Donglin Bai in their unitary channel conductance (School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.

... changes in the Vj-gating properties, but displayed a significantly reduced mutants in N2A cell pairs indicate that the Cx50Cx36E1 channel showed little Cx50 was replaced by the E1 of Cx36, and 4 point mutations in E1 of Cx50... not clear. Experimental evidence showed that residues in the first extracellular domain play an important role in the molecular basis by which connexin channels select among (potential) permeating molecules, and how mutations alter the permeation process. A signal property of connexin channels is the ability to mediate selective diffusive movement of molecules through plasma membrane, yet the movement of biological molecules through these channels has yet to be well-characterized in mechanistic or energetic terms. Different connexin channels have distinct molecular selectivities that cannot be explained on the basis of size or charge of the permeants; the forces that molecules experience within the pore determines which molecules are permeable and to what degree. The energetics of the movement of twelve derivatives of sucrose, one permeable and one impermeable, through an experimentally validated connexin26 (Cx26) structural model were explored using Hamiltonian Replica Exchange MD Umbrella Sampling (US/H-REMD) and Steered Molecular Dynamics (SMD), and associated analytic tools. Crucially, the Cx26 channel model, in explicit membrane/solvent, incorporates key post-translational charge changes shown by Brownian Dynamics to be required to reproduce the electrical conductance characteristics of the native channel [Kwon J.Gen.Physiol. 138:4751]. The results show energy profiles consistent with experimental results. The energetic barriers extend through most of the pore length, rather than being highly localized, as in ion-specific channels. There is little evidence for binding within the pore. Force decomposition reveals how, for each test molecule, interactions with water and with the Cx26 protein vary over the length of the pore, and reveal a significant contribution of interaction with K+ ions. The flexibility of pore width varies along its length, and the test molecules tend to widen the pore as they pass through. This work highlights factors involved in selective molecular permeation that may not be significant for atomic ions. The results suggest that this system can be used to explore the molecular basis by which connexin channels select among (potential) permeating molecules, and how mutations alter the permeation process.

The Residues in the First Extracellular Domain Play an Important Role in Trans junctional-Voltage Dependent Gating and Unitary Channel Conductance of Cx50 Gap Junction Channels

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1Physiol. and Pharm., Western University, London, ON, Canada, 2Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan. Gap junctions (GJs) are intercellular channels that connect the cytoplasm of neighboring cells. GJ channels form by Cx50 and Cx36 show drastic disparity in their unitary channel conductance (γj) and trans junctional voltage-dependent gating (Vgating), but the important underlying molecular domains/residues are not clear. Experimental evidence showed that residues in the first extracellular domain (E1) of Cx50 likely line the GJ channel pore and may play an important role in determining γj and Vgating. We aligned the E1 sequence of Cx50 with that of Cx36 and found 10 different residues (4 out of 10 residues involve a change in charge). We generated a chimera Cx50Cx36E1, in which the E1 of Cx50 was replaced by the E1 of Cx36, and 4 point mutations in E1 of Cx50 (where a charge change occurs, i.e. G46D, D51M, E62N and E68R). Due patch clamp study on the homotypic GJ channels formed by the chimera or the point mutants in N2A cell pairs indicate that the Cx50CxC36E1 channel showed little change in the Vgating properties, but displayed a significantly reduced γj. Surprisingly, homotypic G46D, E62N and E68R channels all increased γj and showed little change in Vgating properties, except E62N. D51M failed to form functional GJ channels. Our homology structure models of the chimera and the mutants indicate that the electrostatic properties of the pore lining residues are a key parameter in facilitating ion permeation and play a role in the Vgating properties of Cx50 and possibly other GJ channels.

Residues Involved in Cx26 Hemichannel Voltage Dependent Gating

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Centro Interdisciplinario de Neurociencias, Valparaiso, Chile. The most well-known role of Connexins (Cxs) is to communicate the cytoplasm of two adjacent cells forming a gap junction channel in the apposition zone. On the other hand, Cx3 forms the so-called “hemichannels”, a fully functional voltage-gated non-selective ion channel, which present two gating mechanism called “slow” and “fast” gating. Hemichannels consist of 6 monomers, each one composed of four transmembrane domains. Despite the fact that hemichannels are voltage-activated, they lack the classical voltage-sensing domain described for canonical voltage-gated domains. Furthermore, the molecular determinants associated to the voltage activation in connexins hemichannels are still elusive. In this work we search for the molecular determinants associated to voltage detection in human Cx26 hemichannels. Using two electrode voltage-clamp in Xenopus laevis oocytes, we study how the steady-state conductance-voltage (G/V) relationship and kinetics of gating are affected by the neutralization of different charged residues located within the first transmembrane domain. We have found that Cx26 conductance-voltage curve shows an apparent number of gating charges (zδ) of 1.4 e0 for the slow gating mechanism. On the other hand, we obtained a zδ of 3.9 e0 for the fast gate, which is in agreement with the zδ of Cx26 gap junctions. A simple three-state closed-open-open2 kinetic model is able to account for the channel voltage dependence. Furthermore, our data shows that neutralizing the K41 residue (K41N) increases the zδ of the slow gate to 2.5 e0, and decreases the zδ of the fast gate to 2.3 e0.

Understanding the Conformational Dynamics of the Porin Ock5 Karunakar Reddy Pothula.

The outer membrane of Gram-negative bacteria contains various porins to permit the passage of water soluble molecules into the periplasm of bacteria. Compared to the Gram-negative bacterium Escherichia coli, the pathogenic organism Pseudomonas aeruginosa mainly contains substrate-specific, relatively small-sized porins making the transport of various substrates through its outer membrane difficult. Small molecules containing carboxylic acid group are taken up by the members of the Ock family (1). Thus, understanding the structure-function relationship of these porins is crucial to rationally design new antibiotics with better permeability. Recent electrophysiological experiments have shown that OckC5 is a member of the Ock family of proteins - exists in two conducting substates, i.e., O2 and O1 (2). Our goal is to study the conformational dynamics of OckC5 porin using molecular dynamics (MD) simulations (3). To this end, accelerated MD simulations have been used to characterize the structural and dynamic properties of the OckC5 porin. Using accelerated simulations, transitions between O2 and O1 substates have been achieved. Our study provides the first atomistic view of the transition between O2 and O1 substates in OckC5. Particularly, the role of specific arginine side chain conformations in defining the different substates of OckC5 is described. To further confirm these findings a temperature-dependent single channel analysis has been performed. Experimental data reveal kinetic and thermodynamic constants of the transitions between the observed major sub-states and probable transition intermediates.

Cyclodextrin Interaction with Specific Channel CymA from K. Oxytoca Satya Pratyusha Bhamidimarri2, Jing Lu1, Jigeshkumar Dahyabhai Prajapati3, Ivan Barcena Urribari1, Bert van den Berg2, Ulrich Kleinekathofer1, Matthias Winterhalter1
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The outer membrane acts as a selective uptake barrier in Gram negative bacteria. It contains protein channels (porins) which provide an entry pathway for...
hydrophilic molecules like small nutrient molecules and β-lactam antibiotics. However the CymA channel is known to take up cyclodextrin molecules giving bacteria the ability to survive on cyclodextrins. Hence regulating uptake of these molecules via porins is vital to comprehend the transport mechanism across the cell membrane. Electrophysiology forms a promising approach to study the permeation of molecules across outer membrane and thereby understanding molecular interactions with the channel. Here we present cyclodextrin interaction studies of CymA from K. oxytoca using single channel electrophysiology. Detailed single channel analysis revealed inherent asymmetric gating characteristics of the channel. Analysis of the ion current reduction through CymA in presence of cyclodextrin led revealed kinetic parameters of substrate binding. To further elucidate the affinity sites of substrate to the channel, mutation of certain channel residues has been performed. An altered channel gating behaviour is observed. To obtain an atomistic view we complement our studies with all-atom molecular dynamics simulation to study the previous conductance states of the channel in the absence of cyclodextrin and to get molecular insight into the uptake of cyclodextrins as well.

References:

2227-Pos Board B364 Mimicking Biology with Nanomaterials: Carbon Nanotube Porins in Lipid Membranes
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Living systems control transport of ions or small molecules across biological membranes using ion channels that form highly efficient and selective pores in lipid bilayers. Although bottom-up synthesis and top-down fabrication could produce pores of comparable size, an unresolved challenge remains to build nanopore scaffolds that fully replicate transport properties of membrane channels. We will show that pores formed by ultra-short carbon nanotubes (CNTs) assembled in the lipid membranes have transport properties that come remarkably close to that goal. These CNT porins can transport water, protons, small ions, and DNA and their ion-rejection properties can be controlled by the charge at the pore mouth. Interestingly, these pores also display the stochastic “gating” behavior common for biological ion channels. Overall, CNT porins represent a simplified biomimetic system that is ideal for studying fundamentals of transport in biological channels, and for building engineered mesoscale structures, such as artificial cells.

2228-Pos Board B365 Understanding the Translocation of Fluoroquinolones through OmpC using the Metadynamics
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The outer membrane of Gram-negative bacteria such as Escherichia coli acts as a selective permeable barrier between cell and external environment. Water filled outer membrane proteins called as porins were identified for exchange of hydrophilic solutes and hydrophilic antibiotics. One of the most abundant outer membrane porins in E. coli is OmpC and many studies revealed that down-regulation or mutation of this porin shows reduced accumulation of antibiotics in bacterial cells [1]. Fluoroquinolones, used since 1980, are the most common treatment for urinary tract infection caused by E. coli and today this treatment is ineffective in more than half of the patients globally due to drug-resistant bacteria. So far the influx kinetics of fluoroquinolones with OmpC has been characterized on free standing lipid bilayers formed on a glass substrate [2]. In particular, detailed analysis of antibiotic interaction with a single OmpC channel using electrophysiology can provide a kinetic description. Here we have investigated two fluoroquinolones, Ciprofloxacin and Enrofloxacin, using an advanced molecular dynamics technique, i.e., metadynamics [3,4]. These free energy calculations help to identify the most favorable paths and activation energies required for molecules to translocate through the OmpC channel. Furthermore, we have also investigated the translocation of the same molecules in the presence of different salts to understand the altered translocation kinetics [5]. Moreover, the identification of favorable interactions networks is important to determine the most prominent residues required for translocation.