ION BINDING STUDY OF CARBONYL OXYGENS BY SOLID-STATE ^{17}O NMR SPECTROSCOPY IN A MODEL PEPTIDE SYSTEM GLY-(^{17}O-GLY)-GLY

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Introduction

^{17}O spectroscopy has not been extensively exploited due to sensitivity and resolution difficulties, while ^{17}O Chemical Shielding (CS) and Quadrupole Coupling (QC) are very direct probes to assess intermolecular interactions in a variety of biomolecules. The advancements in high field NMR instrumentation and MAS techniques have significantly improved both spectral resolution and sensitivity enabling studies, which were difficult to implement before. This work assesses the ion binding effects in the model tripeptide Gly-Gly-Gly that crystallizes in at least four known completely different conformations. Two of these conformations contain Li^+ and Ca^{2+} cations strongly interacting with the central carbonyl oxygen, providing a unique opportunity to study ion binding by solid-state NMR spectroscopy and Density Functional Theory (DFT) calculations, which take advantage of the reported X-ray structures. This is the first systematic study of site specific ion binding by oxygen-17 solid-state NMR spectroscopy.

Experimental

Gly-(^{17}O-gly)-gly was prepared by Fmoc solid-phase peptide synthesis using Fmoc-^{17}O-Gly (50% enrichment) and then crystallized in four conformations. ^{17}O NMR parameters are obtained primarily at 19.6 T and 14.1 T by MAS and static experiments and show substantial field dependence of ^{17}O CS and QC parameters of peptide carbonyl oxygen. *Ab Initio* calculations provided the initial guess for spectral simulations, MAS (20 kHz) experiments were used to obtain χ, η and δ_{iso}, while static experiments were employed to find the other CS and QC parameters.

Results and Discussion

The NMR parameters obtained by simulations shown in Fig.1 provided the following conclusions for NMR parameters: (i) δ_{11} (the most deshielded component of the CS tensor) is reduced by up to 100 ppm, while δ_{iso} decreases by 50 ppm only upon interaction with ions, (ii) δ_{22} experiences changes similar to δ_{11}, (iii) quadrupole coupling constant, χ, decreases by almost 1 MHz and asymmetry, η, somewhat increases by 0.2-0.4, when ions are bound and (iv) orientations of the CS tensor with respect to the QC tensor remain almost the same in all four compounds. The results will be submitted to Journal of The American Chemical Society as a full paper.

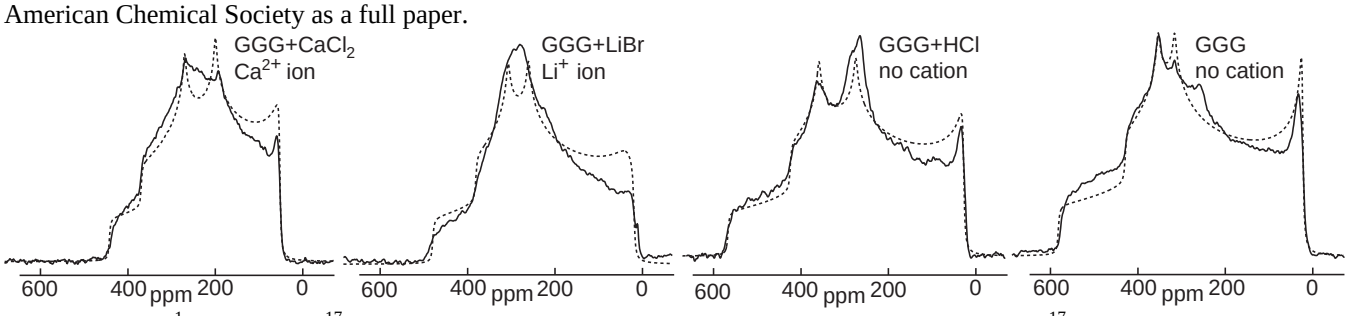

Figure 1. Static ^{1}H decoupled ^{17}O spectra and simulations (dashed line) of single site labeled gly-(^{17}O-gly)-gly in various environments obtained at 19.6 T. The spectra are referenced to external water at 0 ppm.

Conclusions

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