

Peptide ^{17}O Chemical Shielding and Electric Field Gradient Tensors

Kevin W. Waddell^{1,2}; Eduard Y. Chekmenev^{3,4}; Richard J. Wittebort¹

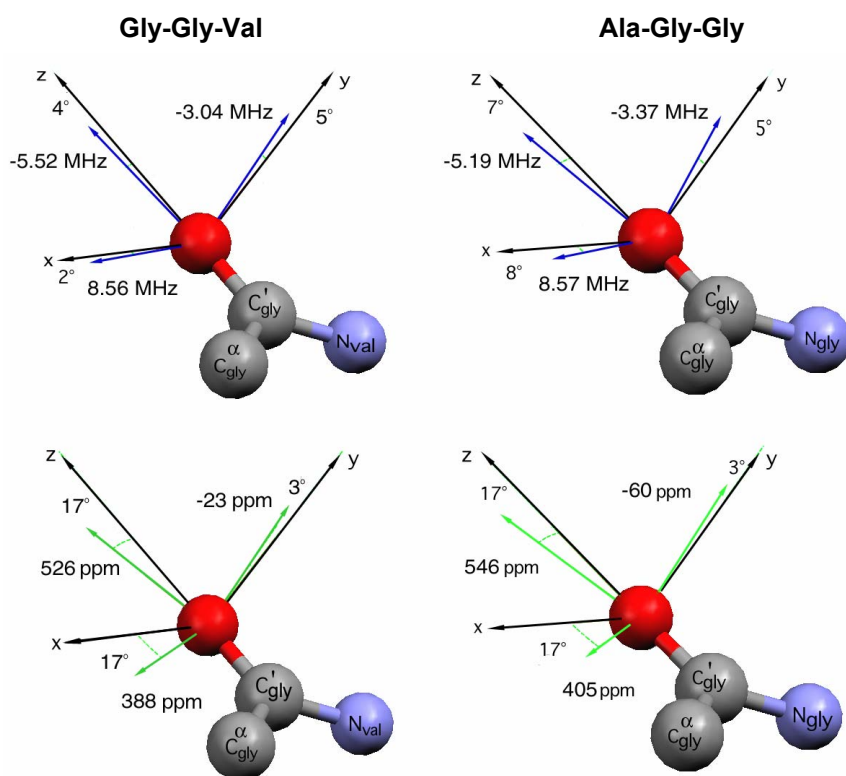
¹University of Louisville, Louisville, KY 40292 USA

²Vanderbilt University, Institute of Imaging Science, Nashville, TN 37232 USA

³MR Spectroscopy Unit, Huntington Medical Research Institutes, Pasadena, CA 91105 USA

⁴National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL 32310 USA

Carbonyl oxygen atoms stabilize biomolecular structure through hydrogen bonding and mediate dynamic processes such as water and ion transport. The strategic location of oxygen combined with large chemical shift dispersion and a quadrupole moment suggest ^{17}O NMR could provide valuable insight into biomolecular structure and function. We have used ^{17}O NMR to study central carbonyl oxygens in 6 model peptide polymorphs. Ala-Gly-Gly and Gly-Gly-Val single crystals were used to rigorously establish all 11 parameters that characterize chemical shielding (CS) and quadrupole coupling (QC) tensors. Additionally, four polymorphs of Gly-Gly-Gly not amenable to single crystal NMR were used to assess the influence of ion-binding on ^{17}O parameters.



To establish a guide for the interpretation of ^{17}O CS and QC tensors in biomolecules, we have systematically analyzed these parameters in the crystallographically characterized model peptides, Ala-Gly-Gly and Gly-Gly-Val, by analyzing single crystal rotations at 11.7 T, static powder patterns at 21.2 T, and magic-angle spinning at 14.1 T. Density Functional Theory (DFT) calculations were completed in parallel and facilitated interpretation of trends that relate noncovalent interactions to CS and QC parameters. We find that DFT calculations in peptide clusters provide reliable initial guesses for extracting NMR parameters from powder data in these peptides at high field. Differences in central residue CS principal components between the two peptides are in the range of 15-30 ppm and correlate with the intermolecular interactions of the peptide plane. In contrast, orientations of CS and QC tensor principal components are almost identical, with the latter set of axes coincident with a molecular axis system defined by the carbonyl bond, the peptide plane, and the plane normal. DFT calculations of CS and QC PAS orientations were within experimental error ($\sim 3^\circ$),

suggesting that DFT can be used to calculate tensor orientations that are a prerequisite for interpreting data from oriented samples, such as membrane bound proteins. Results from Ala-Gly-Gly and Gly-Gly-Val indicate that CS and QC tensor orientations are not highly variable, extracting the set of eight CS and QC parameters from powder patterns at high field is feasible (despite the presence of at most five features), and DFT calculations on trimer clusters provide excellent initial guesses for these simulations and accurately predict absolute orientations. The latter observation was also seen in peptide amide nitrogen shielding tensors.

Four polymorphs of Gly-Gly-Gly with known X-ray structures were also studied using static and spinning crystalline powders at 19.6 T and DFT calculations. Two of the polymorphs feature the central carbonyl oxygen in distinct but ordinary hydrogen bonds, while the other two are bound to ions, Li^+ in one case and Ca^{2+} in the other. Hence, these model systems offer a rare opportunity to study the effects of ion-binding and hydrogen bonding on ^{17}O CS and QC parameters against a constant covalent background. Ion-bound carbonyl ^{17}O CS and QC components differed substantially from their H-bonded counterparts. In summary, ion-binding decreases the χ_{zz} component of the traceless symmetric quadrupole coupling tensor by ~ 0.7 MHz, increases the QC tensor asymmetry parameter by 0.2, decreases shifts: δ_{iso} by ~ 50 ppm and δ_{11} by ~ 100 ppm.