agreement with published FRET data. Cross-correlation matrices revealed correlated motions between the A and phosphorylation (P) domains in both E1 and E2 states. In contrast, anticorrelated motions were observed between N and A/P domains, with slight differences between E1 and E2, suggesting a Ca^{2+} effect. Solvent-accessible surface area around the ATP site increased in the simulated E2 state. Conclusions: (a) Crystal structures of E1 and E2 states of SERCA are not representative of the populations under physiological conditions, where the two structures differ much less than in the crystal. (b) The simulation indicates a much more accessible ATP-binding site than observed in the E2 crystal structure. (c) Calcium-induced modulation of interdomain anticorrelated motions involving the N domain may be important for ATP binding, catalysis and gating. This work was supported by NIH (GM27906, AR007612) and the Minnesota Supercomputing Institute.

747-Pos Board B626

Investigation Of Electrogenic Partial Reactions In Detergent-solubilized Na,K-ATPase

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Electrochromic styryl dyes are in use now for almost two decades to detect ion movements in various P-type ATPases. The extremely hydrophobic dye molecules have a high partition coefficient in favor of the hydrophobic core of lipid phase of membrane preparations. Fluorescence changes are obtained by modification of local electric fields in the membrane dielectric produced by ions bound to or released from binding sites of the ion pumps. To obtain significant signals a prerequisite is a high density of active proteins in the membranes. This limitation could be overcome by solubilization of the Na,K-ATPase in mixed micelles of protein/lipid/detergent obtained by incubation of microsomal membranes from rabbit kidney with dodecyl maltoside. In this preparation the specific enzyme activity of the Na,K-ATPase was reduced compared to that in native membranes. This effect was assigned to the highly affected lipid environment of the singularized proteins which may be depleted of specific lipid components and the content of dodecyl maltoside. The fluorescence changes which were detected with the styryl dye RH421 showed smaller amplitudes than in the case of purified membrane preparations, however, the responses on Na^+ binding, Na^+ release upon enzyme phosphorylation and conformation transition, and subsequent K^+ binding in the E_2P conformation were clearly detectable. Na^+ binding affinity and its dependence on Mg^{2+} concentration and buffer pH, as well as K^+ affinity were comparable to the results obtained with native preparations. The transfer of the method to solubilized ion pumps will allow investigations of mutants isolated from cell in which they were expressed in low density. In addition, this approach will possibly extend method also to the investigation of ion channel molecules by electrochromic styryl dyes.

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Influence of Phosphate Analogs on Palytoxin-opened Na,K-ATPase Pump-channel

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Na,K-ATPase pumps generate steep transmembrane Na and K gradients by behaving like ion channels with gates constrained to open and close alternately. Palytoxin binding disrupts this coupling between the gates, allowing both to sometimes be open, transforming pumps into ion channels. The gates of these pump-channels still respond to extracellular Na and K ions and to cytoplasmic nucleotides. Here we test phosphate mimics. Saturating, 50 nM, palytoxin induced large Na,K pump-channel currents in outside-out patches from *Xenopus* oocytes with 5 mM ATP in the pipette, and smaller currents with no ATP, but failed to induce any currents when pipettes contained BeF_x (200 μM $BeSO_4$ plus 5 mM NaF). In inside-out patches, with 100 nM palytoxin in the pipette, BeF_x or AlF_x (200 μM $AlCl_3$ plus 5 mM NaF) strongly decreased (by at least 70%) the small pump-channel currents seen without ATP and severely impaired (by ~90%) the large current increase by 1 mM ATP that reflects internal gate opening. Be ions alone (200 μM $BeSO_4$) somewhat diminished pump-channel currents without ATP, but did not affect activation by ATP; however, gate closure upon ATP removal was markedly slowed (>100-fold). As subsequent BeF_x application almost abolished activation by ATP, the effects of BeF_x and of Be ions are distinct. Al ions alone (200 μM $AlCl_3$) had similar effects to Be ions, though weaker. In contrast, MgF_x (5 mM $MgCl_2$ plus 5 mM NaF), or even just $MgCl_2$, simply reversibly decreased pump-channel currents in the absence of ATP, without affecting activation by ATP. These results suggest that, despite the presence of palytoxin, phosphate mimics BeF_x and AlF_x stabilize an Na,K pump-channel conformation with the internal gate firmly shut, like that in X-ray crystal structures of E2-BeF_3^- SERCA ATPase. [HL36783]

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Truncation Of The Na/K Pump's C-terminus Attenuates Voltage-dependent Binding Of External Na^+ By Destabilizing Na^+ Occlusion

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In the recent Na/K pump crystal structure the C-terminus directly contacts the transmembrane region, and deletion of its last five residues (KETYY in pig Na/K pumps) strongly lowered (26-fold) apparent affinity for Na^+-dependent phosphorylation, which reflects cytoplasmic-side Na^+ binding (Morth et al., 2007). Here we use two-microelectrode voltage-clamp recordings in *Xenopus* oocytes to investigate the consequence of this C-terminal truncation on the external Na^+ dependence of steady-state, and transient, Na/K pump-mediated currents. The corresponding deletion (ΔKESYY in *Xenopus α1*) was introduced in *Xenopus α1β3* pumps made ouabain resistant by either Q120R-N131D (RD) or C113Y (C-Y) mutations. All extracellular solutions then contained 1 μM ouabain to silence the endogenous *Xenopus* Na/K pumps, and the mutant pump currents were determined by addition of 10 mM ouabain. Inhibition of steady outward Na/K pump current (at 15 mM external K^+) by 125 mM extracellular Na^+ at negative voltages was weaker in both RD and C-Y pumps than in wild-type *Xenopus* Na/K pumps, but was almost completely absent (at -120 mV) in RD-ΔKESYY or C-Y-ΔKESYY pumps. Transient, external Na^+-dependent, pump currents were similarly measured as 10 mM ouabain-sensitive currents, but in K^+-free solution. The voltage dependence of these transient charge movements was shifted to more negative potentials for RD-ΔKESYY and C-Y-ΔKESYY pumps compared to RD or C-Y pumps, similar to the shift caused in wild-type pumps by decreasing the extracellular Na^+ concentration. Together, these results suggest that the C-terminal deletion lowers the apparent affinity with which extracellular Na^+ ions bind to the Na/K pump to reverse Na^+-ion deocclusion. The C-terminus therefore seems important for stabilizing the occluded conformation of the phosphorylated Na/K pump containing 3 bound Na^+ ions. [NIH HL36783]

750-Pos Board B629

Temporal and Steric Analysis Of Ionic Permeation and Binding in Na+,K+-ATPase via Molecular Dynamic Simulations

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Abstract: The Na^+, K^+-ATPase is ubiquitous in animal cells. Yet, many atomic-level details of the structure/function relationship of its electrogenic translocation process remain unanswered. This work employs computational methods to investigate the specific amino acid residues that constitute the two K^+ and three Na^+ ion binding sites. Putative lumenal and cytoplasmic ion permeation pathways are also determined. Homology models of the human α1 isoform of the Na^+, K^+-ATPase based on X-ray structures of the SERCA Ca^{2+}-ATPase in several conformations (E1, E2, and E2P) were created using the Modeller homology modeling software. The sequence alignment incorporated an array of experimental results and consensus with similar proteins (e.g. H^+, K^+-ATPase). The E2P homology model presented here agrees well with the recent X-ray crystallographic structure of the Na^+, K^+-ATPase. The three models were simulated in a water/lipid environment with the GROMACS molecular dynamics package. Established equilibration techniques were followed by several nanoseconds of analyzable production run trajectories for each conformation. Atomic trajectories were analyzed with the steric pathway tool, CAVER, to provide putative ion permeation pathways. These pathways were consistent with regions of negative potential determined via time-averaged electrostatic calculations of the same trajectories. The electrostatic calculations provide a 3D view of the potential landscape encountered by cations. Amino acid residues (as suggested by SERCA structures and mutagenesis studies) involved in the creation of putative binding sites were investigated by placing Na^+ and K^+ ions at these locations and evaluating protein-ion interactions during simulation trajectories. In addition to the results of the Na^+, K^+-ATPase simulations, the homology models and methodology presented here provide a blueprint for the study of the larger class of P-type ATPases.

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Relative Movement Of The α-Subunit's First And Last External Loops Throughout Na/K-Pump Cycle

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Structural models of the Na/K-pump α-subunit show that conserved residues D121 (loop 1-2, pig-α1 numbering) and R972 (loop 9-10) are 5-7 Å apart in E2, and more than 12 Å away in E1. To check whether these residues reach