Chapter 7
^{77}Se NMR Spectroscopy for Speciation Analysis of Selenium Compounds

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Abstract Nuclear magnetic resonance (NMR) spectroscopy is widely used for the determination of the chemical structure of an organic compound. NMR spectroscopy is theoretically applicable to all metallic elements except cerium. In this chapter, we demonstrate selenium (Se) speciation in animal Se metabolites (selenometabolites) by NMR spectroscopy and review hitherto performed NMR spectroscopic studies of Se detection in biological samples. ^{77}Se, an NMR-active isotope of Se, was directly observed, but its NMR receptivity was lower than its sensitivity in mass spectrometry. However, the use of enriched stable isotope improved the receptivity. Each selenometabolite had its own chemical shift, suggesting that the chemical shift of ^{77}Se could be used as a fingerprint. Indirect measurement by heteronuclear multiple bond correlation (HMBC) spectroscopy with ^{1}H nuclides was also effective for the Se speciation.

Keywords NMR • Selenium • Speciation • HMBC • Stable isotope • Selenosugar • Selenomethionine • Selenohomolanthionine

7.1 Introduction

Nuclear magnetic resonance (NMR) spectroscopy is used for the investigation of typical elements, such as ^{1}H, ^{13}C, ^{19}F, and ^{31}P, and is, in principle, applicable to almost all of the metallic elements on the periodic table. All metals except cerium (Ce) have at least one NMR-active isotope, and therefore, NMR spectroscopy can be used for the direct detection of organometallic compounds and inorganic metals bound to a protein, peptide, or another biomolecule [1]. Differences in nuclear properties, such as resonance frequency and nuclear spin, among NMR-active nuclides can yield specific receptivity (sensitivity to NMR measurement), linewidth, and chemical shift (Table 7.1) [2]. However, few nuclides are practically observable due to their physicochemical properties.

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Table 7.1 Basic properties of ^{77}Se for NMR spectroscopy

Spin	1/2
Natural abundance	7.63 %
Chemical shift range	from −1000 to 2000
Reference compound	Me_2Se
T1	~30 s
Receptivity (to ^{1}H at natural abundance)	5.37×10^{-4}
Receptivity (to ^{1}H when enriched)	7.04×10^{-3}

Selenium (Se) is an element belonging to the same group 16 on the periodic table as oxygen, sulfur, and tellurium. Se has chemically and biologically ambivalent characteristics; it has similar chemical properties to sulfur as a typical element, yet possesses chemical properties characteristic of a metal. Se is an essential element in animals but can become highly toxic when ingested in an amount that exceeds the nutritional level. Se is required as the active center of selenoproteins that function as an antioxidant and participate in thyroid hormone production, DNA synthesis, and fertilization [3, 4]. It acts as an active center in the formation of a selenol group (−SeH) on a selenocysteinyl (SeCys) residue in a selenoprotein sequence [5, 6]. Se ingested at physiological levels is excreted in urine as a selenosugar (*Se*-methylseleno-*N*-acetyl-galactosamine, selenosugar 1) and a trimethylselenonium ion (TMSe). In addition, *Se*-methylseleno-*N*-acetyl-glucosamine (selenosugar 2) and *Se*-methylseleno-galactosamine (selenosugar 3) are detected in urine as minor metabolites [7–9]. The ingestion of excess amounts of Se would yield volatile selenometabolites, such as dimethyldiselenide and dimethylselenide, in exhaled breath. On the other hand, Se is not essential in plants and is a bystander mineral for plant growth. Se-containing metabolites (selenometabolites), such as methylselenocysteine (MeSeCys), γ-glutamylmethylselenocysteine (GluMeSeCys), selenohomolanthionine (SeHLan), and selenomethionine (SeMet), are biosynthesized in certain plants [10, 11] and fungi [12].

^{77}Se is one of the nuclides detectable by NMR spectroscopy because of its relatively high NMR receptivity. Thus, ^{77}Se NMR spectroscopy has been utilized for the identification of Se compounds in organic chemistry [2]. Although ^{77}Se NMR spectroscopy is a reliable tool for the identification of organoselenium compounds and inorganic Se compounds, it has been rarely used in biological samples because the compounds are present in trace amounts.

Recent advances in NMR instrumentation and measurement techniques have enabled us to investigate Se compounds routinely without the need for special knowledge or equipment. Because Se is an ultra-trace element in organisms, it seems suitable to evaluate recent NMR techniques for metallomics research. In this chapter, we demonstrate the speciation of selenometabolites by ^{77}Se NMR spectroscopy.

7.2 Advantages of NMR Speciation Over Hyphenated Techniques

At the initial stage of Se analysis, much attention was given to the determination of total Se concentration in biological samples. Recent studies, however, have concentrated on the elucidation of the chemical structures of selenometabolites to clarify the physiological role of Se. In other words, both quantitative and qualitative analyses are required to understand the biological and toxicological roles of Se in biological samples. In this regard, the development of reliable techniques for Se speciation in biological samples is necessary to understand the absorption, distribution, metabolism, and excretion of Se. One of the most powerful methods for Se speciation in biological samples is high-performance liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (LC-ICP-MS) because it has high sensitivity and selectivity for the determination of Se and is robust to biological matrices. Indeed, LC-ICP-MS has provided exceptional results [13–17]. However, although LC-ICP-MS has merits, it also has limitations for Se speciation in biological samples, namely, HPLC cannot detect unstable metabolic intermediates, and LC-ICP-MS is not suitable for the identification of unknown metabolites. In contrast, NMR speciation is applicable to these samples. An alternate hyphenated technique, HPLC coupled with electrospray ionization mass spectrometry, i.e., LC-ESI-MS, was developed for Se speciation [18]. In contrast to LC-ICP-MS, LC-ESI-MS can be used for the identification of unknown metabolites. However, exhaustive pretreatment is required to reduce the matrix in a biological sample. On the other hand, NMR spectroscopy is applicable to crude sample analysis without the need for any pretreatment or HPLC separation in the presence of a matrix because of its selectivity and specificity to each Se compound. In addition, NMR spectroscopy is a powerful method for directly probing the chemical structure and redox properties of Se atom. The X-ray absorption fine structure (XAFS) technique can also be used for Se speciation because it can provide the oxidation number and the coordination number of an element on the basis of the X-ray absorption near-edge structure (XANES) spectrum, as well as the structural information of neighboring atoms of an element, such as the number of ligand atoms and the distance between the element and each ligand atom, on the basis of the extended X-ray absorption fine structure (EXAFS) region [19]. In the same manner as NMR spectroscopy, the XAFS technique does not require any sample pretreatment; however, XAFS data are less informative than NMR data from the point of view of identification of Se compounds. Understandably, the major disadvantage of NMR spectroscopy compared to mass spectrometry is its low sensitivity, and because of this, it has not been used for Se speciation in biological samples in studies conducted to date. In the following sections, we highlight unique NMR techniques for Se speciation.

7.3 Observation of ^{77}Se Nuclide in Authentic Standards

7.3.1 Chemical Shifts of Bio-selenocompounds by Direct Detection

Due to the low natural abundance (7.6 %) of the NMR-active isotope ^{77}Se and the long relaxation time, Se was considered to be not an ideal element for NMR spectroscopy. In the direct detection of ^{77}Se nuclide, the low NMR receptivity of Se could be overcome by increasing the scan number. The relationships between the scan number and the signal-to-noise (S/N) ratio of ^{77}Se are shown in Fig. 7.1a. In this experiment, sodium selenite dissolved in sodium phosphate buffer (pH 7.4) at various concentrations was directly analyzed by an NMR instrument (ECZ600, JEOL, Tokyo, Japan), and the lowest concentration where ^{77}Se at natural abundance was detected was 50 mM under our analytical conditions. The data offer clues to knowing intuitively the NMR receptivity of Se. The S/N ratios were improved by enrichment (99.8 %) of ^{77}Se (Fig. 7.1b).

Similarly to ^{1}H and ^{13}C NMR chemical shifts, the ^{77}Se NMR chemical shifts are definitive for compounds containing ^{77}Se [20]. The chemical shifts of Se compounds having reduced Se (−II) are observed in the relatively high-field region, and those having oxidized Se (from + IV to + VI) are observed in the low-field region. This can be explained by the fact that nuclei in the high electron density region are more shielded from the applied field than those in the low electron density region. For the Se speciation by NMR spectroscopy, dimethylselenide is used as chemical shift reference ($\delta = 0$) [21]. The chemical structures of Se compounds in our library of selenometabolites are shown in Fig. 7.2. The chemical shifts measured by NMR ECZ600 are also shown in Fig. 7.2. Important selenometabolites, such as selenoamino acids, a selenosugar, and methylated Se metabolite, were observed in the narrow region from 0 to 300 ppm. The full width at half maximum of each ^{77}

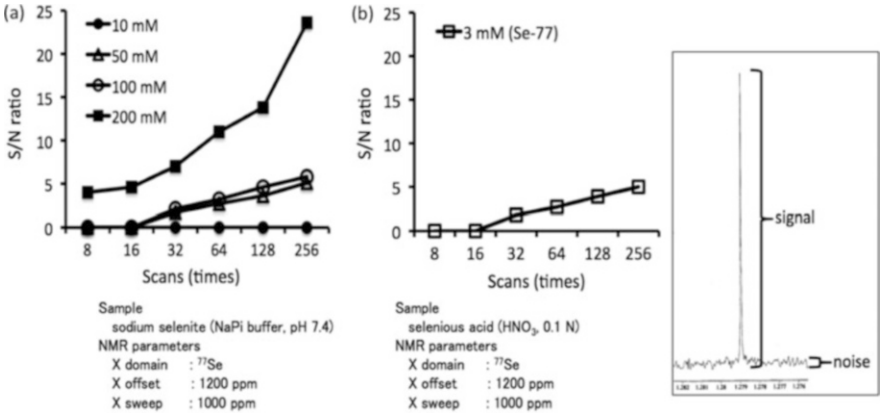

Fig. 7.1 Relationship between scan number and signal-to-noise (S/N) ratio of ^{77}Se

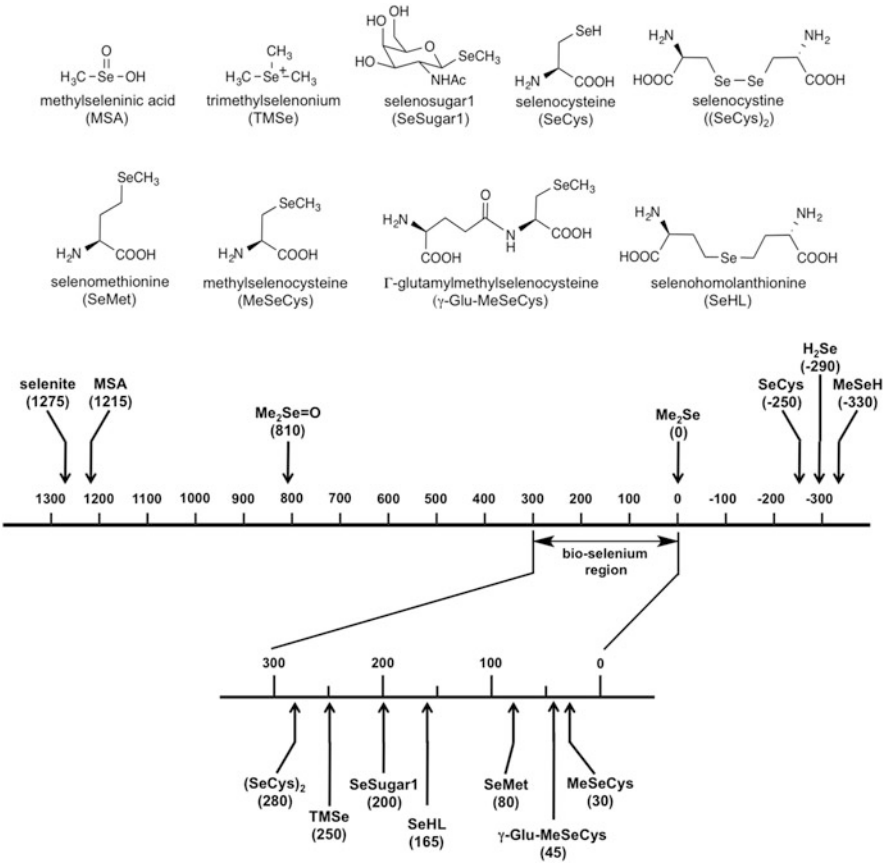

Fig. 7.2 Chemical structures and chemical shifts of Se compounds in our library of selenometabolites measured by NMR (ECZ600, JEOL)

Se signal was so narrow that each Se compound could be identified on the basis of its own chemical shift like a fingerprint. Hence, we propose that the region be named "bio-selenium region."

7.3.2 Advanced Techniques for Detection of Bio-selenocompounds

Because most selenometabolites contain a Se-C-H or a Se-C-C-H bond in their chemical structures, the S/N ratio would be reduced by geminal and vicinal ^{77}Se-^{1}H coupling when the detection is conducted in the single pulse mode without ^{1}H decoupling. Indeed, the S/N ratio was markedly increased in the complete ^{1}H

decoupling mode by proton irradiation, although signal enhancement by the nuclear Overhauser effect was hardly observed.

Another method for enhancing ^{77}Se signal intensity is the indirect measurement by heteronuclear multiple bond correlation (HMBC) spectroscopy with ^{1}H nuclides [22–25]. As an example, the HMBC spectrum of selenosugar 1 is shown in Fig. 7.3. Because the two-dimensional correlation spectrum of ^{77}Se and ^{1}H could be obtained, this method would provide decisive information for chemical structure determination. Furthermore, because this technique is an inverse detection experiment using ^{1}H nuclei, the S/N ratio is markedly increased, namely, the sensitivity obtained at 50 mM would be the same as that obtained at 200 μM.

These advanced techniques for ^{77}Se detection by NMR spectroscopy are useful for Se speciation in biological samples.

7.4 Application of ^{77}Se NMR Spectroscopy to Se Speciation in Biological Samples

As an example of an application of ^{77}Se NMR spectroscopy to Se speciation, the HMBC spectrum of ^{77}Se and ^{1}H of Se compound(s) in a dietary Se supplement ("Yeast-Free" Selenium 200 mcg, Vitamin World®, New York, USA) is shown in Fig. 7.4. The procedures for sample preparation are summarized below.

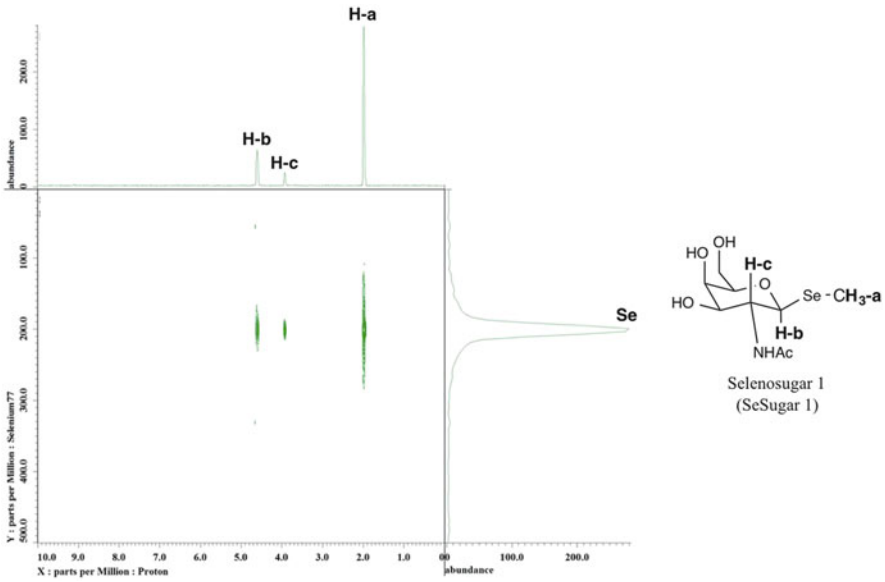

Fig. 7.3 Heteronuclear multiple bond correlation spectrum of selenosugar 1 (50 mM in D_2O)

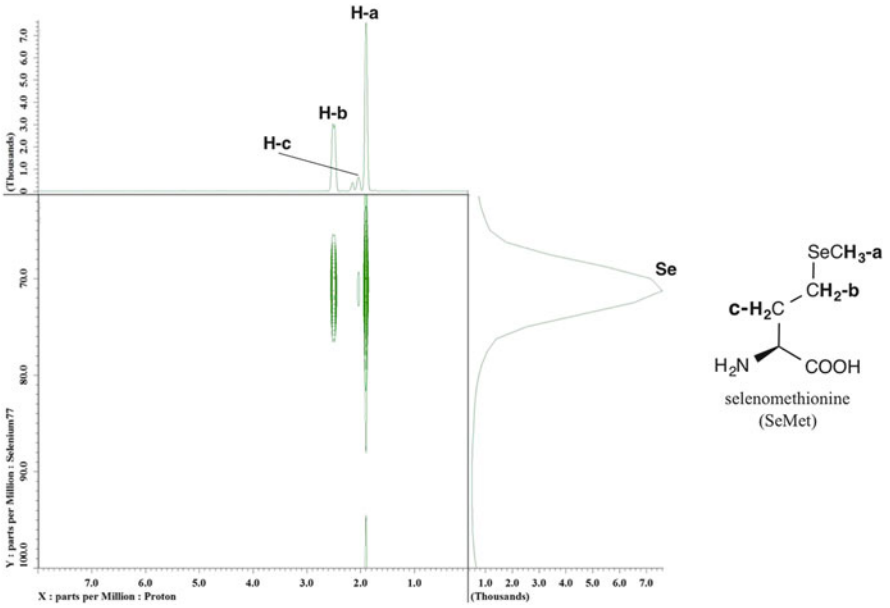

Fig. 7.4 Heteronuclear multiple bond correlation spectrum of Se compound in dietary supplement in D_2O

Five tablets of the dietary Se supplement were ground and extracted with a water and methanol mixture (1:1). The extract was lyophilized, and the lyophilizate was dissolved in D_2O. The HMBC spectrum of the lyophilizate corresponded to that of selenomethionine. The result coincided with the ingredient label showing that selenomethionine is the major Se species in the supplement (Fig. 7.4).

Recent studies have used ^{77}Se NMR spectroscopy for Se speciation in biological samples. Mobli et al. carried out ^{77}Se NMR measurements to determine the p*K*a values of selenocysteine residues incorporated into a bioactive peptide hormone and a neurotransmitter and demonstrated that the p*K*a values of selenocysteine residues in peptide were substantially lower than that of free selenocysteine [26]. Schaefer et al. employed the ^{77}Se NMR technique to probe the local electronic environment of reactive selenocysteine residues by preparing ^{77}Se-enriched proteins [27].

7.5 Conclusions

^{77}Se NMR spectroscopy provides concrete information of the chemical structures of selenometabolites, although the NMR receptivity of ^{77}Se in a biological sample is unsatisfactory compared with the sensitivity in mass spectrometry. The use of

enriched stable isotopes is one of the ways to overcome the disadvantages of NMR spectroscopy. The complementary use of LC-ICP-MS, LC-ESI-MS(-MS), and NMR would be the best analytical approach for the identification of unknown selenometabolites. In addition, we expect that speciation analysis by NMR spectroscopy increasingly become an important technique in metallomics research of not only ^{77}Se but also other nuclides of metals/metalloids. Further development of the measurement technique and instrumentation is expected to lead to the widespread use of NMR spectroscopy in metallomics.

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