by the resting electric field is a compact, ordered phase with behavior, such as critical temperature, hysteresis, fractional dispersion exponent (constant phase angle) and Curie-Weiss law, similar to that of phases seen in ferroelectric liquid crystals (VSIC, pp. 355-383). In the absence of a toxin molecule, threshold membrane depolarization indirectly brings about a stochastic phase transition to a less ordered phase in which S4 segments expand by the mutual repulsion of their positively charged residues. The resulting wider pitch of permeation pathway α helices elastically linked to the S4s permits ion replacement in the interloop H bonds and the subsequent percolation of permeant ions through the channel (VSIC, 477f, 506f). With an externally applied tetrodotoxin (TTX) or analog molecule complexed in the channel, however, the ordered phase is pinned by the toxin, inhibiting the transition to the ion-conducting phase (VSIC, 76, 382f). Phase pinning by impurities is an established effect in ferroelectric liquid crystals. In the toxin, a guanidinium group, $H_2N^+{=}C(NH_2)_2$, a highly resonant, planar, positive ion, is active in pinning the closed phase. The fact that guanidinium is also found in ferroelectric crystals such as guanidinium aluminum sulfate hexahydrate suggests that TTX enhances the spontaneous polarization of the resting phase. This explanation by the gateless gating model of specific toxin action is based on physical principles; in contrast, the phrase ''TTX blocks the pore'' offered by the gated pore model is vague and at the macroscopic rather than molecular scale.

1295-Pos Board B139

Self-organized Models of Selectivity in Ca and Na Channels

Bob Eisenberg[1], Dezso Boda[2], Janhavi Giri[1], James Fonseca[1], Dirk Gillespie[1], Douglas Henderson[3], Wolfgang Nonner[4].
[1]Rush University Medical Center, Chicago, IL, USA, [2]University of Pannonia, Veszprém, Hungary, [3]Brigham Young University, Provo, UT, USA, [4]Miller School of Medicine, Miami, FL, USA.

A simple pillbox model with two adjustable parameters accounts for selectivity of both DEEA Ca channels and DEKA Na channels in many solutions of different composition and concentration. Only side chains are different in the model of Ca and Na channels. Parameters are the same for both channels in all solutions. 'Pauling' radii are used for ions. No information from crystal structures is used. Side chains are over-approximated as spheres. Predicted properties of Na and Ca channels are very different. How can a simple model give such powerful results when chemical intuition says that selectivity depends on the precise relation of ions and side chains? We use Monte Carlo simulations of this model that determine the most stable — the lowest free energy — structure of ions and side chains. Structure is the computed consequence of the forces in this model. Forces are steric repulsion and electrostatic attraction of ions crowded into a small space, modified by protein polarization. The relationship of ions and side chains varies with ionic solution and is very different in Na and Ca channels. Selectivity is a consequence of the 'induced fit' of side chains to ions and depends on flexibility (entropy) of side chains as well as their location. The model captures the relation of side chains and ions well enough to account for selectivity of both Na and Ca channels in the many conditions measured in experiments. Evidently, the structures in the real Na and Ca channels responsible for selectivity are self-organized, at their free energy minimum, close to the positions computed in our model. Oversimplified models are enough to account for selectivity if the models calculate the 'most stable' structure, as it changes from solution to solution, and mutation to mutation.

1296-Pos Board B140

Design, Production and Characterisation of a Thermally-stable Mutant of the Bacterial Voltage Gated Sodium Channel Nachbac

Andrias O. O'Reilly, Kalypso Charalambous, Ghasem Nurani, B.A. Wallace.
Birkbeck College, London, United Kingdom.

NaChBac from *B. halodurans* is a bacterial homologue of the eukaryotic voltage-gated sodium channels which has been expressed and purified from *E. coli*. We have previously shown (Nurani et al (2008) Biochemistry 31:8114-8121) that this membrane protein,, purified from E. coli, forms a mostly helical, tetrameric detergent-solubilisable protein that is capable of binding the drug mibefradil and inducing sodium flux when reconstituted into vesicles. The tetrameric quaternary structure of NaChBac differentiates it from the single-chain eukaryotic sodium channels.

The aim of the present study was to produce a more thermally-stable version of this ion channel which would be suitable for a wide range of structural and functional studies. Using molecular modelling techniques, we have designed a mutant, G219S, which incorporates a serine instead of a glycine at the proposed site which is proposed to form the hinge which enables opening and closing of the channel. The aim was to reduce flexibility and ''lock'' the protein in a single state. Mutant protein was cloned, expressed and purified from E. coli and compared with the wild type protein isolated in the same manner. Whilst it had a similar secondary structure, thermal melting curves monitored by circular dichroism spectroscopy indicated that the mutant was considerably more stable than the wild type protein, although it is still capable of binding mibefradil. Thus the protein produced had the properties as designed and is a particularly suitable candidate for new structural, functional and drug binding studies.
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1297-Pos Board B141

Ion Pair Formation During Activation of the NaChBac Voltage Sensor

Paul G. DeCaen, Todd Scheuer, William A. Catterall.
University of Washington, Seattle, WA, USA.

S4 transmembrane segments of voltage-gated ion channels move outward upon depolarization initiating a conformational change that opens the pore. Formation of ion pairs between gating-charge-carrying arginine residues in S4 and negatively charged amino acid residues in neighboring transmembrane segments is an essential feature of the *sliding helix* model of gating (Catterall, 1986; Guy and Seetharamulu, 1986; Yarov-Yarovoy et al., PNAS, 2006). We studied NaChBac mutants in which E70 in the S2 segment and the fourth gating charge of S4 (R4) were replaced with cysteines. As previously reported for D60:R3 (DeCaen et al. PNAS, 2008), activation of the E70C:R4C reduced I_{Na} irreversibly but had no effect on WT or single mutants. Application of the reducing agent β-mercaptoethanol restored I_{Na}, suggesting reversal of disulfide bond formation between E70 and R4. The voltage dependence of disulfide locking matched the voltage dependence of activation ($V_{1/2} \approx$ -75 mV). Fast deactivation was blocked, and the loss of current upon repolarization was slowed to the rate of inactivation ($\approx$330ms). Evidently, depolarization drives movement of the S4 segment that allows disulfide locking of R4C and E70C, and this activated state of the voltage sensor signals opening of the pore and then inactivation of the channel. These data suggest that gating charge R4 forms an ion pair with E70 during activation and that the side chains of these residues approach within ~2 Å, as required for rapid formation of disulfide bonds in the E70C:R4C mutant. This new molecular interaction allows further refinement of the ROSETTA sliding helix model of gating (see adjacent poster by Yarov-Yarovoy et al). Supported by NIH Grants T32 GM07270 (PGD) and R01 NS157561 (WAC).

1298-Pos Board B142

Structural Modeling of Intermediate States of the Gating Pore of NaChBac

Vladimir M. Yarov-Yarovoy, Paul DeCaen, Todd Scheuer, William A. Catterall.
University of Washington, Seattle, WA, USA.

Voltage-gated sodium channels initiate action potentials in excitable cells. Despite progress in determining the structures of voltage-gated potassium channels, the high-resolution structure of the voltage-gated sodium channels remains unknown. We used the Rosetta-Membrane method (Yarov-Yarovoy et al. Proteins 62, 1010, PNAS 103, 7292) and experimental data suggesting proximity between E70 in S2 and R4 in S4 during activation of NaChBac to construct structural models of intermediate states during channel gating. The structure of the Kv1.2-Kv2.1 chimera channel in the open state (Long et al. (2007) Nature 450, 376) was used as a template and proximity between Cβ atoms of E70 in S2 and R4 in S4 was favored during modeling. The resulting structural models suggest a molecular mechanism of the voltage-dependent activation of NaChBac in which S4 rotates clockwise (as viewed from the extracellular side of the membrane) and translates outward, as proposed in the 'sliding helix' model of gating, while gating-charge-carrying arginines in S4 sequentially interact with negatively charged residues in the S1, S2, and S3 segments. Transition through a local 3-10 helical conformation of a short segment of S4 containing two gating-charge-carrying arginines in the narrow part of the gating pore is required for simultaneous interaction with their ion pair partners during activation. The side chain of highly conserved F67 in S2 is oriented sideways away from the gating pore to allow the long side chains of arginines in S4 to pass through the middle of the gating pore. Outward motion of the S4 segment is coupled to lateral movement of the S4-S5 linker and movements of the S5 and S6 segments that open the intracellular gate of the pore-forming module. Supported by NIMH Grant K01 MH67625 (to V.Y.-Y.) and NIH Grant R01 NS15751 (to W.A.C.).

1299-Pos Board B143

Biophysical And Pharmacological Profiling Of Multiple Na_V Subtypes On QPatch HT

M. Knirke Jensen, Rikke Schrøder, Dorthe Nielsen, Morten Sunesen.
Sophion Bioscience A/S, Ballerup, Denmark.

The voltage dependent sodium channel is responsible for the upstroke and directed propagation of action potentials in nerve and muscle cells, and is therefore a central ion channel in excitable tissues. The implication of voltage gated sodium channels in pain mediation, and diseases such as epilepsy and cardiac arrythmia has made them very important targets for drug discovery. Nine

functional mammalian isoforms have been discovered so far with different functional and pharmacological properties. In our study, eight subtypes of the voltage gated sodium channel were tested in parallel on the automated patch clamp system QPatch HT. The new clone screening feature developed for QPatch 16 and QPatch HT allows running up to eight different cell lines (clones or subtypes) at the same time, thus ensuring that the exact same conditions (temperature, Ringer's, pH etc.) are applied for each of the cell lines tested. $Na_v1.1$, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8 were tested together in a range of experiments on QPatch HT. All but $Na_v1.2$ and $Na_v1.8$ were co-expressed with the β1 subunit. Three types of experiments were designed to explore 1) TTX sensitivity, 2) IV-relationship for activation and inactivation, and 3) recovery from inactivation, for the entire panel of Na_v channel subtypes in a single experiment. It was shown that QPatch experiments using the cell clone screening feature together with the QPatch Assay Software data analysis package, enables the experimenter to obtain IC_{50} values for TTX, IV-relationships and time constants for recovery from inactivation which are very similar to manual patch clamp data, for all Nav subtypes, thus successfully distinguishing one subtype from another.

Mechanosensitive Channels

1300-Pos Board B144

Analysis of Gating Process Associated with Water Permeation of the E-coli Mechanosensitive Channel MscL Using Molecular Dynamics Simulations

Yasuyuki Sawada[1], Masaki Murase[2], Masahiro Sokabe[1,2].
[1]Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]ICORP/SORST Cell Mechanosensing, JST, Nagoya, Japan.

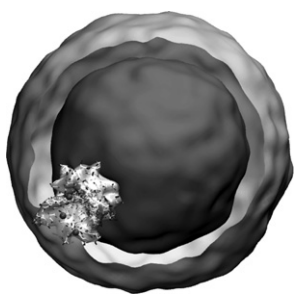
The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer α-helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. Although several models for the opening process have been proposed with Molecular Dynamics (MD) simulations, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. We performed MD simulations for the mechano-gating of MscL embedded in the lipid bilayer. Upon tension generation in the bilayer, Phe78 in the outer helix was dragged by lipids, leading to a tilting of the helices. Among amino acids in the outer helix facing the bilayer, Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. As tension increases, the crossings move toward the cytoplasm associated with an expansion of the constricted part. During the movement, a hydrophobic water block environment around the constricted part was broken followed by water penetration and permeation. We modeled G22N mutant, known to have an ability to permeate ions without increasing membrane tension, and performed 5 ns equilibrium simulations. We analyzed movements of water molecules around the block and found that the asparagine substitution resulted in spontaneous water flow due to a hydrophilic side chain of asparagine, leading to partial channel opening. Thus a change in the environment around the most constricted part from hydrophobic to hydrophilic promotes MscL opening.

1301-Pos Board B145

MscL Gating In Liposomes

Martti Louhivuori, Jelger Risselada, Erik van der Giessen, Siewert-Jan Marrink.
University of Groningen, Groningen, Netherlands.

Mechano-sensitive channels function as safety valves of a cell by controlling the permeability of the plasma membrane. They are non-selective and respond rapidly to sudden changes in the tension of the membrane. During a hypo-osmotic shock mechano-sensitive channels sense the growing turgor pressure and start to gate, thereby releasing the tension and preventing the rupture of the cell membrane.



Mechano-sensitive channel of large conductance (MscL) forms a large, non-selective channel when activated. The crystal structure of MscL in its deactive state extracted from Mycobacterium tuberculosis has given insights into the possible activation mechanism of MscL and has enabled the tentative mapping of the closed-open transition pathway by molecular dynamics simulations. Nonetheless, the role and importance of e.g. membrane curvature, cytoplasmic helix-bundle and directional ion flux are still unclear.

Liposomes, i.e. tiny lipid vesicles, offer unprecedented possibilities to study the effects of membrane curvature and directional ion flux on MscL gating. We have studied in near-atomistic detail liposome embedded Tb-MscL using the recently developed MARTINI coarse-grained model for biomolecules. Various pressure gradients were inflicted across the liposomal membrane to map the tension-activation response of MscL and to obtain fully activated channels capable of rapid release of liposomal stress.

1302-Pos Board B146

Multi-scale Modelling Of Tb-Mscl Gating In Its Native Environment

Elvis Pandzic, Allan M. Haldane, Maria L. Kilfoil.
McGill University, Montreal, QC, Canada.

Here we explore the possibility of combining Molecular Dynamics (MD) simulation together with Monte-Carlo simulation in order to predict the gating pathway for a tension-gated pore protein, the Mechanosensitive Channel of Large Conductance from Mycobacterium Tuberculosis (Tb-MscL). To mimic its native environment, we embed the channel protein in a native-like lipid membrane, itself first equilibrated by MD, and the whole system is then equilibrated using MD, followed by rigid cluster decomposition by FIRST software and Monte-Carlo simulation of channel opening using FRODA software. Our goal was to explore in a more atomistic level protein-lipid interactions that were explored by continuum models, and uncover the role played by various components of the protein-membrane system during the channel gating. Our results suggest that protein-lipid interactions are necessary in order to produce an asymmetric motion of channel subunits, which was observed in previous experimental studies.

1303-Pos Board B147

Spandex Proteins: Mechanosensitive Closed-closed Transitions Suitable for Osmoprotector and for Tension Damper Functions in Large Membrane Proteins

Pierre-Alexandre Boucher[1], Bela Joos[1], Catherine E. Morris[2].
[1]Department of Physics, University of Ottawa, Ottawa, ON, Canada, [2]Neurosciences, Ottawa Health Research Institute, Ottawa, ON, Canada.

Large membrane proteins are potentially more expansible than bilayers. We therefore term membrane proteins with stochastic tension-sensitive transitions between closed states "spandex proteins" and ask what design features might allow spandex proteins to act as tension relievers. Spandex is modeled with two states (contracted, expanded). Its barrier state location strongly impacts the timescale of the expansion transition. Expansion depends on spandex concentration, with the apparent midpoint tension shifting to larger tensions as the membrane density of spandex increases.

In a cell, there are two ways spandex might be advantageous. In the case of an abrupt tension increase, spandex expansion could reduce bilayer tension enough to prevent unnecessary opening of osmotic valve channels. To achieve this safely, a protein that expands at a very precise tension before the channels can open is required. This requires a large spandex protein, whose barrier is located close to the expanded state, ensuring that if tension is high, the spandex will react rapidly.

Secondly, spandex proteins could be used to maintain a steady bilayer tension. However, a single species of spandex could not be both a good partner for osmotic valves and a good tension damper. For reliable tension damping, the spandex tension midpoint must equal the target tension. To ensure the spandex reacted rapidly to tension fluctuations, its barrier would need to be located halfway between the contracted and expanded states. Also, the larger the change in area of the protein, the more precisely the target tension will be maintained in the bilayer. The concentration needed depends on the strain amplitude that is to be dealt with. We discuss possible interactions among the tension sensitive closed-closed and closed-open transitions of different bacterial membrane proteins. *NSERC funded.*

1304-Pos Board B148

A Site-directed FRET Confocal Microscopy Approach for Studying Conformational Changes in the Mechanosensitive Ion-channels, MscL and MscS

Ben Corry[1], **Prithwish Pal**[1,2], Annette Hurst[2], Paul Rigby[1], Boris Martinac[2].
[1]Univ of Western Australia, Perth, Australia, [2]University of Queensland, Brisbane, Australia.

Bacterial mechanosensitive channels act as safety valves that protect cells from hypo-osmotic shock by opening under membrane tension to relieve pressure within the cell. Although the crystal structures of two such ion channels - the mechanosensitive channels of large (MscL) and small (MscS) conductance - are known, the mechanism by which bilayer deformations are transduced into channel opening is still being worked out. Here we describe a method to study conformational changes associated with the channel opening (of both