Molecular Properties of Ca^{2+} Transport Through TRPV2 Channel: A Molecular Dynamic Simulations Study

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Abstract

TRPV channels are a category of nonselective cation channels that activated by heat and ligands, and permeate monovalent and divalent ions. The mechanism of Ca^{2+} transfer through TRPV2 channel is not well known. Here, we investigated the reaction coordination and energy fluctuation of Ca^{2+} transition in TRPV2 channel by steered molecular dynamics (SMD) simulations and potential of mean force (PMF) calculation. Results showed that electrostatic interactions between Ca^{2+} and residues of the first and second gates had main roles in ions transfer through the channel, and also, we recognized important amino acids in this path. Moreover, results indicated that enter and exit of calcium ions needed to overcome barrier energies in first and second gates.

Introduction

Transient receptor potential (TRP) channels are a heterogeneous superfamily of nonselective cation channels that are classified in six groups including TRPA, TRPC, TRPL, TRPML, TRPP and TRPV. The channels are regulated by various physical and chemical stimuli and inhibitors which involve in various actions in cells ¹⁻³. Many TRP channels trigger and regulate signaling pathways via monovalent and divalent cationic fluxes (e.g., Ca^{2+} , Mg^{2+} , and Na^+). TRP channels generally are similar to voltage-gated potassium or sodium channels in structural core as transmembrane topology ^{4,5}.

Calcium as an important second messenger regulates different signaling pathways and events in cell like gene expression, differentiation, proliferation, cell death, sensing, fertilization and etc. Changes in intracellular calcium concentration through channels (calcium influx) is very fast and localized process near the cytosolic mouth of the channels. Indeed, some physiological processes depend on uniform and/or localized distribution of calcium in cells ⁶⁻⁸.

The first Transient Receptor Potential Vanilloid (TRPV) family member is TRPV1 that is known to be the target of vanilloid compounds as capsaicin. They contain six members based on similarity of amino acid sequence, that the first four action of them (TRPV1-TRPV4) relate to change of heat ^{4,9}. In structure, they are embedded in the membrane as tetramers so that each monomer consists of six transmembrane domains (S1 to S6). S1-S4 domains form a package that contribute to thermosensing of channels while S5 and S6 segments form first gate (selectivity filter). Likewise, distal end of S6 helixes form a hydrophobic region in second gate. Indeed, ankyrin repeat domain (ARD) is one of the most common motifs that are very conserve in TRPV channels. ARD consists of 6 ankyrin repeats that contain two antiparallel α helices followed by a finger loop ¹⁰⁻¹².

Here we focus on the TRPV2 channels, one of the interesting members of vanilloid super family with widely gene expression in both neuronal and non-neuronal tissues. In contrast to TRPV1, TRPV2 is not activated through capsaicin but there are some reported compounds comprising 2-Aminoethoxydiphenyl Borate, Cannabinoid class (Cannabidiol, Cannabigerol and Cannabidivarin, etc.) and Uricosuric compounds which active this channel ^{1,13}. Also, previous studies showed that thermal stimuli near 52°C

can active it and cause monovalent and divalent ions pass across the channels ($\text{PCa}^{2+} / \text{PNa}^+ = 2.9$). However, thermal sensitivity of TRPV2 remains unknown in vivo. Therefore, further investigations require to determine the function details of this channel ^{1,14}.

Full-length Structure of rabbit TRPV2 have been determined at high resolution by Cryo-electron microscopy ($\sim 4.4 \text{ \AA}$). Huynh et al. in this study shown that the upper and lower gates of apo-TRPV2 is wider than the closed form of TRPV1 ¹⁵. It seems that, this structure is in a partially open state ¹⁶. Our study aimed to determine how calcium ions pass through the TRPV2 channel and recognized the important residues in this path.

Results And Discussion

RMSD

After embedding TRPV2 in the membrane, molecular dynamics (MD) simulation was done for 50 ns. The value of the root mean square deviations (RMSD) is a good parameter for evaluating structure stability because it make structural comparison between initial structure and following structure during simulations ¹⁷. As shown in Fig. 1a, the system reached equilibration with RMSD value around 0.55 nm that indicated the overall stability and good convergence during MD simulations. After that, RMSD value of steered molecular dynamics (SMD) simulation was calculated during 6 ns that was stabilized around 0.4 nm. The results indicated that the channel was stable when calcium passed through it (Fig. 1b). RMSD of MD and SMD were calculated for backbone of TRPV2 channel.

Ion conduction pathway

The molecular architecture and gates region of TRPV2 channel has been described in some previous studies. The length of TRPV2 ion-conduction pathway from the beginning first gate to end of second gate is approximately $50 \text{ \AA}$ ^{9,12,15}. As shown in Fig. 2a, b, the pore dimensions of TRPV2 were calculated after simulations. The results indicated that radius of first gate had been wide than second gate and showed a big cavity (about a radius of $6.5 \text{ \AA}$) between first gate to the second gate. The length of the first gate was about $10 \text{ \AA}$ that located in range of 9 to $19 \text{ \AA}$ and consisted of four residues (Gly606, Met607, Gly608 and Glu609). Also, the length of the second gate was about $12 \text{ \AA}$ that located in range of 32 to $44 \text{ \AA}$ in ion conduction pathway. The second gate contained distal end of S6 that Met640 and Ile642 were identified as important residues ¹². Previous studies showed that the first and second gates are open state with van der Waals radius about $3.5 \text{ \AA}$ and $3 \text{ \AA}$, respectively ^{16,18}. The Results demonstrated that the channel was in the Apo-state and first and second gates were open after simulations.

Radial distribution functions:

Radial distribution function (RDF) is a function of distance from a specific point which describes density of atoms surrounding the point in MD simulation ¹⁹. RDF was calculated to investigate the important

residues in transport mechanism of calcium ion through the TRPV2 channel. As shown in Fig. 3a, b, RDF showed a sharp peak in monomer C of first gate, and monomer A and D of S6 segments (AA630- AA650) that confirmed ion passed close to these monomers. Moreover, results showed that maximum $g(r)$ in first gate was significantly higher than S6 segments (about 3000 and 70, respectively) because the monomers of first gate had an angle to Z direction (Fig. 3c, d). In the other hand, by placing the ion at a distance of around 1 nanometer from another atoms, they can interact with each other by electrostatic and Van der Waals interactions. So, these results showed that all monomer in first gate and monomer A and D in S6 segments can be considered as effective regions for passing ion through the channel.

Interaction's energy analysis

To determine which residues were important for passing calcium ion through TRPV2 channel, interactions energy was calculated for each residue in the path of ion transport. Non-bonded energies that describe interactions between different atoms arise due to van der Waals and charged (electrostatic) interactions which were modeled using the Lennard-Jones potential and Coulomb's law, respectively. Non-bonded energies between two stationary charged particles are calculated with below formula in equation (1).

$$E = \underbrace{\sum_i \sum_j \left[\frac{A_{ij}}{r^{12}} - \frac{B_{ij}}{r^6} \right]}_{\text{Van der Waals term}} + \underbrace{\sum_i \sum_j \frac{q_i q_j}{Dr}}_{\text{Electrostatic term}} \quad (1)$$

Where A and B are Lennard-Jones parameters, r is the interatomic distance, q_i, q_j are the atomic charges and $D (= 4\epsilon r)$ is a dielectric function. Moreover, interactions energy can be attractive or repulsive that are shown as negative and positive values, respectively²⁰⁻²². Indeed, the net interactions energy between atoms is obtained from the sum of these energies. In first gate, while Lennard-Jones energy was positive in monomers A, C, D but Coulomb energy was extremely negative. These results indicated that, generally, interactions energy between calcium and first gate were attractive. In addition, in the presented residues in calcium ion pathway (S6 segments; AA630 - AA650), Lennard-Jones and coulomb energies were calculated, so that these energies in the monomer D were positive and negative, respectively and the net energy of interactions was negative. A comparison of these results showed that the attraction force (that are determined through interactions energy) between calcium ions and first gate was more than other areas (Fig. 4).

To determine which residues from the channel monomers participate in interactions, Lennard-Jones and coulomb energies were calculated for each residue in first gate and S6 segments (Data not shown). Results demonstrated that the following residues played an important role to conduct the calcium ion through the channel including Gly606, Met607, Gly608, Glu609, Tyr634, Leu637, 638, Met640, Leu641, Ile642, Ala643, Leu644, Met645, Ser646, Gly647, Thr648, Val649 and Asn650.

Diffusion of water in trpv2 channel:

In many channels, diffusion of water molecules play an important role in the transfer of ions^{23,24}. So, water density and interactions of water with calcium ion were determined during SMD simulation. Our results showed that water density in first gate was lower than the created hole by S6 segments. Indeed, the Lennard-Jones energies between calcium ion and water molecules were positive in first gate and its values were lower than S6 segments. Unlike the Lennard-Jones energies, coulomb energies were negative in first gate and its values were higher than S6 segments. Moreover, fluctuation of coulomb energy during 4000 to 4300 ns can be caused by change of radius in this region, so that radius of this region was found to decrease from 6.5 Å in middle channel to 2.5 Å in near the end of the channel (second gate region) (Fig. 5). Results in Figs. 4, 5, demonstrated that interactions of water molecules with calcium ion caused to decrease interactions of Ca²⁺ with residues in the ion pathway. So, interaction of Ca²⁺ with residues in first gate were more than the rest of the ion path.

Ion permeation in TRPV2 channel

Constant velocity pulling is a method in SMD simulation that an atom, molecule or compound is moved with a constant velocity by applying external forces. The force is calculated below formula in Eq. (2).

$$F = -k(x - vt) \quad (2)$$

where k is the spring constant, x and v are the coordinate and velocity of the pulling atom respectively and t is time^{25,26}. Absolutely, if we want to keep the velocity constant, the force must change during simulation because there are some interactions that affect the force. In the other hand, the force indicates whether the interactions are attractive or repulsive in direction of coordination^{27,28}.

As shown in Fig. 1b, first gate was located 1 nm away from the mouth of the channel and its length was about 1 nm. Also, Fig. 6b, showed that the calcium ion had traveled this path about 1.7 ns. Moreover, the force increased to pass ion through first gate because of steric effect and abundance of interaction between calcium ion and residues. When the ion passed through first gate, the force slumped and calcium ion traveled a distance of about 1 nm during a few time steps because radius in this region (middle of S6 segments) increased to 6.5 Å and interaction between calcium ion and its amino acid decreased. Furthermore, as mentioned earlier, effective residues in this path and second gate caused to fluctuate in the force (Figs. 4, 6). Generally, these results indicated that required forces for passing calcium ion through first gate were more than second gate which their values were about 1000 and 400 KJ/mol/nm, respectively.

Potential of mean force (PMF)

PMF is an important parameter in computational studies that reflects all interactions of the desired molecule with its environment as ion, amino acids and solvent, etc. Moreover, dehydration at the entrance and inside the channel strongly affects PMF. It should be noted that PMF is not an average of interactions and potentials, but is equivalent to the Helmholtz free energy^{29,30}.

Existence of two gates on path of Ca^{+2} conduction had caused a force fluctuation pattern with two big force jumps. (Figs. 1, 6). Therefore, as shown in Fig. 7, there were two large barriers at first and second gates. In the first gate, energies for two main steps of reaction mechanism were about 10 and 15 Kcal/mol as well as in second gate, which occurred a leap of energy from 3.2 nm to 3.8 nm with energy value about 28 Kcal/mol and another energy step was about 3 Kcal/mol. The obtained results indicated that barrier energy in second gate was higher than first gate because in addition to the interaction energy, ion dehydration occurred at time 4000 to 4300 ps in the second gate (Figs. 5e, 7). Also, radius of second gate was less than first gate that was an important parameter in the energy calculation. Ultimately, the large barriers of energy at the gates support this suggestion that calcium ion cannot easily enter and/or leave the TRPV2 channel.

Conclusion

SMD simulation of calcium ion pass through TRPV2 channel was used to analyze the conduction mechanisms. Our results show that there are four big energy barriers in the calcium ion passageway, two steps in first gate and the other two in second gate. Due to the interaction between calcium ion and residues in the first and second gates, as well as the decrement of channel diameter in these areas. Indeed, the transfer of calcium ion needs to overcome these deterrent forces. The forces in this path are of electrostatic type. Energy barrier in second gate is higher than first gate, because in addition to attractive forces between calcium ion and the residues, ion dehydration occurs. Also, radius of second gate was less than first gate as an effective parameter in energy calculation. Indeed, transport of hydrated calcium ion through first gate and dehydrated form in narrow pore of second gate are important parameters in conductance of ion in TRPV2 channel. Moreover, the role of each residue in the conduction determined by calculation of Lennard-Jones and Coulomb energies. Generally, this study shows that calcium ion can't enter or exit the TRPV2 channel easily.

Materials And Methods

Preparation of simulation-system

3D structure of the TRPV2, was taken from the Protein Data Bank database (PDB ID: 5hi9). The Membrane Builder in CHARMM-GUI software was used to embed TRPV2 into mixed lipid bilayers (POPC 256; POPE 121; CHOL 148) ¹¹. In this system, 34806 TIP3 water molecules and 0.15 mol/L of NaCl (physiological ions concentration) were added to model the experimental conditions and net charge of system was zero. So, the system was prepared for energy minimization.

Classical molecular dynamic simulation

In this work, all-atom classical molecular dynamics simulations were performed using the GROMACS 4.5.6 package and CHARMM36 force field parameters. After minimization, NVT ensemble was done for desired temperature of 326 K with periodic boundary conditions and using Langevin algorithm. Then, NPT equilibration was done for coupling pressure by Parrinello-Rahman barostat around 1 bar. Finally, MD simulation was run with time step of 2 fs for 50 ns.

Steered molecular dynamic simulations

Umbrella sampling is a free energy calculation method that computes the potential of mean force (PMF) in some processes such as binding affinity of ligand-receptor and passing ions or molecules through channels, etc. PMF indicate changes of energy for any point of reaction coordinate through series of conformational windows^{31,32}. So, for obtaining reaction coordinate path, we used SMD simulation that is based on time-dependent external forces. For SMD simulation, Ca^{2+} was initially placed at the entrance of TRPV2 channel (with 1nm distance from the entrance of the geometry of channel axis). Then, a constant force of $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ was applied to the Ca^{2+} ion so that ion didn't move during energy minimization and 1000 ps NPT equilibration. Thereafter, for pulling Ca^{2+} ion in the Z direction, a constant force of $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ was applied, while no constant force was imposed on the Ca^{2+} ion. So, Ca^{2+} ion was pulled parallel to the channel axis at constant velocities of 0.001 nm/ ps from the mouth to the end of the channel. The total simulation time for the SMD was about 6 ns for the system.

Declarations

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Author contributions

M.A. and B.H.V. concepted and designed the study and the simulations. M.A. performed the simulations and analyzed the data. P.A. as first corresponding author conducted research, sponsored the study and made all the arrangements. M.J. as second corresponding author conceived and proposed the study. M.A. wrote the manuscript. B.H.V., P.A. and M.J. reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Backbone RMSD graph of embedded TRPV2 channel in lipid bilayer. (a) RMSD of TRPV2 was computed and stabilized around 0.55 nm during 50 ns MD simulation. (b) RMSD of channel during SMD simulation was stable around 0.42 nm when Ca^{2+} passed.

Figure 2

Ion conduction pathway of TRPV2 channel. (a) Snapshots of transport calcium (red) through the channel during SMD simulation time. (b) Pore radius along the central axis and calculated with Mole software. The first gate is wider than second gate and also the gates are in open state.

Figure 3

RDF analysis of calcium ion passing through TRPV2 channel. RDF analysis show that calcium ion passes close to the (a) monomer A of first gate and (b) monomer A, D of S6 segments. The position of (c) first gate and (d) S6 segments and second gate in the channel were presented. Monomer A is yellow, monomer B is red, monomer C is blue and monomer D is pink.

Figure 4

Interactions energy analysis of calcium passing through TRPV2 channel during 6 ns SMD simulation. Lennard-Jones and coulomb energies were calculated in (a, b) first gate (AA606-AA609) and (c, d) S6 segments (AA630- AA650).

Figure 5

The role of water in ion transport during 6 ns SMD simulation. (a) 2D graphical density plot and (b) partial density profile in the z direction for water molecules in the TRPV2 channel. (c) Close-up on Present of water molecules (yellow) around calcium ion (red) in the first gate (green). (d) Lennard-Jones and (e) coulomb energies were calculated between water molecules and calcium ion.

Figure 6

Transfer of Ca^{2+} through TRPV2 channel by SMD simulation along 6 ns time. (a) The Pulling force diagram of Ca^{2+} show change in first gate, S6 sigments and second gates. The pulling rate is 0.001 nm/ps and the constant force of 1000 kJ mol⁻¹nm⁻². (b) Distance between reference group and Ca^{2+} along the z-direction. (c) Image show step by step of calcium ion (green) passage.

Figure 7

PMF along the TRPV2 channel in the z-direction during 6 ns SMD simulation. First gate is located from 0.9 to 1.9 nm and second gate is located from 3.2 to 4.4 nm.