The rat TRPV1 is activated by allicin reacting with the cisteine at position 157 generating calcium entry into cells. C157 is located in the N-terminal Ankyrin Repeat Domain (ARD). The ARD crystallographic structure showed a hidden C157, but reactivity of this supposes an alternative conformation were C157 is more accessible. The conformational changes of the TRPV1 structure after the modification with allicin are not known. In this study we used the wild type TRPV1 ARD to investigate the reaction of allicin with the ARD using circular dichroism, fluorescence spectroscopy and molecular modeling to assess the reaction rate and the degree of perturbation to the ARD structure. Our findings show that pure allicin in complex with ARD gives a reaction that showed changes into the far-UV circular dichroism spectra. Mainly the peak at 190 nm shows an increment, presumably due to an increment in alpha helical content after covalent modification of C157. Fluorescence spectra showed a shifted signal from tryptophane 272 from 332 nm to 339 nm, suggesting a conformational change that exposed W272. Accessible surface area analysis of C157 in crystallographic ARD showed a hidden cysteine into the second motif of the ARD. We used molecular dynamics of the ARD in solution to look for a conformation with an accessible path for allicin.

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Regulation of TRPV1 by Phosphoinositides and other Negatively Charged Lipids
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The capsaicin receptor Transient Receptor Potential Vanilloid 1 (TRPV1) is a polymodal, Ca^{2+}-permeable ion channel essential for nociception. Like most members of the TRP family, it is known to be regulated by phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$]. Although the majority of $PI(4,5)P_2$-regulated ion channels are activated by this phosphoinositide, TRPV1 was initially suggested to experience inhibition. Data from cellular systems support the idea that TRPV1 activity depends on the presence of phosphoinositides in the membrane. The purified TRPV1, on the other hand, was recently shown to be fully functional in artificial liposomes in the absence of phosphoinositides. We expressed the rat TRPV1 channel in *Xenopus* oocytes and performed patch-clamp experiments in the excised inside-out configuration. We found that in addition to $PI(4,5)P_2$, several other negatively charged phospholipids, including phosphatidylglycerol, could also support TRPV1 activity in excised patches. When we incorporated TRPV1 into planar lipid bilayers consisting of neutral lipids, capsaicin-induced activity depended on $PI(4,5)P_2$. We also found that TRPV1 activity in excised patches ran down upon excision and that perfusing the patch with MgATP restored channel activity. Inhibition of phosphatidylinositol 4-Kinases or enzymatic removal of phosphatidylinositol abolished this effect, suggesting that MgATP activated TRPV1 by generating endogenous phosphoinositides. We conclude that endogenous phosphoinositides are positive cofactors for TRPV1 activity. Our data highlight the importance of specificity in lipid regulation of ion channels, and may reconcile discordant data obtained in various experimental settings.

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Structural Characterization of Double-Knot Toxin, an Activator of TRPV1 Channels
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Venom from poisonous organisms is a rich source of peptide toxins interacting with different ion channels proteins. These peptide toxins modulate ion channels by different mechanisms, and have been widely used as tools for investigating ion channel mechanisms. Double-knot toxin (DkTx) is a novel peptide toxin that activates TRPV1 channels, and contains two inhibitory cysteine knot (ICK) motifs, as its name suggests. Previous studies show that DkTx activates TRPV1 channels, and suggest that the avidity of the toxin (slow unbinding) arises from its bivalent nature. Here we use solid-phase peptide synthesis to individually produce the two knots of DkTx (K1 and K2), fold each *in vitro*, and find that they exhibit different binding affinities for the channel even though they share high sequence homology. As a first step toward understanding the structural and functional relationship of DkTx binding to TRPV1 channels, we determined solution structures of each knot in using NMR. The structures show that DkTx is composed of two notably amphipathic ICK motifs (each with two beta-strands) that are connected by a flexible linker, and that K2 has a larger hydrophobic surface compared to K1. In addition, the single conserved Trp residue in each knot show different orientations, with that in K1 exhibiting greater solvent exposure. Interestingly, using intrinsic Trp fluorescence, we observe strong partitioning of DkTx and K1, but see no evidence of membrane partitioning for K2. We also made a series of K1/K2 chimeras, and identified variant residues in two loops and the C-terminus that are responsible for the higher activity of K2. From these results we propose that membrane interactions are involved in the mechanisms of DkTx activation of TRPV1, and identify surfaces of the two knots that likely involved in binding to the channel.

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TRPV1 Measured in Lipid Bilayers
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TRPV1 measured in lipid bilayers The TRPV1 ion channel is a member of the transient receptor potential ion channel family which is known to respond to temperature, voltage and certain chemicals such as capsaicin. We have engineered a HEK cell line expressing FLAG-tagged TRPV1. Cellular measurements of expressed TRPV1 with capsaicin resulted in dose dependent activation with an IC50 of approximately 10 nM. Reconstitution of purified TRPV1 and membrane preparations containing TRPV1 in lipid bilayers resulted in conductance measurements of single and multiple channels. We present bilayer measurements of TRPV1 conductance as a function of voltage, temperature, and presence of agonist and antagonist chemicals.

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Structural Insight into the Assembly of TRPV Channels
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Transient receptor potential (TRP) proteins are a large family of polymodal non-selective cation channels. The TRP vanilloid (TRPV) subfamily consists of six homologous members with diverse functions: TRPV1-4 are non-selective cation channels proposed to play a role in nociception, while TRPV5 and TRPV6 are involved in epithelial Ca^{2+} homeostasis. Here we present the cryo-electron microscopy (EM) structure of functional, full-length TRPV2 at 13.6 Å resolution. The map reveals that the TRPV2 cytoplasmic domain displays a four-fold petal-like shape in which high-resolution N-terminal ankyrin repeat domain (ARD) structures can be unambiguously fitted. Fitting of the available ARD structures for other TRPV subfamily members into the TRPV2 EM map suggests that TRPV subfamily members have highly homologous structural topologies. These new results allowed us to postulate a structural explanation for the functional diversity amongst TRPV channels and their differential regulation by proteins and ligands.

Cyclic Nucleotide-gated Channels

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State Dependent and Site Directed Photodynamic Transformation of HCN2 Channel by Singlet Oxygen
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Singlet oxygen (1O2) is a little known signaling factor but a useful tool for both basic research and clinical practice. 1O2 is the central player in the Chromophore Assisted Light Inactivation (CALI) for ablating the function of protein molecules or cells and the Photodynamic therapy (PDT) for treating cancer and other diseases. However, most of the studies on 1O2 have been carried out at cell and tissue levels and yielded little information about 1O2 modification at molecular level. Here we report that the hyperpolarization-activated cAMP-gated (HCN) channels are sensitive to 1O2 modification. To increase the site-specificity of 1O2 generation, fluorescein conjugated cAMP (FITC-cAMP) that specifically binds to the HCN channels or in-frame inserted singlet-oxygen-generator (SOG) protein was used as photosensitizer. Laser pulses in milliseconds transformed the channel biophysics by slowing down the channel deactivation and increasing the voltage-insensitive component in the macroscopic current. The dependence on dissolved oxygen in the solutions, the inhibitory effect by a 1O2 scavenger and the results with HCN2-SOG fusion protein supported the involvement of 1O2. Intriguingly, 1O2 modification of HCN2 channel was state-dependent and had distinct effects on the open and closed channels. Following this insight, we located a critical Histidine residue (H434) near the activation