Solid-state ^{14}N NMR of amino acids and polypeptides

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Amino acids play a vital role as the building blocks of proteins and their arrangement in the polypeptide chain controls protein structure and folding. These systems have been extensively investigated by NMR using ^{13}C and ^{15}N as the probe nuclei. Unfortunately, due to the low natural abundances of the probe nuclei (especially of ^{15}N), costly isotopic labelling is often necessary to obtain high quality spectra. Nitrogen has two NMR active nuclei (^{14}N and ^{15}N), the former is 99.63% naturally abundant and perhaps may have use as an alternative probe nucleus, despite difficulties associated with ^{14}N NMR.

Nitrogen-14 is an integer-spin nucleus (I = 1) with a non-zero electric quadrupole moment (eQ = 20.44 mb) and a low gyromagnetic ratio (γ = 1.93 × 10^7 rad T^{-1} s^{-1}) [1]. Due to the quadrupolar nature of this nucleus, it couples to the surrounding electric field gradient (EFG), which is dependent upon the local ground state electronic environment. Solid-state ^{14}N NMR spectra of micro-crystalline samples often yield powder patterns with widths exceeding 1 MHz [1]. Spectral broadening low Larmor frequencies make acquisitions of ^{14}N powder patterns quite difficult.

Utilizing the 21.1 T NMR spectrometer and the WURST-QCPMG pulse sequence [2,3], we have been able to acquire high quality powder patterns of the majority of known amino acids with generally short acquisition times. In particular, we have found that pseudo-tetrahedral nitrogen environments, R-NH_3^+ (L-serine and L-threonine) and R_2-NH_2^+ (*trans*-4-hydroxy-L-proline), are amenable to ^{14}N NMR experiments.

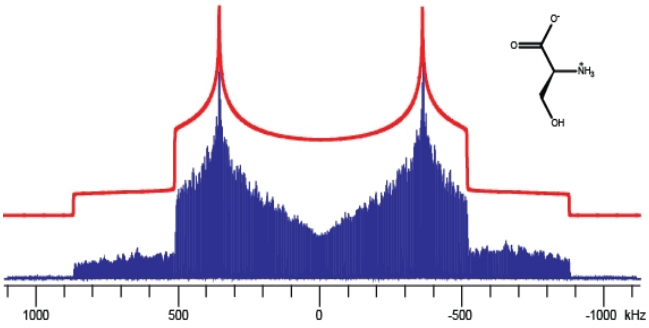

Figure 1: ^{14}N powder pattern of L-serine (blue) at 21.1 T. This spectrum is composed of 4 sub-spectra and took *ca.* 1.5 hours to acquire. Spectral simulation (red) yields C_Q = 1.165 MHz and η_Q = 0.18.

Pseudo-tetrahedral nitrogen environments have quadrupolar coupling constant (C_Q) values ranging from 0.8 to 2 MHz, and can't be differentiated based on C_Q alone, however, there is a clear variation in the asymmetry

parameter (η_Q). We have found that pseudo-tetrahedral nitrogen environments may be grouped into low (0.0 – 0.25), intermediate (0.26 – 0.6), and high (0.61 – 1.0) η_Q classes.

The sensitivity of the ^{14}N NMR spectrum to surrounding EFG is effectively demonstrated with three representative examples. Figure 1 depicts the ^{14}N powder pattern of L-serine (η_Q = 0.18). This type of spectrum is characteristic of C-NH_3^+ environments which have cylindrical pseudo-C_3 symmetry about the C-N bond due to absence of intermolecular interactions.

Figure 2 depicts the ^{14}N powder pattern of L-threonine (η_Q = 0.385). The intermediate η_Q values arise because of varying degrees of intermolecular interactions which cause deviation from cylindrical symmetry about the C-N bond.

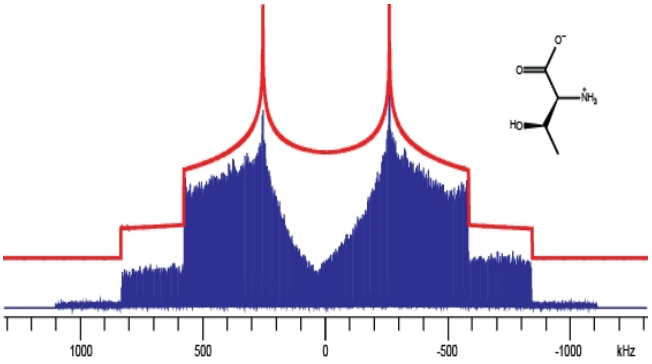

Figure 2: ^{14}N powder pattern of L-threonine (blue) at 21.1 T. This spectrum is composed of 4 sub-spectra and took *ca.* 2 hours to acquire. Spectral simulation (red) yields C_Q = 1.12 MHz and η_Q = 0.385.

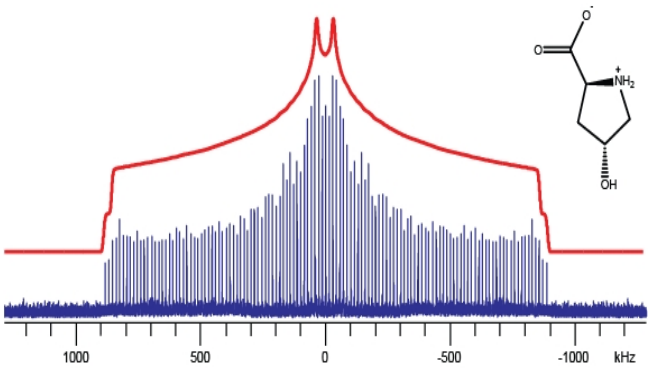

Figure 3: ^{14}N powder pattern of *trans*-4-hydroxy-L-proline (blue) at 21.1 T. This spectrum is composed of 2 sub-spectra mirrored about 0 ppm and took *ca.* 3 hours to acquire. Spectral simulation (red) yields C_Q = 1.19 MHz and η_Q = 0.93.

A ^{14}N powder pattern of *trans*-4-hydroxy-L-proline (η_Q = 0.93) is depicted in Figure 3. High η_Q values are characteristic of an entirely different type of nitrogen environment, with two protons and two carbon atoms bound to the nitrogen, and no pseudo-C_3 rotational axis. ^{14}N EFG tensor calculations using plane-wave DFT methods (i.e., CASTEP [4]) are in good agreement with experimental findings. Further investigations of the remaining amino acids are underway. We are expanding this study to amino acids containing side-group nitrogen atoms (histidine, lysine, arginine, etc.) as well as larger peptide systems (di- and tripeptides).

References

[1] P. Pyykkö, *Mol. Phys.* **99** (2001) 1617.
[2] L.A. O'Dell and R.W. Schurko, *Chem. Phys. Lett.* **464** (2008) 97.
[3] L.A. O'Dell and R.W. Schurko, *J. Am. Chem. Soc.* **131** (2009) 6658.
[4] S.J. Clark, M.D. Segall, C.J. Pickard et al. *Z. Kristallogr.* **220** (2005) 567.