AuPS/ASB Meeting - Adelaide 2010

Free communications: Ion channel gating

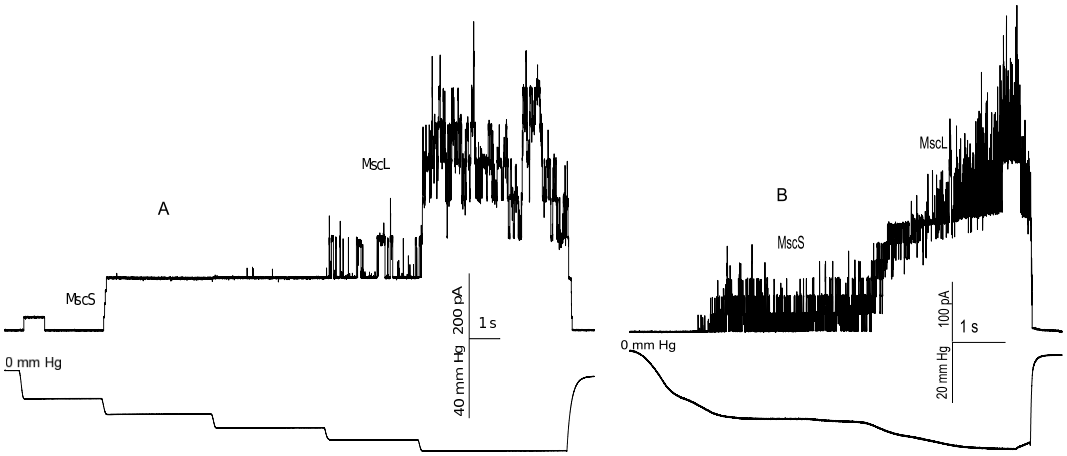
Tuesday 30th November 2010 - The Gallery - 14:30

Chair: Angelo Keramidas

Lipid effects on the gating behaviour and reconstitution of MscL and MscS

A.R. Battle,[1,2] T. Nomura[2] and B. Martinac,[2,3] [1]School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia, [2]The Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia and [3]St Vincent's Clinical School, The University of New South Wales, NSW 2052, Australia.

The *m*echanosensitive *c*hannels of *l*arge (MscL) and *s*mall (MscS) conductance act as osmosensors in bacterial cells against hypo-osmotic shock (Martinac, 2007). MscL has been extensively studied by reconstitution into liposomes (Häse *et al.*, 1995; Moe & Blount, 2005), however MscS has proved more difficult to reconstitute, requiring high protein-lipid ratios (Sukharev, 2002; Vásquez *et al.*, 2007). We recently published an improved reconstitution method for both MscL and MscS in soy azolectin (Battle *et al.*, 2009), a mixture that contains lipids, sugars and sterols. We have expanded these results and show here the effect of both individual and mixtures of lipids on the reconstitution and channel gating behaviour of co-reconstituted MscL and MscS. Introduction of the highly charged lipid cardiolipin causes rapid gating of MscS (Figure, A) in comparison to soy azolectin (Figure, B), indicating that lipid charge may play a significant role on channel gating dynamics.



***MscS/MscL co-reconstitution**. **A**: in soy azolectin. **B**: in mixture of phosphatidyl ethanolamine/ phosphatidyl choline/cardiolipin at a wt/wt ratio of 7:2:1, both recordings at a pipette voltage of +30 mV.*

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Häse CC, Le Dain AC, Martinac B. (1995) *Journal of Biological Chemistry* **270**: 18329-34.
Martinac, B. (2007) *Current Topics in Membranes* **58**: 25-57.
Moe P & Blount P. (2005) *Biochemistry* **44**: 12239-44.
Sukharev S. (2002) *Biophysical Journal* **83**: 290-8.
Vásquez V, Cortes DM, Furukawa H, Perozo E. (2007) *Biochemistry* **46**: 6766-73.

Mapping the sequence of conformational changes underlying selectivity filter gating in a potassium channel

A.P. Hill,[1,2] D. Wang,[1,2] S. Mann,[1,2] M. Perry,[1] P. Tan[1] and J.I. Vandenberg,[1,2] [1]Mark Cowley Lidwill Research Program in Cardiac Electrophysiology, Victor Chang Cardiac Research Institute, 405 Liverpool Street, Darlinghurst, NSW 2010, Australia and [2]St. Vincent's Clinical School, UNSW Faculty of Medicine, Victoria Street, Darlinghurst, NSW 2010, Australia.

The potassium channel selectivity filter both discriminates between K^+ and sodium ions and contributes to gating of ion flow. Static structures of conducting (open) and non-conducting (inactivated) conformations of this filter are known, however the sequence of protein rearrangements that underlie interconversion between these two states is not. Using ϕ-value analysis we have studied the macromolecular rearrangements associated with selectivity filter gating in the human ether-a-go-go-related gene (hERG) K^+ channel, a key regulator of the rhythm of the heartbeat. We have found that closure of the selectivity filter gate is initiated by K^+ exit and then followed in sequence by conformational rearrangements of the pore domain outer helix, extracellular turret region, voltage sensor domain, intracellular domains and pore domain inner helix. In contrast to the simple linear models proposed for opening and closing of ligand-gated ion channels, a much more complex spatial and temporal sequence of widespread domain motions connects the open and inactivated states of the hERG K^+ channel.

Rigid body Brownian dynamics simulations of ion channels and channel blockers

D. Gordon and S.H. Chung, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia.

Ion channels allow cells to regulate the flow of ions across cell membranes in a controlled manner. This ionic flow is responsible for a host of key functions in the organism, including nerve signaling, muscle contraction, chemical signaling, and the regulation of metabolism. The presence of various kinds of channel blocker molecules can impede the normal flow of ions through ion channels, leading to important physiological consequences, both beneficial (*e.g.* calcium channel blocker drugs for high blood pressure) and detrimental (*e.g.* various polypeptide toxins). Therefore, it is important to be able to computationally model the dynamics and energetics of various channel blockers interacting with channels. We report on the development of a system that uses rigid body Brownian dynamics to simulate the interaction between blockers, ion channels, and ions. This allows us to elucidate binding, unbinding and blocking mechanisms, and to directly simulate the effects that blocker molecules have on ionic currents. The use of Brownian dynamics, rigid body dynamics and macroscopic electrostatics means that our simulation can be run on long timescales, allowing the direct measurement of ionic currents.

Our model system contains an ion channel embedded in a lipid bilayer membrane as well as one or more channel blocker molecules, and is solvated by water and ions. The channel is represented as a fixed rigid body and the blocker molecules as mobile rigid bodies. The ions are explicitly represented as spherical charged particles, whereas the water is implicit. The force field for the system contains various terms for short range interactions between the ions, channel and blocker molecules, frictional and random forces that drive the Brownian motion of the ions and blockers, and long range electrostatic forces which are given by the solution to Poisson's equation. The latter are the most challenging to model, and we have developed new methods for efficiently solving Poisson's equation in our molecular system and applying these solutions to our simulation.

The other major component of our simulation is the motion algorithm. We have developed a new algorithm for simulating the rigid body Brownian motion of the blockers (Gordon, Hoyles & Chung, 2009). A rotational and translational Langevin equation is formulated, and a numerical solution algorithm is proposed, based on the velocity Verlet algorithm, with additional steps being needed to handle the more complicated rotational algebra and extra frictional terms.

We have tested our simulation in various applications involving voltage gated potassium channels. We have examined a number of candidate blockers, including small classical blocker molecules like 4-aminopyridine (4AP) and tetraethylammonium (TEA), polypeptide toxins such as charybdotoxin (CTX), and other small charged molecules. Our aim is to elucidate the binding, unbinding and blocking mechanisms for a range of different channels and blockers.

Gordon D, Hoyles M, Chung SH. (2009) An algorithm for rigid-body Brownian dynamics. *Physical Review. E, Statistical, Nonlinear, and Soft Matter Physics* **80,** 066703-1 - 066703-12.

Metamorphic chloride intracellular channel proteins: evidence for transmembrane extension and membrane induced oligomerisation of CLIC1

S.C. Goodchild,[1] S.N. Breit,[2] P.M. Curmi[3] and L.J. Brown,[1] [1]Department of Chemistry and Biomolecular Sciences, Macquarie University, NSW 2109, Australia, [2]St. Vincent's Centre for Applied Medical Research, St. Vincent Hospital and University of New South Wales, NSW 2010, Australia and [3]School of Physics, University of New South Wales, NSW 2052, Australia.

Classically, the study of protein structure is based on the assumption that the native protein fold is unique, with at most small structural alterations to facilitate function. However, the existence of several proteins capable of independently interchanging between two or more vastly different but stable folds arising from the same amino acid sequence have been shown. These proteins have collectively been termed "metamorphic" (Murzin, 2008).

The highly conserved Chloride Intracellular Channel (CLIC) protein family is an example of the metamorphic protein class. While the function of the CLIC proteins is not well understood, the CLICs are expressed as soluble proteins but can also reversibly auto-insert into the membrane to form active ion channels. This conformational transition has previously been shown to involve a large-scale unfolding between the C- and N-domains for CLIC1 (Goodchild *et al.*, 2010). The CLIC1 homologue also displays the unique ability to undergo a dramatic structural metamorphosis from a monomeric state, displaying a classic glutathione-S-transferase fold, to a soluble all α-helical dimer solution upon oxidation (Littler *et al.*, 2009). Furthermore, in the presence of membranes, the effect of oxidation has been shown to increase the interaction of CLIC1 with the lipid bilayer (Goodchild *et al.*, 2009). However to date, experimental evidence characterising the dramatic structural rearrangements that must occur within CLIC1 to confer favourable interactions with the membrane and enable formation of an ion channel pore are lacking.

In the current study, site-directed fluorescence labeling of a series of single cysteine residues (T44C, T45C, K49C, C89) within the vicinity of the single putative transmembrane domain (aa24-46) of CLIC1 was performed using a novel labeling strategy described recently (Goodchild *et al.*, 2010). Fluorescence Resonance Energy Transfer (FRET) was used to monitor for changes in the distance from a single native Trp35, located within the transmembrane, to each of the 1,5-IAEDANS acceptor labeled cysteine residues. This was performed in both the soluble CLIC1 form and upon the addition of lipid bilayers. The FRET changes observed indicate that an extension of residues 24-46 occurs upon interaction with the membrane. This result is consistent with the current model of a single extended helical transmembrane region. To test the hypothesis that the CLIC1 forms an oligomeric channel structure in the presence of membranes, a population of CLIC1 labeled with a donor fluorescent label (1,5-IAEDANS) was mixed with a population of CLIC1 labeled with an acceptor fluorescent label (5-IAF). Appreciable FRET interaction and thus evidence for oligomerisation was only detected upon oxidation of the CLIC1 in the presence of the membrane. Together, these two FRET results reinforce the notion of the CLIC protein family as dynamic and metamorphic entities and challenge many accepted views of protein structure. Currently, our labeling scheme is being extended to further refine our model for the structural transitions and environmental triggers of CLIC1 membrane-induced metamorphosis.

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Goodchild, SC, Howell, MW, Littler, DR, Mandyam, RA, Sale, KL, Mazzanti, M, Breit, SN, Curmi, PM, Brown, LJ (2010), *Biochemistry* **49**, 5278-89.

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pH dependence of the Ca^{2+} release activated Ca^{2+} (CRAC) channel

N.R. Scrimgeour, D.P. Wilson and G.Y. Rychkov, School of Medical Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

CRAC channels activated by the depletion of intracellular Ca^{2+} stores provide a major pathway for Ca^{2+} entry in many cell types. Characteristic properties of CRAC channels include high selectivity for Ca^{2+} over monovalent cations, feedback inhibition by permeating Ca^{2+}, known as fast Ca^{2+} dependent inactivation (FCDI), and block by low external pH (Malayev & Nelson, 1995). The functional CRAC channels are composed of a tetramer of the Orai1 proteins, which forms the channel pore, and a protein called stromal interaction molecule 1 (STIM1), a Ca^{2+} binding protein that plays the role of Ca^{2+} sensor in the endoplasmic reticulum (Soboloff *et al*., 2006). The glutamate 106 residue (E106) in a predicted transmembrane domain of Orai1 has been reported to act as the selectivity filter and to play a role in FCDI of CRAC channels (Yamashita *et al*., 2008). In this work we show that glutamate 106 is also a protonation site responsible for I_{CRAC} block at low pH.

STIM1 and Orai1 were previously subcloned into pCMV-Sport6 and the GFP co-expressing vector pAdTrack-CMV (Scrimgeour *et al.*, 2009). The Orai1 E106D mutation was generated using pCMV-Sport6-Orai1 as a template according to the protocol specified by the QuikChange II site-directed mutagenesis kit (Stratagene). Whole-cell patch clamping was performed at room temperature using a computer based patch-clamp amplifier (EPC-9, HEKA Elektronik) and PULSE software (HEKA Elektronik).

I_{CRAC} mediated by heterologously expressed Orai1 and STIM1 was inhibited by low pH reaching virtually complete block at pH 5.5. The apparent pKa of CRAC channel pH dependence was 7.8±0.1 (n=4). The E106D Orai1 mutant, which has higher selectivity for Na^+ over Ca^{2+} and is blocked by Ca^{2+} in time and voltage dependent manner (Yamashita *et al*., 2008), showed no such dependence on pH. In contrast, lowering pH from 7.4 to 6.3 or below increased the amplitude of the current and reduced the extent of inactivation at negative potentials suggesting that the Ca^{2+} block of Na^+ current was reduced. The apparent pKa of the block of Na^+ conductance through E106D mutant by Ca^{2+} was 6.1±0.1 (n=4). Investigation of Ca^{2+} currents mediated by this mutant in the absence of all permeable monovalent cations in the external solution revealed that FCDI of E106D is much faster than that of WT Orai1 and is progressively reduced at lower external pH.

Overall, these results suggest that ring of negative charges at position 106 in the Orai1 pore controls not only the selectivity of the channel, but also contributes to a complex mechanism of FCDI and accounts for the pH dependence of CRAC channel.

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Yamashita M, Navarro-Borelly L, McNally BA & Prakriya M (2007). *Journal of General Physiology* **130,** 525-540.