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ScienceDirect
Journal of Molecular Structure 843 (2007) 45–56
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

Experimental determination of the carboxylate oxygen electric-field-gradient and chemical shielding tensors in L-alanine and L-phenylalanine

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Received 21 October 2006; received in revised form 12 December 2006; accepted 20 December 2006
Available online 12 January 2007

Abstract

We report a solid-state ^{17}O NMR study of the ^{17}O electric-field-gradient (EFG) and chemical shielding (CS) tensors for each carboxylate group in polycrystalline L-alanine and L-phenylalanine. The magic angle spinning (MAS) and stationary ^{17}O NMR spectra of these compounds were obtained at 9.4, 14.1, and 16.4 T. Analyzes of these ^{17}O NMR spectra yielded reliable experimental NMR parameters including ^{17}O CS tensor components, ^{17}O quadrupole coupling parameters, and the relative orientations between the ^{17}O CS and EFG tensors. The extensive quantum chemical calculations at both the restricted Hartree–Fock and density-functional theories were carried out with various basis sets to evaluate the quality of quantum chemical calculations for the ^{17}O NMR tensors in L-alanine. For ^{17}O CS tensors, the calculations at the B3LYP/D95** level could reasonably reproduce ^{17}O CS tensors, but they still showed some discrepancies in the δ_{11} components by approximately 36 ppm. For ^{17}O EFG calculations, it was advantageous to use calibrated Q value to give acceptable C_Q values. The calculated results also demonstrated that not only complete intermolecular hydrogen-bonding networks to target oxygen in L-alanine, but also intermolecular interactions around the NH_3^+ group were significant to reproduce the ^{17}O NMR tensors.

Keywords: Solid-state ^{17}O NMR; Chemical shielding tensor; Electric-field-gradient tensor; L-Alanine; L-Phenylalanine

1. Introduction

Oxygen is a key element in biological systems and very often plays major roles in a number of biological activates. Thus, solid-state ^{17}O nuclear magnetic resonance (NMR) is expected to become an efficient tool to investigate the physiochemical properties of biological compounds. Compared with conventional solid-state NMR such as ^{1}H, ^{13}C, and ^{15}N, however, very little is known about solid-state ^{17}O NMR of biological systems. This may be due to the fact that the only NMR accessible nucleus among the oxygen stable isotopes, ^{17}O, contains unfavorable properties for NMR measurements (natural abundance = 0.038%, $\gamma = -3.62808 \times 10^7$ rad T^{-1} s^{-1}, $Q = -2.558$ fm^2). Nevertheless, several applications of solid-state ^{17}O NMR to biological molecules have been reported thanks to the recent development of solid-state NMR techniques [1–4].

One of the advantages of solid-state NMR is to make it feasible to handle quadrupolar nuclei in contrast to the cases of solution NMR in which it is generally difficult to deal with quadrupolar nuclei due to rapid quadrupole relaxation properties. It is also known [5,6] that solid-state NMR spectra contain more information than the solution NMR spectra. More specifically, solid-state NMR provides an opportunity to obtain the anisotropic information of all

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doi:10.1016/j.molstruc.2006.12.034

NMR parameters. In principle, NMR parameters are expressed by second-rank tensors and the anisotropy is capable of yielding more detailed information concerning the three-dimensional molecular and electronic structure. The relevant NMR tensors for solid-state ^{17}O NMR of biological solids are the electric-field-gradient (EFG) tensor and the chemical shielding (CS) tensor.

Previously, some solid-state ^{17}O NMR studies [7,8] were reported on amino acids, which are the fundamental monomers of peptides and proteins. It was shown that the ^{17}O NMR parameters such as isotropic chemical shifts were sensitive to the hydrogen-bonding strengths. A few theoretical works of ^{17}O NMR tensors for amino acids and amino acid hydrochlorides were reported previously [9,10]. As far as we know, however, experimental determination of ^{17}O NMR tensors for amino acids was not reported. Recently, our group demonstrated that ^{17}O NMR tensors for γ-glycine could be unambiguously derived by analyzing the ^{17}O stationary NMR spectra measured at multiple magnetic fields [11]. We believe that, to truly understand the ^{17}O NMR parameters of amino acids, it is mandatory to deal with ^{17}O NMR tensors instead of isotropic values. The detailed analysis of tensor components may offer new insights into mechanisms of hydrogen bonds for the future applications to biological macromolecules such as proteins, and the accumulation of experimental data for ^{17}O NMR tensors in amino acids is of importance. In this paper, we will present the experimental determination of ^{17}O CS and EFG tensors in polycrystalline L-alanine and L-phenylalanine. This work is part of a systematic investigation of amino acids, peptides, and proteins by solid-state ^{17}O NMR performed at RIKEN Genomic Sciences Center.

2. Experimental

Water at 80–85% ^{17}O atom was purchased from Taiyo Nippon Sanso Corp. (Tokyo, Japan). [^{17}O]-enriched L-alanine and L-phenylalanine were independently obtained by the saponification of the corresponding *N*-α-Fmoc amino acid pentafluorophenyl esters with the enriched water (80–85% atom %). Deprotection of the N-terminal protecting group was achieved by trifluoroacetic acid in dichloromethane in order to yield free L-alanine and L-phenylalanine. The white crystalline crudes were purified by ion-exchange chromatography. The detailed procedures have been described elsewhere [12]. The solution NMR and MS results were in good agreement with the literature values. The powder X-ray diffraction was taken on Rigaku Ultima+ diffractometer using Cu Kα radiation at room temperature. The diffraction patterns were obtained from $2\theta = 3.00$ deg to $2\theta = 90.00$ deg in 0.02 deg steps in a scan rate of 10 deg/min. From the diffraction patterns, the space groups of the [^{17}O]-enriched L-alanine and L-phenylalanine were confirmed to be $P2_12_12_1$ ($Z = 4$, $a = 6.031$ Å, $b = 12.351$ Å, $c = 5.782$ Å) [13] and $P2_12_12_1$ ($Z = 8$, $a = 12.6$ Å, $b = 30.8$ Å, $c = 4.8$ Å) [14], respectively.

Solid-state ^{17}O NMR spectra were recorded on JEOL ECA-700, Bruker Avance-600, and Chemagnetics Infinity-400 spectrometers, operating at 94.9, 81.4, and 54.2 MHz for ^{17}O nuclei, respectively. Proton decoupling with the TPPM scheme [15] was employed during the data acquisition in all the NMR experiments. Polycrystalline samples were packed into either zirconium oxide or silicon nitride rotor (4 mm o.d.). Typical sample spinning frequencies for MAS experiments were 13–20 kHz. In the static ^{17}O NMR experiments at 14.1 T, the echo sequence of Oldfield and coworkers [16] was employed. Typical recycle delays were between 5 and 10 s. An external sample of liquid water was used for chemical shift referencing. Spectral simulations were carried out with the program written by the authors on MATLAB (The MathWorks, Inc.) on a Pentium IV personal computer (3.00 GHz, 1 GB memory, 200 GB disk space).

All *ab initio* calculations were performed with the Gaussian03 program [17] on RIKEN Super Combined Cluster (RSCC). The quantum chemical calculations were carried out using the restricted Hartree–Fock (RHF) and density-functional theory (DFT). In the DFT calculations, the B3LYP exchange functional was employed [18–20]. The following basis sets were used: STO-3G, 6-31G, D95**, TZVP, 6-311G**, 6-311+G**, 6-311++G**, and cc-pVTZ. The experimental geometry of L-alanine determined by a neutron diffraction study [21] was derived from the Cambridge Structural Database [22].

The gauge-induced atomic orbital (GIAO) approach [23,24] was used for chemical shielding calculations. In NMR experiments, the frequency of an NMR signal is observed relative to that in a reference system. For ^{17}O NMR, liquid water is generally used as the reference. Because the quantum calculations give absolute chemical shielding values, σ_{ii}, it is convenient to convert them into chemical shifts relative to water, δ_{ii}, by using

$$\delta_{ii} = 307.9 - \sigma_{ii} \text{ (ppm)} \tag{1}$$

where the value of 307.9 ppm is the absolute chemical shielding constant for the ^{17}O nucleus in liquid H_2O [25].

The quantum chemical calculations yield EFG tensors in atomic units (a.u.), q_{ii}. In solid-state NMR experiments, quadrupolar coupling interactions are expressed as EFG tensors in frequency whose principal components are defined as $|V_{XX}| < |V_{YY}| < |V_{ZZ}|$. To describe a traceless EFG tensor, one uses two NMR parameters, namely, the quadrupole coupling constant, C_Q, and the asymmetry parameter, η_Q. The following equations were employed for making a direct comparison between theoretical and experimental data:

$$C_Q \text{ (MHz)} = eV_{ZZ}Qh^{-1} = -2.3496Q \text{ (fm}^2\text{)}\ q_{ZZ} \text{ (a.u)} \tag{2}$$

and

$$\eta_Q = (V_{XX} - V_{YY})/V_{ZZ} \tag{3}$$

where Q is the electric quadrupole moment of the ^{17}O nucleus and the factor of 2.3496 comes from unit