Interhelical Correlations for a Helical Membrane Protein from Magic Angle Spinning Solid State NMR

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Introduction

There has been limited success in the structural characterization of small helical membrane proteins. Solid state Nuclear Magnetic Resonance (ssNMR) is a technique capable of determining the structures for these proteins. The most promising strategy [1] involves measuring orientational restraints for the protein backbone to determine secondary structure and then to determine a few interhelical correlations, which fix the tertiary structure of the protein. Such data has not yet been obtained for a protein with more than two helices. To illustrate the utility of ssNMR to characterize proteins with three helices we study the Rv1861 protein from *M. tuberculosis* that is involved in cell wall reorganization. A series of experiments were previously performed at the NHMFL NMR facility to obtain orientational restraints for Rv1861 [2]. The remaining obstacle in the structure calculation was to obtain the interhelical correlations that define the tertiary structure for the protein.

Experimental

Interhelical contacts were determined using Magic Angle Spinning ssNMR. 3D correlation experiments were performed on isotopically labeled Rv1861 to measure isotropic nitrogen and carbon frequencies for sequence specific sites in the protein. 2D carbon correlation experiments were then employed to observe correlations between frequencies associated with sites on different helices of the protein. Experiments were performed at the NHMFL NMR facility using the 14.1 T and 21.1 T superconducting magnets and probe technology engineered and constructed by the RF Program.

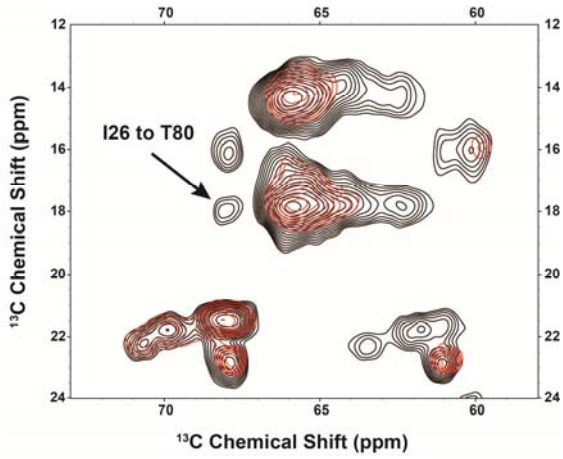

Fig. 1. Carbon correlation spectra at 50 ms, red, and 800 ms, black, mixing times. A correlation representing an interhelical contact is indicated with an arrow.

Results and Discussion

In the 2D carbon correlation experiment a mixing time is used to allow magnetization transfer between carbon nuclei. New signals appearing at long mixing times are interpreted as long distance (~5 Å) correlations. Fig. 1 illustrates a new signal observed at long mixing times for the Rv1861 protein that represents an interhelical correlation between a Threonine and an Isoleucine residue. We observed an additional correlation between an Asparagine and a Lysine residue. Fig. 2 illustrates the results of the experiments performed at the NHMFL. The helix orientations are fixed but the whole helix can rotate about a vertical axis. When combined with the interhelical correlations the data suggest there is a unique structure solution with two helices approximately parallel with the third laying over the top. Restrained molecular dynamics structure calculations are underway.

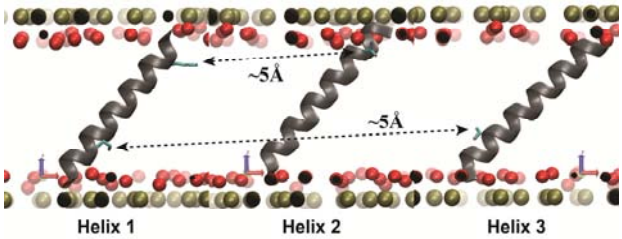

Fig. 2. Helix models based on orientational restraints. Interhelical correlations bring the helices together and define a unique tertiary fold for the Rv1861 protein.

Acknowledgements

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References

[1] Murray, D.T., *et al*., Accounts of Chemical Research, **46**, 2172-2181 (2013).
[2] Murray, D.T., *et al*., Journal of Magnetic Resonance, Under Review (2013).