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.

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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.

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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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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).

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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.).

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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