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stabilized by surrounding hydrophobic residues. We propose that mutations of the asparagines affect activation-inactivation of the channels and drug action through destabilizing the open state. Diversity of H-bonding partners explains different effects of mutations of individual asparagines on the channel activation, inactivation, and drug sensitivity. Supported by NSERC grant to BSZ, the RAS program "Molecular and Cell Biology" to DBT, and CIHR award to IB.

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Binding of Isoflurane to a Bacterial Voltage-Gated Sodium Channel: Structure and Accessibility of Distinct Interaction Sites

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The most likely targets for volatile anesthetics (VA) are ion channels. The mechanism of volatile anesthetic action is not completely understood. Identifying the molecular pathway for drug binding is crucial to understand the effect of VA on voltage gated sodium channels. We use Molecular Dynamics simulations to identify the binding sites for a hydrophobic general anesthetic isoflurane, on voltage gated bacterial sodium channel NaChBac. Apart from the voltage sensing domain (S1-S4), linker and the pore domain (S5-S6), bacterial sodium channels also have fenestrations, which provide a hydrophobic tunnel through the lipid-embedded portion of the channel to the central cavity, where the known local anesthetic site is located. Unbiased "flooding" simulations were performed on the activated open confirmation of NaChBac. We performed a cluster analysis to identify all the possible binding sites of isoflurane. The three most important ones among them are: a region near the selectivity filter, called the extracellular site, a region near the S4-S5 linker, called the linker site, and a region within the cavity, called the cavity site. The most important observation is that isoflurane enters the central cavity through the fenestrations. Free energy perturbation method was employed to calculate the binding affinities of isoflurane for each of these sites. We also studied the interactions between isoflurane and the amino acids in these three binding sites.

696-Pos Board B465

A Key Gating Charge Interaction Required for Slow Inactivation of the Navab Bacterial Sodium Channel

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Bacterial voltage-gated sodium (Nav) channels are considered an ideal model for structure-function studies. The elucidation of the crystal structure of the bacterial channel NavAb (Payandeh et al., Nature 486, 135-139, 2011) opened an avenue to understand electrical signaling in excitable cells at the structural level. NavAb expressed in Hi5 insect cells as for structural studies has unusually negative voltage-dependent activation (Va ~ 130 mV). NavAb also has three phases of inactivation, a biphasic early inactivation process (τ1~103 ms, τ2~3.8 s, at −180 mV) followed by an unusually strong and use-dependent slow-inactivation process. To search for the molecular basis for negative activation and slow inactivation of NavAb, we mutated the outermost gating charge partner in the S2 segment, Asn49, to Lys. This mutation shifted the activation curve ~ 75 mV toward more positive potentials. Surprisingly, it also completely abolished use-dependent slow inactivation. We showed previously that the equivalent residue in NaChBac (Asp60) interacts with the R3 gating charge in the S4 segment during activation using the disulfide locking method (DeCaen et al, PNAS 2008 105 (39) 15142-15147). To test whether this interaction between R3 and N49 was critical for slow inactivation, we mutated NavAb R3 to Cys. The resulting mutant NavAb_R3C also had positively shifted channel activation (+75 mV) and no use-dependent slow inactivation. The fact that these reciprocal mutations have the same functional effects suggests that interaction between R3 in the S4 segment and N49 in the S2 segment is an important link that stabilizes the activated state of the voltage sensor and triggers the slow inactivation process.

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Computational Study of the Prokaryotic Sodium Channel

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Ion-selective voltage-gated ion channels allow ions of a specific element or set of elements to pass, but exclude other ions. Recently, the crystal structure of NavAb was published (Payandeh et al (2011) Nature 475, 353), giving an unambiguous picture of the structure of the sodium channel selectivity filter (SF) of the prokaryote *Arcobacter butzleri.* The filter is much wider than that of the narrow, carbonyl-lined potassium channels, allowing ions to penetrate with minimal dehydration, multiple pathways, and even to pass each other. Previous computational studies have attempted to explain sodium selectivity, and claimed consensus (Corry and Thomas (2011) JACS 134, 1840, Furini and Domene (2012) PLOS 8, e1002476). Experimental ion selectivity determination from pore-only constructs of a sodium channel with the same SF sequence shows stronger ion selectivity from reversal potential than from the ratio of the fluxes of sodium and potassium (Shaya et al (2011) PNAS 108, 12313), but selectivity, Na^+/K^+, was still less than five. Simulations based upon the closed-pore crystal structure could not determine selectivity directly from conduction. To investigate this issue, we first calculated multi-ion potentials of mean force in the selectivity filter of pore-domain-only NavAb with an extended equilibration period, and a replica-exchange molecular dynamics scheme, which allows for improved sampling. We then explored the non-equilibrium conduction of sodium and potassium in the presence of an applied electric field through a model NavAb pore truncated to mimic the conformation of the open pore. We also present multi-ion pmf calculations to illustrate similarities and differences between this model and the intact pore. When taken together, these two methods provide complementary views of ion selectivity and conduction based upon the conformation of the NavAb crystal structure of Payandeh et al (2011).

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Volatile General Anesthetic Interactions with a Bacterial Sodium Channel

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General anesthesia results from complex interactions involving ion channels in the brain. Voltage-gated ion channels are modulated by halogenated inhaled general anesthetics, however the drug-channel interactions are generally believed to be non-specific making it difficult to investigate the molecular mechanisms of anesthetic action. We find, however, that the bacterial sodium channel NaChBac exhibits differential regulation by different general anesthetics, suggesting that there may be specific interactions. Molecular dynamics (MD) simulations have identified several possible binding sites for isoflurane in the NaChBac channel structure. Here, we have characterized the affinity, occupancy and hydrogen bonding for these sites and identified key interactions. In addition, experimental evidence suggests that both isoflurane and sevoflurane have dual actions on NaChBac, possibly involving action at two different sites. Consistent with pore block, sevoflurane accelerates the decay of the current (20% faster at 0.4 mM sevoflurane) but, at the same time, increases the peak current (10% increase with 0.4 mM sevoflurane), which argues against the blocking mechanism. We hypothesize that an additional mechanism involving a distinct site is necessary to explain the latter effect. We are currently employing MD simulation and structural modeling combined with mutagenesis and electrophysiology to test this two-site hypothesis and investigate the molecular mechanisms of these opposing effects. Supported by NINDS F31 NS077689 (AFB) and NIGMS P01 GM0558 (MLK).

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Polymodal, High Affinity Actions of μ-Conotoxins on a Bacterial Voltage-Gated Sodium Channel

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μ-Conotoxins (μCTXs) are potent blockers of eukaryotic sodium channels, and individual members of the μCTX family are highly specific for particular Nav1 isoforms. We have begun to explore μCTX interactions with NaChBac, a prokaryotic Nav channel closely related to NavAb, whose crystal structure was recently determined (Payandeh et al, 2011, Nature 475:353). Our data reveal actions on both ion conduction and gating, consistent with polymodal actions on the pore domain.

Under voltage clamp, whole-cell currents from NaChBac expressed in tsA-201 cells were reduced, up to nearly complete block, by concentrations in the pM to μM range, for wildtype toxins and derivatives of μCTX PIIIA and KIIIA. For wildtype PIIIA, dose-response data (0.001-10,000nM) yielded the following: IC50=0.005nM; maximal fraction of current blocked = 0.95; Hill coefficient = 0.7. Chen & Chung (2012, Biophys. J. 102:483) predicted an IC50 of 0.1nM for PIIIA when the NavAb channel is occupied by 2 sodium ions.

Even at very low [PIIIA] (1pM), the unblocked currents showed increasing rates of inactivation as the peptides were washed in, suggesting a gating modulation, that was not tightly associated with pore block. For μCTX PIIIA (0.1nM), or KIIIA (30nM), inactivation accelerated by ~10-fold. Substitution of key basic residues (PIIIA-R14A and KIIIA-K7A) reduced blocking potency and decreased the speeding of inactivation to less than 2-fold.

Given the remarkably high selectivity and affinity that μCTXs show for certain eukaryotic Nav channels with highly asymmetric pores, it may seem surprising that they bind to symmetric bacterial Nav channels with such high affinity.