DEER MEASUREMENTS ON THE PHOSPHOLAMBNAN PENTAMER

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Introduction

We are using EPR to resolve a disparity in two recently reported structural models of the PLB homopentamer. One of these models suggests that the subunits of the pentamer have an L-shaped structure similar to that of the monomer, with the N-terminal cytoplasmic domains making contact with the lipid bilayer surface (Robia, et al, 2005), and another suggesting that the cytoplasmic domain of PLB extends up away from the membrane surface (Oxenoid, et. al, 2005). Previous unpublished experiments with paramagnetic relaxation agents show that the cytoplasmic domain of PLB spends most of its time interacting with the lipid bilayer surface, supporting the former model (Robia). We are using double electron electron resonance (DEER) to measure distance distributions of the PLB pentamer spin labeled at different positions in the cytoplasmic domain.

Experimental

Solid-phase peptide synthesis was used to synthesize phospholamban which was then spin labeled at either the N-terminus, K3 or residue 11 (where Ala was mutated to Cys). Free spin label was then removed using reverse-phase HPLC and the purified PLB was lyophilized. PLB was then reconstituted in DOPC/DOPE lipid bilayers with a buffer containing 50mM MOPS, 50mM KCl, 5mM MgCl$_2$, 210uM CaCl$_2$, 0.5mM EGTA pH 7.0.

DEER spectra were acquired using a Bruker 680 pulsed spectrometer using a 4 pulse sequence (90°-τ$_1$-180°-τ$_2$-180°) with a MD5 cavity (Pannier M et al. 2000). The experiments were performed at 65 K. The data were then analyzed using the fitting program developed by Dr. Fajer’s lab. This program uses a Monte Carlo approach to select various distance distributions and simulate the corresponding spectra. Chi squared values and error surfaces are generated to compare simulated and experimental spectra.

Results and Discussion

Initial results indicate the pentamer is most likely a dynamic structure with the cytoplasmic domains spending some of the time in contact with the lipid bilayer surface (Robia model) and some of the time extended up and away from the bilayer surface (Oxenoid model). We are currently selecting different labels and labeling sites to acquire additional data for further analysis.

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References

Oxenoid et al. PNAS 120, 10870-5 (2005)