DEVELOPMENT OF A JOINT SOLUTION AND SOLID-STATE NMR BASED METHODOLOGY FOR THE BACKBONE STRUCTURAL CHARACTERIZATION OF HELICAL INTEGRAL MEMBRANE PROTEINS

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Introduction

Solving the three-dimensional backbone structure of helical integral membrane proteins currently poses one of the most significant challenges to high throughput structural characterization of proteomes. The relatively simple folds of helical membrane proteins produce a surprisingly diverse group of structures with a range of sizes and functions. This inherent diversity necessitates a multidisciplinary approach to structural characterization. We have proposed a joint solution and solid-state NMR based methodology. The proposed approach takes advantage of multiple data sources including: residual dipolar couplings, paramagnetic relaxation enhancement effects and PISEMA (polarization inversion spin exchange at the magic angle) correlations. To test the development of the protocol for helical integral membrane protein backbone structure determination, several suitable proteins have been selected from the *Mycobacterium tuberculosis* genome. The proteins have been purified, and initial solution and solid-state NMR spectra collected.

Experimental

Three proteins, Rv1031 (KdpC), Rv2433c, and Rv1342c (pks14) have been selected for testing the proposed structure determination approach. KdpC is the 21.2kDa C-subunit of the potassium transporting ATPase complex Kdp. KdpC has one transmembrane helix. Rv2433c is a 10.6 kDa conserved hypothetical protein with two transmembrane helices. The pks14 protein is a 13.4 kDa polyketide synthase with three transmembrane helices. KdpC and Rv2433c have been cloned and expressed using a modified pET16b vector (Novagen), creating an expressed fusion protein consisting of the entire encoded protein and a small 9 amino acid affinity tag including six histidine residues. The pks14 protein has been cloned with a modified pET29b vector (Novagen) creating a fusion protein with a carboxy-terminal histidine tag. Purification of all proteins has being achieved via nickel affinity chromatography. Initial characterization by solution NMR spectroscopy has been performed upon uniformly ^{15}N-Rv2433c, ^{15}N-KdpC and ^{15}N-pks14.

Results and Discussion

Conditions are being screened for each protein to determine conditions appropriate for structural characterization. Initial spectra of Rv2433c have yielded 96% of expected backbone amide resonances in ^{15}N-HSQC spectra. Shown below is an ^{15}N/^{1}H HSQC spectra (Fig. 1A) of uniformly labeled ^{15}N-Rv2433c collected on a 600MHz spectrometer at the University of California, San Diego. This project has also taken advantage of the 720MHz and 500MHz solution NMR spectrometers at NHMFL for screening conditions. Initial solid-state NMR of uniformly ^{15}N-Rv2433c in oriented POPC:POPG lipid bilayers have also yielded promising spectra (Fig. 1B,C).

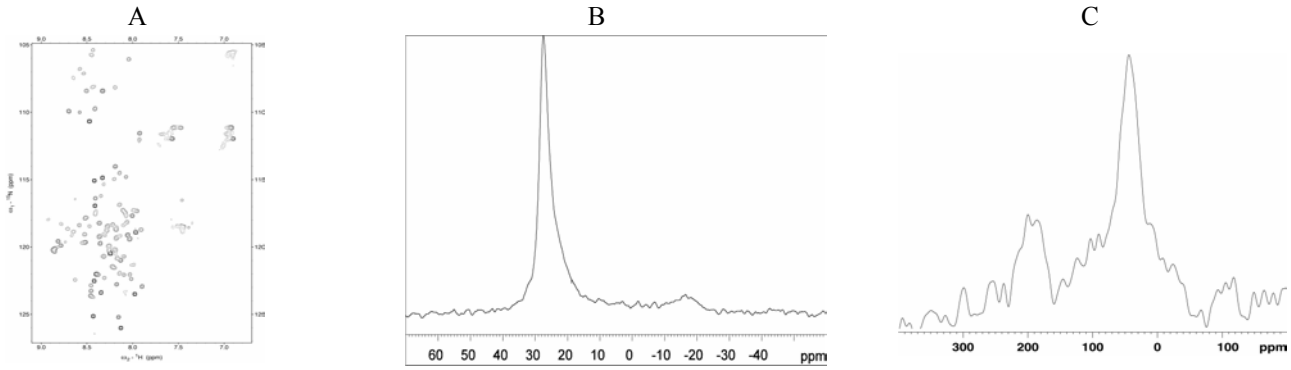

Figure 1: (A) ^{15}N-^{1}H HSQC of Rv2433c in 150mM LMPG (lyso-1-myristoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)]) collected on a 600MHz spectrometer at UCSD. (B) ^{31}P spectrum of Rv2433c in POPC:POPG, (4:1) oriented bilayers with ^{1}H cw decoupling recorded at 7.0 T. (C) ^{15}N CP spectra of uniformly ^{15}N-Rv2433c in oriented 4:1 POPC:POPG bilayers. Recorded at 600 MHz spectrometer with 9300 scans.