Chemical Shift Anisotropy Tensors of the Amide Proton Nucleus in a Peptide Bond using High Resolution Heteronuclear Correlation NMR Spectroscopy

M. L. Truong (NHMFL, FSU), R. Fu (NHMFL), T. A. Cross (NHMFL, FSU)

Introduction

Chemical shift anisotropy (CSA) tensors are important to interpret orientation-dependent interactions in solid state NMR. In the PISEMA (Polarization Inversion Spin Exchange at the Magic Angle) experiment, spectra of membrane proteins embedded in aligned lipid bilayers depend largely on the orientation of nitrogen CSA tensors with respect to the amide bonds. The CSA tensors of the amide proton in proteins may also provide similar orientational information. However, information on the CSA tensors of amide protons in peptides and proteins is limited. Only a few solid-state studies and a few studies based on the transverse CSA/DD cross-correlation rate in solution have been published (1). The aim of this research is to obtain accurate amide proton CSA tensors and a framework for amide proton chemical shifts as structural restraints for membrane proteins using solids state NMR.

Experimental

Two-dimensional heteronuclear correlation (HETCOR) spectroscopy offers an alternative to more commonly used separated local field experiments such as PISEMA to obtain high-resolution ^{1}H chemical shifts, ^{15}N chemical shifts, as well as ^{15}N-^{1}H dipolar couplings. HETCOR has been previously shown (2) to produce spectra of high-resolution for ^{15}N chemical shifts, ^{15}N-^{1}H dipolar couplings, and ^{1}H chemical shifts in NMR of aligned samples. The HETCOR sequence uses an MSHOT (Magic Sandwich High Order Truncation) homonuclear decoupling sequence and was modified to use the WIM12 (Windowless Isotropic Mixing) sequence in place of the standard Hartmann-Hahn cross polarization sequence. Isotropic mixing sequences allow for the transfer of any magnetization component of one nucleus to the corresponding component of its dipolar coupled partner (3). WIM12 provides a more efficient polarization transfer, and therefore aid in the overall HETCOR sequence in obtaining quality spectra and measurement of the CSA tensors of the amide protons.

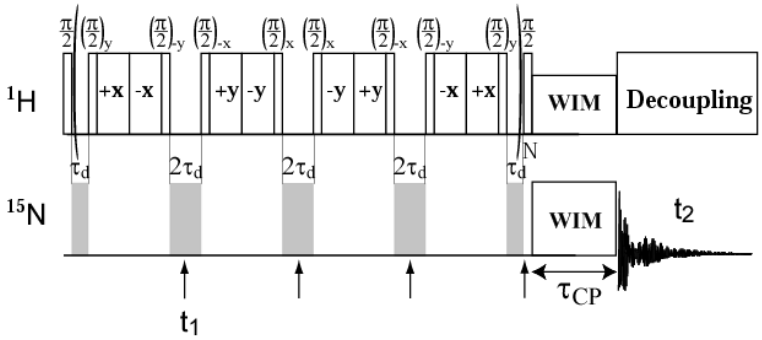

Figure 1: HETCOR NMR pulse sequence using the MSHOT homonuclear decoupling sequence, and the WIM sequence for cross polarization

A static powder sample of the dipeptide, alanyl-leucine, was used to obtain proton CSA measurements. The HETCOR experiment was performed on the 400 MHz High Resolution Wide Bore NMR magnet, using a low-E double-tuned (nitrogen, proton) probe built at the NHMFL. Preliminary HETCOR spectra show the principal values of the proton CSA tensors to be different from the published values (4). These CSA values will soon be extracted and applied in the structural determination of larger peptides and proteins. We hope that we will soon have the ability to use the proton CSA tensors as structural restraints, along with the dipolar couplings and ^{15}N chemical shift restraints that are readily obtainable to produce a three-fold methodology in solving membrane peptides and protein structures using solid state NMR.

Acknowledgements

This work was supported in part by the NIH AI23007. All experiments were performed at the National High Magnetic Field Laboratory, supported by the NSF Cooperative Agreement DMR-0084173 and the State of Florida.

References

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