TOWARDS STRUCTURE DETERMINATION OF *MYCOBACTERIUM TUBERCULOSIS* CELL WALL HYDROLASE RV2719C VIA SOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Introduction

Rv2719c is a 17kDa single-span membrane protein from *Mycobacterium tuberculosis* shown to exhibit cell wall hydrolase activity[1]. This enzyme consists of three separate domains: C-terminal, transmembrane, and N-terminal. Structure determination of this protein is being performed based on a "divide and conquer" approach in which fragments of the various domains are constructed and their structure solved individually. Domain structures will then be pieced together to create a picture of the entire protein. The C-terminal domain of this protein has been shown to be nonessential for proper enzyme functioning and therefore is not being considered in protein structure determination.

Experimental

HSQC, CBCA(CO)NH, and CBCANH solution NMR spectra of $^{15}N/^{13}C$-Rv2719c were acquired on the 720 MHz spectrometer at NHMFL. An HNCA spectrum of $^{15}N/^{13}C$-Rv2719c was acquired on the 600 MHz spectrometer at NHMFL. HSQC spectra of specific labeled proteins (^{15}N-Val, ^{15}N-Ala, and ^{15}N-Leu) were acquired on the 500 MHz spectrometer at NHMFL. All spectra were acquired on approximately 0.5mM protein sample in 20mM sodium phosphate buffer pH4.0, 1mM EDTA, 50mM glutamate, 50mM arginine, and 100mM NaCl.

Results and Discussion

Significant progress has been made towards determining the structure of the N-terminal domain of Rv2719c using solution NMR spectroscopy. The spectra mentioned above have been analyzed for sequential backbone connectivities based on NH, ^{15}N, Cα, and Cβ chemical shifts in addition to known residue types (Gly, Ala, Val, Leu). Using these experiments, full backbone assignments for the N-terminal domain of Rv2719c have been made (Figure 1). Side chain assignments will be obtained using CCONH and HCCONH experiments collected at the NHMFL. The trans-membrane domain has been cloned for structural studies and a construct containing both the N-terminal and transmembrane domains has been created for both structural and functional studies.

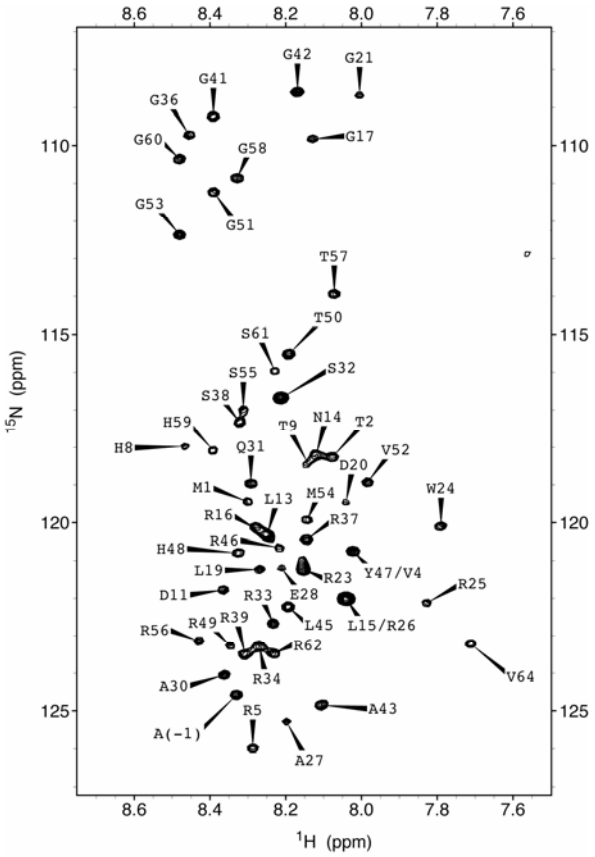

Figure 1: HSQC spectrum of Rv2719c N-terminal domain with labeled residue assignments

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References

[1] Chauhan, A, *et al.*, Molecular Microbiology, **62(1)**, 132-147 (2006).