Computational Study of the Electrostatic Coupling of Membrane-spanning α-Helices Controlled by Dielectric Media

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Keyword: Voltage Gated Ion Channel.

Abstract: Voltage-gated potassium ion-channels (Kv) play a key role in neurons. The ion-channel is a tetramer each having two domains: voltage senor-domain (VSD) and pore-domain (PD), with four (S1-S4) and two (S5-S6) α-helices respectively, which behave like macrodipoles. The VSD appears capable of adopting different orientations relative to the pore-domain of Kv channels in response to the variable (-70V to +30V) transmembrane-voltage controlling the passage-way of the K+ ions across the membrane. There is an immense progress in the study of voltage-gated channel; however the molecular mechanism underlying voltage sensing is still a matter of debate. Here, we have used a novel theoretical approach using electrostatic theory to identify the possible stable conformation of the voltage gated potassium ion-channel of *Aeropyrum pernix* (KvAP) at zero transmembrane-voltage by computing the minimum potential energy of the system embedded in hybrid dielectric environment. We have set up an algorithm to generate data, which is presented graphically and then analyzed to study the configuration of the biological system of KvAP. It is observed that in ion-channel protein two adjacent α-helices behaving like a macrodipole conform to antiparallel arrangement and the involvement of the charged residues with the multidielectric environment gives the ion-channel protein different conformations.

1 INTRODUCTION

The key process underlying the electrical activity of excitable tissue (neuron) is the voltage-dependent opening and closing of tetrameric Na^+, Ca^{2+}, and K^+ channels. This gating action is mediated by a voltage sensor whose movement is somehow coupled to an intracellular activation gate contained within the channel's pore domain (Yellen, 1998). The K^+ channel is composed of four identical or homologous subunits, each containing six transmembrane α-helices: S1–S6 (Figure 1). α-helices S1–S4 form the voltage-sensing domain (VSD), and α-helices S5 and S6 connected by the P loop, which is involved in ion selectivity, comprise the pore-forming α-helices domain (PD). The most mobile S4 has four gating-charge-carrying arginines (R1–R4) spaced at intervals of three amino acid residues, which are highly conserved and are thought to play a key role in coupling changes in membrane voltage to opening and closing of the pore.

The structure and the function of different voltage-gated potassium ion channels have been reviewed (Borjesson and Elinder, 2008).

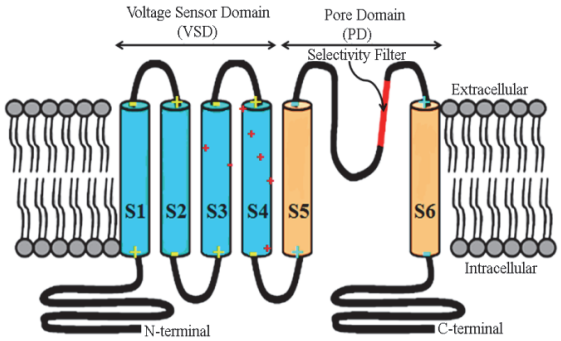

Figure 1: Schematic diagram of KvAP ion channel monomer at membrane-spanning orientation. The helices ion of VSD (S1, S2, S3, S4) (blue) and PD (S5, S6) orange. The (+) and (-) are position of charges; charged amino acids (reds), N-terminal charge (+) and C-terminal charges (-). (The figure is generated by 3D Max).

S4 always stay together, while the other helices of the voltage sensor domain (VSD) present different spatial In the structures of KvAP obtained

Mapder T. and Adhya L..
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DOI: 10.5220/0004193601110115
In *Proceedings of the International Conference on Bioinformatics Models, Methods and Algorithms* (BIOINFORMATICS-2013), pages 111-115
ISBN: 978-989-8565-35-8

by different methods (Jiang et al., 2003a); (Lee et al., 2005); (Butterwick and MacKinnon, 2010) the S3b and orientations. Each of these helices forms a macrodipole and can potentially contribute to the electrostatic field.

Here, we have made use of an application of information technologies and computational systems to understand the configuration of a pair of helices (S3b-S4) of the voltage gated potassium ion channel of *Aeropyrum pernix* (KvAP) at zero transmembrane voltage.

2 THEORY

2.1 Model of a Pair of Antiparallel α-Helix

The voltage-gated ion channel protein (KvAP) which is a transmembrane helix-turn-helix protein in lipid and ionic environment is modeled as an aggregation of macrodipoles in a hybrid or complex dielectric environment. A α-helix possesses a dipole moment by virtue of the alignment of its peptide bonds having half-positive and negative charges at their ends respectively. For this fractional charge separation, a single peptide unit behaves like a microdipole (Wada, 1976). When these microdipoles align along the axis of the α-helix, making hydrogen bonds with the neighboring peptide units, it behaves like a macrodipole (Hol 1978) with positive C-terminal and negative N-terminal on either end.

The VSD has four such antiparallel α-helix macrodipoles (S1, S2, S3a-S3b, S4). The S3b-S4 helix-pair "paddle" of the voltage sensor domain (PDB: 1ORQ) in the x-ray crystallography structure of KvAP is selected for the simulation of the helix-pair. The S3b contains a positively charged Histidine (H109) and a negatively charged Glutamic acid (E107) (Figure 2). On the other hand S4 contains five positively charged Arginines (R117, R120, R123, R126 and R133). Except Histidine which is half, all the residues have unit charge. Apart from the charged residues, the contributions of the terminal charges of the helix dipoles are half unit at two positive N-terminals (N3, N4) and two negative C-terminals (C3, C4). The length of the S3b and S4 helices are 19.5Å and 24.0Å respectively. The Histidine (H109) is 3.0Å above and 200º apart (clockwise) from E107. On S4 the first charged residue R117 is at the positive N-terminal (N4), while the others are placed 60º, 120º, 180º, and 200º clockwise away from and 4.5Å, 9.0Å, 13.5Å, and 24.0Å below R117 respectively.

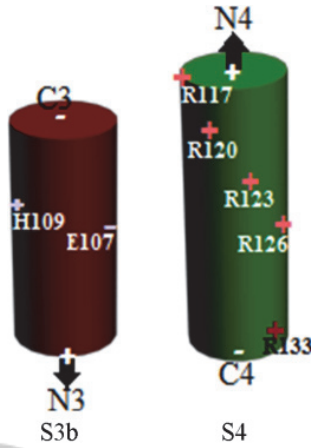

Figure 2: The S3b and S4 helix pair with the charges. C3, N3, N4 and C4 are the terminal dipole charges and the arrows specify the direction of the dipole moments of the two helices. R17, R120, R123, R126 and R133 are the positively charged arginines on S4 and on S3b, H109 and E107 are positively charged Histidine and negatively charged glutamic acid respectively. (The figure is generated by 3D Max).

The interaction potential energy of the system of charges on S3b-S4 macrodipoles is calculated to study the mutual configuration between S3b and S4 helices. Three parameters of motion of S4 are considered. 1) The angle of rotation (θ°) about its own axis; 2) the relative translational (x Å) with respect to S3b; 3) the oscillation (β°) in the plane parallel to S3b. At θ=0.0°, x=0.0Å and β=0.0°, R117 of S4 helix faces E107 of S3b, which is perfectly parallel to S4. With the variation of x, S4 slides along S3b. At different combinations of theta and x values different residues come in front of E107.

2.2 Electrostatic Principle Holding the Macrodipoles Together

On the basis of the principle of electrostatic theory, the antiparallel arrangement is best understood by dipolar interactions in which the mutual potential energy (PE) of two interacting adjacent macrodipoles depends upon their dipole moment ($\vec{p}$) and varies with their relative angular separation (θ) (Mahajan, 1988). The two dipoles tend to orient so as to achieve the minimum PE of the system. Lower the PE, more stable is the conformation. When they are parallel (θ=0°) the PE is maximum; when perpendicular (θ=90°) PE is zero, and when antiparallel (θ=180°), the PE reaches a minimum value (Mapder and Adhya, 2012).

When the dipoles are very close to each other (i.e. the distance between the two dipoles is smaller

than their length) the interaction of the pole charges plays a dominant role. Due to electrostatic attraction, the two opposite poles of the antiparallel dipoles possess a negative Coulombic potential energy ($PE_{coulomb}$) (Eqn 1a), while a positive potential energy is possessed by the two similar poles of parallel dipoles.

Coulomb Energy;

$$PE_{coulomb} = \frac{1}{2}\frac{1}{(4\pi\epsilon_0)}\sum_{\substack{i=1\\ j=1\\ i\neq j}}^{n,m}\frac{q_i q_j}{(d_{ij})}\left\{\frac{1}{\epsilon_i}+\frac{1}{\epsilon_j}\right\}, \quad (1a)$$

When the charges are near the boundary of two different dielectric media, opposite charges are induced on the dielectric interface. These induced charges interact with the original charges by the method of image charges (Jackson, 1975), contributing (a) self energy (PE_{self}) (Eqn 1b) and (b) shielding energy (PE_{shield}) (Eqn.1c). PE_{self} is due to the dielectric screening of the charge in its own medium. When there is an interaction between two charges in two different media, then both charges are shielded by their respective media creating a shielding effect to the columbic interaction energy (PE_{shield}).

Self Energy;

$$PE_{self} = \frac{1}{(4\pi\epsilon_0)}\sum_{\substack{i=1\\ j=1}}^{n,m}\frac{(\epsilon_i-\epsilon_j)}{(\epsilon_i+\epsilon_j)}\left\{\frac{{q_i}^2}{2\epsilon_i\, d_i}\right\}; \quad (1b)$$

Shielding Energy;

$$PE_{shield} = \frac{1}{2}\frac{1}{(4\pi\epsilon_0)}\sum_{\substack{i=1\\ j=1}}^{n,m}\frac{q_i q_j}{(d_{ij})}\frac{(\epsilon_i-\epsilon_j)}{(\epsilon_j+\epsilon_i)}\left\{\frac{1}{\epsilon_i}-\frac{1}{\epsilon_j}\right\}; \quad (1c)$$

where q_i, q_j are the charged residues in dielectric medium of dielectric constant, ϵ_i, ϵ_j respectively and d_i, d_j are the distances of the respective charged residues from the dielectric interface and d_{ij} is the distance between two charges. The ½ factor in the coulomb energy and the shield energy is to eliminate the duplicity of the summation on i^{th} and the j^{th} particles.

From the superposition principle, the total electrostatic potential energy PE_{total} of the system of charges present on S3b and S4 helices will have three prominent contributions.

$$PE_{total} = PE_{coulomb} + PE_{self} + PE_{shield}.$$

3 RESULTS AND DISCUSSION

The computation study of the voltage gated ion channel of KvAP follows the flow chart (Figure 3) to predict the probable conformation of the S3b-S4 pair at zero transmembrane potential. From the Protein Data Bank (PDB 1ORQ) the sequence of the voltage gated potassium ion channel of KvAP is selected. A section of the VSD of 34 residues is considered forming helix-turn-helix S3b-S4 pair. The S4 α-helix is presumed to be more mobile than S3b. With all probability the S4 helix can have all possible motions; (1) rotational motion (θº) about its helix axis, (2) translational motion (x Å) along its axis and (c) oscillatory motion (βº) in the plane parallel to S3b. The interaction potential energy between the charged residues of S3b (+N3, -E107, +H109, -C3) and S4 (+N4, +R117, +R120, +R123, +R126, +R133, -C4) α-helices is calculated by rotating, translating and oscillating S4 with respect to the negative charged residue E107 of S3b. The interaction potential energy varies with the motion of S4 as all the charges between S3b and S4 move closer to or farther away from each other. The opposite charges produce negative or attractive interaction energy while the similar charges produce positive or repulsive energy. The total potential energy is the summation of all the interactions.

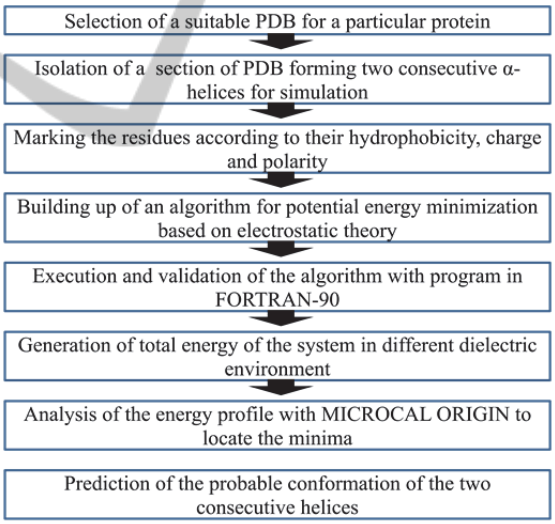

Figure 3: The flow chart showing the pathway to predict the probable conformation of protein.

The S3b-S4 helix pair is a part of the VSD which is embedded in protein. However, helix S4 is particularly being too mobile gets partially exposed to lipid. The variation of the exposure depends on the inclination of S4 into the hydrophobic lipid (Figure 4). Therefore the charged residues get exposed to either protein or lipid. Depending upon the exposure to different dielectric membrane the total energy varies even though the inclination of the S4 is fixed. At different angular rotation (θ) of S4

different arginines is at the lipid-protein interface e.g. residues R1, R2, R3 and R4 are at 120° and 240°, 195° and 285°, 270° and 330° and 15° and 345° respectively (Figure 4). The potential energy (PE) of the S3b-S4 system of charges is generated by the algorithm run by the program in Fortran-90 keeping S3b stationary and S4 in motion by rotation, translation and oscillation. S4 helices laden with positively charged residues have a strong attractive interaction with E107 of S3b comparable to the interaction with other charges (Mapder, 2012).

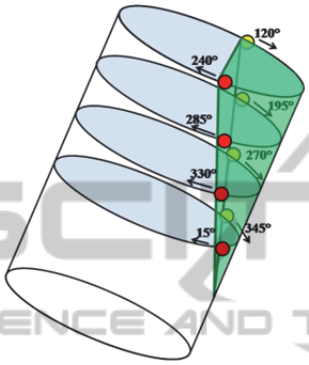

Figure 4: Schematic diagram of S4 helix partially exposed to lipid (green). R1, R2, R3 and R4 (different levels from the top) at the lipid-protein interface at an angular rotation (labeled) of S4 about its axis; (red dot-in front; yellow dot-at the back). (The figure is generated by 3D Max).

The contour (Figure 5) shows the gradation of PE of S3b-S4 α-helix pair at different configuration. The interaction is attractive and comparatively stronger when the positive arginines residues (R1, R2, R3, R4, R5) are at the vicinity of the negative E107 of S3b, showing five low energy contour-line at different (θ°, xÅ) coordinates e.g. (0°,0.0Å), (60°, 4.5Å), (120°, 9.0Å), (180°, 13.5Å) and (200°, 24.0Å) respectively. The S3b-S4 pair in different dielectric medium has different conformations. When the helix pair is in uniform dielectric (e.g. protein ε=10.0) environment (Figure 5a) the arginine (R123) of S4 is at the vicinity of the negative E107 of S3b, while when this pair is in a hybrid dielectric environment (lipid ε_l=2.0, protein ε_p=10.0 and water ε_w=80.0) (Figure 5b) the S4 takes a new conformation, with R126 of S4 facing the E107 of S3b.

There are various evidences (Borjesson, 2008) showing that the arginine of S4 has a vital role in the gating process. Our theory explains that these charged residues are also responsible for the mutual conformation of S3b-S4 pair at zero potential. By virtual mutagenesis of individual arginine of S4 the energy range and its gradation changed. On mutating each charged residues, each contour plot (Figures 5c-5f) shows only four such low energy contours indicating that there is no energy contribution of the missing charged residue at their respective positions. In absence of R117 the energy of S3b-S4 has energy minimum at θ=285° i.e. none of the charges are facing S3b, while in absence of R120 the energy minimum is at 175° i.e. R126 is at the vicinity of the S3b. Virtual mutagenesis explains that each residue has a role in conforming S3b-S4 together at zero potential.

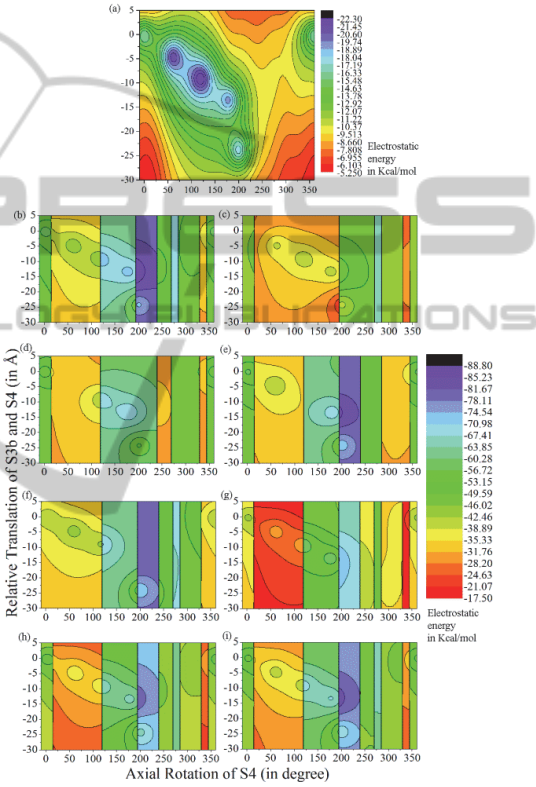

Figure 5: Electrostatic potential energy contour of the system of charges of S3b-S4 pair with axial rotation (θ°) and translation (xÅ) of S4; in (a) uniform medium (b) hybrid medium. With virtual mutagenesis of (c) R1, (d) R2, (e) R3, (f) R4, (g) R5 (h) N4, (i) C4. The vertical lines indicate the lipid-protein interface faced by the charged residues. (The figure is generated by Microcal Origin).

The figures (5g and 5h) shows the change in the range of the total energy when the dipolar terminal charges are neutralized. Therefore, these end terminal charges even though they are weaker; they do have a contribution in the stabilization of the S3b-S4 conformation.

When S3b-S4 pair is in hybrid dielectric medium environment, the vertical contour explains the position of the respective charges near the lipid-

protein interface. Comparing Figure 3 and Figure 5(b-i), it is quite apparent that whenever the environment of any one of the charge changes the total energy also changes. If the change in the dielectric constant is from a lower to a higher value (e.g. from lipid to protein) the potential energy of the system, changes from a lower to a higher range and vice versa. It is an inherent property of any system is to minimize its potential energy to attain stability. This explains that the interaction of the charge with the hydrophobic lipid membrane is favored.

4 CONCLUSIONS

The helix-turn-helix S3b-S4 pair in voltage sensor domain of KvAP tries to accommodate, in a membrane-spanning multi-dielectric environment at zero potential, in such a fashion that the electrostatic coupling of the inter-helix charges can attain a minimum potential energy electromechanical equilibrium. The stable conformations of the helix pair is dependent not only on the inter helix charge interaction but also on the hybrid dielectric environment, which supports the conformation. For proper understanding of the mechanism of voltage sensor, it is important to know the zero potential conformation of the S3b-S4 couple in the appropriate hybrid dielectric environment. This can give an insight to the researchers working in this field to trace the movement of the VSD under the influence of the variable transmembrane voltage. Our program can be applied to all types of protein which are in the form of amphipathic helices.

ACKNOWLEDGEMENTS

This work is sponsored by SR/SO/BB/0080/2009 of the Department of Science and Technology, Govt. of India.

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