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The role of CatSper1 and CatSper2 ion channels in male fertility and infertility

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Abstract

Ion channels in human body play multiple roles in many important biological processes. They regulate sperm maturation in the female reproductive tract and facilitates in hyperactivated motility, maturity through capacitation and acrosome reaction. The four sperm-associated cation channel, known as CatSpers and different ligand gated and voltage gated ion channels have been found to play crucial role for sperm physiology preparation for fertilization. The major function for maintaining Ca^{2+} concentration in flagellum is governed by CatSper channels in plasma membrane. Until now the role of these channels are not very clear. Here we have analysed the PDB structure (generated so far) of CatSper1 and CatSper2 channel proteins and found their ligand binding sites. The calculations are done by Charmm potential. Energies are calculated before and after ligand binding. We have also carried out Molecular Dynamics (MD) simulation for the protein model after filling up the simulation box with water and a number of K^+ and Cl^- ions. We see that the opening and closing of the channel pores depend on the binding of ligands.

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Keywords: CatSper ion channel; male fertility; ligand binding; MD simulation

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1. Introduction

CatSper is a sperm specific calcium ion channel in the flagellum which was discovered in 2001 as a new family of calcium selective ion channel. Before this discovery it was considered that voltage gated Ca^{2+} channel, Ca_V and voltage gated cation channels (VGCC) were responsible for Ca^{2+} level increment. But after the advent of CatSpers which are cation channels of spermatozoa, the concept is completely changed to revolutionize the molecular biology field. At present 4 members of CatSper ion channel have been identified and seven CatSper subunits corresponding to heteromeric CatSper channel are addressed by Ren et al. (2001), Quill et al. (2001), Lobley et al. (2003), Jin et al.(2005) and Qi et al. (2007). CatSper channels are putative 6TM, voltage gated calcium permeate channels that are presumed to assemble as a tetramer of α like subunits and mediate the current $I_{Caatsper}$. CatSper1, CatSper2, CatSper3 and CatSper4 are restricted to testis and localized to the principal piece of sperm tail. But recently, putative 2TM auxiliary CatSperβ [Liu et al., (2007)] protein and two putative 1TM associated CatSperγ [Chung et al., (2011)] and CatSperδ [Wang et al., (2009)] proteins have been noticed in the same part of the sperm. But for male fertilization hyperactivation of sperm cell mobility plays an important role and from this point of view CatSper1 and 2 are the most suitable candidates. CatSper1 and 2 are essential for the hyperactivation of sperm cell motility, which is required for fertility. Sequence identities among these CatSper family members, range between 22 and 27% across the ion transport domain [Jin et al. (2005)].

Ca^{2+} signalling mechanism is well studied by many authors − both experimentally and theoretically. The dependence of the triggering of hyperactivation is evident from the experimental studies, but in vivo mechanism is not known successfully. The hyperactivated motility of spermatozoa is a very complex process that involves chemical signaling, dynein force generation dynamics, passive elastic properties of the sperm structure and external fluid dynamics. However, theoretical investigations of Arti et al. (1993), Blum et al. (2000), Dougherty et al. (2005), Wang et al., (2008) can be mentioned regarding the structure-function relationship of ion channels. These studies include many cell types like pituitary gland, vertebrate olfactory receptor neurons, muscle and Xenopus oocyte. Mathematical model of Olson et al., (2010), gives the Ca^{2+} flux due to channels, stores and also the diffusion of ions along the length of the flagellum. Recently, detail studies on CaSper channels [Mohammadi et al. (2013)], molecular mechanism of sperm chemotaxis and influence of these channels regarding chemotactic movement [Lu et al. (2014)] are reported. However, the detailed knowledge about the working principle of the CatSper protein channels is still unrevealed.

Many unknown facts still remain about these ion channels and hence it is necessary to study their structure-function relationship in more detail. Here we have analyzed the structure of CatSper1 and CatSper2 of human in detail and their ligand binding mechanism which are responsible for changing the protein channel transport properties. We have simulated the structures and their implicit water models to get energy minimized values. MD simulation of the proteins has given us valuable information about the internal change of pressure and energy with time. Also we have simulated the protein with ion and water to get the energy minimized value.

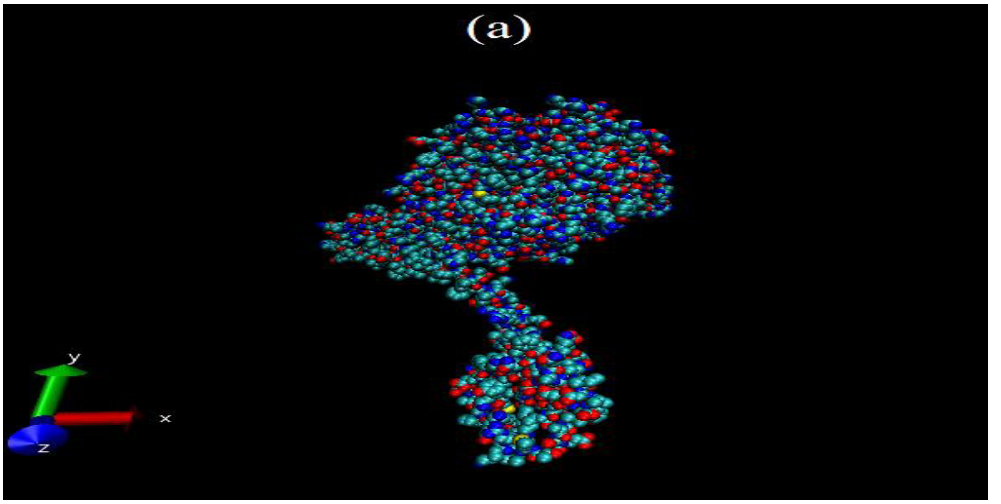
2. Methods and modeling

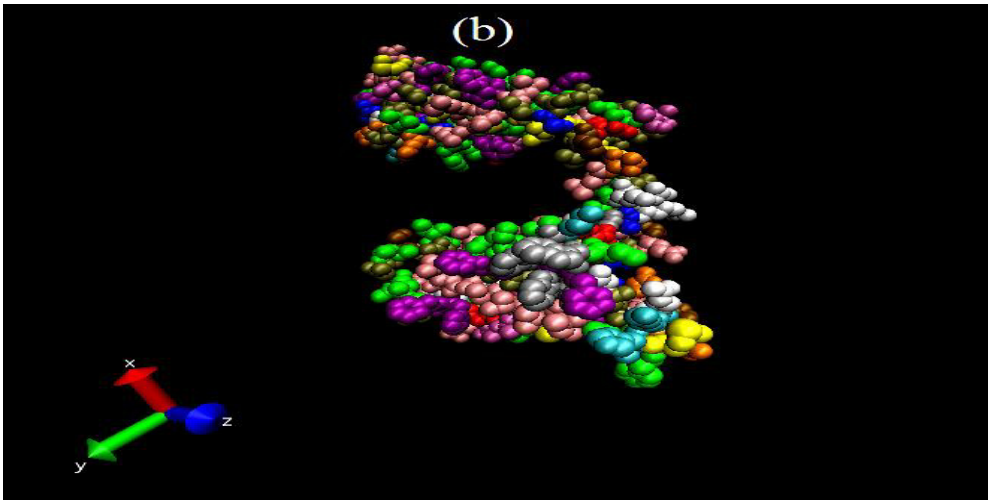
The PDB structure of CatSper1 and CatSper2 are generated by Protein Homology/Analogy Recognition Engine V 2.0, Phyre2 [Kelly et al. (2009)] from the amino acid sequence of Homo sapience. Ligand binding sites are predicted by 3D ligand Site- Ligand Binding Site prediction Server [Wass et al. (2010)]. Modeling is done with VMD, Visual Molecular Dynamics software [Dalke and Schulten (1996)]. Open source software Abalone and Xeno View [Shenogin and Ozisic (2007)] and online simulation CHARMM-GUI [Jo et al. (2008)] are used for energy minimization and MD. For energy minimization hybrid LS and CD method is employed [Fletcher (1987), Liu and Storey (1991)].

3. Results and discussion

The identifier names of these proteins are sp_Q8NEC5_CTSR1 and sp_Q96P56_CTSR2 respectively. Both the proteins contain 20 aligned regions. Several transport proteins are found in their domain, namely, metal transport protein, several voltage gated sodium channel, potassium channel, proton channel, bacterial sodium channel (in high Calcium), membrane protein, transient receptor potential cation channel, cyclic nucleotide gated potassium channel,

ion transport protein, ion transport 2 domain protein etc. Ligand Binding site for CatSper1 is predicted with residue 456, 483, 486, 487 and 490 with amino acid VAL, LEU, ILE, PHE and ILE respectively. The average distances of contact are 0.30 Å, 0.33 Å, 0.40 Å, 0.03 Å and 0.00 Å respectively. Interestingly, for residue 490, perfect contact is established. Heterogenes present in predicted binding site are HEM and ZN with count 4 and 40. For CatSper2, the predicted binding sites are found for residue 157, 184, 185, 216, 219 for amino acid PHE, VAL, PHR, ARG, LYS respectively. Here, the shortest distance is obtained for residue 219 with average value of 0.00 Å indicating perfect contact. Heterogens are only ZN with count 60. The ligand binding is also verified by simulating in the software Abalone. The protein structure of CatSper1, shown in Fig.1(a) and 1(b), depicts the ligand binding mechanism. The structural changes of the channel pore before and after ligand binding are depicted in Fig. 1(c) and 1(d).





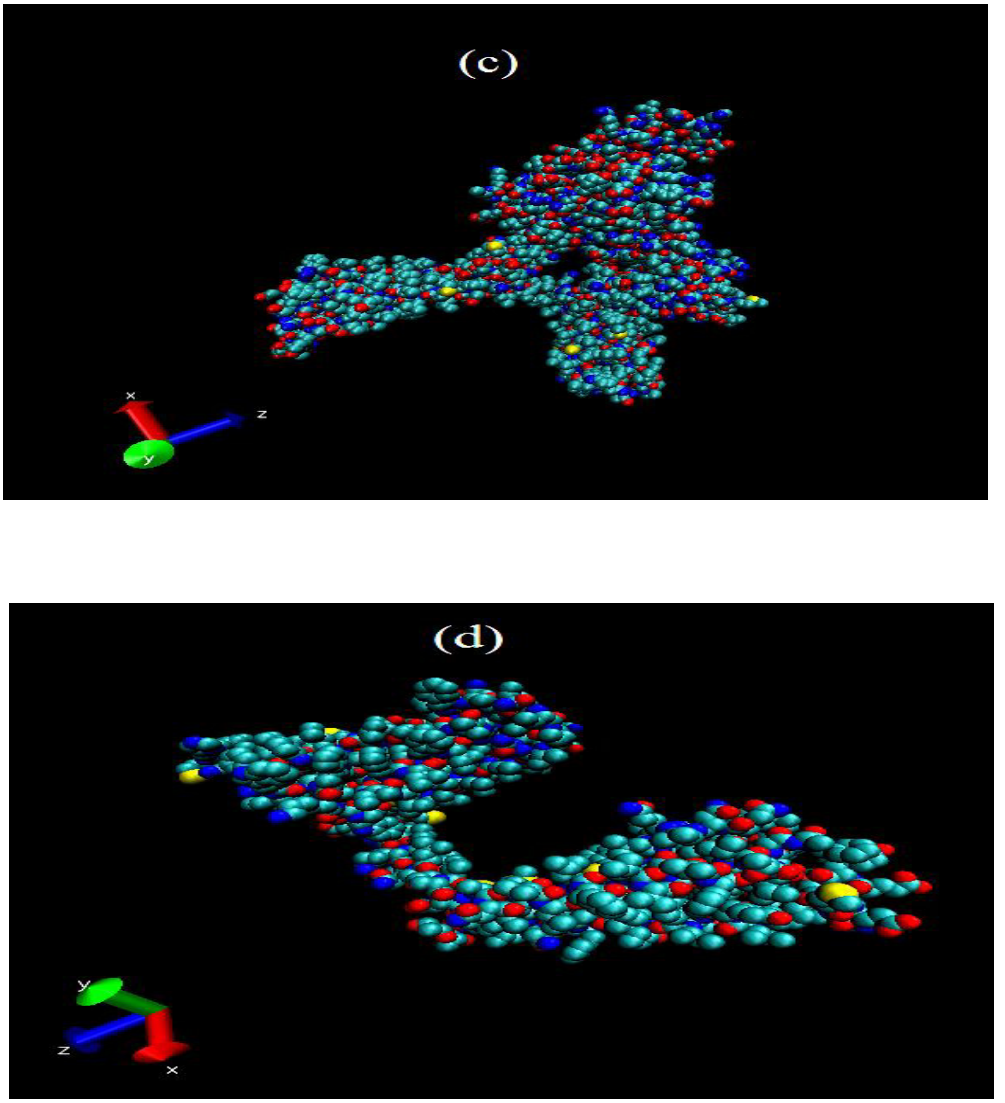


Fig.1. (a) Structure of CatSper1 protein (b) Ligand binding of CatSper1(residues are coloured by their name) (c) Structure of CatSper2 protein (d) Opening of CatSper2 channel on ligand binding

In our study, we have found that binding of ligand opens the pore of the CatSper1 and CatSper2 protein channels which usually remain closed. So it can be said that ligand binding plays a vital role in opening and closing of the channels which in turn must affect the rate of flow of ions through the pores.

Next, we have optimized the structure of the proteins with Charmm potential in the online Charmm-GUI interface and also with the software Abalone. Final energy is calculated as 18415.0 kcal/mol. To confirm the results energy optimization is also done in Xeno-View software with pcff.frc force field. MD Simulation of the protein of 100000 steps is carried out taking the same force field in Xeno View software. Berendsen Thermostat is used to maintain the temperature constant, allowing only a small change to rescale the velocities of the atoms. The variations of energy and internal pressure with time are shown in Fig 2(a) and 2(b) respectively.

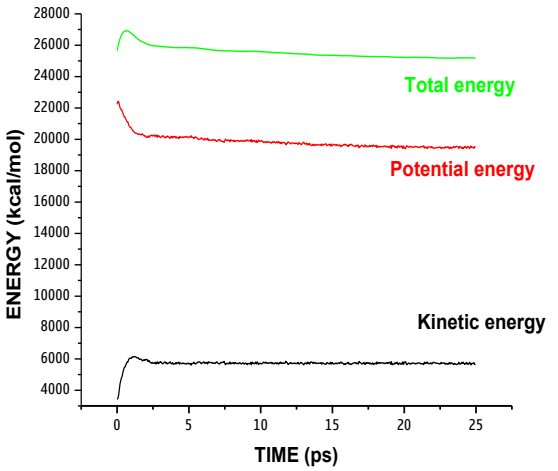
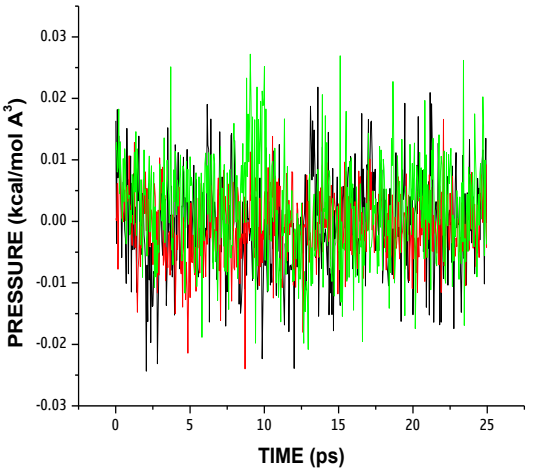


Fig. 2. (a): Variation of Kinetic energy, potential energy and total energy with time



(b): Variation of pressure in [1, 1] (black), [2, 2] (red) and [3, 3] (green) direction

The implicit water model of the protein CatSper1 is also simulated to get energy optimized value. A Barendsen thermostat is used to maintain the temperature as 300K. Generalized Born method is utilized to find the optimised energy as 19730.3 kcal/mol for the implicit water model where the time per step is 2.5 fs and duration 100000 ps. In this model the channel pore is filled up with water and then simulated. The maximum and minimum radii of the channel pore have been calculated as 11.446 Å and 5.614 Å respectively using online simulation using CHARMM-GUI. Cross sectional area of the protein and the pore radius are shown in Fig 3 (a) and 3 (b), respectively.

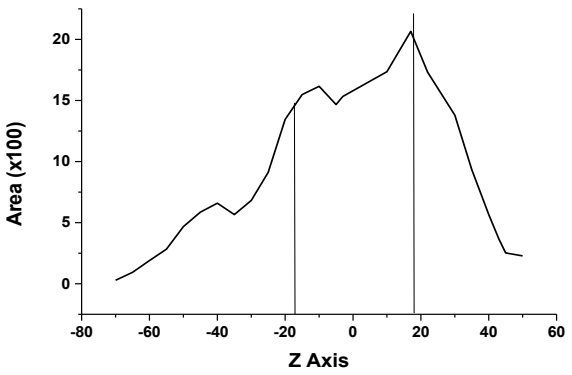
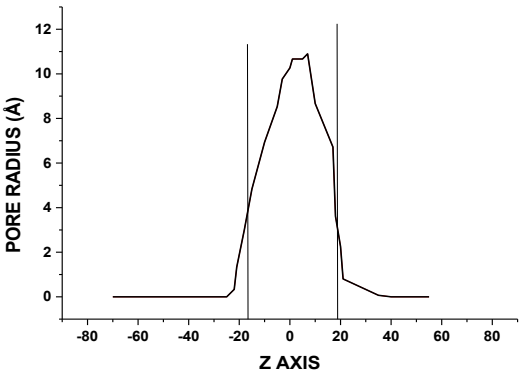


Fig. 3: (a) Cross-sectional area of the Catsper 2 protein,



(b): Pore radius of CatSper2 ion channel protein

The energy minimization of the water-ion model of the protein is performed by hybrid LS and CD method. Before simulation, a simulation box is created with water molecules and 600 K^+ and 600 Cl^- ions. This is performed to get realistic results as in human body proteins are associated with water and ions. The model is generated in VMD [Humphrey et al. (1996)] software and the structure is minimized in Abalone. As a whole the structure contains approximately 65000 molecules which is shown in Fig. 4. The minimized energy of the model is 26478. 5 kcal/mol. Gradient tolerance is reached after 23095.7 sec. This value is higher than the energy value of implicit water model.

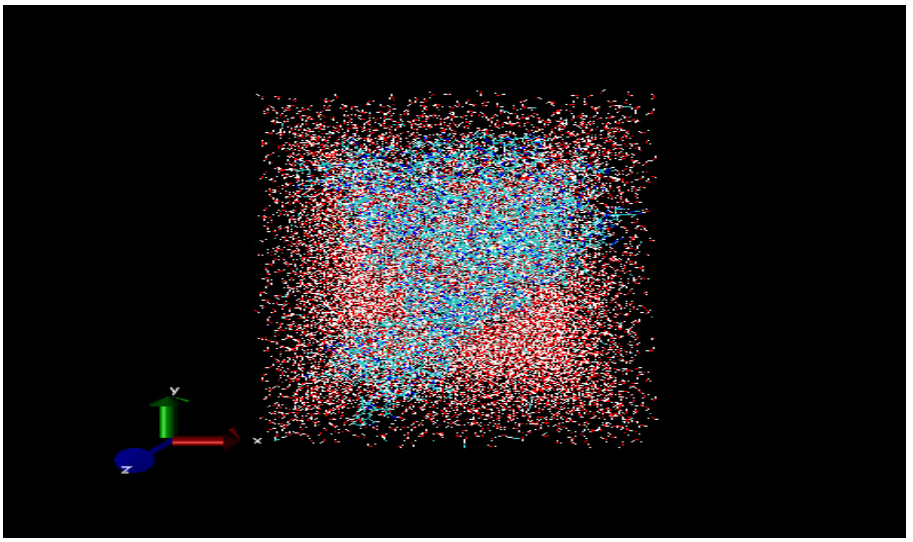


Fig. 4: Water ion model of CatSper1 protein. (Light green-protein molecule, blue-K^+ ion, green- Cl^- ion, red and white-water molecule)

Conclusions:

We have performed a detailed computational study of the CatSper1 and CatSper2 proteins, analyzed their structure and found their ligand binding sites using MD. We have observed that ligand binding plays a crucial role in passage of ions through the pores. The channel pore is opened on ligand binding which facilitates the passage of ions through the channel pore. The maximum and minimum channel radii are calculated as 11.446 Å and 5.614 Å respectively. The simulation of the protein, implicit water model of the proteins and the water-ion model give some important properties of the ion channel proteins. The minimum energy of the protein is computed as 18415.0 kcal/mol whereas the minimum energy of implicit water model and water ion model are found as 19730.3 kcal/mol and 26478.5 kcal/mol respectively.

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