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Effects of deuterium in octopole reaction and collision cell ICP-MS on detection of selenium in extracellular fluids

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Abstract

Inductively coupled plasma-mass spectrometry (ICP-MS) equipped with a reaction/collision cell has been successfully used for dissociating polyatomic interferences. Hydrogen (H_2) is one of the most effective and frequently used reaction/collision gases. However, the H_2 reaction produces interference in the detection of selenium (Se) in bromine (Br)-containing samples, such as extracellular fluids. In this study, deuterium (D_2) was evaluated for possible use as a reaction gas instead of H_2. Although Se concentration in serum and urine was over-estimated in the H_2 reaction mode, it was determined accurately in the D_2 one. In speciation analyses, the background counts at *m*/*z* 77, 78 and 80 were reduced and the signal-to-noise (S/N) ratios were improved by either the H_2 or the D_2 reaction. The $^{79}Br^1H^+$ and $^{81}Br^1H^+$ interferences appearing at *m*/*z* 80 and 82, respectively, were decreased by changing from the H_2 reaction mode to the D_2 one. Thus, D_2 was effective in dissociating polyatomic interferences and removing Br interferences during Se determination and speciation, suggesting that the D_2 reaction mode is useful for selenometallomics, particularly in samples containing Br, such as serum, urine and cell culture medium.

Keywords: Selenium; ICP-MS; Reaction/collision; Deuterium; Bromine; Speciation

1. Introduction

Selenium (Se) is an essential micronutrient that is known to function as the active center of such selenoenzymes as glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinase [1]. Both inorganic and organic forms of Se can be utilized as a nutritional source [2–4]. In fact, nutritional supplements consist of inorganic Se (selenite or selenate) or extracts from selenized yeast containing selenomethionine (SeMet) mainly and diverse selenocompounds [5,6]. As it has been suggested that a low Se concentration in the diet results in an increased risk of cancer [7], Se-accumulating vegetables, such as garlic, onion and mushroom, are available in market. These selenized vegetables contain selenoamino acid derivatives, such as Se-methylselenocysteine and γ-glutamyl Se-methylselenocysteine, as the major Se compounds [8–11].

The physiological and toxicological effects of Se on the absorption, metabolism and excretion process highly depend on its chemical form. Hence, Se speciation techniques are essential to reveal the biological effects of Se on the human body. Inductively coupled plasma-mass spectrometry (ICP-MS) is one of the most frequently used methods for detecting Se because of its high sensitivity and capability to discriminate endogenous elements from exogenous ones with the use of enriched stable isotopes (SIs) [12]. Indeed, SI-labeled selenocompounds coupled with ICP-MS detection have provided novel insights into Se metabolism [13,14]. However, certain problems exist in the detection of Se by ICP-MS. The most (^{80}Se, 49.6%) and second most (^{78}Se, 23.8%) abundant isotopes are markedly affected by polyatomic interferences from the argon (Ar) plasma source, i.e., $^{40}Ar^{40}Ar^+$ and $^{40}Ar^{38}Ar^+$, respectively. Other isotopes, including ^{74}Se (0.89%), ^{76}Se (9.37%), ^{77}Se (7.63%) and ^{82}Se (8.73%), are also affected by the polyatomic interferences originating from the Ar plasma source and biomatrices, such as $^{38}Ar^{36}Ar^+$, $^{40}Ar^{36}Ar^+$, $^{40}Ar^{37}Cl^+$ and $^{40}Ar^{42}Ca^+$,

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respectively [15,16]. Although ICP with high-resolution MS can separate these interferences from Se, it is less commercial than a conventional ICP with low resolution owing to its considerable high price. To this end, ICP-MS equipped with a reaction/collision cell was developed as an alternative technique to ICP with high-resolution MS, in order to overcome this problem posed by the polyatomic interferences.

Among the gases evaluated so far, including helium (He), ammonia (NH_3) and oxygen (O_2), hydrogen (H_2) gas may be the most effective reaction/collision gas for dissociating the polyatomic Ar compounds [17–20]. However, the H_2 reaction presents a problem in the detection of Se in biological samples, particularly extracellular fluids. Extracellular fluids, such as blood plasma and urine, contain a certain amount of bromine (Br). Despite the fact that Br is not an essential element in mammals, it has been found to be a contaminant of chloride. The Br-containing medicine, bromohexine hydrochloride, is frequently ingested as an expectorant. Br that is excreted into the extracellular fluid interferes with Se detection by ICP-H_2 reaction-MS by producing novel polyatomic interferences, $^{79}Br^1H^+$ and $^{81}Br^1H^+$, in the reaction of H_2 with Br. These polyatomic interferences affect the detection of $^{80}Se^+$ and $^{82}Se^+$, respectively. The use of a mixture of H_2 and He as the reaction/collision gas has been suggested for the efficient removal of $ArAr^+$ [20]. However, the mixture cannot avoid generating BrH^+, and He tends to decrease Se intensity more than H_2. Therefore, an alternative method is needed to analyze Se in the presence of Br.

In this study, the utility of deuterium (D_2) in place of H_2 as a reaction gas was clarified. D_2 is expected to have the same chemical properties as H_2 as a reaction gas and not to form the polyatomic interferences that adversely affect the Se detection. Hence, the effects of D_2 not only on the determination but also on the speciation of Se in ICP-MS were evaluated.

2. Experimental

2.1. Reagents

Sodium selenite, nitric acid of analytical grade and ammonium acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Standard Se solution (1000 μg mL^{-1}) was purchased from Kanto Chemicals (Tokyo, Japan) and diluted with 0.1 M nitric acid prior to use. TRIZMA® base, TRIZMA® HCl and RPMI-1640 cell culture medium were purchased from Sigma (St. Louis, MO, USA). Trimethylselenonium (TMSe) iodide ion, penicillin–streptomycin solution, fetal bovine serum (FBS) and deuterium (99.6 atom%) were purchased from Trichemical Laboratories Inc. (Yamanashi, Japan), Invitrogen Corporation (Carlsbad, CA, USA), Biotrace International (Bridgend, UK) and Showa Denko (Tokyo, Japan), respectively. 1β-Methylseleno-*N*-acetyl-D-galactosamine (Se sugar) was synthesized in our laboratory [21].

2.2. Animal experiment and sample preparation

One male Wistar rat at 5 weeks of age was purchased from Clea Japan Inc. (Tokyo, Japan) and fed a standard diet (CE-2; Clea Japan Inc.) and tap water ad libitum. After acclimation for 1 week, blood was collected under ether anesthesia and centrifuged at 1600 × *g* for 10 min after clotting to obtain serum. Another male Wistar rat at 5 weeks of age (Clea Japan Inc.) was fed a standard diet and water containing sodium selenite at 5.0 μg mL^{-1} ad libitum after the acclimation. The 24-h urine sample collected at 14 days after the induction of Se toxicosis was used for the analyses. For quantitative analysis, a 100-μL aliquot of serum from the non-treated rat or urine from the Se-toxicosis rat was wet-ashed with nitric acid, and the residue was diluted with deionized water to 3.0 mL.

2.3. Instrumentation for ICP-MS and HPLC system

An Agilent7500cs ICP-MS (Yokogawa Analytical Systems, Hachiouji, Japan) equipped with an octopole reaction system (ORS) was used. The operating conditions are summarized in Table 1. The concentrations of Se in the samples were determined with the calibration curve method and compared among the non-, H_2 and D_2 reaction modes. The ICP-MS was coupled to an HPLC system as the detector for the Se speciation. The HPLC system consisted of an on-line degasser, an HPLC pump (PU713; GL Science Co., Ltd., Tokyo, Japan), a Rheodyne six-port injector with a 100- or 20-μL sample loop, and a column. Multi-mode gel filtration columns, Shodex Asahipak GS-520 7G (7.5 mm i.d. × 500 mm, with a guard column, 7.5 mm i.d. × 75 mm, Showa Denko, Tokyo, Japan) for serum separation and GS-

Table 1
Operating conditions of ICP-MS for the speciation of Se

Plasma setting	
RF power (W)	1500
Nebulizer type	Babington
Nebulizer gas flow (L min^{-1})	1.05
Make-up gas flow (L min^{-1})	0.25
Auxiliary gas flow (L min^{-1})	1.15
Plasma gas flow (L min^{-1})	15.0
Reaction/collision cell	
H_2 gas flow (mL min^{-1})	4.5
D_2 gas flow (mL min^{-1})	3.0
Data acquisition	
m/*z* monitored	74–84 (except 80 in non-reaction mode)
Dwell time (ms)	100 for Se isotopes and 75 300 for Br isotopes, 83 and 84
Point per peak	3 for the determination 1 for the speciation