Probing the interaction between psalmotoxin 1 and acid sensing ion channel 1a, an analgesic drug target

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Acid sensing ion channel 1a (ASIC1a) is one of the primary acid sensors in the peripheral and central nervous system, and it has emerged as a novel target for the development of drugs to treat chronic pain, neurodegeneration, and possibly depression (Sluka *et al.*, 2009).The only known selective inhibitor of ASIC1a is psalmotoxin-1 (PcTx1), a 40-residue disulfide-rich peptide isolated from the venom of the Trinidad chevron tarantula *Psalmopoeus cambridgei*. PcTx1 is a potent blocker of ASIC1a (IC_{50} ~0.5nM) but it does not inhibit other ASIC subtypes (Escoubas *et al.*, 2000). Remarkably, PcTx1 has analgesic activity comparable to morphine in rat models of acute pain (Mazzuca *et al.*, 2007). With a view to using PcTx1 as a lead for development of novel analgesics, we developed an efficient bacterial expression system for production of recombinant toxin and determined a high-precision structure using 3D/4D triple resonance NMR spectroscopy. Site-directed mutagenesis revealed a highly cationic pharmacophore located within one of the intercystine loops. Molecular dynamics simulations in combination with NMR spin relaxation and relaxation dispersion measurements revealed significant motion in this loop over a wide range of timescales (ps to ms), thus precluding the use of rigid body docking protocols for modelling the toxin:channel complex. Instead, we used HADDOCK (Dominguez *et al.*, 2003) to dock the toxin onto a homology model of rat ASIC1a (rASIC1a). Key interacting residues identified from mutagenesis of the toxin and the channel were used as ambiguous interaction restraints and the sidechains of residues at the interaction interface were allowed to move during simulated annealing and refinement. The resulting model of the PcTx1:rASIC1a complex reveals a novel mode of interaction dominated by ion pair interactions involving arginine residues in the β-hairpin loop containing the toxin pharmacophore. The toxin:channel model is currently being used for *in silico* screening of chemical libraries to find nonpeptide mimetics of PcTx1.

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