Biophysics of Ion Permeation

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Molecular Dynamics Studies of Ion Permeation in VDAC

Huan Rui, Kyu Il Lee, Richard W. Pastor, Wonpil Im.

The voltage dependent anion channel (VDAC) in the outer membrane of mitochondria serves an essential role in transport of metabolites and electrolytes between the cell matrix and mitochondria. To examine its structure, dynamics, and mechanisms underlying its electrophysiological properties, we have performed a total of 1.77 μs molecular dynamics simulations of human VDAC isoform 1 in DOPE/DOPC mixed bilayers in 1M KCl solution with transmembrane potentials of 0, ±25, ±50, ±75, and ±100 mV. The calculated conductance and ion selectivity are in good agreement with the experimental measurements. In addition, ion density distributions inside the channel reveal possible pathways for different ion species. Based on these observations, a mechanism underlying the anion selectivity is proposed; both ion species are transported across the channel, but the rate for K+ is smaller than that of Cl- because of the attractive interactions between K+ and residues on the channel wall. This difference leads to the anion selectivity of VDAC.

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Effects of Divalent Cations on the Single-Channel Conductance of the OmpF Channel: Linearity, Saturation and Blocking

M. Lidón López, Elena García-Giménez, Vicente M. Aguilella, Antonio Alcaraz.

Previous studies on the bacterial porin OmpF (Biophys. J. 96 (2009) 55-66) have shown that the channel moderate cationic selectivity of OmpF in NaCl and KCl solutions is reversed to anionic selectivity in solutions of $CaCl_2$ and $MgCl_2$. We study here the effect of salts of divalent cations on the channel conductance with a particular emphasis in dissecting the role of the electrolyte, the role of the counterion accumulation induced by the protein channel charges and any other effect not found in salts of monovalent cations. Single-channel conductance measurements are performed over a wide range of salt concentrations (up to 3 M).

We find that the change of channel conductance with salt activity in bulk solution exhibits different features in salts of monovalent cations and in salts of divalent cations. In order to separate channel and electrolyte effects we analyse the correlation between channel conductance and bulk solution conductivity. While one scales with the other in solutions of NaCl and KCl over the whole concentration range studied, the conductance for $CaCl_2$ and $MgCl_2$ has two regimes. At salt concentrations below 1 M we found the same pattern as in solutions of NaCl and KCl. However, for higher concentrations such linearity between conductivity and conductance vanishes. Moreover, surprisingly, at high concentrations of $MgCl_2$ and $CaCl_2$ the conductance decreases as conductivity increases. Once, one accounts for the big variation of activity coefficients of divalent salts at high concentrations, the experimental results suggest that at low concentrations the pore conductance is controlled mainly by the electrolyte properties. The current recordings in salts of divalent cations reveal the existence of substates of lower conductance as one of the causes of the conductance decrease in the high concentration regime.

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Sialic Acid Transport in E. coli: Role of Outer Membrane Porin NanC

Janhavi Giri, John M. Tang, Christophe Wirth, Caroline M. Peneff, Tilman Schirmer, Bob Eisenberg.

Sialic acid is a nutrient of bacteria important in host-pathogen interactions. The mechanism of transport of sialic acid from outer membrane to periplasmic space of *Escherichia coli* is not known. N-acetylneuraminic acid (Neu5Ac) - the most abundant form of sialic acid - induces a specific porin NanC (**N-a**cetyl**n**euraminic acid **C**hannel) in the outer membrane of *E. coli*. Recently, a high resolution structure of NanC (Wirth et al., J.Mol.Biol., (2009) 394:718) revealed unique structural features that support Neu5Ac transport. However, patch-clamp experiments seemed to show that NanC conductance is unaffected by sialic acid (Condemine et al., J.Bacteriol., (2005) 187:1959). We report single channel current measurements of NanC in bilayers in the presence of Neu5Ac. Neu5Ac changes gating and considerably increases the ionic conductance of NanC in 250 mM KCl, pH 7.0. (See our other NanC poster.) The unitary current through NanC increases when 7-12 mM of Neu5Ac is added to the grounded side of the bilayer. A distinct steady voltage dependent current (sublevel) is observed that seems to add to the unitary current. The single channel slope conductance of NanC increases by 51% in the presence of 7 mM Neu5Ac and by 74% in 55 mM. The effect of Neu5Ac on the unitary current through NanC seems to saturate at higher Neu5Ac concentrations. The unit conductance of NanC also increases when 20 mM Neu5Ac is added to both sides of the bilayer. It is likely that some of the current is carried by Neu5Ac. Interestingly, Neu5Ac reduces the ionic conductance of trimeric OmpF (Outer membrane porin F) under the same conditions: frequent, long closures are seen. Thus, we provide evidence that sialic acid translocation is specifically facilitated by NanC, and not by the general porin OmpF.

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Measurement and Interpretation of Ion Selectivity in Wide Channels: Merging Information from Different Approaches

Antonio Alcaraz, Elena García-Giménez, María L. López, Vicente M. Aguilella.

The ion selectivity of a channel can be quantified in several ways by using different experimental protocols. A wide, mesoscopic channel, the OmpF porin of the outer membrane of E. Coli, serves as a case study to compare and analyze several measures of the channel cation-anion permeability in chlorides of alkali metals (LiCl, NaCl, KCl, CsCl). We show how different insights can be gained and integrated to rationalize the global image of channel selectivity. To this end, reversal potential, channel conductance and bi-ionic potential (two different salts with a common anion on each side of the channel but with the same concentration) experiments are discussed in the light of an electrodiffusion model based on the Poisson-Nernst-Planck (PNP) formalism. Measurements and calculations based on the atomic crystal structure of the channel show that each protocol displays a particular balance between the different sources of selectivity.

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Numerical Simulation of Molecular Delivery via Electroporation

Hao Lin, Mohamed Sadik, Jianbo Li, Jerry W. Shan, David I. Shreiber.

A numerical simulation to study molecular delivery via electroporation is presented. The Nernst-Planck equations for species transport are solved in both the intra- and extra-cellular spaces, and are coupled at the cell membrane through an asymptotic Smoluchowski equation for membrane permeabilization. The delivery of calcium ions into a Chinese Hamster Ovary cell is simulated. To facilitate comparison with fluorescence measurement, the simulation includes three species (Ca2+, Fluo-3, and CaFluo) and their reactive kinetics. The results agree well with experimental data from the literature (Gabriel and Teissié, Biophys. J., 1999), and reveal that ion electrophoresis plays an important role in the process. Furthermore, the maximum achievable concentration within the cell is reciprocally correlated with the extracellular electrical conductivity. This observation corroborates well with both previous data (Djuzenova et al., Biochim. Biophys. Acta, 1996) and our own recent measurements. The root-cause of this behavior is an electrokinetic mechanism known as field-amplified sample stacking. Through this mechanism, the intracellular ion concentration can reach a level higher than the extracellular one provided that the intra-to-extra-cellular conductivity ratio is greater than unity. This work is a step toward the quantification of electroporation-mediated molecular delivery.

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A Pressure-Polish Setup to Fabricate Patch Pipettes Yielding Low Access Resistance and Efficient Intracellular Perfusion

Mascia Benedusi, Alberto Milani, Marco Aquila, **Giorgio Rispoli**.

When performing whole-cell configuration recordings, it is very important to minimize series resistance. This goal is achieved here by using a method able to enlarge the cone-shape section of the pipette, without increasing the tip opening diameter, by using a calibrated combination of heat and air pressure. The heat was produced by passing current in a glass-coated platinum wire, shaped appropriately to ensure an homogeneous heating of pipette shank, connected to a regulated high-current generator. The pressurized air (~4 atm) was applied to the pipette lumen through a 0.2 μm filter (to avoid pipette clogging), via the pressure port of a modified commercial holder. The pipette reshaping was viewed on an LCD monitor (and recorded on-line on a computer), connected to a contrast-intensified CCD camera coupled to a bright-field stereomicroscope. The hot halogen lamp of the microscope illuminator was replaced with a variable white LED source, to avoid the loss of fine control of the platinum wire temperature. By pressurizing the pipette lumen during fire-polishing, the pipette shank was widened as desired, without increasing the tip opening diameter: these pressure-polished pipettes, tested on many cell types, yielded routinely access resistances ~4-fold smaller than the ones attained with conventional pipettes. The pressure-polished pipettes minimized therefore intracellular ion accumulation or depletion, and errors in membrane potential control, in the presence of large membrane currents. Moreover, they allowed to study rapid voltage-activated currents (by reducing the time constant of charging the cell membrane capacitance), the efficient incorporation in the cytosol of large molecules (that was followed with fast fluorescence imaging), and to position pulled quartz capillaries inside the pipette very close to its tip, resulting in fast intracellular perfusion.