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

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

Dan S. Bolintineanu, Yiannis N. Kaznessis, H Ted Davis.
University of Minnesota, Minneapolis, MN, USA.

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.

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Quantitative Modeling of Passive Permeation through the Blood Brain Barrier

Hugo Filipe, Armindo Salvador, Winchil Vaz, **Maria Joao Moreno**.
Universidade de Coimbra, Coimbra, Portugal.

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

Jia Zeng[1], Heather Eckenrode[1], Hai-Lung Dai[2].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Temple University, Philadelphia, PA, USA.

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

Melody L. Mak-Jurkauskas[1], Alexander B. Barnes[1], Yoh Matsuki[2], Marina Belenky[2], Robert G. Griffin[1], Judith Herzfeld[2].
[1]Massachusetts Inst Technology, Cambridge, MA, USA, [2]Brandeis University, Waltham, MA, USA.

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.