and the possible mechanisms of ammonia re-protonation and how side chains are reset back to original state are presented.

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Permeation Mechanism in the AmtB Ammonium Transporter: Putative Electrogenic Co-Transport of NH3 and H+

Shihao Wang, Sefer Baday, Esam A. Orabi, Simon Bernèche, **Guillaume Lamoureux**.

Despite the growing amount of structural information, the molecular details of the mechanism by which membrane proteins of the Amt/Rh family mediate ammonium transport remain elusive. For instance, in protein AmtB from Escherichia coli, it is not known whether NH3 is diffusing passively through the protein pore or is involved in an NH3/H+ co-transport mechanism.

Using state-of-the-art computational methods (polarizable force fields and hybrid QM/MM molecular dynamics simulations combined with free energy calculations) we investigate the thermodynamics and kinetics of various mechanisms for proton co-transport. Based on these simulations we propose a plausible NH3/H+ co-transport mechanism in which the twin-histidines dyad lining the pore plays a central role.

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Investigating Ammonium Transport Mechanisms in AmtB and RhCG by Molecular Dynamics Simulations

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Membrane proteins of the ubiquitous Amt/Rh family mediate the transport of ammonium. Despite the availability of different X-ray structures that provide many insights on the ammonium permeation process, the molecular details of its mechanism remain controversial. Functional experiments on plant ammonium transporters and rhesus proteins suggest a variety of permeation mechanisms including the passive diffusion of NH3, the antiport of NH4+/H+, the transport of NH4+, or the cotransport of NH3/H+. The X-ray structures have revealed that the pores of the prokaryotic AmtB and the eukaryotic RhCG proteins share a similar architecture suggesting that they might both catalyze the diffusion of NH3. However, molecular mechanics simulations of both proteins reveal that small differences in the pore lining residues might actually alter the properties of the pore. We notably find that the pore of the AmtB transporter can stabilize water molecules at much greater extent than the pore of RhCG. The possible presence of water molecules in the pore lumen of AmtB opens the door to alternative permeation mechanisms, notably involving the co-transport of H+. We discuss the possible permeation mechanisms in both the AmtB and RhCG proteins in light of some recent functional studies, and illustrate how closely related proteins can support quite different mechanisms.

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A View of Hydrogen/Hydroxide Flux Across Lipid Membranes

J. Wylie Nichols, **Ronald F. Abercrombie**.

A topic emerging roughly thirty years ago and engendering an incompletely resolved controversy is addressed: the relatively high permeability and pH independence associated with H^+/OH^- passive movements across lipid membranes. The expected characteristics of simple H^+/OH^- diffusion and those of a reaction between H^+ and OH^- being attracted from opposite surfaces and condensing in an interfacial region of the membrane are considered. An interfacial H^+/OH^- reaction mechanism predicts the experimentally observed behavior of a H^+/OH^- flux that is independent of the pH measurement range. In order to obtain the correct magnitude of flux, it is assumed that H^+ and OH^- within the interfacial zone become electrostatically aligned on opposite sides of the hydrophobic membrane core. Electrostatic attraction combined with charge delocalization among a small cluster of water molecules surrounding the ions sufficiently reduce the Born energy for insertion into lipid, accounting for the experimentally determined magnitude of this flux. The pH independence associated with H^+/OH^- passive movements across membranes could have satisfied a requirement for pH homeostasis in emerging life forms and provided stability for natural selection.

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Single Channel Measurements of N-Acetylneuraminic Acid-Inducible Channel (NANC) in E. coli

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Escherichia coli can use *N*-acetylneuraminic acid (Neu5Ac) as its sole carbon source even if the general outer membrane proteins OmpF and OmpC are not expressed: NanC - a monomeric outer membrane channel - allows Neu5Ac to move into the bacterial periplasm. Recently, a high resolution structure of NanC in two different crystal forms was reported by Wirth et al., J.Mol.Biol., (2009) 394:718 (PDB codes: 2WJQ and 2WJR). Our goal is to determine appropriate 'baseline' ionic conditions to study the transport of Neu5Ac through NanC using single channels in lipid bilayers. Measurements of single channel currents showed that NanC has two modes of time dependent behavior ('gating'). In the many situations we have tested, the modes are not induced or changed by surrounding ionic conditions or voltage. Single channels of NanC at pH 7.0 have: (1) a large conductance (around 100 pS to 800 pS in 100 mM KCl to 3M KCl) that varies with the polarity of the applied voltage; (2) anion over cation selectivity ($V_{reversal}$ around +16 mV in 250 mM KCl || 1 M KCl); (3) voltage-dependent gating (channel closures above ±200 mV). Single channel conductance of NanC decreases about 50% when HEPES concentration is increased from 100 μM to 100 mM in 250 mM KCl at pH 7.4, consistent with the two HEPES binding sites observed in the crystal structure (PDB code: 2WJR). Studying alternative buffers, we found that phosphate interferes with the channel conductance, whereas TRIS could not be used because it reacts with Ag/AgCl electrodes producing artifacts even in the presence of Agar-KCl bridges. Our further studies of NanC will use no pH buffers, but low concentration (250 mM) salt solutions adjusted to neutral pH 7.0.

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Blebbistatin Protects Rodent Myocytes from Death in Primary Culture via Inhibiting Na/Ca Exchange

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Introduction: It has been long recognized that rodent myoyctes die during long-term primary culture, which limits the use of genetically altered myocytes for signaling studies. Blebbistatin (BLB), a myosin II ATPase inhibitor, has been used to protect rodent myocytes. However, the mechanisms underlying the protective effects of this drug are not clear and are the topics of this study.

Materials & Methods: Adult rat ventricular myocytes (ARVM) were isolated and cultured with or without BLB (10μM) and BDM(10mM) for 72 hours. Myocyte death was evaluated by trypan blue staining. The effects of these two drugs on myocyte contraction, intracellular Ca transient ([Ca]i, Indo-1,410/480), SR Ca content, L-type calcium and Na/Ca exchanger currents were studied acutely.

Results: 1, Both BDM (61.5 ± 6.4%) and BLB (74.0 ± 3.2%) promoted myocyte survival in culture at 72 hours (control: 7.0 ± 1.8%); 2. ARVM fractional shortening was reduced by BLB (1.7 ± 0.4%) and BDM (0.5 ± 0.1%, control: 6.5 ± 0.7%); 3, Acutely, the amplitude of $[Ca]_i$ ($\Delta[Ca]_i$) was depressed by both BDM (0.038 ± 0.005) and BLB (0.065 ± 0.008) comparing to control (0.130 ± 0.010). 4, Diastolic Ca was significantly increased by BLB (0.90 ± 0.06) but not by BDM (0.73 ± 0.06) comparing to control (0.70 ± 0.05). 5. BLB and BDM significantly reduced the SR Ca content(Δ[Ca]i in BLB vs. BDM vs. control: 0.16 ± 0.016, 0.09 ± 0.01, 0.24 ± 0.03). 5. The mechanisms of the protective effect of BDM and BLB are different in that BDM mainly reduced Ca influx through the L-type Ca channel (85% reduction) and Na/Ca exchanger (60% reduction) while BLB inhibited Na/Ca exchanger (100% inhibition) without altering the LTCC(<5% reduction).

Conclusion: These results suggest both BDM and BLB protects rodent myocytes in culture by preventing cytosolic and SR Ca overload by both common and different mechanisms: both BDM and BLB inhibit NCX while BDM, but not BLB, reduces $I_{Ca\text{-}L}$.

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On Conduction and Gating in K^+-Channels

Carmen Domene, Simone Furini.

Potassium channels can conduct passively K^+ ions with rates of up to ~ 10^8 ions per second at physiological conditions, and they are selective to these species by a factor of 10^4 over Na^+ ions. Ion conduction has been proposed to involve transitions between two main states, with two or three K^+ ions occupying the selectivity filter separated by an intervening water molecule. The largest free energy barrier of such a process was reported to be of the order of 2-3kcal mol^{-1}. Here, we present an alternative mechanism for conduction of K^+ in K^+ channels where site vacancies are involved, and we propose that coexistence of several ion permeation mechanisms is energetically possible. Conduction can be described as a more anarchic phenomenon than previously characterized by the concerted translocations of K^+-water-K^+. Experiments also suggest that local structural changes in the selectivity filter may act as the a gate referred to as C-type inactivation. An extensive computational study on KirBac, is presented which supports the existence of a physical gate or constriction in the selectivity filter of K^+ channels. Our computations identify a new selectivity filter structure, which is likely associated with C-type inactivation.