TWO-DIMENSIONAL NMR SPECTROSCOPY OF SARCOLIPIN IN ORIENTED LIPID BILAYERS

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Introduction

Sarcolipin (SLN), a 31 amino acid integral membrane protein, regulates SERCA1a and SERCA2a, two isoforms of the sarco(endo)plasmic Ca-ATPase, by lowering their apparent Ca^{2+} affinity and thereby enabling muscle relaxation. Previous 1D solid-state NMR experiments on selectively ^{15}N-labeled sites showed that SLN crosses the lipid bilayer with an orientation nearly parallel to the bilayer normal. With a view toward the characterization of SLN structure and its interactions with both lipids and SERCA, we started the analysis of SLN in mechanically oriented DOPC/DOPE lipid bilayers as mapped by two-dimensional ^{15}N PISEMA experiments.

Experimental

NMR experiments were performed on a Bruker DMX-600 operating at a frequency of 600.14 MHz for ^{1}H and 60.82 MHz for ^{15}N. Prior to ^{15}N data acquisition, the integrity of the oriented lipid bilayer was verified using ^{31}P NMR. PISEMA experiments (18) were performed on a probe designed and constructed at the National High Magnetic Field Laboratory. In order to reduce sample heating, the probe utilizes a separate, low inductance ^{1}H resonator outside the ^{15}N detection coil. Typical NMR parameters used in the PISEMA experiments include a ^{1}H pulse length of 3.7 us, decoupling field of 50 kHz, rf field strengths of 48 kHz for ^{1}H and 67.6 kHz during the SEMA element, and a recycle delay of 3.5-5 s. PISEMA spectra on selectively ^{15}N-labeled SLN were acquired at 5°C with 9-12 t_1 increments and ~3k scans, while uniformly ^{15}N-labeled SLN samples required 32 t_1 increments and ~2k scans. .

Results and Discussion

PISEMA spectra mapped the structure and topology of SLN in oriented DOPC/DOPE bilayers. Under our experimental conditions, this single-pass membrane protein adopts a helical conformation and crosses the membrane with a tilt angle of ~ 23°. PISEMA spectra obtained with both uniformly and selectively ^{15}N-labeled SLN clearly indicate a preferential orientation of the helix face with Leu-10, 21, 25 and Ile-17 pointing toward the bilayer surface. Remarkably, PISEMA spectra suggest a topological interconversion on a slow time scale (i.e., slower that 10^{-4}s) with the presence of a second population of resonances. This additional population exhibits roughly the same tilt angle (θ) of the SLN helix with respect to the membrane bilayer, but a slightly different rotational angle (ρ). These two topologies may represent the two states whose information is totally lost in the isotropic micellar environments and may represent a pre-equilibrium state of SLN that constitutes a requirement for SERCA recognition.

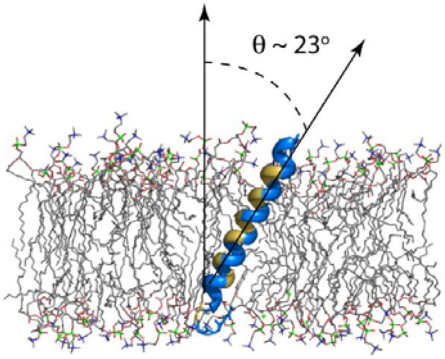

Figure 1. Proposed structural model of SLN in lipid membranes. The Leu residues are highlighted in orange and Ile residues are highlighted in yellow. Notice that Leu and Ile residues fall on the same face of SLN. SLN is rotated around the helix axis so that the majority of the Leu and Ile residues face the bilayer surface, as indicated by the asymmetry of the ^{15}N-Leu and ^{15}N-Ile PISEMA pattern.

Conclusions

SLN crosses the lipid bilayer with an angle of approximately 23°, echoing the conclusions of a similar investigation carried out with phospholamban (another Ca-ATPase inhibitor) under identical experimental conditions. This result demonstrates that high homologous primary sequences (are likely to have the same interactions with lipid bilayers.

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References

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