Research Article

Effect of dielectric interface on charge aggregation in the voltage-gated K^+ ion channel

Lipika Adhya, Tarunendu Mapder, Samit Adhya

Department of Engineering Physics, B. P. Poddar Institute of Management and Technology, Kolkata, West Bengal, India

Address for correspondence:
Dr. Lipika Adhya, No. 137, VIP Road, Kolkata - 700 052, West Bengal, India.
E-mail: ladhya.bppimt@gmail.com

Abstract

Background: There is experimental evidence of many cases of stable macromolecular conformations with charged amino-acids facing lipid, an arrangement thought to be energetically unfavourable. **Methods and Objectives:** Employing classical electrostatics, we show that, this is not necessarily the case and studied the physical basis of the specific role of proximity of charges to the dielectric interface between two different environments. We illustrate how self and induced energies due to the dielectric medium polarization, on either side of the interface, contribute differentially to the stability of a pair of charges and hence the mutual conformation of the S3b-S4 α-helix pair of the voltage-gated K^+ channel. **Results and Conclusion:** We show that (1) a pair of opposite charges on either side of lipid-protein interface confers significant stability; (2) hydrophobic media has an important role in holding together two similar repelling charges; (3) dielectric interface has stabilizing effect on a pair of charges, when an ion is closer to its interface than its neighboring charge; (4) in spite of the presence of dielectric interface, there is a nonexistence of any dielectric effect, when an ion is equidistant from its image and neighboring charge. We also demonstrate that, variation in dielectric media of the surrounding environment confers new mutual conformations to S3b-S4 α-helices of voltage sensor domain at zero potential, especially lipid environment on the helix side, which improved stability to the configuration by lowering the potential energy. Our results provide an answer to the long standing question of why charges face hydrophobic lipid membranes in the stable conformation of a protein.

Key words: Electrostatic theory, induced energy, lipid, self energy

INTRODUCTION

Biological systems (e.g., cell membranes in cellular solution) are viewed as dielectric material with gradual variation in dielectric constants[1] with lipid (ε_l~2.0), protein with charged residues (ε_p~10.0),[2] the phosphate head group (ε_{ph}~30.0)[3,4] and the cellular ionic solution (ε_w = 80.0). In K^+ ion channel, alpha helices are embedded in a hydrophobic lipid membrane with phosphate head groups and ionic solution on top and bottom. Since a dielectric discontinuity exists at the interface between any two different media, the interactions between charges and dielectric media can be described by incorporating image charges[5] to account for the polarization of the medium.

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.149120

The electrostatic basis of interaction between charges present on the surface or interior of the protein affects its structure and function. Proteins with α-helix secondary structure possess inherent extracellular and intracellular terminal dipole charges,[6] which interact, with the charged amino-acids[7] and with the multi-dielectric environment[8] to affect the minimum energy and bring stability to the system of charges of S3b-S4 helix pair of the voltage sensor domain (VSD) of voltage-gated K^+ ion channel. The accommodation of the protein and its changing conformation in the lipid membrane is crucial to many biological processes, e.g., gating of ion channels and signal transduction.[9] However, the specific role of the dielectric medium on the aggregation of a pair of charge, which is one of the basic principal of the accommodation of

a protein in its hybrid dielectric environment, is still not clear until date.

Here, we have schematically presented interaction between charge pairs and multi-dielectric media to study the variation of different energies (coulomb, self and induced) due to polarization of neighboring dielectric environment, leading to the stability of aggregation of a pair of charges. The S3b-S4 α-helix pair of VSD of the K^+ ion channel is a highly mobile pair which senses trans-membrane voltage and changes its conformation to initiate a gating process,[10] but at zero potential their different mutual conformation is dependent on the physical property of the surrounding membrane.[11,12] Using S3b-S4 α-helix pair at zero potential as an example of a protein in a composite dielectric surrounding, we have studied the variation in the conformation of S4 exposing the hydrophilic side of S4, laden with charged residues, to hybrid dielectric environment, especially hydrophobic lipid.

MATERIALS AND METHODS

Dielectric media are electrically neutral unless there is an external electric field or any other charges present to polarize it. Due to this polarization, the total potential energy (PE) of the system either gets reduced or enhanced, affecting the stability. When charges are in a single dielectric medium (ε); the coulomb energy Eq. (1) predominates, but when they are near a dielectric interface, the induced (image) charges interact with original charges producing self and induced energy Eqs. (2 and 3). The total PE Eq. (4) of a pair of charges at the vicinity of a dielectric interface is the sum of all the following energies:

Coulomb energy $$C_{ij}(\theta, x) = \frac{1}{2}\sum_{i,j}^{n,m} \frac{1}{4\pi\varepsilon_0\varepsilon_i} \frac{q_i q_j}{d_{ij}(\theta, x)} \quad (1)$$

Induced energy $$I_{ij}(\theta, x) = \frac{1}{2} \frac{1}{4\pi\varepsilon_0} \sum_{i,j}^{n,m} \frac{q_i q_j}{d_{ij}(\theta, x)} \frac{(\varepsilon_i - \varepsilon_j)}{\varepsilon_i(\varepsilon_i + \varepsilon_j)} \quad (2)$$

Self-energy $$S_{ij}(\theta, x) = -\frac{1}{4\pi\varepsilon_0} \sum_{i,j}^{n,m} \frac{q_i^2}{2d_i(\theta, x)} \left(\frac{\varepsilon_i - \varepsilon_j}{\varepsilon_i + \varepsilon_j}\right) \frac{1}{2\varepsilon_i} \quad (3)$$

Total potential energy $$PE\ (\theta, x) = C_{ij}(\theta, x) + I_{ij}(\theta, x) + S_{ij}(\theta, x) \quad (4)$$

ε_0 is the dielectric constant of free space and d_{ij} is the distance between charges q_i and q_j. The helices are rotated by θ° and translated by xÅ. d_i and d_j are the distances of the dielectric interface from the respective charges (q_i, q_j) and their images. Dielectric constants ε_i and ε_j are on either side of the interface.

To understand the effect of dielectric interface on the interaction between two opposite or similar charges (OC or SC), we have considered two regions; region-I having fixed (ε_1 = 10.0 i.e., protein) and region-II having variable (ε_2 = 1.0–80.0) dielectric constant respectively [Figure 1]. In the first scheme, OC and SC are embedded in the same medium (SM) on one side of the interface (region-I), while in the second scheme two charges are in different media (DM) on either side of the interface (one in each region). Therefore, the four possible combination of interactions of a pair of charges at the proximity of the interface are:

1. Opposite charges in the same medium (OCSM),
2. Similar charges in the same medium (SCSM),
3. Opposite charges in different media (OCDM) and.
4. Similar charges in different media (SCDM). The purpose of selecting these schemes is to simulate the interactions between charges embedded in or on the surface of the membrane protein (ε_p = 10.0) with their hybrid dielectric environment ranging gradually from hydrophobic ε_l = 2.0 to hydrophilic ε_w = 80.0.

Due to the changing conformations of the helices in an ion channel, the distances 'dc' between two neighboring charges and 'ds' between charge and its image (ds/2 is the distance of the charge from the interface) can vary. Therefore, the variation of the PE with dielectric constant is studied when charge is:

1. Closer to (ds = 4, dc = 8);
2. Equidistant from (ds = 8, dc = 8); and
3. Farther away from (ds = 8, dc = 4) its image than the neighboring charge.

The current approach is based on the most important universal principles Eq. (1-3) of the electrostatic theory between a pair of charges in the hybrid dielectric environment. This approach giving an exact solution has been applied to the voltage-gated K^+ channel of Aeropyrum pernix (KvAP). Following the sequence of pdb 1ORQ of KvAP,[11] we have considered the interaction between all the charged residues and the dipolar charges of two α-helices (S3b and S4) with their actual charges and position in their appropriate hybrid dielectric environment. We have also compared this structure to that of the X-ray and to the nuclear magnetic resonance (NMR) structure of the KvAP VSD. The S4 α-helix with two inherent terminal dipolar charges (+N4 and -C4) carries five positive arginine residues R1-R5 (R117, R120, R123, R126 and R133) interact with S3b α-helix possessing two dipole charges (−C3 and +N3), negative glutamic acid (−E107) and positive histidine (+H109). All these charges interact with surrounding media; the S3b-S4 macrodipole pair of VSD being at the periphery of the ion channel, it has a probability of being exposed to all types of media at least partially. The total PE of the system of charges for S3b-S4 α-helix pair is the summation of the above interactions between all

the charges of the helix pair and with their respective composite dielectric environment.

To compute the total interaction PE of a pair of charges at the dielectric interface as a summation of coulomb, self and induced energy, we have developed a Linux based program in Fortran 90, in two dimensions and have customized a three-dimension program to evaluate the interactions between the charged residues of the S3b-S4 α-helix pair with their multi-dielectric environment from all sides at the closest proximity. This program is a numeric solver that includes dielectric shielding or screening Eq. (3) for any type of ion channel with ampiphilic α-helices. Using this program we have shown previously[7,8] that apart from the positive charged arginines of the S4 α-helix, the terminal dipole charges have considerable role and the negative charged residue Glutamic acid of S3b α-helix has a dominant role in the aggregation of the S3b-S4 α-helix pair as a paddle. The overall protein shape is considered irregular due to the mobility of the α-helices conforming to variable configuration in hybrid dielectric environment. However, the dielectric interface at the proximity of the charges is assumed to be planar to evaluate image charge. The individual S3b and S4 α-helices are considered as three-dimensional cylinders with charged residues on the cylindrical surface and dipolar charges on the center of the cross-section on the either ends. The position of the charged residues is taken on the basis of the pitch (1.5Å/residue) and the diameter (14Å) of an alpha helix. The distances between the charges and from the dielectric interfaces are computed for all possible positions of the charges on S3b and S4 helices, when S3b is kept fixed and S4 translated (xÅ) along and rotated (θ°) about its axis. The coulomb, self, shield energies, their summed total and the minimum PE of the system of charges of the S3b-S4 helix pair is computed in a three dimensional matrix. The data are analyzed and graphically represented by the software origin pro 8.0.

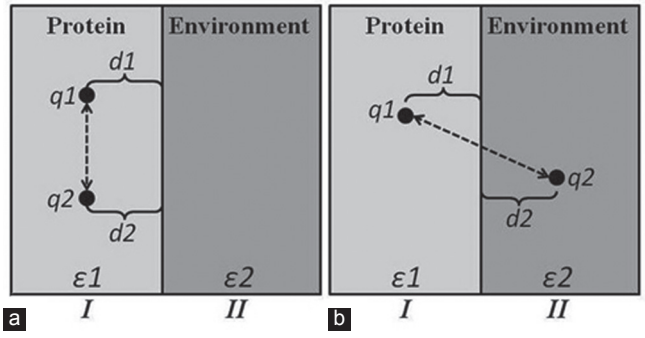

Figure 1: Electrostatic interaction between two charges, q_1 and q_2 in the presence of dielectric interface: Region-I (ε_1 = 10) and region-II (ε_2 = 1-80). d_1 and d_2 are distances from the interface. Charges are (a) on the same side or (b) on the opposite sides of the interface

RESULTS AND DISCUSSIONS

Role of the dielectric environment on the aggregation of a pair of charges

Figure 2 illustrates the profiles of coulomb energy (*C*), sum of coulomb and induced energy (*C* + *I*), sum of coulomb and self energy (*C* + *S*) and total PE (sum of coulomb, induced and self-energy (*C* + *I* + *S*). As a function of the variable dielectric constant ε_2 of the environment, the profiles show how coulomb energy gets modulated by the induced and self energies, in the presence of dielectric interface, resulting in a new profile of PE, which varies from positive to negative values. At $\varepsilon_1 = \varepsilon_2 = \varepsilon_p = 10.0$, the two regions have identical medium, therefore, the interface does not exist and the induced and self energies due to dielectric interface, vanishes. Hence, at this coordinate, all the four profiles of (*C*), (*C* + *I*), (*C* + *S*) and (*C* + *I* + *S*) converge and PE is same as the coulomb energy.

The interacting OC or SC pair 8Å apart, possesses negative or positive coulomb energy respectively. With both the charges in the SM of fixed dielectric constant ($\varepsilon_1 = \varepsilon_p$) and 2Å away from the interface, the system has constant coulomb energy (OCSM [Figure 2a] and SCSM [Figure 2b]). But when two charges are on either side of the interface, with q_1 and q_2 embedded in ε_1 and ε_2 dielectric medium respectively, the magnitude of this energy decreases sharply for $\varepsilon_1 > \varepsilon_2$ and gradually for $\varepsilon_1 < \varepsilon_2$, (OCDM [Figure 2c] and SCDM [Figure 2d]). With ε_1 = 10.0 (protein) and ε_2 = 2.0 (lipid); the coulomb energy can be as low as −12 Kcal/mol [Figure 2c] for OC and can be as high as +12 Kcal/mol [Figure 2d] for SM. Thus the coulomb effect is stronger when at least one of the charges is in the lower dielectric medium and the protein-lipid interface is favored by the charge to attain stability.

The profile (*C* + *I*) shows modulation of coulomb energy by the induced energy, which in general diminishes the magnitude of the coulomb energy illustrating a shielding effect or solvent screening effect when the dielectric medium is ionic or ε = 80.0. The solvent screening or shielding effect of the induced energy of OC in protein, interacting with the protein-water interface ($\varepsilon_1 < \varepsilon_2$), [Figure 2a] has been described previously.[13,14] Hence, here we report that when the dielectric medium of the environment is lower ($\varepsilon_1 > \varepsilon_2$), with both the charges in the SM (OCSM, SCSM) (Figure 2a and b), the induced energy magnifies the coulomb energy (energy enhancement). We show that for a pair of OC facing the protein-lipid interfaces [Figure 2a], the energy gets minimized assisting stabilization. This illustrates that induced energy does not always shields the coulomb effect, but supports aggregation of charge pairs, improving stability in presence of lipid.

The effect of the self energy on the coulomb energy ($C + S$) illustrates that, in general, the self energy helps to lower the coulomb energy improving stabilization, except for $\varepsilon_1 > \varepsilon_2$, when both charges are in the SM facing hydrophobic environment (OCSM, SCSM), the selfenergy raises the coulomb energy [Figure 2b]. But, when at least one of the charges on either side of the interface ($\varepsilon_1 > \varepsilon_2$) is exposed to the lower dielectric medium (OCDM, SCDM), the self energy lowers the coulomb energy even further to as low as −23 Kcal/mol at $\varepsilon_2 = 2.0$ [Figure 2c], facilitating the exposure of charge to the hydrophobic medium (lipid). Therefore, the image charge has both enhancing and reducing effects on the coulomb energy profile.

The profile of the total PE of a pair of charges ($C + I + S$) shows that irrespective of the nature of the charges, the total energy is always negative signifying stability at the protein-water ($\varepsilon_p - \varepsilon_w$; $\varepsilon_2 = 80.0$) dielectric interfaces. When at least one of the charges is exposed to hydrophobic medium (lipid; $\varepsilon_2 = 2.0$) [Figure 2c and d], PE is as low as −17 Kcal/mol [Figure 2c], which is lower than the total PE when charges are in the vicinity of hydrophilic medium. This is justified by the well-known fact[15] that a charge pair when exposed to water gets solvated gaining stability, but here we show that the stability is enhanced when exposed to lipid. Therefore, charges have the tendency to prefer hydrophobic over hydrophilic environments.

Moreover, SM on either side of the interface SCDM having positive coulomb energy, possess a negative total PE in the presence of the hydrophobic interface ($\varepsilon_1 > \varepsilon_2$) [Figure 2d]. At ($\varepsilon_2 = 2.0$) the combined effect of induced and self energy lowers the repulsive coulomb energy from +12.0 Kcal/mol to attractive PE -5.0 Kcal/mol, supporting aggregation. This is a new finding that highlights the importance of the hydrophobic medium holding together two similar repelling charges.

Hence, the role of the hydrophobic interface is substantially important in establishing stability not only between OC but also between a pair of SM.

Effect of the distance of the charges from the dielectric interface

The voltage-gated K^+ ion channel has conformational variability, as the solution and X-ray crystal structures[11,12] exhibit several differences throughout the VSD, due to which the charged residues on the α-helices, not only are exposed to different dielectric environment, but their distances from the interface and charges of the neighboring helices also change. Hence, the PE at the vicinity of the dielectric interface is dependent on the distances (dc) between two charges and their distance (ds) from their image charges. We have computed PE by considering three cases with distance of a charge from its image being greater than (ds > dc), equal to (ds = dc) or less than (ds < dc) the distance between two charges.

In general, for a pair of charges with hydrophobic environment ($\varepsilon_1 > \varepsilon_2$) (e.g., lipid) it is observed that distance of a charge from its image charge when lesser than (ds = 4.0, dc = 8.0), equal to (ds = 4.0, dc = 4.0) or greater than (ds = 8.0, dc = 4.0) the distance between two charges, the self energy is greater, equal or lesser in magnitude than the induced energy respectively [Figure 2].

With OCSM, the self energy is higher but the induced energy is lower than the coulomb energy. As a result, when ds > dc, PE is as low as −14 Kcal/mol [Figure 2i] bringing more stability, in comparison to −2.5 Kcal/mol [Figure 2a] when ds < dc. In contrast, with SM SCSM; irrespective of the magnitude of ds and dc, the effect of induced and self energy is always additive, raising PE above coulomb energy, [Figure 2b and f] and producing instability (+17 kcal/mol) with ds > dc [Figure 2j]. But with ds = dc, PE is equal to coulomb energy [Figure 2e], the effects of the induced and self energy nullifying each other. This is an interesting and new observation, i.e., in spite of the presence of the dielectric interface; there is nonexistence of any dielectric effect.

Generally, with two charges on either side of the interface (OCDM, SCDM) and one of the charges in the hydrophobic medium, PE is lower than the coulomb energy, stabilizing the aggregation, irrespective of the distance ds and dc. However, it is interesting to note that opposite charges closer to the low dielectric interface have lower interaction energy conferring better stability, that is, for OCDM at ds = 4.0Å and 8.0Å the energies are PE = −17 Kcal/mol and -12 Kcal/mol respectively [Figure 2c and g] and for SCDM at ds = 4.0Å and 8.0Å, the energies are PE = −4.0 Kcal/mol and +1.0 Kcal/mol [Figure 2d and h] respectively. This shows that the distance from the dielectric interface not only improves stability but can trigger stabilization of an unstable system.

Effect of multi-dielectric exposure of the S3b-S4 helix pair in KvAP

Assuming the antiparallel S3b-S4 α-helix macrodipole pair of VSD is a "paddle,"[16] with S3b beside S4, the dielectric medium on the helix side of both S3b and S4 is considered to be the same for both. There is no hard evidence for this assumption except that S3b and S4 are stuck together in all available crystal structures, and our theoretical study has also shown that there is a strong electrostatic interaction between them.[8] But the exposure of the extracellular and intracellular end of the S3b and S4 helices can be different, due to the flexibility of the paddle and deep

water-filled crevices, which have been found by electron paramagnetic resonance (EPR) spectroscopy of an isolated VSD of KvAP.[17] S3b being in the interior, all the charges (+N3, −C3, -E107 and +H109) are embedded in protein, while on the periphery of the ion channel, S4 α-helix is assumed to be half exposed to the protein and the other half to the dielectric medium of the environment on the helix side. Figure 3 illustrates a schematic molecular picture of the possible minimum PE stable S3b-S4 conformation of KvAP in the hybrid dielectric medium as tabulated in Table 1. Therefore, in S3b-S4 pair, each α-helix experiences three interfaces on three sides (i.e., extracellular end, helix side and intracellular end). The interfaces on three sides of S3b are ε_p-ε_{3e}, ε_p-ε_s and $\varepsilon_p\varepsilon_{3i}$ and of S4 are ε_p-ε_{4e}, ε_p-ε_s and ε_p-ε_{4i} [Figure 3a]. Protein (ε_p) being on one side of each interface; the other side of the three interfaces of S3b and S4 summarily are $\varepsilon_{3e}\varepsilon_s\varepsilon_{3i}$ and $\varepsilon_{4e}\varepsilon_s\varepsilon_{4i}$ respectively (e.g., for row 1 LLL of S3b and column 1 LLW of S4) [Table 1a]. The different possible interfaces experienced by S3b-S4 pair are protein-lipid (ε_p-ε_l), protein-water (ε_p-ε_w) and protein-protein (ε_p-ε_p; i.e., nonexistence of interface).

Each of the interactions between the charges of S3b and S4 α-helices, belong to any one of the classifications described in the previous section, e.g., OCSM is the type of interaction of negative charges (−E107 and −C3) of S3b α-helix with positive charges (+R1, +R2, +R3, +R4, +R5 and +N4) of S4 α-helix within the SM (protein; region-I) but when some of these charges are exposed to a different dielectric media of the environment (region-II), it is OCDM; similarly positive charges (+H109 and +N3) of S3b interacting with positive charges (+R1 to +R5 and +N4) of S4 within the protein, it is SCSM type of interaction but when exposed to two different dielectric medium, it is SCDM type. Hence, the total PE of S3b-S4 pair is the resultant effect of the interaction of the charged residues with dielectric interfaces from three sides (e.g., extracellular, helix side, and intracellular).

The electrostatic interaction predominates over other weaker interactions (e.g., covalent bond, Vander Waals etc.). The sum total PE, for combinations of six interfaces (three each for S3b (rows) and S4 (columns) α-helices) is always

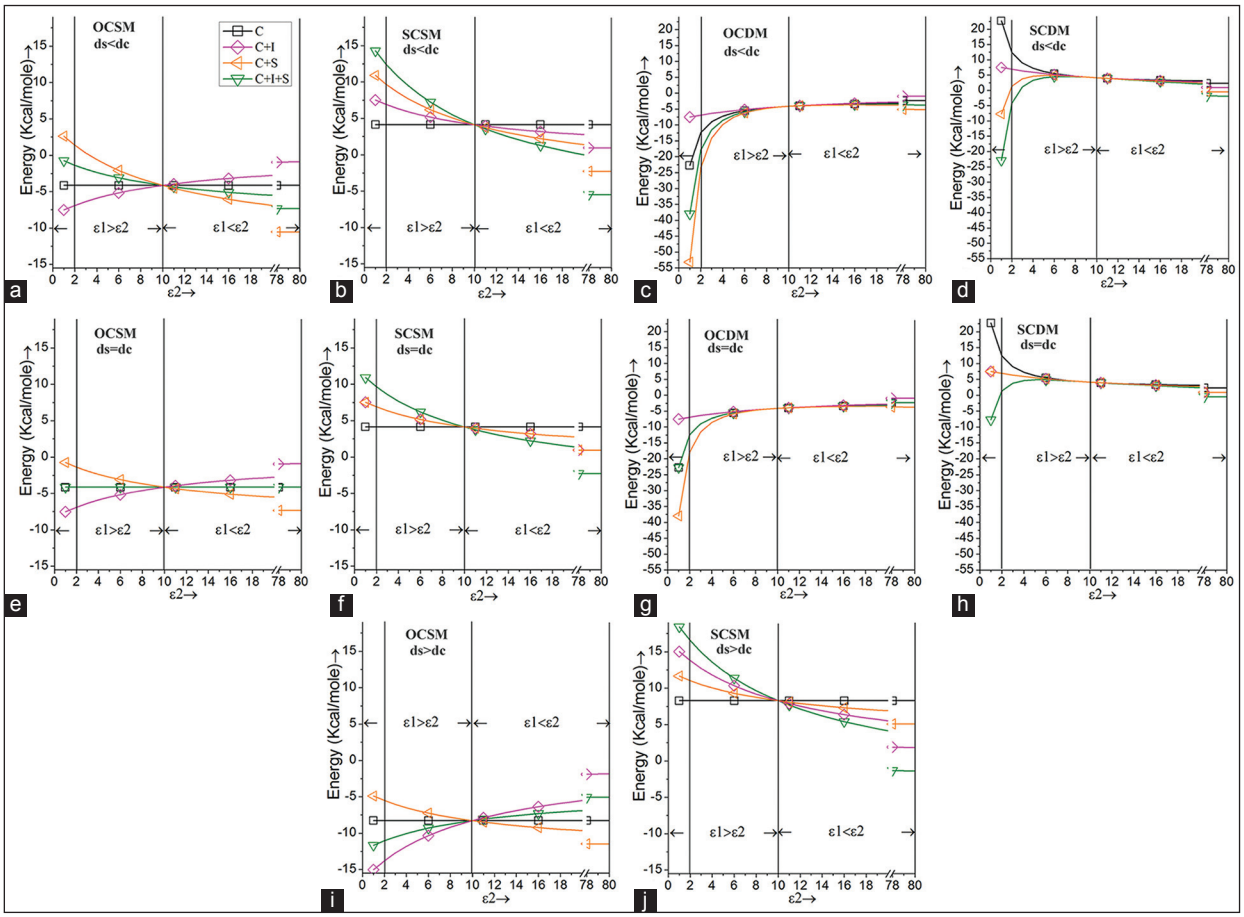

Figure 2: Electrostatic energy profiles with respect to the dielectric constant (ε_2) of the environment: Coulomb energy (black square), coulomb and induced energy (pink diamond), coulomb and self-energy (orange side triangle), total potential energy (green down triangle) at three different sets of distances: Column I, ds < dc (a) opposite charges in same medium (OCSM), (b) similar charges in same medium (SCSM), (c) opposite charges in different media (OCDM), (d) similar charges in different media (SCDM); Column II, ds = dc (e) OCSM, (f) SCSM, (g) OCDM, (h) SCDM; Column-III, ds > dc (i) OCSM, (j) SCSM

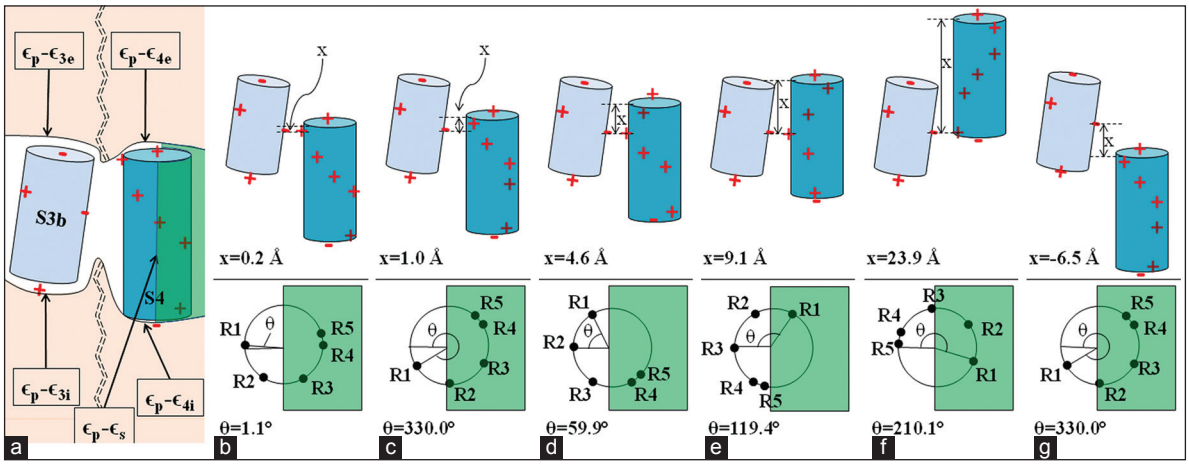

Figure 3: Schematic diagram of S3b-S4 α-helix (a) extracellular/intracellular medium (off white), lipid (green); dielectric interface (i) at the extracellular end (ε_p–ε_{3e}, ε_p–ε_{4e}) (ii) protein-helix side (ε_p–ε_s), (iii) protein-intracellular (ε_p–ε_{3i}, ε_p–ε_{4i}), charged residues (+ and –) (red); and different mutual positions of S4 helix (x, θ): (b) 0.2Å, 1.1°, (c) 1.0Å, 330°, (d) 4.6Å, 59.9°, (e) 9.1Å, 119.4°, (f) 23.9Å, 210.1°, (g) −6.5Å, 330°. Lighter shaded charges are behind the helices

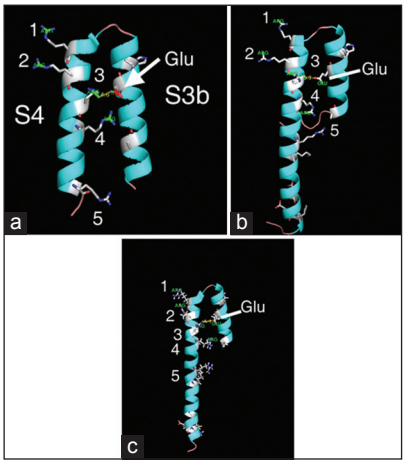

Figure 4: Orientation of S4 helix in the voltage sensor domain (VSD) of KvAP. (a) The S3b-S4 pair in the X-ray structure of intact KvAP (PDB code 1ORQ). (b) X-ray structure of isolated VSD of KvAP (1ORS). (c) Nuclear magnetic resonance structure of isolated VSD of KvAP (2KYH). The arginine residues 1-5 of S4, and the central glutamic acid residue of S3b, are indicated

negative [Table 1], which is a signature of stability. Here we show that from three sides, when S3b-S4 α-helix pair is exposed to a different combination of dielectric interfaces ($\varepsilon_{3e}\varepsilon_s\varepsilon_{3i}$ of S3b and $\varepsilon_{4e}\varepsilon_s\varepsilon_{4i}$ of S4), the S4 helix attains a new conformation (x, θ) [Table 1]. Tables 1a, 1b and 1c illustrate the minimum energy of stabilization (PE Kcal/mol) with lipid, water and protein dielectric environment on the helix side, respectively. There are some important observations.

When the S3b-S4 is exposed to lipid ($\varepsilon_s = \varepsilon_l$) from the helix side the energy of stabilization is the lowest, ranging from −96.307 Kcal/mol to −75.129 Kcal/mol [Table 1a]; in comparison to when it is exposed to protein ($\varepsilon_s = \varepsilon_p$) (−40.65 Kcal/mol to -7.80 Kcal/mol) [Table 1c] or water ($\varepsilon_s = \varepsilon_w$) (−29.56 to -5.40 Kcal/mol) [Table 1b]. Hence, partial exposure of the S3b-S4 pair to lipid from the side is preferred, for maximizing stability.

With lipid on the helix side and a variable cellular end environment, the S4 α-helix settles at different configurations ranging θ = 1.0° to 330° and x = -6.5Å to 23.9Å, with respect to S3b [Table 1a]. The most probable angular orientations of S4 with respect to S3b are θ = 330°, 210.1° and 1.1° [Figure 3b, f and c], which accommodate four (R2, R3, R4 and R5), two (R1 and R2) and three (R3, R4 andR5) arginine residues respectively in the lipid. For several hybrid environments, the angular orientation θ = 330° may be same but the translational shift x can be different (x = −1.1Å, −1.9Å, −6.5Å etc.,); hence the mutual position of S3b-S4 becomes different [Figures 3c and g]. Therefore with varied composite dielectric environment, S3b-S4 helix couple assumes different stable configuration, with at least one residue exposed to lipid.

There are experimental evidences of S4, that is not only in close contact with the lipid bilayer but the extracellular residues of S4 of KvAP are exposed to lipid.[16,18,19] Our theory shows that in several types of composite dielectric environment, more than one residue are exposed to the lipid and due to the helical position of the charged residues; there can be a possibility of the deformation of the membrane to accommodate all the charges. This is in agreement with the theoretical evidence that the S4 helix is stable if the membrane is deformable and the peptide insertion is highly unstable when no membrane bending is allowed, but considerably improved when the membrane bends to expose charged and polar amino-acids.[20]

With water on the helix side ($\varepsilon_s = \varepsilon_w$), the minimum energy angular orientation and translational position varies from

Table 1a: Conformational stability of S3b-S4 in hybrid dielectric environment

S3b (ε_{3e} ε_{s} ε_{3i})	S4 (ε_{4e} ε_{s} ε_{4i})								
	LLW	WLW	LLP	PLW	WLL	WLP	PLL	PLP	LLL
LLL									
E	−96.307	−96.010	−95.215	−93.964	−93.585	−92.960	−91.897	−91.563	−89.479
θ	1.1	330.0	1.1	330.0	210.1	330.0	210.1	1.0	1.2
x	0.2	−1.1	0.2	−0.4	23.9	−0.4	23.8	0.2	0.3
PLL									
E	−92.739	−93.966	−90.897	−91.763	−89.980	−90.760	−88.289	−89.087	−85.159
θ	330.0	330.0	1.1	330.0	210.1	330.0	210.1	330.0	1.2
x	0.2	−1.9	0.2	−0.8	23.9	−0.8	23.9	−0.2	0.3
LLP									
E	−92.540	−93.768	−90.699	−91.565	−89.781	−90.561	−88.091	−88.889	−84.960
θ	330.0	330.0	1.1	330.0	210.1	330.0	210.1	330.0	1.2
x	0.2	−1.9	0.2	−0.8	23.9	−0.8	23.9	−0.2	0.3
PLP									
E	−90.250	−92.366	−87.114	−89.451	−86.176	−88.447	−84.485	−86.655	−80.824
θ	330.0	330.0	330.0	330.0	210.1	330.0	210.1	330.0	330.0
x	0.0	−6.5	0.4	−1.4	23.9	−1.4	23.9	−0.5	1.0
WLL									
E	−90.069	−92.445	−86.928	−89.312	−85.774	−88.309	−84.083	−86.487	−80.632
θ	330.0	330.0	330.0	330.0	210.1	330.0	210.1	330.0	330.0
x	0.0	−6.5	0.3	−1.6	23.9	−1.6	23.9	−0.6	1.0
LLW									
E	−89.639	−92.014	−86.498	−88.882	−85.343	−87.878	−83.653	−86.056	−80.202
θ	330.0	330.0	330.0	330.0	210.1	330.0	210.1	330.0	330.0
x	0.0	−6.5	0.3	−1.6	23.9	−1.6	23.9	−0.6	0.0
WLP									
E	−87.617	−91.527	−84.427	−87.511	−81.975	−86.507	−80.279	−84.130	−78.091
θ	330.0	330.0	330.0	330.0	210.1	330.0	210.1	330.0	330.0
x	−0.4	−6.5	0.1	−6.5	23.9	−6.5	23.9	−1.1	1.0
PLW									
E	−87.385	−91.295	−84.195	−87.279	−81.743	−86.276	−80.047	−83.899	−77.859
θ	330.0	330.0	330.0	330.0	210.1	330.0	210.1	330.0	330.0
x	−0.4	−6.5	0.1	−6.5	24.0	−6.5	23.9	−1.1	1.0
WLW									
E	−84.851	−90.785	−81.539	−86.441	−77.550	−85.437	−75.842	−81.638	−75.129
θ	330.0	290.1	330.0	330.0	210.1	330.0	210.1	330.0	330.0
x	−1.2	−6.5	−0.2	−6.5	24.1	−6.5	24.0	−3.3	0.9

Minimum potential energy (E-Kcal/mol), angular position (θ°) and translational shift (xÅ) of S_4 with respect to S3b: $\varepsilon_{3e}\varepsilon_{s}\varepsilon_{3i}$ (row) and $\varepsilon_{4e}\varepsilon_{s}\varepsilon_{4i}$ (Column): Dielectric media on three sides of S3b and S_4 respectively, L: Lipid, P: Protein, W: Water, The dielectric medium of helix side is lipid ε_{s}=L

θ = 30.6° to 270.1° and x = 6.5Å to 33.5Å [Table 1b]. The most probable orientations of S4 are at 119.0° and 60.0°, with one (R1) or two (R4 and R5) arginine residues respectively exposed to water [Figure 3d and 3e]. Deep water-filled crevices at the extracellular and intracellular sides have been found by EPR spectroscopy[17] and in the X-ray and solution structures[11,12] of isolated VSD of KvAP. On both sides of the channel protein, water can penetrate these deep crevices, close to the center of the VSD, exposing the charged residues near both the terminals. In general, the Coulomb interaction is a long-range force, with next-nearest neighbours also contributing, so that the salt-bridge positions are not a priori obligatory equilibrium positions.[21]

With protein on the side (i.e., nonexistence of dielectric interface from the side), a narrow range of 1° of angular rotation (199.5° to 200.5°) and 2Å of translational shift (8.9Å and 9.1Å) is observed [Table 1c] with charged residue R3 of S3b α-helix in front of E107 [Figure 3e]. This exemplifies the predominance of the coulomb effect. The small variation is due to the effect of extracellular and intracellular dielectric interfaces. Therefore, we observed that the absence of an interface from the helix side has restricted the variation of the angular orientation as well as the translational position [Table 1c]. When the S3b and S4 are exposed to the protein from the helix side [Table 1c] with at least one of the cellular terminals exposed to the lipid, the stability is enhanced. This is consistent with our previous report on the role of dipolar terminal charges, which explained that the dipole charges exposed to lipid lowers the PE and these positive and negative half electronic charges on either end of a α-helix are not insignificant.[8]

Recent experimental studies suggest that changing the lipid composition alone, without a change in transmembrane voltage, causes the VSD to switch conformations.[22] There is also evidence of structures

Table 1b: Conformational stability of S3b-S4 in hybrid dielectric environment

S3b (ε_{3e} ε_s ε_{3i})	S4 (ε_{4e} ε_s ε_{4i})								
	LWL	LWP	PWL	LWW	WWL	PWP	PWW	WWP	WWW
LWL									
E	−29.555	−24.094	−23.881	−22.047	−21.310	−16.750	−14.147	−13.569	−9.946
θ	119.4	119.4	59.9	119.1	60.0	119.2	118.7	60.1	110.1
x	9.1	9.1	4.6	9.0	4.7	9.1	9.0	4.7	8.1
PWL									
E	−24.509	−19.049	−18.905	−17.004	−16.334	−11.706	−9.115	−8.593	−8.566
θ	119.4	119.3	59.9	118.9	59.9	119	116.7	60.3	270.1
x	9.1	9.1	4.6	9.0	4.6	9.0	8.8	4.7	9.3
LWP									
E	−24.310	−18.850	−18.706	−16.806	−16.135	−11.508	−8.917	−8.394	−8.367
θ	119.4	119.3	59.9	118.9	59.9	119.0	116.7	60.3	270.1
x	9.1	9.1	4.6	9.0	4.6	9.0	8.8	4.7	9.3
PWP									
E	−19.265	−13.805	−13.730	−11.769	−11.159	−6.467	−6.372	−5.700	−7.756
θ	119.4	119.2	59.9	117.5	60.0	118.1	110.1	90	110.1
x	9.11	9.1	4.6	8.9	4.6	9.0	−6.5	33.5	−6.5
WWL									
E	−18.646	−13.163	−13.099	−11.135	−10.528	−5.828	−6.537	−5.864	−7.921
θ	110.0	119.1	59.9	116.0	60.0	117.5	110.1	90.0	110.1
x	9.0	9.0	4.6	8.8	4.6	8.9	−6.5	33.5	−6.5
LWW									
E	−18.216	−12.732	−12.669	−10.705	−10.098	−5.398	−6.107	−5.434	−7.491
θ	110.0	119.1	59.9	116.0	60.0	117.5	110.1	230	110.1
x	9.0	9.0	4.6	8.8	4.6	8.9	−6.5	33.5	−6.5
WWP									
E	−13.763	−7.923	−7.927	−9.108	−8.578	−3.138	−6.157	−5.564	−7.629
θ	110.0	118.1	59.7	110.1	90.0	110.1	150.0	30.6	150.0
x	9.0	9.0	4.5	−6.5	−6.5	−6.5	−6.5	33.5	−6.5
PWW									
E	−13.531	−7.691	−7.695	−8.877	−8.347	−2.907	−5.925	−5.338	−7.397
θ	110.0	118.1	59.7	110.1	90.0	110.1	150.0	30.6	150.0
x	9.0	9.0	4.5	−6.5	−6.5	−6.5	−6.5	33.5	−6.5
WWW									
E	−8.066	−5.228	−5.255	−8.743	−7.977	−2.754	−5.840	−5.296	−7.312
θ	110.0	150.0	90.0	150.0	90.0	150.0	150.0	30.6	150.0
x	8.9	−6.5	−6.5	−6.5	−6.5	−6.5	−6.5	33.5	−6.5

Minimum potential energy (E-Kcal/mol), angular position (θ°) and translational shift (xÅ) of S_4 with respect to S_{3b}: $\varepsilon_{3e}\varepsilon_s\varepsilon_{3i}$ (row) and $\varepsilon_{4e}\varepsilon_s\varepsilon_{4i}$ (column) – dielectric media on three sides of S_{3b} and S_4 respectively. L: Lipid, P: Protein, W: Water. The dielectric medium of helix side is water ε_s=W

of the VSD of KvAP (PDB-1ORQ, 1ORS and 2KYH) achieved by different methods[11,12] in different hybrid environment, showing that the mutual conformation of S3b-S4 couple is not the same. In the 2KYH structure closest to the mean coordinates, S4 is shifted closer to S2 by ~3Å, while S3b is further away from S1 by ~5Å resulting in a ~23° twist in the orientation of the paddle with respect to S1 and S2 in comparison to the structure of 1ORS. This complies with our theoretical results of varied S4 conformation in hybrid dielectric environment without any transmembrane voltage.

Our computations revealed energy minima of the S3b-S4 helix pair at specific values of the rotational (θ) and translational (x) coordinates, that is, stable conformations of S4 in different hybrid dielectric media. A number of discrete conformational states (θ, x) were observed [Table 1a-c]: (1.2, 0.3), (60, 4.6), (119, 9), (210, 24), and (330, 0.2 to -6.5). In each state, a specific Arg side chain of S4 was appose to the lone Glu residue of S3b [Figure 3b-g]. We compared these theoretical conformations to the experimentally derived S3b-S4 structures of KvAP obtained by X-ray crystallography and NMR. In the conformation (119, 9), residue R3 of S4 is closest to the Glu107 of S3b [Figure 3e]. In both the X-ray[11] and NMR[12] structures of KvAP, this is what has been observed: In all the cases (1ORQ, 1ORS, 2KYH) R3 is apposed to the Glu of S3b [Figure 4]. Thus the theoretical (119, 9) conformation closely resembles the observed structures, validating the results obtained in the Table 1c.

The (119,9) conformation was observed in all cases when protein is on the side of S4, irrespective of the dielectric medium above or below the helix [Table 1c]; in the KvAP crystal structure, the molecules presumably would be close packed, with the peripheral S4 helix facing the protein of an adjacent molecule, thus favoring the (119,9) conformation. The (119, 9) state is stabilized in most cases when S4 is

Table 1c: Conformational stability of S3b-S4 in hybrid dielectric environment

S3b (ε_{3e} ε_s ε_{3i})	S4 (ε_{4e} ε_s ε_{4i})								
	LPL	PPL	LPP	WPL	LPW	PPP	WPP	PPW	WPW
LPL									
E	−40.647	−38.890	−38.207	−36.215	−35.838	−33.020	−30.887	−30.090	−28.082
θ	119.5	119.6	119.6	119.7	119.6	119.6	119.6	119.6	119.7
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
PPL									
E	−38.926	−30.520	−33.161	−31.169	−30.792	−27.974	−25.842	−25.044	−23.036
θ	119.6	119.5	119.6	119.6	119.6	119.6	119.7	119.7	119.7
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
LPP									
E	−38.727	−33.646	−29.638	−30.971	−30.593	−27.775	−25.643	−24.846	−22.837
θ	119.6	119.6	119.5	119.6	119.6	119.6	119.7	119.7	119.7
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
PPP									
E	−33.681	−28.600	−27.917	−25.925	−25.548	−19.405	−20.597	−19.800	−17.793
θ	119.6	119.6	119.6	119.7	119.7	119.5	119.7	119.7	119.8
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.0
WPL									
E	−33.039	−27.958	−27.274	−21.958	−24.905	−22.087	−19.955	−19.158	−17.151
θ	119.6	119.6	119.6	119.5	119.7	119.7	119.7	119.7	119.9
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.0
LPW									
E	−32.609	−27.527	−26.844	−24.853	−21.151	−21.657	−19.525	−18.727	−16.721
θ	119.6	119.6	119.6	119.7	119.5	119.7	119.7	119.7	119.9
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.0
WPP									
E	−27.795	−22.713	−22.030	−20.039	−19.661	−16.843	−11.386	−13.914	−11.912
θ	119.6	119.7	119.7	119.7	119.7	119.7	119.5	119.8	120.6
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	8.9
PPW									
E	−27.563	−22.482	−21.798	−19.807	−19.429	−16.611	−14.479	−10.357	−11.680
θ	119.6	119.7	119.7	119.7	119.7	119.7	119.8	119.5	120.5
x	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	8.9
WPW									
E	−21.676	−16.595	−15.912	−13.921	−13.543	−10.725	−8.594	−7.797	−10.863
θ	119.7	119.7	119.7	119.9	119.9	119.8	120.5	120.5	179.8
x	9.1	9.1	9.1	9.1	9.1	9.1	9.0	9.0	−6.5

Minimum potential energy (E-Kcal/mol), angular position (θ°) and translational shift (xÅ) of S4 with respect to S3b: $\varepsilon_{3e}\varepsilon_s\varepsilon_{3i}$ (row) and $\varepsilon_{4e}\varepsilon_s\varepsilon_{4i}$ (Column): Dielectric media on three sides of S3b and S4 respectively, L: Lipid, P: Protein, W: Water. The dielectric medium of helix side is protein ε_s=P

exposed to water on the side [Table 1b]. Remarkably, with the S4 helix exposed to lipid at the side, the (119, 9) state was never observed [Table 1a]. The available X-ray structures of KvAP have not detected lipid associated with the VSD. In a chimaeric Kv1.2 channel co-crystallized with lipid,[23] patches of lipid were observed to be associated with the tetramer, but the VSD appears to be largely lipid-free. NMR studies of VSD-lipid micelles indicate only an overall association without revealing the specific environment of S4 with respect to S3b. Exposure of S4 to the aqueous medium would explain the observation of the (119, 9)-like conformation in this structure also. In the native membrane-bound state, the periphery of the ion channel, including the S4 helix, would be in direct contact with the lipid bilayer, and not surrounded by protein, as in X-ray crystals. Thus, the S4 conformation revealed by these studies is likely to be an artifact of the observation method rather than a reflection of the native state.

With lipid on the side of S4, a number of stable states were observed, depending upon the medium to which either end is exposed [Table 1a]. With lipid at the intracellular end, and water or protein at the extracellular end (column 5 and 7) of the S4, the (210, 24) conformation was favored. With the water at the intracellular end, a number of states with the same angular rotation (θ = 330°) but varying translational coordinates (x = 0.2Å to 6.5Å) were stabilized (Column 1, 2 and3). Along the translation axis, a range of displacement of −6.5Å to +24Å, that is, close to the bilayer thickness was observed. These results suggest that, during translation across the bilayer (e.g., under the influence of an external electric field), the lipid-exposed S4 helix can stabilize at different positions without rotation (θ = 330°), or jump with rotation from a low x value (0.2Å to −6.5Å) at θ = 330° to a high value (+24Å) at θ = 210°. The observation of energy minima at discrete (θ, x) values is in accordance with translational as well as rotational motion of S4.

CONCLUSION

On investigating different combinations of all possible hybrid environments, we could conclude summarily that:

1. The protein-lipid dielectric interface has a profound effect on lowering total PE, with self and induced energies contributing to better stabilization of opposite ion pairs closer to the interface than to each other.
2. The hydrophobic medium has an important role in holding together two similar repelling charges on either side of the interface.
3. The self and induced energies can neutralize each other's effects, when ds = dc, so that PE is equal to coulomb energy, which exemplifies the virtual nonexistence of the dielectric interface.
4. The interactive pair of charges favors low hydrophobic dielectric interface to stabilize their aggregation.
5. When applied to the S3b-S4 voltage sensing paddle of the KvAP channel, the theory predicts several stable conformations in different hybrid dielectric environments.
6. With the S4 helix facing lipid at the side, several states stabilize along the translational and rotational axes.
7. The spatial relation between S4 and S3b observed in the X-ray crystallographic and NMR structures of KvAP corresponds to the conformational state stabilized with S4 in contact with protein or water at the side.

This report can potentially give an insight to experimentalists and theoreticians in finding the missing link between the natural conformation of the VSD of K^+ ion channel protein at zero potential and their X-ray or solution structures or the molecular dynamics simulations.

ACKNOWLEDGMENTS

This work is sponsored by the project no. SR/SO/BB/0080/2009 of the Department of Science and Technology (SERB), Government of India.

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How to cite this article: Adhya L, Mapder T, Adhya S. Effect of dielectric interface on charge aggregation in the voltage-gated K + ion channel. J Nat Sc Biol Med 2015;6:188-97.

Source of Support: Nil. **Conflict of Interest:** None declared.