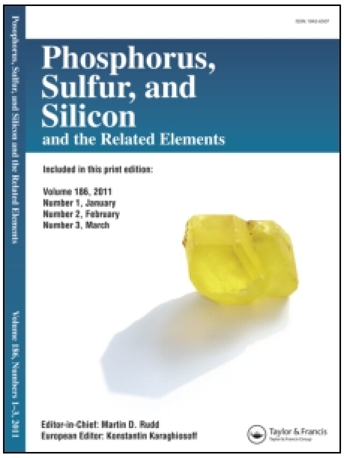

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Application of the ^{77}Se Solid State NMR for Investigation of Bioorganic Compounds—the Case of Selenomethionine

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Variable Temperature ^{77}Se Cross Polarization Magic Angle Spinning (CP/MAS) NMR experiment was used to study of structure and dynamics of L-selenomethionine (L-SeMet) in the solid state. The ^{77}Se principal elements of chemical shift tensor δ_{ii} and shielding parameters were calculated employing SIMPSON fitting procedure. Correlation between experimental and computed ^{77}Se δ_{ii} parameters and molecular geometry of L-SeMet in the crystal lattice is discussed.

Keywords ^{77}Se solid state NMR; DSC; L-Selenomethionine; phase transitions

INTRODUCTION

Selenium is an important trace element involved in a number of metabolic processes in the living organisms.[1] In the human body, the natural source of selenium is food. Products containing two important amino acids, selenocysteine (SeCys) and selenomethionine (SeMet) are significant supplement of diet. SeMet is often incorporated into protein in place of methionine (Met), because tRNA$^{\mathrm{Met}}$ does not discriminate between Met and SeMet.[2] This replacement does not significantly alter protein structure but may influence the activity of some enzymes (e.g. oxyreductases).

We reviewed the structural studies of L-SeMet in different environments.[3] A number of experimental techniques were employed

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to study the structure and molecular dynamics in the liquid phase.[4] So far, less attention has been focused on investigation in the condensed phases. In particular, it is surprising that X-ray structure of L-SeMet has not been reported, despite the fact that selenomethionyl proteins now account for about two-thirds of all new protein crystal structures phased by multiwavelength anomalous diffraction (MAD).[5]

In our recent paper, we reported first X-ray diffraction data of L-SeMet collected at 130 K.[6] The cryo-cooling of crystals has now become standard procedure in crystallography of biological samples.[7] However, there is a risk that structures determined at low temperature may suggest conclusions based on aspects of the structure that not necessarily relevant at room temperature.[8] Hence, the knowledge about room-temperature structures obtained from X-ray data collection or employing other complementary techniques are strongly desired. In this work, we present a systematic study of the thermal processes in L-SeMet crystals using variable temperature (VT) solid-state NMR techniques and differential scanning calorimetry (DSC).

RESULTS AND DISCUSSION

^{77}Se nucleus (spin ½) was found to be useful probe in structural studies of seleno-organic and seleno-inorganic compounds.[9,10] The selenium nucleus characterizes wide chemical shifts range over 3000 ppm and is very sensitive to change in molecular structure. The interpretation of NMR spectra recorded in liquid phase is usually straightforward. The isotropic chemical shifts ^{77}Se δ_{iso} and J-couplings are commonly employed for structure refinement. In the solid state, the analysis of NMR spectra is more complex. Due to large chemical shift, anisotropy (CSA) the isotropic line is flanked by number of spinning sidebands and assignment of ^{77}Se δ_{iso} is sometimes ambiguous. On the other hand, solid-state NMR, which can yield the symmetric part of the chemical shift tensor (CST), is potentially more informative than its solution counterpart. Analysis of principal components of CST ^{77}Se δ_{ii} is source of very detailed information about electronic surrounding of the nucleus and deformation of molecular geometry.

Figure 1 shows ^{77}Se CP/MAS spectra of SeMet at 183 K (Figure 1a), at room temperature (Figure 1b) and at 313 K (Figure 1c). The ^{77}Se δ_{ii} CST have been established employing SIMPSON program.[11] From inspection of spectra at 183 K and 298 K it is apparent that the asymmetric unit consists of two molecules (A and B). At higher temperature

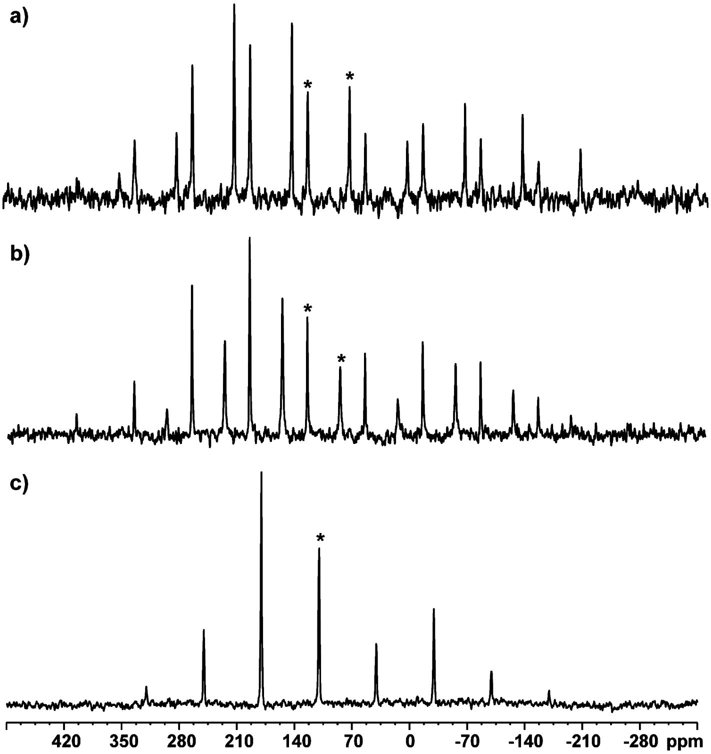

FIGURE 1 Experimental ^{77}Se CP/MAS NMR spectrum of 1 recorded at (a) 183 K, (b) 298 K, (c) 313 K with the spinning rate of 4 kHz. Asterisks denote the isotropic lines.

(Figure 1c) the phase transition takes place and symmetry of the unit cell is changed. Only one molecule in the unit cell exists. It is noteworthy that this process is reversible.

The analysis of the values of the CST[6], shows that the shielding parameters of the ^{77}Se nuclei are dependent on the temperature of measurement. At 183 K the anisotropy parameter values (Ω) are larger compared to those observed at 298 K. The difference was found to be ca 70 ppm for molecule B. A more spectacular narrowing of the ^{77}Se line is observed at 313 K, after the phase transition. The difference in span values is 200 ppm. This result proves that C_{3V} rotation of the Se-Me group has a significant influence on the ^{77}Se δ_{ii} parameters.

In the last few years, theoretical calculations of NMR parameters for such nuclei as ^{1}H, ^{13}C and ^{15}N has become increasingly useful for spectroscopists. Comparison of experimental and theoretical results can be used for signal assignment, analysis of dynamic process and explanation of nature of intra- and/or intermolecular interactions (e.g., hydrogen bonding). Calculation of selenium NMR shielding parameters is still challenging for theoretical chemistry. The open question is how relativistic effects are important for the calculation of ^{77}Se CST. Several authors have discussed this problem in detail.[12,13,14] Recently, Bayse has presented improved procedures, which allows the calculation of reliable ^{77}Se parameters for selenium proteins in the liquid phase.[15,16] Wasylishen and coworkers employing solid-state ^{77}Se NMR data for several organic, organophosphorus, and inorganic selenium compounds have reported new approach for analysis of computed CST.[17]

In our project, the low-temperature X-ray structure of SeMet was taken as input. The calculations were carried out with a dimer. In general, the advantage of such an approach is related to the fact that it is possible to compare the theoretical and experimental results for molecules with exactly the same geometry of heavy atoms. Analysis of theoretical data[6] reveals that the obtained results only roughly correspond to the experimental values. The difference between isotropic shielding values ($\Delta\delta_{\text{iso}}$) for A and B molecules is 150 ppm while Ω are found to be 731 and 851 ppm, respectively. The appropriate ^{77}Se chemical shift parameters at 297 K are $\Delta\delta_{\text{iso}} = 38.6$ ppm and span 548 and 580 ppm. Variable temperature ^{77}Se measurement shows that the dynamic processes have a significant influence on the chemical shift anisotropy parameters. With decreasing temperature, the difference between ^{77}Se δ_{iso} values increases. A similar trend is observed for span values. Thus, we can conclude that unhindered rotation around C$^{\gamma}$-Se-C$^{\delta}$ bond reduces the CSA. This effect is clearly seen for molecule B. The measurements at very low temperature, much below 183 K, should further improve the consistency between theoretical calculations and measured values.

The variable temperature ^{77}Se results are consistent with ^{13}C CP/MAS data. Figure 2 shows ^{13}C solid state NMR spectra recorded in temperature range 183K-313 K. The standard notation common for amino acids was used to ^{13}C signal labeling in SeMet (presented on top of spectrum). It is apparent from these data that two molecules of SeMet in the crystal are not equivalent, as each signal is split. With the increase of temperature, the $\Delta\delta$ values decrease for γ and δ carbons while α and β are almost the same as those at 183 K.

Such results suggest that carbon atoms of SeMet are under different motional regime, with a relatively rigid backbone (C0 and Cα carbons) and a mobile side chain (β, γ and δ carbons). At 253 K, coalescence of the γ and δ signals is observed, whereas the α and β are still split. The

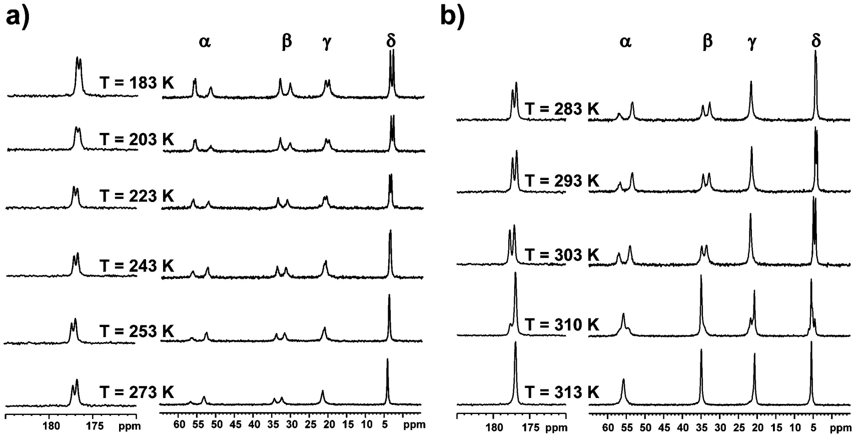

FIGURE 2 Variable temperature (a) (183-273 K); and (b) (283-313 K) ^{13}C CP/MAS NMR spectra of L-selenomethionine.

important information is the apparent change of the spectral pattern for the α and β carbons at 310 K. At 313, after the phase transitions single resonances for each carbons prove that only one molecule is independent part of the unit cell.

Finally, differential scanning calorimetry (DSC) experiment proved that transition of selenomethionine phase occurs at a temperature of 310 K. Figure 3 shows the DSC profile of L-SeMet below the melting temperature.

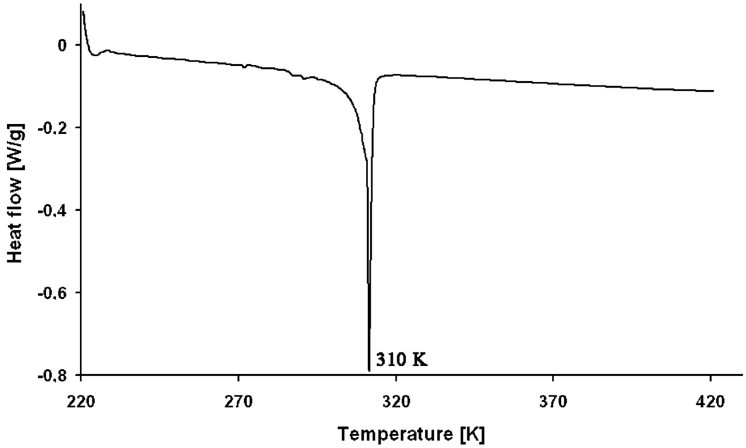

FIGURE 3 DSC profile of L-selenomethionine.

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