Structure and Topology Studies of the Integral Membrane Protein Phospholamban

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Introduction
Phospholamban (PLN) is a 52-residue integral membrane protein playing a major role in the regulation of cardiac cycle (contraction and relaxation), which controls the heartbeat. PLN binds to and inhibits sarcoplasmic reticulum (SR) Ca-ATPase (SERCA) within muscle cells. It inhibits SERCA by shifting its affinity for Ca\(^{2+}\), thereby impeding the translocation of Ca\(^{2+}\) into the SR lumen. PLN exists in equilibrium between monomer and pentamer oligomeric states. Both forms have been shown to bind SERCA \([1, 2]\), indicating the importance of both states. \(^{15}\)N solid-state NMR spectroscopy is a powerful tool for obtaining direct information on the structural topology of such proteins in their native membrane environment \([3]\). However, because the lipid membranes are inherently lossy at high RF frequencies, the resulting RF-induced heating can destroy the sample preparation. A custom low-E probe was constructed at NHMFL in order to overcome this hurdle by drastic reduction of the high frequency electric fields E present in the sample.

Experimental
The low-E NMR probe for mechanically aligned lipid bilayers was based on the design \([4]\) developed at NHMFL in prior years. However, it had to be completely re-designed to fit within the narrow bore and interface with the Varian 700 MHz spectrometer at the University of Minnesota (Figure 1). Wild-type PLN (wt-PLN) selectively labeled with \(^{15}\)N-Cys was reconstituted into mechanically aligned lipid bilayers as previously described \([5]\). The final molar ratio of lipid to protein was \(\sim 125 : 1\). A 2D polarization inversion spin exchange at the magic angle (PISEMA) experiment was acquired with 4k scans and 12 increments at 4\(^\circ\)C with a recycle delay of 4 sec. The spectrum was acquired at 16.4 T (\(^1\)H frequency of 700 MHz) using a Varian solid-state spectrometer at the University of Minnesota.

Results and Discussion
Figure 2 shows the result from the PISEMA experiment acquired using a selectively labeled \(^{15}\)N-Cys wt-PLN (30 kDa). The 2D spectrum shows 3 resonances corresponding to C36, C41, and C46 within wt-PLN. Using this and other selectively labeled spectra, the \(\sim 15^\circ\) tilt angle of the transmembrane domain helix with respect to the lipid bilayer normal was determined \([5]\). This tilt angle differs from that of the PLN monomer by \(\sim 6^\circ\) \([5, 6]\), showing that oligomerization of the pentamer requires smaller tilt angles with respect to the depolymerized species.

Conclusion
With the aid of a low-E solid-state probe constructed at NHMFL, we have applied PISEMA solid-state NMR spectroscopy to determine the structure and topology of wt-PLN protein in its pentameric state.

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References
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