STRUCTURE OF MONOMERIC PHOSPHOLAMBAN IN ORIENTED LIPID BILAYERS

Traaseth, J. Nathaniel (U of Minnesota), Buffy, J. J. (U of Minnesota), Veglia, G. (U of Minnesota)

Introduction
Phospholamban (PLN), a 52-residue integral membrane protein, is the endogenous regulator of Ca-ATPase in cardiac muscle. When unphosphorylated, PLB inhibits Ca-ATPase and calcium translocation into the sarcoplasmic reticulum. Inhibition of the enzyme activity is relieved upon phosphorylation at Ser16 or at micromolar calcium concentrations in the cytosol. The overall goal of this project is to identify the structure and the dynamics of this protein in lipid bilayers.

Experimental
The 2D polarization inversion spin exchange at the magic angle (PISEMA) was performed with TPPM decoupling during acquisition. PISEMA experiments were acquired with 1024 scans and 48 $t_1$ increments for [U-15N]AFA-PLN, 3072 scans and 13 increments for [15N-Leu] and [15N-Ile]AFA-PLN, and 12288 scans and eight increments for [15N-Ala]AFA-PLN at 4°C, using a recycling delay of 4 s. All PISEMA data were acquired at a 14.1 T field strength ($^1$H frequency of 600.1 MHz) equipped with a Bruker DMX spectrometer (National High Magnetic Field Laboratory, Tallahassee, FL). Cross-polarization and SEMA were both performed at 63 kHz using a low-E probe utilizing a doubly-tuned, low inductance resonator built by the RF program at the National High Magnetic Field Laboratory.

Results and Discussion
PISEMA spectra using selective labels show the cytoplasmic domain to be helical and make a 93±6° with respect to the bilayer normal, a conformation in which the helix is in contact with surface of the lipid bilayer (Figure 1). The data also reveal two conformational topologies. While some of this dynamics was revealed by previous solution NMR relaxation studies in micelles, these measurements in anisotropic native lipid environment reveal new dynamic features encoded in the free protein that might be crucial for SERCA recognition and the inhibitory mechanism. Our results consistently show that the cytoplasmic domain helix is in contact with the surface of the lipid bilayer, forming an overall L-shape or bent structure.

Conclusions
Our data show unambiguously that the cytoplasmic domain is helical and lies on the surface of the bilayer, giving monomeric PLN an overall dynamic L-shaped conformation. PISEMA data reveal the presence of two topologies for the transmembrane domain. The dynamics of the cytoplasmic domain and the different topologies of the transmembrane domains underscore the importance of a pre-existing dynamic equilibrium necessary for the protein’s biological function.

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References