Indirect Detection of Selenium-77 in Nuclear Magnetic Resonance Spectra of Organoselenium Compounds

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77Se was measured by inverse proton detection using multiple-quantum 1H–{77Se} correlation spectroscopy. One- and two-dimensional heteronuclear multiple quantum coherence (HMQC) experiments are reported for selenophene, benzeneselenol, dimethyl selenide, dimethyl diselenide, and D,L-selenomethionine with a range of 77Se–1H coupling constants from 9.5 to 54.2 Hz. In these compounds having protons with different 77Se–1H coupling constants the different correlations can be selectively enhanced by varying the mixing time. The enhancement for indirect versus direct 77Se detection was estimated for dimethyl selenide yielding a value close to the theoretical enhancement.

KEY WORDS 77Se NMR; indirect detection; selenophene; benzeneselenol; dimethyl selenide; dimethyl diselenide; selenomethionine

INTRODUCTION

Selenium is important in organic synthesis and moreover it is a biologically essential mineral known to occur in several proteins, usually as a selenocysteine residue, and also in transfer RNA. Selenocysteine residues are found to be incorporated cotranslationally into proteins rather than as the result of posttranslational protein modification. Monoselenophosphate has been discovered as the selenium donor for the synthesis of selenium-dependent enzymes and seleno-RNAs. In addition to their biological roles, selenium-containing compounds such as chselen5 and selenazofurin6 show pharmacological promise. The properties of selenium render structural studies of selenium-containing compounds important. For example, selenium has been incorporated into proteins for multiwavelength anomalous diffraction and NMR spectroscopic studies. In principle 77Se NMR spectroscopy affords an attractive avenue for studying systems where selenium is present. The NMR active 77Se isotope has spin I = 1/2 and a chemical shift range of over 3000 ppm, leading to exquisite sensitivity of this nucleus to its environment. This is well illustrated by the advantageous use of 77Se reagents for the quantitative determination of the enantiomeric excess at remote chiral centers up to seven bonds away from the selenium atom and the apparent detection of overoxidation at Met222 of selenobut-12

Nevertheless, the low magnetogyric ratio of 77Se and its relatively low natural isotopic abundance of 7.58% can make its direct detection difficult. Isotopic enrichment, increased concentrations, and the acquisition of a large number of transients can help alleviate these problems. However, such strategies have been to little avail in studying the catalytically active form of 77Se-enriched glutathione peroxidase on account of its limited solubility, although its reduced and denatured forms could be investigated. Consequently, the development of a method to increase further the sensitivity of 77Se NMR spectroscopy is needed. Detection of insensitive spin I = 1/2 nuclei (13C, 15N, 113Cd, 199Hg and others) can be enhanced by taking advantage of the high magnetogyric ratio and high natural isotopic abundance of the proton using inverse detection. Such enhancement of 77Se by inverse proton detection should be possible and sizable because of the $N(y/y_S)^{3/2}$ theoretical enhancement, where $N$ is the number of sensitive nuclei, $y$ the magnetogyric ratio for the sensitive nuclei and $y_S$ the magnetogyric ratio for the insensitive nucleus. This paper describes for the first time the substantial enhancement of 77Se by inverse proton detection in organoselenium compounds employing multiple-quantum 1H–{77Se} correlation spectroscopy. Recently, the use of 13C–{77Se} correlation spectroscopy has also been reported.

EXPERIMENTAL

Selenophene, benzeneselenol and dimethyl diselenide were procured from Aldrich Chemical (Milwaukee, WI, USA) and were used without further purification. Dimethyl selenide was obtained from Strem Chemical (Newburyport, MA, USA) and D,L-selenomethionine from Janssen Chemica (Geel, Belgium) and were also used without further purification. The selenium compounds contained 77Se at natural isotopic abundance (7.58%). All deuterated solvents were freeze-pump-
thawed to exclude molecular oxygen. The solvents and the selenium compounds were transferred to 5 mm NMR tubes in a rigorously inert atmosphere. The concentrations used for each of the compounds were limited by their solubilities in their respective solvents. An effort was made to try to match previously reported literature concentrations.\textsuperscript{10}

Selenium-77 and proton NMR spectra were recorded with spectrometer (carrier) frequencies from 76.31 to 76.36 MHz and 400.1 MHz, respectively, on a Bruker AMX 400 pulsed Fourier transform NMR spectrometer. Both \textsuperscript{77}Se and \textsuperscript{1}H were detected directly and, employed to detect protons directly and for inverse \textsuperscript{77}Se containing other selenium isotopes. In a typical two-dimensional experiment, spectral widths of 1.50-5 s were typical. All \textsuperscript{77}Se and \textsuperscript{1}H NMR spectra were recorded in a 1K by 1K matrix. Recycle times of the order of 1.50-5 s were typical. A total of 16-64 transients were collected in the phase-sensitive mode for each of compounds (ppm) \(A\frac{w1}{w2}\) of 3.0 Hz was determined from the direct \textsuperscript{77}Se NMR spectrum of this compound. Delay times were set as described (1/25)\textsuperscript{21} to optimize either one-bond or more than one-bond couplings, with and without the decoupling of \textsuperscript{77}Se. Delays were set as described (1/2J)\textsuperscript{20,21} to optimize the signals of those protons scalar coupled to \textsuperscript{77}Se while suppressing the signals of protons in molecules containing other selenium isotopes. In a typical two-dimensional experiment, spectral widths of ca 500-2000 Hz were employed for the \(\omega_2\) dimension and 500-5000 Hz for the \(\omega_1\) dimension. These spectra were all processed in a 1K by 1K matrix. Recycle times of the order of 1.50-5 s were typical. A total of 16-64 transients were collected in the phase-sensitive mode for each of \(16-64\ t_1\) values. Some spectra were apodized with sinebell functions in the \(\omega_2\) dimension. Additionally, some samples were run without spinning to minimize any noise. The 90° pulses for \textsuperscript{77}Se were determined to be 7-10 µs for all compounds.

One-dimensional inverse detection experiments (without \textsuperscript{77}Se decoupling) were performed as described by Bax et al.\textsuperscript{20} to estimate the observed experimental enhancement versus that of directly detected \textsuperscript{77}Se (with \textsuperscript{1}H decoupling). One-dimensional experiments using 100 mm CH\textsubscript{3}SeCH\textsubscript{3} in C\textsubscript{2}HCl\textsubscript{3} were used to obtain the relative enhancement seen in inverse detection versus \textsuperscript{77}Se direct detection. Signal-to-noise (S/N) measurements were calculated using the X32 computer of the Bruker AMX 400 spectrometer. The enhancements were calculated from the number of transients, the S/N as calculated by the computer and the filling factors of the respective coils in the inverse probe using the equations\textsuperscript{17-22}

\[
\text{Enhancement} = \left( \frac{(S/N)_{\text{indir}}(N_c)^{1/2}}{(S/N)_{\text{dir}}(N_c)^{1/2}} \right)^2 \eta_{\text{dir}, \text{indir}}
\]

\[
\eta_{\text{dir}, \text{indir}} = \frac{d_1^2}{2 D_c}
\]

where S/N denotes the signal-to-noise ratio, \(N_c\) the number of scans, \(\eta\) the respective filling factors, \(d_1\) the inside diameter of the NMR tube and \(D_c\) the respective coil diameters.

### RESULTS AND DISCUSSION

To validate the usefulness of inverse detection of \textsuperscript{77}Se by \textsuperscript{1}H NMR, a series of organoselenium compounds (1–5) were investigated whose direct \textsuperscript{77}Se NMR spectra had been reported previously.\textsuperscript{10}

\[
\begin{align*}
\text{CH}_3\text{SeCH}_3 & \quad \text{CH}_3\text{SeSeCH}_3 \\
3 & \quad 4 \\
\text{CH}_3\text{SeCH}_2\text{CH}_2\text{CH}(\text{NH}_3^+)\text{CO}_2^- & \quad 5
\end{align*}
\]

Satisfactory one- and two-dimensional \textsuperscript{1}H-(\textsuperscript{77}Se) heteronuclear multiple quantum coherence (HMQC)\textsuperscript{23,24} spectra were obtained for all of these compounds. The coupling constants and concentration-dependent chemical shifts obtained from these experiments are summarized in Table 1. The chemical shifts and scalar couplings of \textsuperscript{77}Se agree reasonably well with previously reported literature values.\textsuperscript{10} Since the efficacy of magnetization transfer depends on the \textsuperscript{77}Se-\textsuperscript{1}H scalar coupling constant, the influence of this variable was investigated. Two-dimensional heteronuclear multiple quantum coherence (HMQC)\textsuperscript{23,24} experiments provided detection of one-bond (compound 2), two-bond (compounds 1 and 3–5), and three-bond (compound 1) scalar proton–selenium correlations.

Selenophene (1) is selected to illustrate the results in detail because it has both a relatively large and a relatively small \textsuperscript{77}Se-\textsuperscript{1}H coupling constant. In Fig. 1(a) the \textsuperscript{1}H NMR spectrum of selenophene is presented and Fig. 1(b) shows the direct \textsuperscript{77}Se NMR spectrum. In the former spectrum H-1,4 are centered at 8.22 ppm and

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<th>Table 1. \textsuperscript{77}Se NMR spectroscopic parameters from HMQC experiments for organoselenium compounds 1–5</th>
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<tr>
<td>Compound</td>
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<tr>
<td>1\textsuperscript{b,c}</td>
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<td>2\textsuperscript{d}</td>
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<td>3\textsuperscript{a}</td>
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\(a\) \(\delta(\textsuperscript{77}Se)\) (ppm) relative to 60% (v/v) solution of 3 in C\textsubscript{2}HCl\textsubscript{3}.

\(b\) C\textsubscript{2}HCl\textsubscript{3} as solvent.

\(c\) \(J(\textsuperscript{77}Se,\textsuperscript{1}H)\) of 9.2 Hz was determined from the direct \textsuperscript{77}Se NMR spectrum of this compound.

\(d\) \(J(\textsuperscript{77}Se,\textsuperscript{1}H)\) of 3.0 Hz was determined from the direct \textsuperscript{77}Se NMR spectrum.

\(e\) H\textsubscript{2}O as solvent.

\(f\) Coupling constant for H-5.
Figure 1. (a) $^1$H NMR spectrum of selenophene (1) at 400.13 MHz. (b) $^{77}$Se NMR spectrum of selenophene (1) at 76.358 MHz. (c) 1D HMQC spectrum of selenophene (1) obtained from eight transients with a spectral width of 2 kHz, 16k data points, a 7.5 $\mu$s 90° selenium pulse and an interpulse delay of 2 s.

H-2,3 are centered at 7.56 ppm. The $^{77}$Se satellites from H-1,4 are seen at 8.16 and 8.28 ppm and the $^{13}$C satellites at 7.98 and 8.45 ppm in this spectrum. However, owing to the small coupling constant between H-2,3 and $^{77}$Se, the $^{77}$Se satellites from H-2,3 lie beneath the $^1$H signal at 7.56 ppm. Nevertheless, the $^{13}$C satellites from H-2,3 are seen at 7.36 and 7.77 ppm. In the $^{77}$Se NMR spectrum in Fig. 1(b) the $^{77}$Se resonance is split by H-1,4 into a triplet with a coupling constant of 47.3 Hz, which is further split by H-2,3 with a coupling constant of 9.2 Hz. Figure 1(c) illustrates the one-dimensional, inverse detected spectrum corresponding to the H-1,4 protons. Comparison of Fig. 1(c) with Fig. 1(a) shows that the $^{77}$Se satellites of the H-1,4 absorption at 8.22 ppm are more apparent because the signal of protons that are adjacent to non-magnetic selenium isotopes is suppressed.

Two-dimensional $^1$H-$^{77}$Se HMQC spectra of selenophene (1) are shown in Fig. 2 optimized for the large $^{77}$Se-1H coupling constant in selenophene, and the $^{13}$C coupling constant. The $^1$H NMR spectrum is shown along the horizontal $\omega_2$ axis and the $^{77}$Se NMR spectrum along the vertical $\omega_1$ axis in each case. These projections demonstrate that both sets of proton signals (H-1,4 and H-2,3) can be seen even though the delay is optimized for either the large or small coupling constants. In Fig. 2(a), the more deshielded H-1,4 protons show the larger signal in the proton dimension ($\omega_2$) whereas the signal due to H-2,3 is still observed. Conversely, in Fig. 2(b) the signal centered at 7.56 ppm due to H-2,3 is enhanced relative to that of H-1,4 but the H-1,4 signal centered at 8.22 ppm is also seen. The results show that both a two-bond (large coupling constant) and a three-bond (small coupling constant) can be seen in the same spectrum. Either correlation can be enhanced by varying the mixing time, thereby optimizing either the two- or three-bond correlation. The signal that is seen in the proton dimension is directly correlated with the delay times used in the mixing period of a two-dimensional experiment. As this delay is usually set as a function of the reciprocal of the scalar coupling constant (1/2J), one would expect resonances with larger $^{77}$Se-1H coupling constants to have a more efficient transfer of magnetization (from the protons to selenium) owing to the shorter delay than those with smaller $^{77}$Se-1H scalar coupling constants. Consequently, the larger $^{77}$Se-1H coupling constants give the larger enhancement values as seen experimentally.

Benzeselenol (2) was selected for study because it features another important functional group and has a one-bond $^{77}$Se-1H coupling. The magnitude of this coupling constant is similar to that of the larger two-bond coupling constant in selenophene, and therefore provides comparable results. On account of the relatively large one-bond coupling constant 1H($^{77}$Se, $^1$H), the polarization transfer is fairly efficient and thus a relatively short delay time (1/2J) is needed to optimize
the corresponding indirect signal. This one-bond coupling constant of 54.2 Hz is clearly evident in the one-dimensional $^1$H--$^{77}$Se HMQC spectrum of 2, which is consistent with a lack of rapid exchange of the selenol hydrogen. In general, the HMQC spectra of benzeneselenophene, but with the distinction that a one-bond coupling is observed versus a two-bond coupling.

For compounds 3-5 the two-bond $^{77}$Se--$^1$H scalar coupling constants are relatively small and comparable to the three-bond $^{77}$Se--$^1$H coupling in selenophene (1) as discussed above. Satisfactory one- and two-dimensional HMQC spectra were obtained in all cases (not shown; cf. Table 1) after optimizing the delay times used in the mixing period for these spectra as described above for selenophene (1). In addition, the one-dimensional HMQC spectrum of 3 allowed a determination of the enhancement for indirect versus direct $^{77}$Se detection, as described in detail below. It is worth noting that the two-dimensional $^1$H--$^{77}$Se HMQC experiment with selenomethionine (5) provides definitive information regarding scalar coupling correlations which can facilitate assignment of the $^1$H NMR spectra of selenomethionine derivatives, and also proteins into which selenomethionine has been incorporated.9

To estimate the enhancement due to indirect versus direct $^{77}$Se detection, a 100 mM sample of dimethyl selenide (3) in $^2$HCl$_2$ was investigated. Because of the correspondence between the magnitude of the scalar coupling constant and the expected enhancement, a representative sample was chosen that would give an enhancement of the order of that expected for a large selenium-containing biomolecule with scalar $^{77}$Se--$^1$H couplings. As the refocusing period (set equal to 1/2J) is longer in this compound because of the small coupling constant, less effective magnetization transfer is expected if the relaxation times of the protons are close in magnitude to those of compounds containing larger scalar coupling constants.

Figure 3(a) displays the direct $^{77}$Se NMR spectrum of this compound without $^1$H decoupling. The $^{77}$Se signal is split into a septet by the six equivalent protons that are scalar coupled to the $^{77}$Se with a coupling constant of 10.2 Hz. Figure 3(b) shows an expansion of the $^1$H NMR spectrum of this compound without $^{77}$Se decoupling. The satellites of the main signal of 1.68 and 1.72 ppm are due to the molecules that contain $^{77}$Se at natural isotopic abundance. This can be verified by decoupling $^{77}$Se, in which case the satellites disappear (not shown). The main peak is due to methyl protons of molecules containing selenium isotopes other than $^{77}$Se. The spectrum shown in Fig. 3(c) was obtained by direct $^{77}$Se detection of the 100 mM dimethyl selenide sample in $^2$HCl$_2$ with proton decoupling. Figure 3(d) presents the inversely detected $^1$H--$^{77}$Se HMQC spectrum of the 100 mM dimethyl selenide sample in $^2$HCl$_2$. Comparison of this spectrum with that in Fig. 3(b) reveals that the center peak in the $^1$H NMR spectrum has been suppressed, revealing the satellites. This result is more meaningful for ascertaining the enhancement in indirect detection, e.g. as opposed to the case with $^{77}$Se decoupled. If $^{77}$Se were decoupled in this experiment, a fraction of the signal obtained could be due to the methyl peak of those protons in molecules having non-magnetic selenium isotopes. By not decoupling $^{77}$Se in the inverse spectrum, one can be assured that all of the methyl signal resulting from protons that are not scalar coupled to $^{77}$Se is suppressed, and that the signal obtained is due solely to indirect detection of $^{77}$Se. The data were corrected for (i) differences in the number of acquisitions, (ii) the differences in S/N [after doubling the S/N for the indirect measurement because a doublet is obtained as seen in Fig. 3(d)] and (iii) the different filling factors due to the different coils used in each detection method. No correction was made for any nuclear Overhauser effect.
inherent sensitivity and high natural abundance of the proton should prove to be generally useful in the detection of dilute selenium samples. When isotopic enrichment is combined with the use of inverse detection, one can realize sensitivity increases of the order of 800-fold. Thus HMQC methods are advantageous for inverse detection of 77Se by 1H NMR, and such methodology may be useful in the future with regard to NMR spectroscopic studies of biomolecules such as selenoproteins.

Acknowledgement

The authors gratefully acknowledge financial support from the American Heart Association (Grant BG-2-13-92 to R.S.G.) and the US National Institutes of Health (Grants GM14113 and RR03529 to M.F.R.), and helpful discussions with Dr Ad Bax and Professor Michael Barfield.

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