MECHANISM OF TEMPERATURE SENSITIVITY IN TRPV1 CHANNEL

Allocation: NSF PRAC/6,510 Knh
PI: Vincenzo Carnevale[1]
Collaborator: Tibor Rohacs[2]

[1]Temple University
[2]Rutgers New Jersey Medical School

BW

EXECUTIVE SUMMARY

TRPV1 is an ion channel crucially responsible for transduction of nociceptive stimuli into pain signals. Accordingly, inhibition of TRPV1 is one of the major strategies for designing next-generation antipain drugs. The polymodal nature of TRPV1 activation, i.e., the fact that a variety of stimuli can open the channel, suggests a complex molecular mechanism of activation whose details are still largely unknown. In particular, we do not currently have a satisfactory microscopic model to explain TRPV1 temperature sensitivity. In this project, we revealed the crucial role played by four nonpolar cavities whose presence and involvement in activation have not been described before. Free energy calculations show that dehydration of these cavities triggers activation of the channel. This observation is able to explain the puzzling response of TRPV1 to diverse environmental factors such as increased cytosolic hydrostatic pressure and osmolarity.

RESEARCH CHALLENGE

The nonselective channel TRPV1 is a crucial player in the human nociceptive system. TRPV1 is responsible for the detection of several harmful stimuli such as heat, low pH, and irritating chemicals that are transduced in painful signals orginating from peripheral nerves. In chronic pain syndromes, this channel is hypersensitized and the threshold for activation is so low that innocuous stimuli result in constant pain. This involvement in the pain pathway makes TRPV1 an appealing target for designing novel antipain drugs: selective modulation of this channel would inhibit the generation of the pain signal without interfering with other physiological pathways. Drug discovery campaigns aimed at this target hold promise to deliver pain killers that are virtually devoid of side effects. However, several promising molecules have failed in late stages of clinical trials. The reason for these failures is that TRPV1 is involved in body temperature regulation, and inhibitors of this channel might cause hyperthermia in patients. To make progress in identifying molecules that aptly modulate the channel without interfering with the mechanism of temperature sensitivity, a microscopic understanding of TRPV1 is needed. This project aimed at filling this gap by using long time-scale molecular dynamics (MD) simulations together with enhanced sampling techniques to estimate free energies.

METHODS & CODES

The structure of the TRPV1 capsaicin-bound (CAP-bound) state was taken from the Protein Data Bank: the PDB code is 3j5r [1]. The structure was refined and the missing residues were modeled using Rosetta software [2]. Four capsaicin molecules were docked following the protocol described in [3]. The protein with the ligands was embedded in a hydrated 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer and surrounded by 150 mM NaCl solution. The overall size of the system was ~170x170x160 Å^3; the total number of atoms was ~400,000. Two MD trajectories were generated with the peripheral cavities (PCs) either empty or hydrated. The CHARMM36 force field [4] was used to describe the protein and the POPC lipids. For capsaicin, we used the parameters derived in [3]. The TIP3P model was used to describe water [5]. An analogous setup was used to simulate the TRPV1 apo state (PDB code 3j5p [6]). The equilibration of the systems (three in total: the CAP-bound state with empty and hydrated PCs, and the apo state) was performed using NAMD 2.10 software [7] in several steps. Simulations were performed at constant temperature and pressure (1 atm) using the Langevin piston approach. For the Van der Waals interaction, we used a cutoff of 11 Å with a switching function between 8 and 11 Å. The long-range component of electrostatic interactions was calculated using the Particle Mesh Ewald approach [8] with a cutoff for the short-range component of 11 Å. The equations of motion were integrated using a multiple time-step algorithm, with a time step of 2 fs (femtoseconds) and long-range interactions calculated every other step.

We performed metadynamics simulations using the preliminary unbiased trajectories to estimate an upper bound for the free energy barrier and the diffusion constant along the biased collective variable. These were used to obtain an *a priori* estimate of the error on the reconstructed free energy profile using the expressions reported in [9], which relate the error to the width, height, and deposition rate of the hills. This estimate informed our choice of the metadynamics parameters. Metadynamics simulations were performed using the collective variable module implemented in NAMD 2.10 [10] at three temperatures: 280°K, 300°K and 340°K.

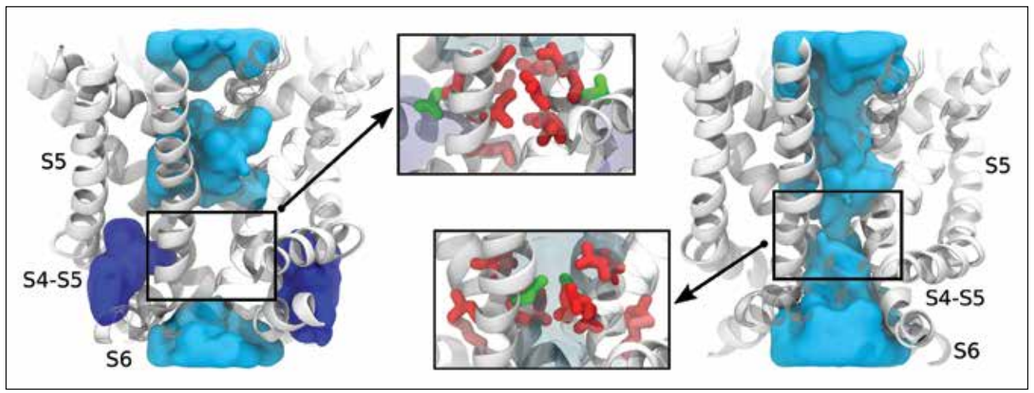

Figure 1: TRPV1 pore domain with hydrated (left) and empty (right) protein cavities (PCs). Water in the PCs and in the central pore is shown in blue and cyan, respectively. In the state with hydrated PCs, there are two interruptions of water density, while with empty PCs the water density is continuous. N676 is shown in green.

RESULTS & IMPACT

We found that the lower gate is open or closed depending on the conformation of N676, an amino acid located on S6 at the edge of a π-helix segment (Fig. 1). This segment is characterized by extreme conformational flexibility [11]: Not all the backbone hydrogen bonds can be simultaneously satisfied and therefore their pattern is dynamic. The presence of the π-helix allows N676 to easily rotate in and out the central pore. This motion is, in turn, controlled by the hydration state of the adjacent protein cavity (PC). Besides extensive accessibility experiments by Salazar, et al. [12], the presence of these cavities is supported by alanine scanning mutagenesis performed on the S6 segment [13]; the residues lining the PCs were shown to produce the greatest perturbation to the channel activation in response to several stimuli, including capsaicin and heat. Interestingly, our molecular mechanism does not entail any large conformational rearrangement of the TRPV1 central pore, whose radius profile is not dramatically altered by the closed-open transition. The hydration/dehydration of this compartment is, in fact, controlled by N676, which upon rotation changes the hydrophobic character of the molecular surface lining the central pore. This susceptibility to perturbations is not uncommon in the pores of ion channels. Wet-to-dry transitions have been reported several times [14] and are, arguably, the result of a precise evolutionary optimization. This microscopic picture provides the basis for rational design of precise modulators of TRPV1.

WHY BLUE WATERS

We investigated a system of approximately 400,000 atoms using MD simulations. The time scales involved in the activation process of TRPV1 dictated trajectory lengths on the microsecond time scale. This was possible thanks to a massively parallel calculation enabled by the computational capabilities of Blue Waters.