and SERCA antibodies, both in the absence and presence of Ca in cardiac myocytes and a heterologous expression system. Overall, the data suggest that PLB and SERCA do not dissociate at high Ca. Planned experiments will investigate the location and functional significance of the proposed novel PLB binding site on SERCA.

652-Pos Board B432

TOAC Spin Label Conformation Resolved by MD Simulation of Membrane-Bound Phospholamban

Andrew Reid, Jesse McCaffrey, Christine Karim, Bengt Svensson.
University of Minnesota, Minneapolis, MN, USA.

We have used all-atom molecular dynamics (MD) simulation to determine the conformational occupancy of the nitroxide spin label TOAC attached to membrane-bound phospholamban (PLB), as needed for interpretation of EPR spectra. EPR spectroscopy is sensitive primarily to the dynamic angular distribution of the probe principal axis relative to the magnetic field, θ_{HP}. MD simulations determine directly the orientation of the probe principal axis relative to the α-helix axis, θ_{MP}. Previously, θ_{MP} has been determined from X-ray crystallography of TOAC on α-helical peptides, which have indicated sole occupancy of a twistboat conformation, with $\theta_{MP} = 13.3°$ (Marsh 2006 JMagRes). We have employed all-atom MD simulations to determine the distribution of θ_{MP} values for TOAC bound to PLB. The PLB structure (PDB ID: 2KB7) was mutated with TOAC at residues 11, 24, 36, or 46, and placed in Monte-Carlo-generated lipid bilayers composed of either DOPC or DMPC:POPC. MD simulations were performed using NAMD with the CHARMM36 force field. Trajectories were computed to 100ns for chair, boat, and twistboat starting conformations of TOAC. Time-averaged geometry of PLB α-helices in the lipid bilayer membrane were calculated, and results agreed with published NMR data, validating the MD results. Furthermore, angle distributions in the probe frame - (θ_{HP}, ϕ_{HP}) and (θ_{MP}, ϕ_{MP}) - were calculated, indicating a bimodal TOAC occupancy model with sensitivity to label location. Coupling MD-derived conformational models with EPR data fitting has improved determination of the conformational distribution of PLB in a lipid bilayer and will allow us to determine PLB orientations in other conditions such as when bound to SERCA.

653-Pos Board B433

Using Molecular Dynamics Simulations to Interpret Fluorescence and EPR Data on Labeled SERCA and Phospholamban

Bengt Svensson[1], Xiaoqiong Dong[1], Karl J. Petersen[1], Zachary M. James[1], Jesse E. McCaffrey[1], Howard S. Young[2], David D. Thomas[1].
[1]Biochemistry, Molecylar Biology and Biophysics, Univ. of Minnesota, Minneapolis, MN, USA, [2]Dept of Biochemistry, Univ. of Alberta, Edmonton, AB, Canada.

We have performed molecular dynamics simulations of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and phospholamban (PLB) labeled with spectroscopic probes in order to establish a foundation for analyzing fluorescence and EPR data from this system. Site-specific labeling of a protein can provide insight into local structural dynamics, based on fluorescence quenching, anisotropy, or EPR measurements. Labelling can also provide structural information like distances to another label as measured with FRET or DEER and orientation in aligned bicelles as measured with EPR. To interpret the experimental results in a structural context, we have undertaken an approach involving X-ray crystallography and computational simulations. To perform these simulations, we developed CHARMM force-field parameters for the probes used. SERCA was labeled at position C674 with the fluorescent probe IAEDANS. A crystal structure of IAEDANS-labeled SERCA was used as a starting point for molecular dynamics simulations. The transition dipole autocorrelation functions were calculated and were shown to agree with experimental measurements of fluorescence anisotropy. Interprobe distance R and orientation factor κ^2, determined from the simulations were used to predict lifetime changes due to FRET. PLB was labeled with the bifunctional methanethiosulfonate spin-label (BSL) at position F32C/A36C, and EPR data was acquired on magnetically aligned bicelles, which allows determining the orientation of spin labels with respect to the applied magnetic field. By combining EPR studies and molecular dynamics simulations, we have shown that this approach is capable of directly reporting the structural topology of monomeric PLB. These results show that we have established reliable frameworks for integrating spectroscopic experiments and molecular simulations in the SERCA-PLB complex. Spectroscopic studies were performed at Minnesota Biophysical Spectroscopy Center and computational work at the Minnesota Supercomputing Institute. This work was supported by NIH (GM27906, AR007612).

654-Pos Board B434

Phospholamban Phosphorylation Alters its Conformational Equilibrium to Regulate SERCA Activity

Leanna McDonald, Gianluigi Veglia.
University of Minnesota, Minneapolis, MN, USA.

Heart muscle contractility is modulated by the interaction of the integral membrane protein, phospholamban (PLN) with the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). Unphosphorylated PLN reversibly inhibits SERCA activity upon binding. Phosphorylation at Ser16 or Thr17 of PLN causes relief of the inhibition. The critical role PLN and SERCA play is cardiac contractility is clear however, the mechanism by which phosphorylation changes PLN's interaction with SERCA is not well understood. It has been hypothesized that phosphorylation induces an otherwise sparsely populated state of PLN in which the cytoplasmic domain is extended and bound to SERCA. Here we investigated PLN's conformational and dynamic changes by solid state NMR. The interaction of PLN with SERCA is shown for phosphorylated PLN at Ser16 or Thr17 and compared to unphosphorylated PLN.

655-Pos Board B435

Atomic-Level Characterization of the Inhibition Mechanism of the Calcium Pump by Phospholamban

L. Michel Espinoza-Fonseca[1], Joseph M. Autry[1], G. Lizbeth Ramírez-Salinas[2], David D. Thomas[1].
[1]Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA, [2]Laboratorio de Modelado Molecular y Bioinformática, Instituto Politecnico Nacional, Mexico City, Mexico.

We have performed protein pK_a calculations and molecular dynamics (MD) simulations of the calcium pump (sarcoplasmic reticulum Ca^{2+}-ATPase, SERCA) in complex with phospholamban (PLB). Regulation of SERCA activity by PLB is central in modulating cardiac contractility. X-ray crystallography studies have suggested that PLB locks SERCA in a low-Ca^{2+} affinity E2 state that is incompatible with metal ion binding, thereby blocking the conversion toward a high-Ca^{2+} affinity E1 state. Estimation of pK_a values of the acidic residues in the Ca^{2+} sites indicates that under normal intracellular pH (7.1-7.2), PLB-bound SERCA populates an E1 state deprotonated at residues E309 and D800 yet protonated at residue E771. We performed microsecond-long MD simulations to evaluate the structural dynamics of SERCA-PLB in a solution containing 100 mM K^+ and 3 mM Mg^{2+}. Principal component analysis showed that PLB-bound SERCA lies exclusively along the structural ensemble of the E1 state. We found that the transport sites of PLB-bound SERCA are completely exposed to the cytosol, and that two K^+ ions bind transiently ($\leq$5 ns), and non-specifically (9 different positions) to the two transport sites; the total occupancy time of the two K^+ ions in the transport sites is 80%. These findings indicate that PLB does not inhibit the E2-to-E1 interconversion but instead populates a novel E1 intermediate, $E1{\bullet}H^+_{771}$, that depresses SERCA activation by Ca^{2+}. We propose that the efficient regulation of SERCA activity by PLB results from structural transitions that occur primarily in the E1 state of the pump. *This work was supported by grants to L.M.E-F. from the American Heart Association (12SDG12060656) and to D.D.T from NIH (GM27906), and by the University of Minnesota Supercomputing Institute.*

656-Pos Board B436

Novel Roles of Gq-Dependent Signal Transduction for Cardiac Pacemaking and Cardiac Impulse Propagation Studied by Gq-KO and a DREADD

Elisabeth Kaiser[1], Laura Schröder[1], Kathrina Wiesen[1], Jürgen Wess[2], Qinghai Tian[1], Lars Kaestner[1], **Peter Lipp**[1].
[1]Institute for Molecular Cell Biology, Saarland University, Homburg/Saar, Germany, [2]Molecular Signaling Section, Laboratory of Bioorganic Chemistry, NIDDK, Bethesda, MD, USA.

Many processes in the heart are modulated via G protein-coupled receptors (GPCRs). While the role of Gi-, Go- and Gs-proteins for pacemaking, impulse propagation and the myocardial function are well understood, the contribution of Gq-coupled signaling to pacemaking and excitation conduction remains largely elusive. We have generated cardiac-specific Gq-KO mice and animals with targeted expression of Gq-coupled DREADDs to address these questions. Animals were investigated from the single cell to the whole organism using a large variety of different techniques including telemetric ECG and blood pressure measurements, echocardiography, single cell imaging and electrophysiology as well as immunofluorescence subcellular localization of receptors. We found that specifically Gq-KO animals displayed chronic cardiac arrhythmias including sinus arrest and AV blocks. A