Molecular Dynamics Simulation of a NaK channel protein

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ABSTRACT

Ion channels control the flow of ions across the membrane in all cells. It is an integral membrane protein. In this study molecular dynamics simulation and principal component analysis of the NaK ion channel in water environment have been performed. From the analysis of the simulation trajectory, it is observed that flexibility presents in the each helixes. RMSF values are seen to be largest in case of the N-terminal residues of B chain which are the part of M0 helix. It is also seen that the filter shows considerable fluctuation, and it does not act as a rigid tube through which selected ions pass.

Keywords: Ion channel, Molecular dynamics simulation, Root mean square deviation, Principal component analysis.

INTRODUCTION

Ion channels are protein, present in cell membrane, contains water filled pores that pass millions of ions per second between the cell exterior and interior [1]. The movement of ions through these channels controls excitation and electrical signaling in the nervous system. One of the most interesting properties of ion channel is their ability to distinguish between ions. Ion channels are usually selective i.e., only certain ions are allowed to flow through the central pore because they are equipped with some kind of ion filter. Ion filter has a signature sequence of several amino acids.

Potassium channels are a large family of ion channels that play an important role in the membrane physiology of shells and share a common property of selectivity for K^+ over Na^+ ions[1-2]. All potassium channels contain the highly conserved signature sequence TVGYG (threonine-valine-glycine-tyrosine-glycine), which is essential for K^+ ion selectivity [3-4]. The cyclic nucleotide gated (CNG) channel pore shares high sequence homology with K^+ channels but is non selective and permeable to most group IA monovalent cations [5-8].

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Shai et al., identified two two-transmembrane channels from Bacillus cereus and bacillus anthracis that have sequences very similar to the Kcs K channel, except for their selectivity filters, which resemble those of CNG channels with the sequence TVGDG or TVGDA. They have established the crystal structure of the NaK channel from Bacillus cereus. It consists of 110 amino acids per chain. In the crystal, it is found in homodimeric state containing two chains A & B but coordinates of some residues are missing in both the chains. Each chain contains some helixes (like M0, M1 etc). In this protein, filter has a sequence of $_{63}$TVGDG$_{67}$ (threonine-valine-glycine-aspartate-glycine) with the hydroxyl oxygen atom from threonine 63 and backbone carbonyl oxygen atoms from threonine 63 and valine 64 forming two ion –binding sites. In comparison to K channel ion filter sequence, only tyrosine is replaced by aspartate residue in NaK channel filter. The replacement of tyrosine by acidic aspartate residue causes pronounced changes in the structure and also in the selectivity of the NaK filter [9].

In this work molecular dynamics (MD) simulation is applied to explore the dynamic properties of the whole protein, chainA, chainB and helixes of the protein and in the conformational change of the filter region taking 2AHY.PDB code as starting structure which contains Na$^+$ ions. MD simulation of the protein containing K$^+$ ion has been done by Vora et al., [10].

EXPERIMENTAL SECTION

In this work molecular mechanics and dynamics were carried out using GROMACS (ver 3.3.1), running on a Pentium IV 64 bit computer [11-12]. The coordinates of NaK ion channel (protein data bank code 2AHY.PDB) was used as a starting structure. The protein was solvated with pre equilibrated SPC water molecules for simulation in aqueous environment in a box of size 45*45*45 Å^3.

The molecular dynamics simulation was conducted at a constant temperature (300K), coupling each component separately to a temperature bath using the Berendsen coupling method. Before running simulation, energy minimizations are performed by steepest descent (sd) method followed by conjugate gradient method [13-14]. The GROMACS 96 43a1 official distribution force field was used and all bonds were constrained using the LINCS algorithm [15]. A cutoff of 0.9 nm for Vanderwaals interaction was used and the PME (particle mesh Ewald) method was employed to calculate longer-range electrostatics contribution with 1 nm cutoff for columbic interactions [12]. After that position restrained dynamics are performed at different temperatures for total 400 ps (50K, 100K, 200K and 300K). Finally the restrain is withdrawn and simulation is performed for 1000 pico second with time step 2 fs in water. After completion of the simulation the trajectory file was analyzed with different tools of GROMACS.

RESULTS AND DISCUSSION

The MD simulation of NaK channel protein (PDB code 2AHY.PDB) in water is performed to characterize the behavior of the channel by monitoring a set of structural parameters as a function of simulation time. These parameters provide a general idea of the flexibility and structural behavior of NaK channel protein.

The RMSD of whole protein, RMSD of chainA and chainB is presented in figure 1. From the figure, it is seen that RMSD of chainB is higher than chainA. Again, RMSD of chainB is very much similar to the RMSD of the whole protein. Chain A and chain B both contain helixes like M0, M1, M2 etc. To explore the relative motions of different helixes between two chains, the RMSD of the helixes (M0 and M1 helixes were taken as representatives) have been analyzed and

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compared. The results are shown in figure 2. From the figure it is observed that helix M0 of chain A and M0 of chain B have the highest fluctuation among the helixes and figure also indicates that flexibility presents within the helixes as each helix fluctuates with time. It is very interesting that initially RMSD values of M0 helix of chainA is higher than M0 of chain B but after about 150 ps RMSD of chainB exceeds. Average RMSD of M0 of chainA is 0.4567 nm (standard deviation, STDEV 0.0992)   and RMSD of M0 of chain B average is 0.5385nm (STDEV 0.1113). Figure 2 also reveals that most of the time RMSD of M1 helix of chain B is higher than M1 helix of chain A.   Average RMSD of M1of chainA is 0.2404 nm (STDEV 0.0493) and RMSD of M1 of chain B average is 0.2579 nm (STDEV 0.0585).
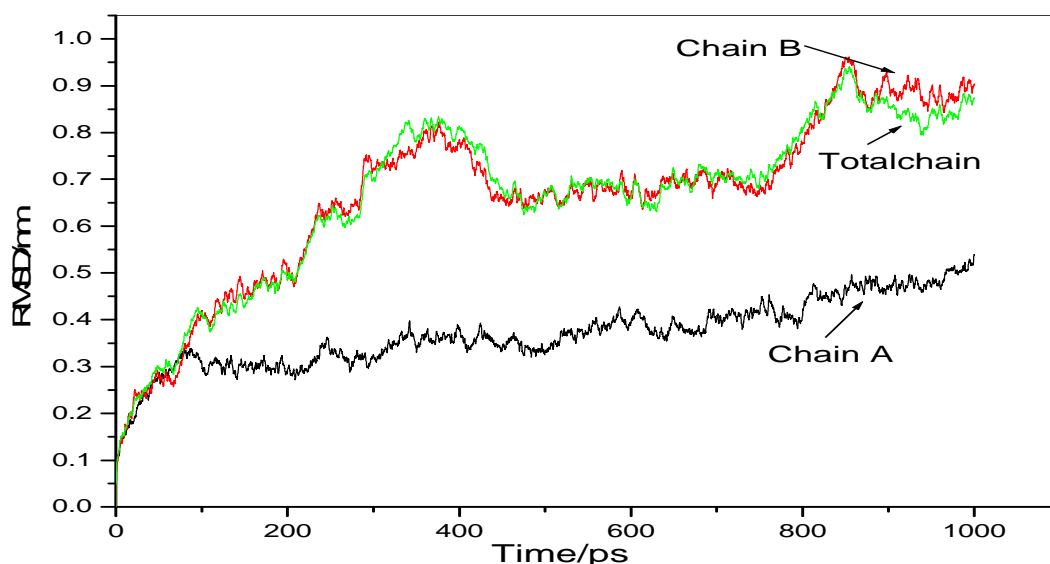
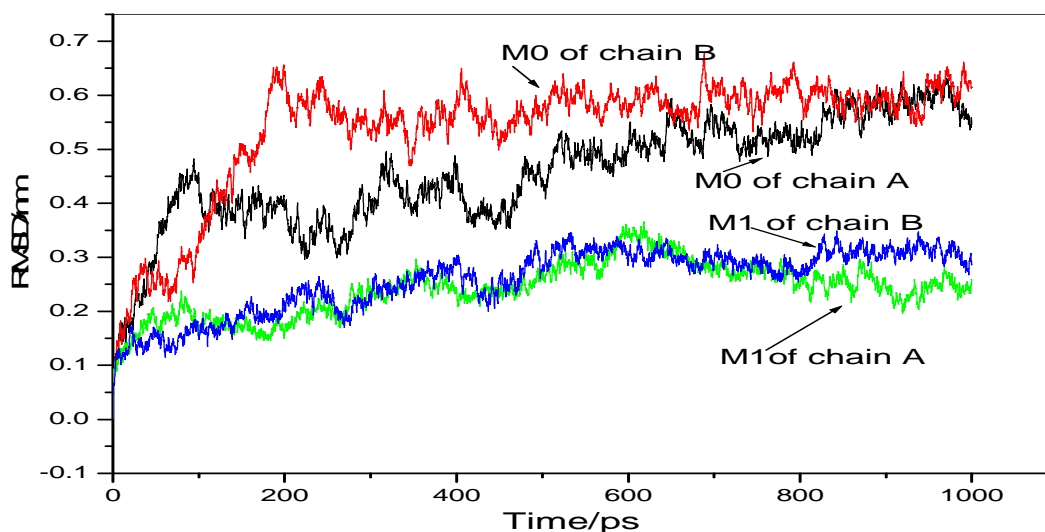**Figure 1: Plot of root mean square deviation (RMSD) with simulation time**

Figure 2: Plot of root mean square deviation (RMSD) of individual helixes with simulation time.

The flexibility of a protein channel is also often explored by the RMSF of each residue. Figure 3 shows the RMSF of C-alpha atom. RMSF values are seen to be largest in case of the N-terminal residues of B chain (residue number: 1 to 8) which are the part of M0 helix and it is also

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supported by RMSD of M0 helix. It may be conclude that the M0 helix of the B chain is the most labile portion of the system.
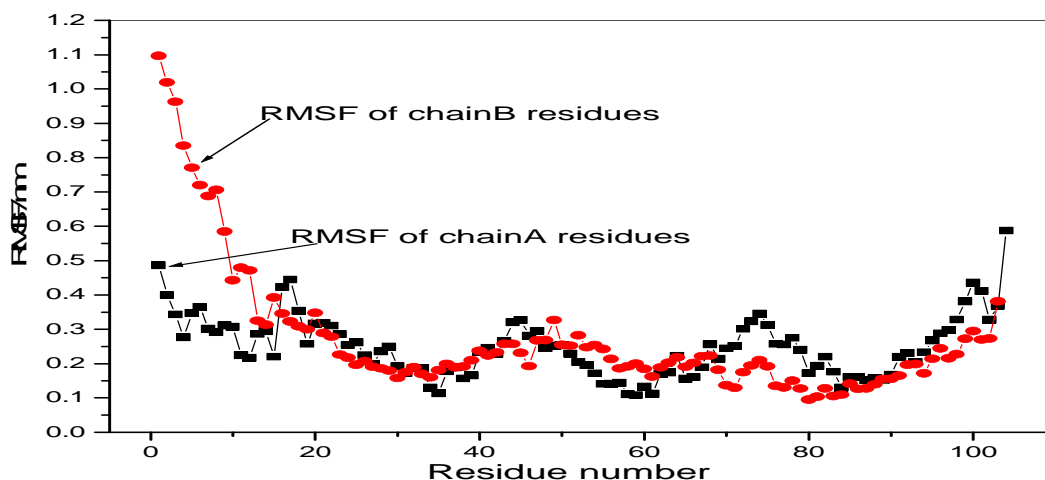

Fig 3. Plot of the RMSF values with C^{α} atom of residues.

The major motions of the protein can be identified by the use of Principal component analysis (PCA) [16-17]. PCA of the dynamic trajectory of NaK channel protein (PDB code 2AHY) in aqueous medium reveals that the first eigenvector corresponds to 59.83% of the total motion and the second to a further 21 % (figure 4). The third eigenvector in aqueous medium corresponds to 7.1%. Thus from the analysis it is concluded that motion is mainly concentrated in two direction.
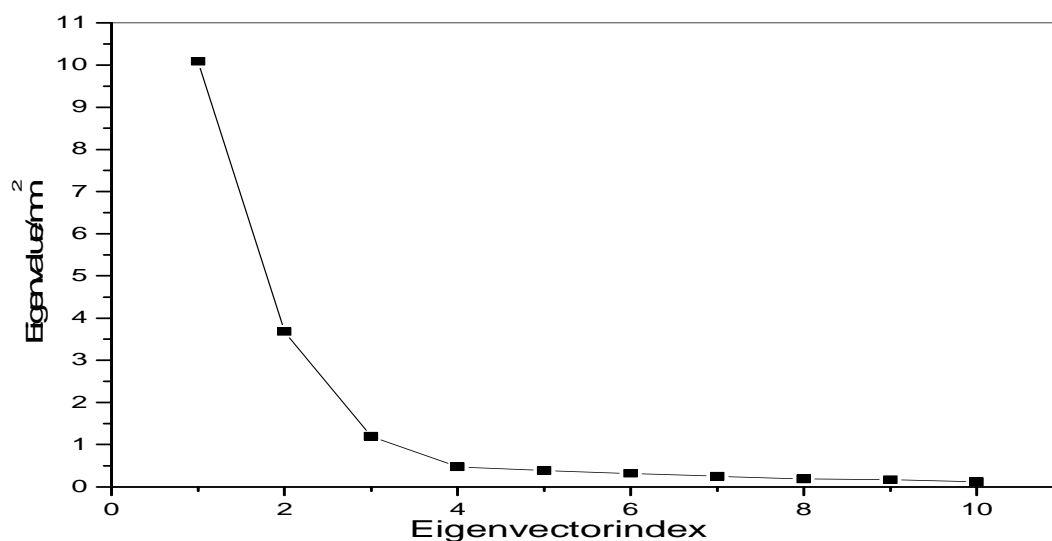
Figure 4: Plot of eigenvalues with eigenvector indexes.

Figure 5 represents RMSF values of filter region of both the chains. It is evident from the figure that valine (residue number 64 of chainA) show the highest RMSF among the filter region residues. From this it may be concluded that it is a pivotal residue for the ion selectivity. The RMSF of the filter region is moderate (average RMSF is 0.2609, range of RMSF of the filter region in between 0.1558 to 0.2212) which indicate moderate flexibility of the residues of that region and it does not act as a rigid tube through which selected ions pass.
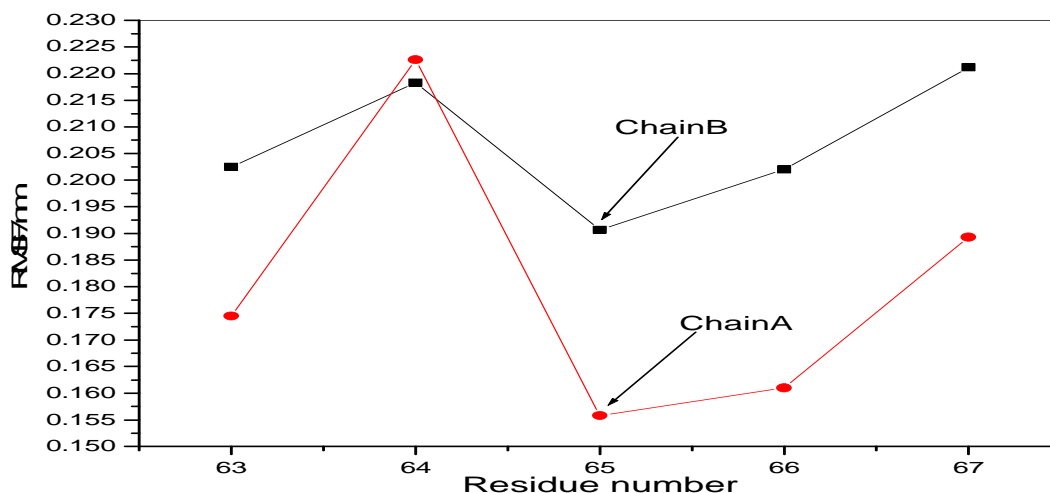
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Figure 5: Plot of the RMSF values with C$^{\alpha}$ atom of residues.

Snapshots of the filter region during the course of the simulation at different time were taken (figure 6a to 6e) and it reveals that the filter undergoes changes its conformation during the simulation time frame. It is also in accordance with the fact that the region is not rigid tube like but has considerable flexibility which induces its proper function.
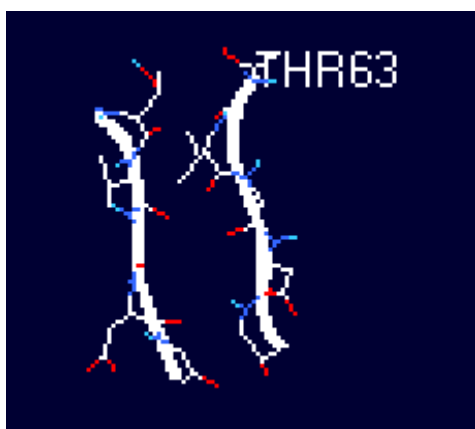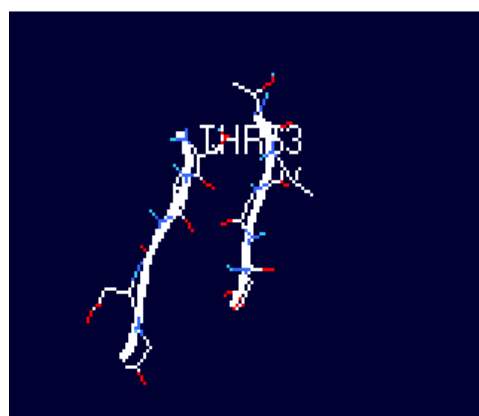

Figure 6a.Snapshot at 0ps
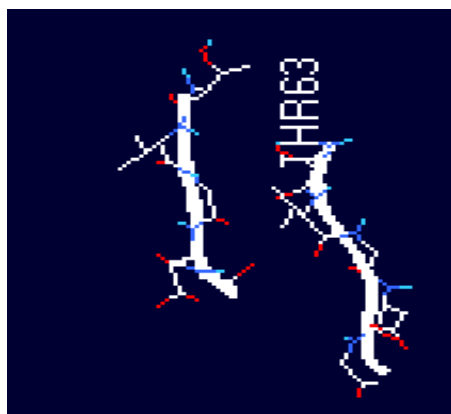

Figure 6b.Snapshot at 308ps
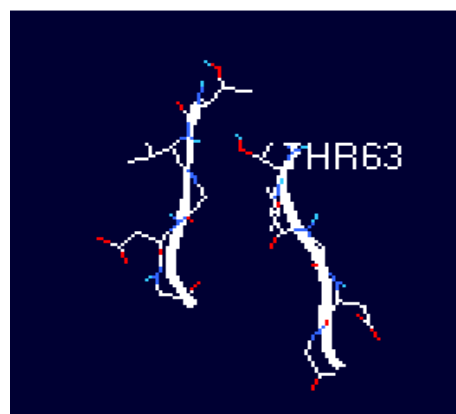

Figure 6c.Snapshot at 422ps

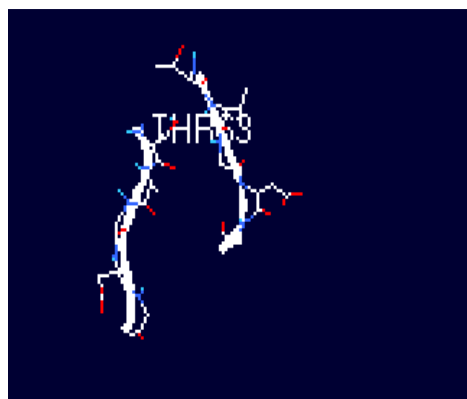**Figure 6d.Snapshot at 517ps**

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Figure 6e.Snapshot at 642ps

Shi et al., showed that at the entryway to the filter from the extracellur solution, the main chain at glycine (residue number 67 of chain A) pinches inwards so that its carbonyl oxygen points towards the pore axis, forming an ion binding site. So the fluctuation of distance with simulation time between alpha carbon atoms of the glycine (residue number 67 of chainA) residue and glycine (residue number 67 of chainB) residue and carbonyl oxygen atoms of the glycine (residue number 67 of chainA) residue and glycine (residue number 67 of chainB) residue are monitored and shown in figure 7 and it is evident from the figure that the fluctuation is considerable in both cases.
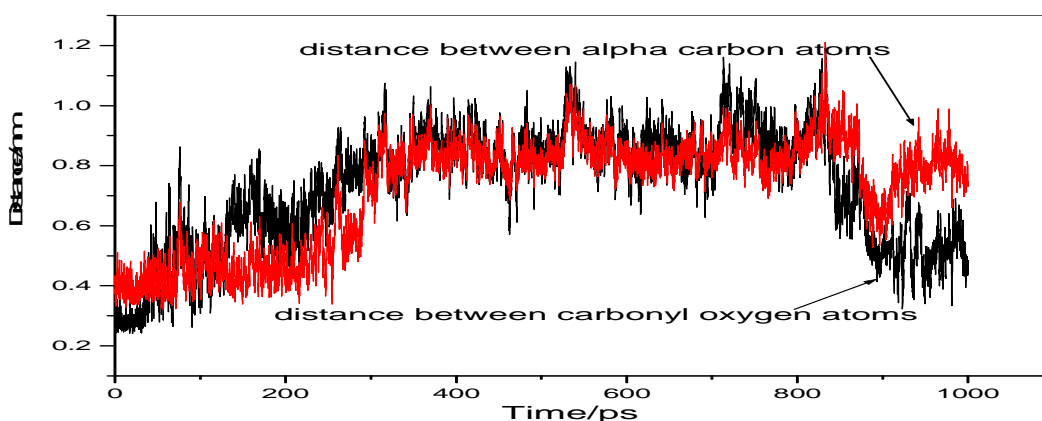

Figure 7: Plot of fluctuation of distance with simulation time.

Acknowledgements

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