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The Theoretical ^{77}Se Chemical Shift as a Probe of Selenium State in Selenoproteins and Their Mimics

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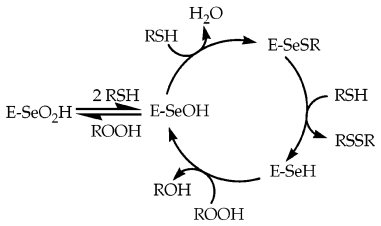
Theoretical ^{77}Se chemical shifts of a series of simple organoselenium compounds are compared to known NMR data for various derivatives of selenoenzymes and selenoamino acids. Since the theoretical data only differs from the biochemical data set by an overall ∼15–30 ppm downfield shift, simple theoretical model studies are suggested as an additional tool for the interpretation of selenoenzyme spectra. Further studies demonstrate that model systems can be extended to incorporate the effects of intramolecular interactions (such as Se···N bonds).

The antioxidant activity of glutathione peroxidase (GPx) arises from selenium's ability to cycle between oxidation states (Scheme 1).[1] Oxidants which may cause cellular damage are consumed by reaction with the selenol form of the selenocysteine (SeCys) residue at the enzyme's active site. The resulting selenenic acid (E-SeOH) returns to the resting state through a selenenyl sulfide (E-SeSR) intermediate by two consecutive reactions with glutathione (GSH). In addition, an overoxidized seleninic acid form (E-SeO_2H) is observed in crystal structures of GPx and the semisynthetic peptide selenosubtilisin (SS) and can be activated by treatment with GSH.[2]

The sensitivity of the selenium nucleus to changes in its electronic environment,[3] such as those in the GPx cycle, makes ^{77}Se NMR an ideal tool for detection of long-lived intermediates over the course of a reaction. Intermediates have been successfully identified for GPx[4] and SS[5] demonstrating the utility of ^{77}Se NMR for large protein molecules.

Dowd and Gettins synthesized model compounds of selenoprotein active sites to help identify resonances corresponding to intermediates in the GPx cycle; however, their approach was limited by the synthetic availability of reasonable model compounds.[6] Models are often used in theoretical studies to truncate large systems to computationally manageable sizes. Since the chemical shielding of selenium in a selenoenzyme will only be affected significantly by interactions at the active site, theoretical studies of small organoselenium compounds should afford the same success as the experimental study of Dowd and Gettins without the synthetic limitations. In the following study, calculations are performed on models of selenoenzyme active sites where the enzyme has been truncated to a methyl group. This truncation assumes that only the oxidation state and functionality of selenium are critical to its chemical shielding. The small size of the model allows one to avoid the limited basis sets required for larger, more realistic systems while providing the flexibility to extend the model to incorporate interactions (such as Se···N bonds) which can significantly contribute to the selenium chemical shift.

Scheme 1



Chemical shieldings can be calculated theoretically using a variety of methods, foremost being gauge-invariant atomic orbitals (GIAO)[7] which have been implemented in the usual theoretical methods (HF, MP2, DFT, etc.). Selenium is among the elements examined using GIAO techniques,[8] and studies have shown that MP2-based methods provide more reliable results than the cheaper DFT-based approaches.[8e,9]

The theoretical data in Table 1 has been obtained by MP2 calculations in two basis sets.[10] Basis set I (BSI) uses the Schafer et al. double-ζ representation for selenium and sulfur[10a] and Dunning's split-valence triple-ζ representation

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Table 1. Comparison of GIAO-MP2 Chemical Shifts of Model Compounds to Their Experimental Counterparts[a]

model	δ ^{77}Se, ppm MP2/BSI (MP2/BSII)	modeled compounds	δ ^{77}Se, ppm exptl	exptl conditions
MeSeH	−128 (−155)	MeSeH	−130,[b] −155[c]	acetone-d_6
		SeCys	−141[b]	D_2O, pD 5
$MeSe^-$	−215 (−233)	NaSeMe	−330[b,c]	D_2O, pD 10,
			−332[d]	H_2O
		E-Se^- (SS)	−215[e]	D_2O, pD 7
		E-Se^- (GPx)	−212[f]	D_2O, pD 8
		SeCys	−267[g]	D_2O, pD 11.7
		AESe	−212[b]	D_2O, pD 8.3
MeSeOH	1153 (1190)	2,4-DNPSeOH	1099[h]	$PhNO_2$-d_5
$MeSeO_2^-$	1199 (1211)	E-SeO_2^- (SS)	1188,[e] 1190[e]	D_2O, pD 7
$MeSeO_2H$	1270 (1281)	$MeSeO_2H$	1216[d]	H_2O
		E-SeO_2H (SS)	1216[e]	D_2O, pD 3.87
		ebselen-SeO_2H	1265[i]	DMF
		$AESeO_2H$	1226[h]	D_2O
MeSeSMe	333 (365)	E-SeSR (SS)	389[e]	D_2O, pD 5
		E-SeSR (GPx)	377[f]	D_2O, pD 8
MeSeSeMe	249 (268)	MeSeSeMe	281,[c] 268,[d] 270,[j] 275[k]	$CDCl_3$
		SeCyx	294[l]	D_2O, pD ∼1
		AESeSeCNP	Se_{Ar}: 517[f]	D_2O, pD 8
			Se_{alk}: 234[f]	
MeSeEt	67 (74)	MeSeEt	108[d]	CH_2Cl_2
		SeMet	75[b]	D_2O, pD 4
		MeAESe	44[b]	$CDCl_3$
		E-SeAcm(GPx)	195[f]	D_2O, pD 8
Me_2SeO	804 (805)	Me_2SeO	819,[b] 812[d]	D_2O, pD 7, H_2O
		SeMet oxide	844,[m] 850[n]	D_2O

[a] Referenced to Me_2Se. [b] Reference 12a. [c] Reference 12b. [d] Reference 12c. [e] Reference 4. [f] Reference 5. [g] Reference 12d. [h] Reference 6. [i] Reference 12e. [j] Reference 12f. [k] Reference 12g. [l] Reference 12h. [m] Reference 12i. [n] Reference 12j.

for all other atoms.[10b] Polarization functions were included for all heavy atoms. BSII adds diffuse functions to all non-carbon heavy atoms and a set of f-type polarization functions to Se ($\zeta_{f,Se} = 5.8$). GIAO-MP2 shifts were calculated from the MP2-optimized geometry and referenced to calculated isotropic shieldings of Me_2Se in BSI and BSII using eq 1.

$$\delta_i^{calc} = \sigma_{ref,Me_2Se}^{calc} - \sigma_{i,Se}^{calc} \quad (1)$$

Structures corresponding to the minimum on the potential energy surface were used to calculate the ^{77}Se chemical shieldings; thus, rovibrational and dynamic effects have been neglected. Relativistic effects are not explicitly considered in these calculations, but effects due to the contraction of the inner shell electrons are uniform[8e] and cancel in the calculation of the relative chemical shift (eq 1). The accuracy of the methods in this Communication will be discussed in more detail separately.[11]

The GIAO-MP2 shifts for the model compounds generally correlate well to known experimental data for MeSeH, MeSeEt, $MeSeO_2H$, MeSeSeMe, and Me_2SeO (Table 1).[12] Plots of the experimental shifts versus theoretical data from Table 1 show that the addition of diffuse and polarization functions in BSII ($R^2 = 0.991$) represents a slight improvement over BSI ($R^2 = 0.988$). The most significant deviation is obtained for methylselenolate $MeSe^-$, which lies 100 ppm downfield of the experimental value of NaSeMe. This error surfaces from comparison of a gas-phase anion with a solvated species as compounds with anionic selenium centers tend to be solvent dependent (for example: HSe^- (δ −495 ppm (EtOH);[13a] −529 ppm (H_2O)[13b]).

Given the excellent overall correlation between theory and experiment for the model compounds, the calculated shifts of the methyl derivatives have been compared to experimental data for derivatives of denatured GPx and SS, SeCys, selenocystine (SeCyx), 2-aminoethylselenol (AESe), selenomethionine (SeMet), and ebselen (Table 1). High correlation between the ^{77}Se chemical shifts of these compounds and the model systems will indicate whether the model is sufficiently accurate to assist interpretation of future NMR studies of selenoproteins such as thioredoxin reductase,[14a] selenoprotein P,[14b,c] or the Se−Mo enzyme carbon monoxide dehydrogenase.[14d]

Substitution of H_{Me} with Me on MeSeH and NaSeMe shifts the selenium resonance downfield by an average of 136 and 154 ppm, respectively, for each additional methyl group (e.g., EtSeH, 39 ppm; ^{i}PrSeH, 159 ppm; ^{t}BuSeH, 278 ppm).[3] However, for enzyme and amino acid derivatives, the ^{77}Se chemical shifts of the selenols and selenolates are very similar to the theoretical shifts of the methyl model compounds. Fortuitous counterbalancing of solvent and inductive effects may allow for good correlation between the theoretical model compounds and the experimental selenolates.

Selenenic (−SeOH) and seleninic acid ($-SeO_2H$) derivatives should be highly deshielded, but the instability of the former has complicated its isolation for NMR study. Known derivatives of the unstable selenenic acids are aryl compounds with ortho nitro,[6] carboxy,[15] or amine[16] groups (for example, 2,4-dinitrophenylselenenic acid (2,4-DNPSeOH[6] in

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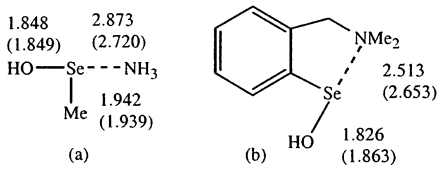

Figure 1. (a) MP2/BSI (MP2/BSII) geometries of the $MeSeOH{\cdot}NH_3$ complex. (b) HF/3-21g* (HF/LANL1DZ) geometry of a model compound with an intramolecular interaction.

Table 1)). These substituents prevent oxidation to the seleninic acid by donating electron density to the selenium. Similar intramolecular interactions are believed to occur at the active site of GPx and have been designed into organoselenium GPx mimics.[17] Barton et al. describe similar interactions as hypervalent three-center four-electron bonds because of short selenium−donor bond distances and near linear ∠N,O−Se−OH bond angles.[18]

These stabilized selenenic acids typically appear between 1020 and 1100 ppm or 100−150 ppm upfield of the calculated shift of MeSeOH. The model system was extended to incorporate intramolecular effects by calculating the shielding of a complex of ammonia and MeSeOH. The amine was placed trans to the −OH group to simulate the 3c−4e interaction. The optimized geometry is shown in Figure 1. While the calculated Se−N bond length (BSI, 2.87 Å; BSII, 2.72 Å) in $MeSeOH{\cdot}NH_3$ is much larger than the intramolecular interaction (HF 2.51−2.65 Å) in an ortho substituted model compound (Figure 1),[16] the shielding for the complex (BSI, 1048 ppm; BSII, 1083 ppm) is in the correct range for a stabilized selenenic acid.

For the seleninic acid derivatives, the observed resonances for E-SeO_2^- (SS) at high pH match well with the computed values for methylseleninate $MeSeO_2^-$.[5] Under acidic conditions, there is similar agreement to known −SeO_2H shieldings, although there is a larger upfield shift upon deprotonation in the $MeSeO_2H/MeSeO_2^-$ conjugate pair (28−30 for E-SeO_2H (SS), 70 for $MeSeO_2H$).

The reduction of E-SeOH by thiol to the selenenyl sulfide E-SeSR shifts the selenium shielding upfield. The GIAO-MP2 shieldings of our methyl model systems show similar shifts and correlate well for both −SeS− compounds and the related diselenides. However, in each case where selenium is attached to a phenyl ring, the experimental resonances appear ∼200−300 ppm downfield of their methyl analogues. Delocalization of charge density through resonance with the aromatic ring causes organoselenium compounds in low oxidation states to be sensitive to the nature of bonded R groups. In higher oxidation states, the selenium shielding is dominated by electronegative groups.

Although SeMet does not appear at the active site of any known enzyme, it is a known antioxidant and, thus, may play an important role in cancer protection.[19] As primary R groups generally result in downfield shifts compared to Me,

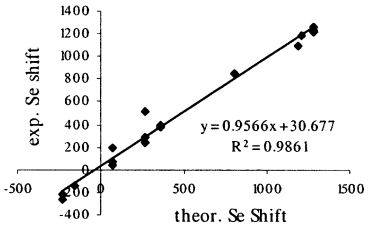

Figure 2. Plot of experimental ^{77}Se shift for selenoenzyme and selenoamino acid derivatives versus theoretical ^{77}Se shifts (GIAO-MP2/BSII) of model compounds. Data are from Table 1.

SeMet has been modeled by MeSeEt to help distinguish it from the reference. Choice of a larger model may be necessary at times to more accurately mimic the chemical environment around selenium.

While the error between the experimental and theoretical shifts of MeSeEt is large, the GIAO-MP2 shift of the model agrees well with the experimental value of SeMet. The alkylation product of GPx with iodoacetamide (E-$SeCH_2CONH_2$ (E-SeAcm))[2a] and *Se*-methyl-AESe (MeAESe) are shifted downfield and upfield, respectively, reflecting the sensitivity of Se to the R groups in low oxidation states. The selenoxide of SeMet appears at 840−850 ppm,[10i,j] 25 ppm upfield of Me_2SeO, but much less than the 75 ppm shift between Me_2Se and SeMet indicating the greater effect electronegative groups have upon shielding in high oxidation states.

A plot of the literature enzyme/amino acid ^{77}Se data versus the calculated shifts of model compounds (Figure 2) demonstrates the potential of the method described in this Communication. Linear regression reflects a near 1:1 correspondence where the theoretical data set is shifted slightly downfield by 30 ppm. The accuracy is further increased if only aliphatic selenium compounds are considered (omission of the aryl Se shift in AESeSeCNP reduces the intercept by 50%).

The parallels between the calculated ^{77}Se shifts of the model compounds and known experimental data for biological selenium species demonstrate the value of theoretical calculations in the interpretation of NMR spectra. Similar models may be useful for mechanistic studies of selenoproteins and their mimics through the identification of unknown resonances or the detection of intramolecular interactions through comparison of complexed and free systems (e.g., MeSeOH vs $MeSeOH{\cdot}NH_3$). These results may also be helpful for studies of the variety of selenium metabolites present in biological systems.[20] Further research is under way to expand and refine this method.

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Supporting Information Available: Theoretical geometries of the model compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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