

# Ion binding Study by $^{17}\text{O}$ MAS NMR Spectroscopy at High Field and by *Ab Initio* Calculations in Model Peptide Systems

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$^{17}\text{O}$  NMR spectroscopy is very attractive and powerful tool to probe various intermolecular interactions in a variety of biomolecules. Carbonyl sites in proteins are interesting to study hydrogen for their hydrogen bonding and ion binding. However,  $^{17}\text{O}$  spectroscopy has not been extensively exploited due to sensitivity and resolution difficulties. While the introduction of MAS techniques has significantly improved the resolution in the majority of NMR experiments by decreasing the line width, the line width in  $^{17}\text{O}$  powders is determined by the quadrupole interactions in addition to chemical shift anisotropy. This limits many experiments and makes them difficult to implement. High magnetic fields, however, have significant advantages for MAS of quadrupole nuclei by narrowing the lines and thus additionally improving the sensitivity and resolution.

Here, we present the study of ion binding to an  $^{17}\text{O}$  carbonyl site in a unique sample of Gly-Gly-Gly (GGG) tripeptide. Glycine is known for torsion angle flexibility, which permits GGG crystallization in a variety of conformations under different crystallization conditions. Two polymorphs, Cambridge Structural Database codes TGLYCY10 and GGGCAC, are particularly interesting, because of the different  $^{17}\text{O}$  central residue carbonyl hydrogen bonding. While the carbonyl interacts with an  $\cdots\text{H-N-}$  group of another GGG molecule in TGLYCY10 lattice, the carbonyl site in GGGCAC is bound to a  $\text{Ca}^{2+}$  ion. We employ  $^{17}\text{O}$  chemical shielding (CS) and quadrupole coupling (QC) tensors to probe the ion binding.

$^{17}\text{O}$  20 kHz MAS and static spectra acquired at 19.6 Tesla with signal-to-noise ratio of  $>70$  are well-resolved. The line shapes side band intensities are utilized to determine CS and QC values. We apply Density Functional Theory (DFT) calculations as a starting point, which correlates well with experimental spectra. The theoretical parameters are then employed to fit the experimental data.

Our results show significant effects introduced by ion binding primarily on the magnitudes of  $^{17}\text{O}$  CS and QC tensor's principal components, suggesting that  $^{17}\text{O}$  CS and QC is a useful probe for ion binding studies. Selective labeling and high isotope enrichment with a combination of high magnetic field and short  $^{17}\text{O}$   $T_1$  relaxation time in proteins has, therefore, a potential for characterizing ion binding in larger biomolecules.