Topology and Structure of Phospholamban Monomer in Oriented DOPC Lipid Bilayers

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Introduction

Phospholamban (PLN) is an essential integral membrane protein within the sarcoplasmic reticulum (SR) of cardiac myocytes. Within the membrane, PLN is an endogenous inhibitor of SR Ca-ATPase (SERCA) that is believed to be important in two oligomeric forms: a) a monomer that directly binds to and inhibits SERCA (1) and b) a pentamer that acts as a storage form within the SR membrane (1). In this work, we looked at the structure and topology of PLN monomer in mechanically oriented lipid bilayers using the solid-state NMR experiment PISEMA (polarization inversion spin exchange at the magic angle).

Experimental

A fully functional monomeric mutant of PLN (C36A, C41F, C46A) was selectively labeled with ^{15}N-Leu and reconstituted into mechanically aligned lipid bilayers as previously described (2). The final lipid/protein molar ratio was ~150/1. A 2D PISEMA experiment was acquired with 4k scans and 12 increments at 35 °C with a recycle delay of 5 sec. The spectrum was acquired at a 14.1 T field strength (^{1}H frequency of 600.1 MHz) equipped with a Bruker DMX spectrometer at the NHMFL in Tallahassee, FL using a low-E probe with a doubly tuned, low-inductance resonator built by the RF program at the NHMFL (3).

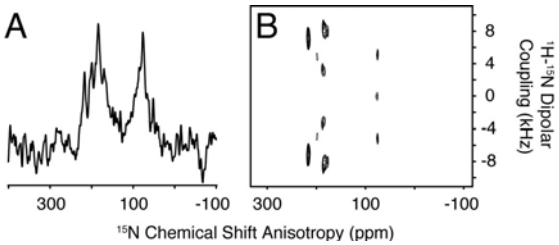

Figure 1. A. 1D cross-polarization spectrum of [^{15}N-Leu] PLN in DOPC lipid bilayers. B. PISEMA spectrum of the same sample in A.

Results and Discussion

Figure 1 shows the results from the 1D cross-polarization and PISEMA experiments acquired using a selectively labeled ^{15}N-Leu PLN. Since 9 of the 10 leucine residues are within the transmembrane portion of the spectrum, the majority of the signals resonate in the downfield portion of the spectrum (~200 ppm), indicating good aligment. Using the results from this spectrum and other selectively labeled samples, we were able to determine the 24° tilt angle that the membrane embedded helical domain makes with respect to the lipid bilayer normal (2, 4).

Conclusions

We have used PISEMA solid-state NMR spectroscopy at the NHMFL to determine the structure and topology of monomeric PLN (Figure 2), which has recently been submitted for publication (4).

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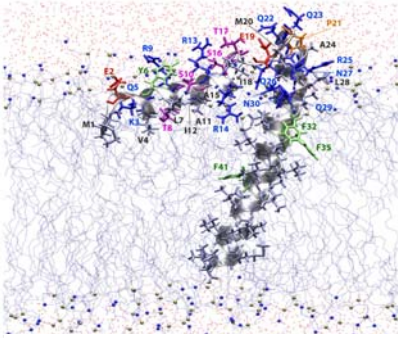

Figure 2. Structure and topology of monomeric PLN refined in explicit DOPC lipid bilayers that agrees with all solid-state and solution NMR restraints.

References

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[4] Traaseth, N.T., *et al.*, Submitted.