_5)_2Se$	1.0	1.7
$(CH_3)_2Se_2$	1.0	1.9

CONCLUSIONS

It appears that naturally occurring sources of selenium biomethylation exist and that anthropogenic emission of inorganic selenium seems necessary for the process. From the limited number of samples analysed, it is not possible to derive quantitative estimates of the importance of the biomethylation source to the atmospheric burden of the element. With the high concentration of dimethyl selenide obtained at the digestion tower of the sewage treatment plant, it is reasonable to conclude that anaerobic micro-organisms are sometimes responsible for the emission. Dimethyl selenide is the only selenium metabolite of mice administered with selenite, while for seleno-amino acids, the experimental animals metabolize at least two species of alkyl selenide more readily, after addition of these to drinking water. Intraperitoneal injection of selenocystine produces two alkyl selenides more rapidly at much higher concentrations. Selenocystine behaves more similarly to selenite than to selenomethionine. For selenomethionine, a third unidentified selenium metabolite contributes to a large extent to the emission of the alkylated element by the lungs. These results appear to be the first that show possible sources for biomethylation of selenium being indicated and identified in ambient air and ground soil, and where a quantitative determination procedure for alkyl selenides in the breath of mice is described.

The adoption of the flash injection mode and the addition directly to the graphite furnace of hydrogen enhance the sensitivity for the determination of the products.

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