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]

749-Pos Board B628

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.

751-Pos Board B630

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

conformation-dependent atomic proximity, the substitutions D121C and R972C were introduced (individually and concurrently) into the ouabain-insensitive C104Y-*Xenopus*-α1. Each mutant was coexpressed with *Xenopus*-β3 in *Xenopus* oocytes and their function tested in Na^+-loaded oocytes under two-electrode voltage clamp. K^+_o-activated Na/K-pump currents were observed in oocytes injected with D121C or R972C, but not in those injected with D121C-R972C, unless the eggs were exposed to TCEP (10-50 mM, ~20 min), consistent with the presence of a pump-inhibiting disulfide.

To identify the conformation locked by the disulfide, we used palytoxin to transform Na/K-pumps into channels. Palytoxin-induced currents (I_{PTX}) in outside-out patches from oocytes expressing D121C-R972C, bathed in Na^+ solutions, were insensitive to $MTSET^+$ application. Patch exposure to DTT restored $MTSET^+$-sensitivity (~65% I_{PTX} reduction) without affecting I_{PTX} amplitude. Palytoxin stabilizes an E2P-like structure; thus, the lack of DTT effect on I_{PTX} suggest that cross-linking between D121C and R972C occurs in E2P, with the external cation pathway open. Moreover, pump inhibition by spontaneous disulfide formation indicates that conformational mobility between these residues is required for the E2 to E1 transition.

The slow component of ouabain-sensitive transient charge movement in 125 mM Na_o^+ was measured in Na^+-loaded oocytes expressing these mutants. The center of the equilibrium distribution of charge (voltage of equal occupancy of E1 and E2) for R972C ($V_{1/2}$= -34 ± 8 mV) was identical to that of the C104Y-α1 template ($V_{1/2}$= -35 ± 3 mV). Therefore, it appears that a D121-R972 salt bridge is not necessary for E2P stabilization among Na^+-occupied states.

Membrane Transport

752-Pos Board B631

Modeling Osmotic Lysis of Cells by Antimicrobial Peptides: Transient Diffusion of Ions and Osmotically-driven Flow

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Antimicrobial peptides (AMPs) are a promising new class of antibiotics that are believed to kill pathogens by permealizing their cell membranes. We present a model for the transient transport that takes place in a bacterial cell as a result of exposure to high concentrations of protegrin, a particularly potent AMP found in porcine leukocytes. In particular, we focus on the efflux of potassium, the decay of the transmembrane potential, and the volume changes associated with osmotic flow across the membrane, all of which are coupled phenomena. The model that we employ is based on the classic nonequilibrium thermodynamics approach for transport of solutes across permeable membranes, commonly referred to as Kedem-Katchalsky formalism. In our model, the cellular interior and the exterior bath are assumed to be well-mixed compartments, separated by a thin homogeneous membrane region. Overall mass balances on each diffusing species and an overall volume balance yield a tractable set of initial-value, ordinary differential equations; some complexities arise in the modeling of the electrostatic potential and the hydrostatic pressure differences across the membrane. The model parameters that relate to membrane properties appear as parameters in the flux expressions. This work allows us to investigate the timeline of events that follow protegrin treatment leading to cell death, as well as assessing the role of osmotic lysis as a mechanism of action for antimicrobial peptides.

753-Pos Board B632

Quantitative Modeling of Passive Permeation through the Blood Brain Barrier

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In the last few years, this research group has obtained the rate constants and equilibrium binding constants for the interaction of several amphiphiles with the blood protein albumin, with lipoproteins and with lipid bilayers in the liquid ordered and liquid disordered phase (Abreu et al, 2003, Biophys. J. 84:386; ibid 2004, 87:353; Estronca et al, 2007, Biophys. J. 93:4244; ibid 2005, 88:557; Moreno et al, 2006, Biophys.J, 91:873; Sampaio et al, 2005, Biophys. J. 88:4064). Those rate constants allowed us to build a "master" kinetic scheme for the equilibration of the amphiphiles with blood components, and to quantitatively model its passive permeation across tight endothelia like the blood brain barrier. The amphiphile is added to the blood, equilibrated with serum albumin, and its sequestration by other blood components, interaction with the endothelium and accumulation in the tissue is followed over time.

The results obtained for two homologous series will be presented: *i*) a fatty amine with a single acyl chain (NBD-Cn; n=8, 10, 12, 14 and 16); and ii) a phospholipid derivative (NBD-diCnPE; n=6, 8, 10, 12 and 14). For the kinetic parameters typical of the amphiphiles considered, the accumulation in the tissue is well described by a mono-exponential curve and the characteristic rate constant ranged from 0.02 s^{-1} (for NBD-C8) to 10^{-8} s^{-1} (for NBD-C16 and NBD-diC14PE).

Contrary to the common expectation, an increase in the hydrophobicity of the amphiphile, along each homologous series, conduced to a decrease in the characteristic rate of accumulation in the tissue. A sensibility analysis was performed and the rate limiting steps for each amphiphile were identified. The extent of sequestration in the blood and the rate of interaction with the apical membrane of the endothelium were found to be the determinant processes for most amphiphiles.

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Time-Resolved Studies of Adsorption and Transport of a Hydrophobic Ion at Escherichia coli Bacterial Membranes by Second Harmonic Generation

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The adsorption and transport processes of a hydrophobic molecular ion Malachite Green (MW 329.4) at E.coli bacterial outer membrane and cytoplasmic membrane, respectively, have been characterized by using a nonlinear optical technique- Second Harmonic Generation. Adsorption isotherms of the MG ion to both membranes of the gram-negative bacteria E.coli cell have been measured for the determination of the maximum adsorption densities and adsorption equilibrium constants. In some of the experiments, the classical permeablizer EDTA was used to eliminate the bacterial outer membrane and enable independent determination for the cytoplasmic membrane. A pH-modified Langmuir adsorption model has been applied to analyze the isotherms obtained under physiological conditions. The effect of solution ionic strength on adsorption has been examined. The nonlinear optical signal also directly allows the observation, with real time resolution, of the transport of the molecular ions through the two membranes and the determination of the respective transport rates.

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15N Chemical Shift Anisotropy of the Schiff Base in Bacteriorhodopsin Intermediates

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Bacteriorhodopsin is a prototypical ion pump with a retinylidine chromophore. Ion translocation involves photo-isomerization and distortion of the chromophore, coupled with deprotonation and reprotonation of the Schiff base (SB) on opposite sides of the transport channel. Thus the SB changes its connectivity between the early and late M states, while the SB is deprotonated. Previous solid-state NMR experiments have shown that in the early M state, the SB is more strongly hydrogen-bonded than in the late M state, as indicated by the isotropic ^{15}N chemical shifts. However, the three principle values of the chemical shift tensor are more sensitive to the environment than the isotropic average, and should yield further insight into differences between the two M states. At sufficiently low spinning frequencies, redistribution of the signal intensity from the center band to the sidebands allows calculation of the chemical shift anisotropy.

The intensity of the weakest detected sideband corresponds to one site in a molecular weight of ~500 kDa. With the signal enhancement provided by dynamic nuclear polarization, we have recorded high signal-to-noise spectra of M (see Figure) and reliably obtained the principal values of the shift tensor.