



# Role of terminal dipole charges in aggregation of $\alpha$ -helix pair in the voltage gated $K^+$ channel

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## ABSTRACT

The voltage sensor domain (VSD) of the potassium ion channel KvAP is comprised of four (S1–S4)  $\alpha$ -helix proteins, which are encompassed by several charged residues. Apart from these charges, each peptide  $\alpha$ -helix having two inherent equal and opposite terminal dipolar charges behave like a macrodipole. The activity of voltage gated ion channel is electrostatic, where all the charges (charged residues and dipolar terminal charges) interact with each other and with the transmembrane potential. There are evidences that the role of the charged residues dominate the stabilization of the conformation and the gating process of the ion channel, but the role of the terminal dipolar charges are never considered in such analysis. Here, using electrostatic theory, we have studied the role of the dipolar terminal charges in aggregation of the S3b–S4 helix pair of KvAP in the absence of any external field ( $V=0$ ). A system attains stability, when its potential energy reaches minimum values. We have shown that the presence of terminal dipole charges (1) change the total potential energy of the charges on S3b–S4, affecting the stabilization of the  $\alpha$ -helix pair within the bilayer lipid membrane and (2) the C- and the N-termini of the  $\alpha$ -helices favor a different dielectric medium for enhanced stability. Thus, the dipolar terminal charges play a significant role in the aggregation of the two neighboring  $\alpha$ -helices.

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## 1. Introduction

The building block of the ion channel protein is the  $\alpha$ -helix.  $\alpha$ -helices are macrodipoles, a fact that has been known for a long time [1] but has often been ignored as an important property of  $\alpha$ -helices in a biological system. Each  $\alpha$ -helix of the ion channel protein, having a backbone of amides all pointing in one direction, from the negative C-terminus towards the positive N-terminus, with  $-0.5$  and  $+0.5$  proton charges at the two termini, respectively, behaves like a macrodipole [1] with a length of about  $1.5$  N Å and a net dipole moment of about  $3.5$  N Debye, where N is the number of residues [2]. In an  $\alpha$ -helix, the peptide backbones are aligned in such a fashion that nearly 97% of the peptide dipole moments point in the direction of the helix axis [3] (Fig. 1). Thus, a long  $\alpha$ -helix can produce a considerable electrostatic field. To obtain electrostatic stabilization, it is an inherent property of electric dipoles in a multimeric aggregate to settle adjacent to each other in anti-parallel orientation, such that the electric lines of force traveling from the positive end of one dipole to the negative end of the other follow the shortest path [4]. When the  $\alpha$ -helix dipoles are in such an anti-parallel sense, they confer significant electrostatic stabilization to the structural motifs of the protein [5].

However, the voltage dependent  $K^+$  ion channel is a homo-tetramer with six  $\alpha$ -helices S1–S6 in each subunit. The six transmembrane helices are primarily hydrophobic, with most of the positive charges of the amino acid side chain on S4 located at every third residue; a few other positive and negative charges are scattered on the other helices. In the structures of KvAP obtained by different methods [6–8] the S3b and S4 always stay together, while the other helices of the voltage sensor domain (VSD) present different spatial orientations. Each of these helices forms a macrodipole and can potentially contribute to the electrostatic field. In various experimental and theoretical studies, emphasis is given to the side-chain interactions [9–12] while the role of the dipolar charges is ignored. Here, we have used an S3b–S4 pair of the VSD as an example to determine the contribution of the terminal dipolar charges of the S3b–S4 pair in stabilization of their aggregation. Furthermore, since the S3b–S4  $\alpha$ -helices are quite mobile and the dipolar charges on either end can potentially get exposed to polar (aqueous medium) or non-polar (membrane) environments, we have considered the role of the dielectric properties of the media in such interactions.

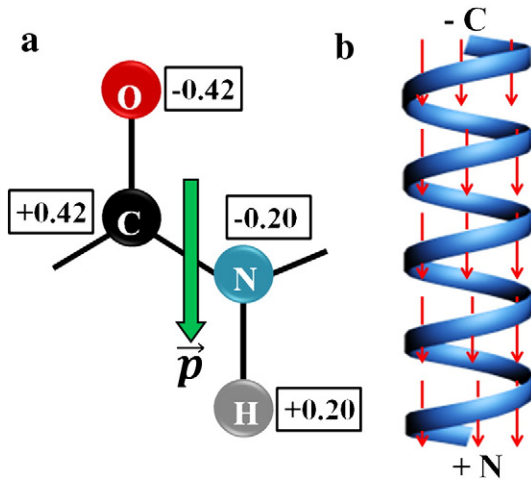
## 2. Theory

### 2.1. 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

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**Fig. 1.** Origin of macrodipoles. (a) Dipolar charges on a peptide bond. (b) Multiple peptide dipoles aligned along the axis of the  $\alpha$ -helix summate to produce a macrodipole.

mutual potential energy (PE) of two interacting adjacent macrodipoles depends upon their dipole moment ( $\vec{p}$ ) and varies with their relative angular separation ( $\theta$ ) [4]. The two dipoles tend to orient so as to achieve the minimum PE of the system. The lower the PE, the more stable is the conformation. When they are parallel ( $\theta=0^\circ$ ) the PE is at the maximum; when perpendicular ( $\theta=90^\circ$ ) PE is zero, and when antiparallel ( $\theta=180^\circ$ ), the PE reaches a minimum value.

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 ( $PE_{\text{coulomb}}$ ) (Eq. (1a)); while a positive potential energy is possessed by the two similar poles of parallel dipoles.

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

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 [13], contributing (a) self energy ( $PE_{\text{self}}$ ) (Eq. (1b)) and (b) shield energy ( $PE_{\text{shield}}$ ) (Eq. (1c)).  $PE_{\text{self}}$  is due to the interaction of a charge with its own (self) image charge. This image charge creates a shielding effect ( $PE_{\text{shield}}$ ) on the Coulombic interaction.

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

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

where  $q_i, q_j$  are the charged residues in medium of dielectric constant  $\epsilon_i, \epsilon_j$  respectively,  $d_i$  is the distance of the respective charge from the dielectric interface and  $d_{ij}$  is the distance between two respective charges. The 1/2 factor in the Coulombic energy and the shield energy is to eliminate the duplicity of the summation on  $i$ th and the  $j$ th particles. When

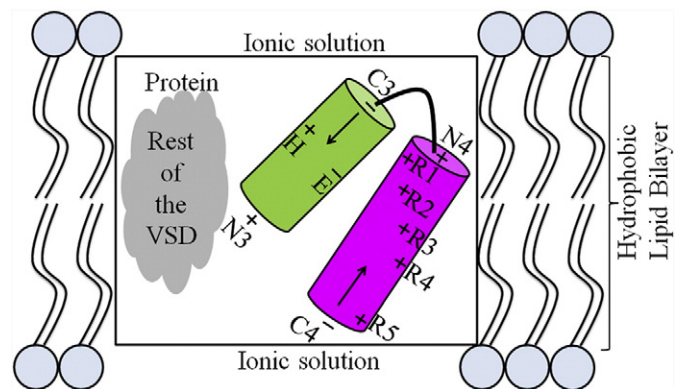
the charges are in the same medium the shielding effect comes from the dielectric constant of that medium as a factor in the denominator of Coulombic interaction ( $PE_{\text{coulomb}}$ ), (Eq. (1a)). When the charges are on either side of the interface an additional shielding effect ( $PE_{\text{shield}}$ ) (Eq. (1c)) gets included due to the induced charges at the interface.

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

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

## 2.2. The S3b–S4 pair

Here we are exploiting the role of the terminal dipolar charges (N3, C3, N4 and C4) in the stabilization of the S3b–S4  $\alpha$ -helix pair. Both S3b and S4 as a macrodipole have positive and negative half unit charges on each of their N- and C-termini respectively, apart from several charged side chains of residues scattered on their surfaces (Fig. 2). There are five charged arginines on S4 (+R117, +R120, +R123, +R126 and +R133) which are predominantly considered as the voltage sensor and two charged residues on S3b (histidine (+H109) and glutamic acid (−E107)). The first four positive arginines are three residue apart (i.e. apart by  $300^\circ$  about the axis and 4.5 Å along the axis) along the helical path on the S4 helix. However, they all lie on one hemispherical surface, making S4 an amphipathic macrodipole. The two charged residues of the S3b helix are almost diametrically ( $200^\circ$ ) apart. At physiological pH, all residues on S3b and S4 carry a unit charge, except histidine (+H107) with a half charge. The linker L34 is non-helical with 3 nonpolar residues. The length of the 3 residue linker L34 if stretched can vary from 13.5 Å to 35 Å. The maximum allowance of the relative translation between S3b and S4 considered in our work is 24 Å which is within the expandable length of the L34 linker. These alpha helix protein, of dielectric constant  $\epsilon_p = 10.0$  is embedded in a multi-dielectric environment like a lipid membrane ( $\epsilon_l = 2.0$ ) on the side (Fig. 2) and an ionic solution ( $\epsilon_w = 80.0$ ) on the extracellular (top) and intracellular (bottom) sides. An S3b–S4 pair is at the periphery of the VSD which is a part of the ion channel. The terminal charges being at the top and bottom end of the helices, we have considered the immediate dielectric boundary to be linear. Crevices in the calculation are introduced by considering the dielectric interface of a charge on the side to be water instead of lipid. Thus, this macrodipole pair has the probability of being partially exposed to lipid, protein and ionic media. The total interaction potential energy of the system of charges of S3b–S4 macrodipole pair is a balance between the attractive and



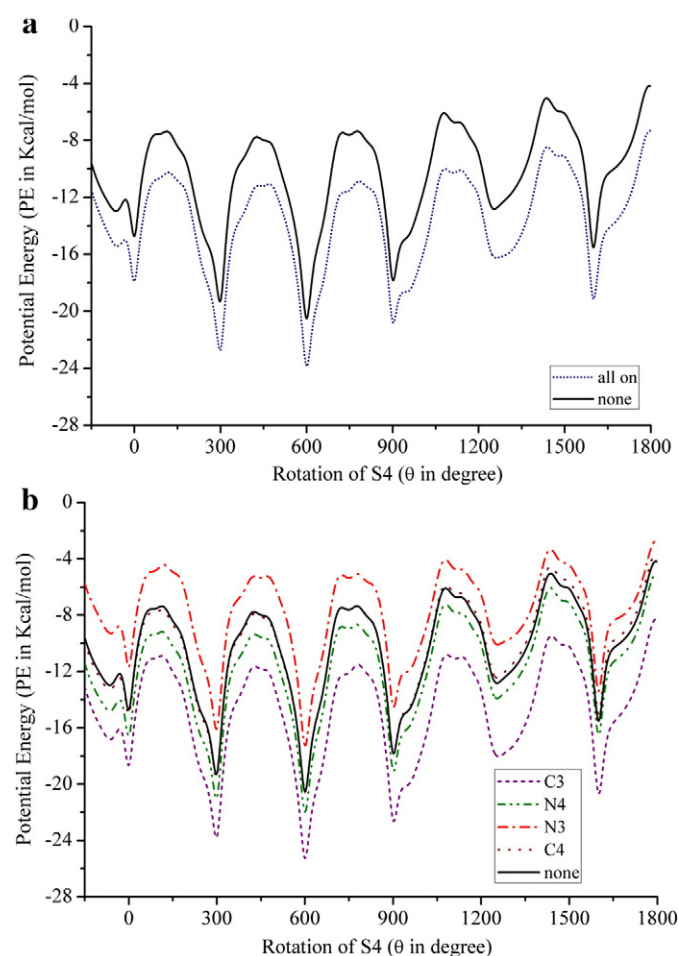
**Fig. 2.** The Voltage Sensor Domain of the KvAP channel. The S3b–S4  $\alpha$ -helix pair, with dipolar charges (N3, C3, N4 and C4) and side chain charges (R1–R117, R2–R120, R3–R123, R4–R126, R5–R133), are surrounded by the lipid bilayer and ionic solution. The rest of the VSD is shaded gray.

repulsive interactions and the minimum potential energy condition gives the most stable situation.

### 3. Result

#### 3.1. Role of the dipole charges in stabilization of the antiparallel S3b–S4 $\alpha$ -helix pair

The energy profiles (Fig. 3) show the convolution of the potential energy due to the interaction of the system of charges on S3b and S4, assuming all the charges (charged residues and terminal dipolar) are in protein. The charge residue E107 of S3b faces the S4  $\alpha$ -helix, which rotates helically clockwise by  $\theta^\circ$  about its axis and translates upwards by  $x = 0.015\theta$  Å along its axis, with respect to the position of E107. The consecutive residues in an  $\alpha$ -helix are separated from each other by  $\theta = 100^\circ$  about its axis and  $x = 1.5$  Å vertically. The energy profile has local minima whenever each positive side chain (R117–R126) of S4, which are three residues apart, comes closer to the negative charged residue (E107) of S3b, by virtue of the helical rotation of the S4 helix by  $0^\circ, 300^\circ, 600^\circ, 900^\circ$  and translation by  $0$  Å,  $4.5$  Å,  $9.0$  Å,  $13.5$  Å respectively. This explains that the positive arginines locally get stabilized at these positions in front of the negative glutamic acid. The local minimum at  $1600^\circ$  is due to R133 of S4. However, an additional minimum at  $1200^\circ$  is due to the resultant effect of R126 and R133.



**Fig. 3.** The potential energy profile of the system of charges on S3b–S4 helix pair with respect to the helical angular rotation of S4 about its axis, (a) with all (blue-all) or without any (black-none) dipolar charges (b) without any (black-none), or with only C3 (violet), or with only N4 (green), or with only N3 (red), or with only C4 (brown) single dipolar charge/s.

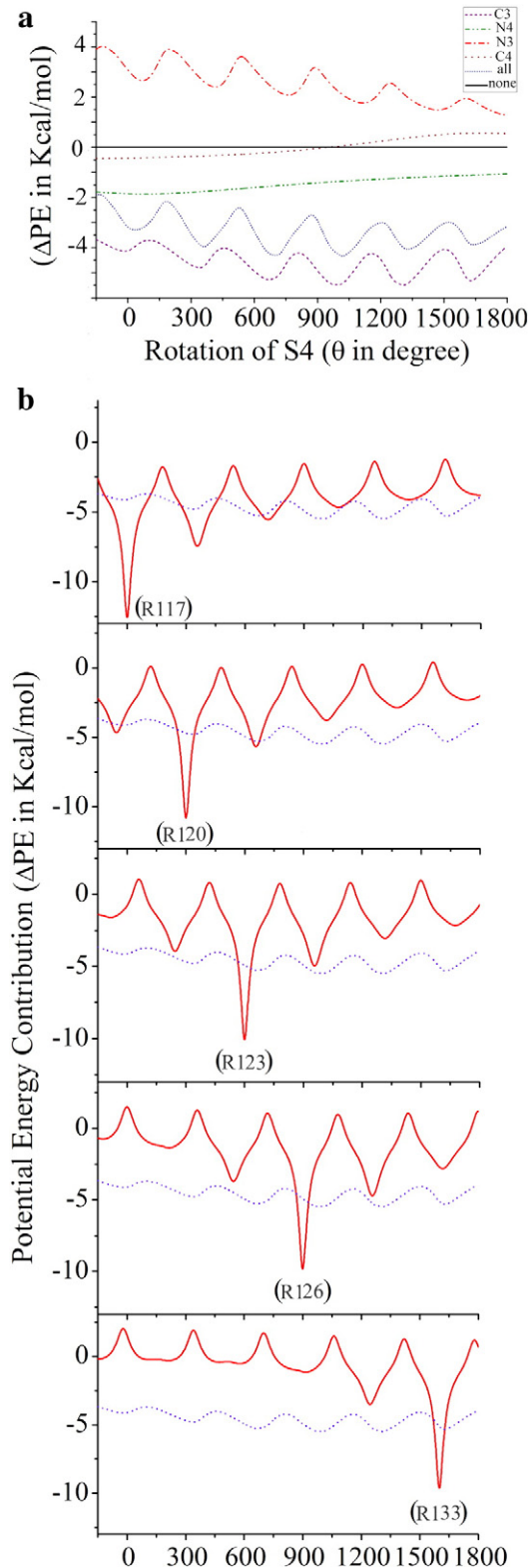
The profile (Fig. 3a) projects the potential energy of the interaction between the charges including and excluding all the terminal dipolar charges (i.e. N3, C3, N4 and C4). The interaction between the charges of the S3b–S4 pair including all the dipolar charges has a lower potential energy profile and local minima positions than the profile excluding them. This shows that the presence of the dipolar charges lowers the potential energy, increasing the stability to the system.

To gauge the role of the individual dipolar terminal charges in stabilization of the aggregation of the S3b–S4 pair, the four dipolar charges are added one at a time to the system of side chain charges and the change in the potential energy is studied (Fig. 3b). When C3 or N4, the extracellular dipolar charges are included in the system, the energy profile shifts downwards, and the minima are lower. However, the stabilizing effect of the C-terminal dipolar charge (C3) is stronger than N-terminal charge (N4). When the intracellular terminal charge (N3) is included, the potential energy profile shifts upward, indicating a destabilizing effect and the C4 dipolar charge has a minimal effect on the PE. This indicates that the terminal charges individually have effects on the stability of S3b–S4 pair aggregation.

Fig. 4 gives a comparative study of the contribution of energy ( $\Delta$ PE) by the individual charges of the S3b–S4 helix pair. The extracellular terminal charges (C3 and N4) contribute negative energy supporting the aggregation of the S3b–S4 pair (Fig. 4a). The intracellular terminal charge (N3) contributes positive energy opposing aggregation while the pole charge C4 has a nominal contribution with a mixed effect. The contribution of all the dipole charges is negative explaining the stabilizing property. The individual side chain arginines of S4 have an overall negative energy contribution (Fig. 4b) which maximizes at the local minima point when the respective charge is in the vicinity of the E107 of S3b. Except at the energy minimum position of the individual arginine,  $\Delta$ PE, the contribution of the energy of the individual arginines, is comparable to that of the dipole charge C3.  $\Delta$ PE is the energy difference of the system with and without the respective individual charges.

#### 3.2. Role of the dipole charges in different dielectric medium contributing stability

The VSD is a membrane spanning protein embedded in the bilayer lipid membrane, with the top and bottom near the cellular medium. By virtue of the mobility of the VSD, the charges (side chain and dipolar) on S3b–S4  $\alpha$ -helices are occasionally exposed to media of different dielectric constants ( $\epsilon_i$ ) (Eqs. (1a), (1b) and (1c)) e.g. extracellular and intracellular ionic solution ( $\epsilon_w = 80.0$ ), protein ( $\epsilon_p = 10.0$ ) or bilayer lipid membrane ( $\epsilon_l = 2.0$ ). The phosphate head group of bilayer lipid is hydrophilic in contrast to hydrophobic lipid tail. The phosphate head groups being hydrophilic, in our electrostatic theory at the lipid/water interface, we have considered them to be in continuum with the water. The protein dielectric value is not a constant because it depends on the protein environment. The simple models of proteins as nonpolar and homogeneous is described by a low dielectric constant (i.e.,  $\epsilon_p = 2$  to 4), however with more complex models, considering sources of local heterogeneity, the dielectric constant varied from  $\epsilon = 6$  to  $\epsilon = 21$  [14]. Hence the effective dielectric including the reaction of the solvent, polar residues etc. give the dielectric constant of protein as  $\epsilon_p \approx 10.0$  [15]. Since the  $\alpha$ -helices S3b and S4 have several polar residues and are exposed to solvent therefore we have considered the above value.  $\epsilon_p \approx 10.0$  for our calculation. The four dipolar charges (N3, C3, N4, and C4), being at the terminals of the  $\alpha$ -helices; can possibly have exposure to all these three media. To understand the effect of the exposure of the four terminal dipolar charges to different media in all possible combinations, we have assumed that all the charged residues are in protein medium in the absence of any external field. It was observed that the exposure of the dipolar charges to a different combination of media changes the total interactive energy and acquire different minimum PE (Table 1).



**Fig. 4.** The contribution of the potential energy ( $\Delta PE$ ) of the individual charges with respect to helical angular rotation of S4 about its axis or its different orientation. (a)  $\Delta PE$  of terminal dipole charges (color coding same as in Fig. 3a). (b)  $\Delta PE$  of side chain charged arginines of S4 (red) in comparison to C3 (violet) dipole charge.

Some interesting observations are:

- (a) For all combinations of the exposure of the intracellular pole charges (N3–C4), the energy of the system is minimum when

the extracellular pole charge C3 is exposed to protein and N4 is exposed to lipid (column marked P–L, Table 1). This indicates that in the absence of any external field, the C-terminal (C3) of S3b favors the protein environment while the N-terminal (N4) of S4 favors the lipid environment to maintain stability, i.e. the  $\alpha$ -helix S4 is partially exposed to the lipid bilayer from Table 1 (column 1, row1), with C3 in protein ( $\epsilon=10.0$ ), and N3, N4 and C4 exposed to lipid ( $\epsilon=2.0$ ), the system attains a minimum of  $-42.15$  kcal/mol of energy, but when the extracellular N4 is exposed to a higher dielectric like protein and water, keeping the medium of the other three terminals fixed, the stability decreases with energy  $-40.4$  kcal/mol and  $-39.3$  kcal/mol respectively. In general, if the exposure of one of the terminal changes from the higher to lower dielectric medium keeping the exposure of other three terminals fixed, then the energy minimizes bringing stability to the system.

- (b) Exposure of either N3 or C4 to lipid (Block I) leads to greater stability ( $PE_{\min} = -30$  to  $-42$  kcal/mol, column P–L) than when they are exposed to either water or protein (25–26 kcal/mol). This predicts a tendency of the intracellular terminals of S3b–S4 pair to prefer the lipid medium to aqueous medium under appropriate conditions.
- (c) With C3 and N4 exposed to protein and lipid environment respectively and (i) the intracellular poles N3 and C4 exposed to lipid, the lowest value of PE is  $-42.15$  kcal/mol (Table 1, Block I), indicating that N4 and C4 of S4 are both exposed to lipid while N3 and C3 of S3b are in lipid and protein respectively; (ii) if N3 and C4 are away from lipid, the lowest PE is  $-26$  kcal/mol for which the N3 is exposed to water and C4 to protein (Table 1, Block II), indicating that N4 and C4 of S4 are in lipid and protein respectively and N3 and C3 of S3b are in water and protein respectively.
- (d) Due to water crevices formed at the extracellular and the intracellular ends [16, 17], the terminal charges are exposed to high dielectric water introducing a screening effect and destabilization [18] to the system. In this present work, with a screening effect from solvation of the dipole terminal charges, the magnitude of the potential energy gets raised, introducing destabilization. But that is not always the case. In fact, when either C3 or N4 or both are solvated, the energies are lower i.e. the system attains greater stability (Table 1, columns 4, 5 and 6) than when neither of these terminal charges are solvated (Table 1, columns 7 and 8). Therefore, dipole interaction is not always marginal in the presence of ionic solution.

#### 4. Discussion

Each  $\alpha$ -helix in the voltage gated ion channel protein has two charged poles of a half unit of opposite polarity. Here we showed that these terminal dipolar charges have a substantial role in stabilizing or destabilizing the aggregation of the S3b–S4 pair when exposed to a single medium and then to different combinations of media. Therefore, they cannot be ignored.

##### 4.1. Experimental evidences

Our electrostatic theory explains that due to dipolar interaction the energetically favorable orientation of a pair of helices is the anti-parallel one. This is in accordance to the crystallographic structure of the VSD of different ion channels [7, 19, 20] which shows that on the extracellular side, the C-termini of S1, S3, and S5 are close to the N-termini of S2, S4 and S6 respectively while near the intracellular side the C-termini of S2 and S4 are close to the N-termini of S3 and S5 respectively.

The argument may arise that the linkers between the helices, e.g. extracellular linker L12, L34 and L56 and the intracellular linker L23 and

**Table 1**  
Minimum PE of the system when dipole charges are exposed to different dielectric media.

	C3–N4	P–L	P–P	W–L	P–W	W–P	W–W	L–L	L–P	L–W
N3–C4										
L–L	Block I	–42.15 <sup>a</sup>	–40.4	–40	–39.3	–38.3	–37.2	–35.85	–34.15	–33.05
L–P		–37.4	–35.6	–35.2	–34.6	–33.5	–32.4	–31.1	–29.35	–28.3
L–W		–36.7	–35	–34.6	–33.9	–32.8	–31.8	–30.45	–28.7	–27.65
W–L		–30.8	–29	–28.6	–27.95	–26.85	–25.8	–24.45	–22.7	–21.65
P–L	Block II	–30.45	–28.7	–28.3	–27.6	–26.55	–25.45	–24.15	–22.4	–21.3
W–P		–26	–24.2	–23.85	–23.15	–22.05	–21	–19.7	–17.9	–16.85
P–P		–25.65	–23.9	–23.5	–22.8	–21.75	–20.65	–19.35	–17.6	–16.5
W–W		–25.35	–23.55	–23.15	–22.5	–21.4	–20.35	–19	–17.25	–16.2
P–W		–25	–23.25	–22.85	–22.15	–21.1	–20	–18.7	–16.95	–15.85

<sup>a</sup> Minimum potential energy (kcal/mol) of the system of charges. The rows and the columns describe the exposure of the intracellular ends (N3–C4) and the extracellular ends (C3–N4) respectively of S3b–S4 helices to different media (L–lipid, P–protein and W–water). Block-I (at the intracellular end at least one of the dipole charges is in the lipid). Block II (at the intracellular end none of the dipole charges is in the lipid).

L45, bring the terminals together. However, there is experimental evidence, that a shortening of the linker L34 between S3b and S4 in the Shaker K<sup>+</sup> channel does not abolish the gating mechanism [21] and when the linker is removed [21, 22] 50% of the activity still remains. So the linker L34 is not the only connector between the extracellular end of the S3b and S4 macrodipoles. Our prediction is that the electrostatic attraction between the extracellular dipole charges acts like a virtual linker keeping the C3 and N4 terminals together and probably is linking the movement during gating.

The presence of the terminal dipole charges (N3, C3, N4 and C4) leads to a lower interaction PE of the system, increasing the stability of the aggregation of the S3b–S4 pair (Fig. 3a). The extracellular dipole charges (C3 and N4) of the  $\alpha$ -helix pair S3b–S4 make an appreciable contribution in the total interaction energy in comparison to that of the side chain residues (arginines) (Fig. 4b). Therefore, the role of the two extracellular dipole charges (C3 and N4) cannot be overlooked. In fact, they can possibly have a role in the mechanism of gating in the presence of transmembrane voltage.

Using our electrostatic theory, we have made an attempt to answer one of the existing questions regarding the controversy of the partial exposure of the S3b–S4 helix pair to hydrophobic bilayer lipid. This theory shows that when the terminals of antiparallel macrodipoles are exposed to a multi-dielectric environment, then at least one of the terminal pole charges in the lipid minimizes the total potential energy for stabilization. This is supported by experimental evidence highlighting the importance of lipid in holding the different domains of the channel together [8, 20, 23] and the essentiality of the lipid membrane for proper voltage sensing [5]. According to the paddle model [24] the S3b–S4 pair is partially exposed to lipid. Our theory predicts that when extracellular N4 terminal is exposed to lipid, the energy of the system minimizes (Table 1; column P–L), this is in accordance with the molecular dynamics simulation [25] which indicates that the N-terminal of S4 is immersed in the lipid membrane. Here we have also shown that in addition and preferably the exposure of the intracellular pole (N3 and/or C4) to lipid, the total potential energy gets further minimized, bringing stability to the S3b–S4 pair (Table 1, Block I).

It is generally assumed that the exposure of the terminal side chain charges of the helices is favored by interaction with the phosphate head groups of the lipids [20, 26, 27] and at zero voltage the molecular dynamic simulation has shown that the arginines at the extracellular terminus of S4 forms salt bridges with lipid phosphates at the lipid/water interface [16]. In contrast, we showed that when the terminal dipole charges are exposed to hydrophobic lipid ( $\epsilon_r=2.0$ ) there is a maximum stabilization of their ionic interaction. The dielectric constant of lipid being lower than phosphate head group, the favorable interaction of the dipole charges with lipid opens up another option of lipid–protein interaction. Thus, stabilization of the  $\alpha$ -helix in lipid can occur also through dipolar interaction and is not necessarily dependent only upon the presence of phosphate or other groups in the

lipid environment; although the lipid head groups may also contribute to stability and function [28].

Our theory explains that when the intracellular end dipolar charges (N3 and C4) of S3b–S4 pair are exposed to lipid, the stabilization is at the maximum. But when these charges are barred from lipid (Block II, Table 1), for the most stable configuration the terminals of S4 (N4 and C4) are in lipid and protein and S3b (N3 and C3) are in water and protein respectively. This agrees with the experimental evidence [6, 25] showing that while the extracellular end of the S4 (N4) being inclined outwards into the lipid bilayer membrane away from the pore, the last residue of S4 (R133) interacts with the conserved negatively charged residue in S2 (D62), hence exposing the intracellular pole of S4 helix (C4) to protein [7, 8]. There is not much experimental information about the exposure of S3b termini charges, other than that it has an apparent affinity for lipid (PSPC) [6] and it is always dragged behind by the S4 helix. In several experimental and theoretical studies, the dipolar charge is ignored because in most cases it is presumed to be in the extracellular or in the intracellular ionic solution and therefore solvated, presenting no effect on the gating mechanism. But electrostatic theory has shown (Table 1) that even if the terminals are solvated (i.e. in water medium); there could be changes in the potential energy, and thus stability.

Our electrostatic theory applied on the S3b–S4 helix pair of the VSD of K<sup>+</sup> ion channel predicts that the dipole charges of the  $\alpha$ -helices have a prominent role in stabilization of the aggregation of two macrodipoles. This is in accordance with the experimental evidence of the interaction between the side chain charge and the dipole charge of alpha helices, which has been observed through pH titration experiments in hemoglobin [29] and influenza hemagglutinin [30]. Furthermore, denaturation transition experiments with wild type and mutated lysozymes [31] clearly show the role of charges of the end termini of the alpha helices in stabilizing the structure.

The electrostatic theory used here in understanding the role of dipolar charges in the aggregation of S3b–S4  $\alpha$ -helices, is based on the interaction between charges (charged residues and dipolar charges) on the helices and image (induced) charges formed near the interface of multi-dielectric media (protein, lipid and water) and is explained in all standard books of Classical Electrodynamics [13]. Nakamura [32] has made a simple theoretical examination of the electrostatic energy of a pair of ions in protein at the protein/water interface, which supports our theory.

Our present work is at zero transmembrane voltage ( $V=0$ ) and the movement of the alpha helices will only be apparent when the transmembrane voltage is included in our computational calculation but this is beyond the scope of the present manuscript. Hence, we have not shown any movement mechanism; rather we have calculated the potential energy of the system of charges for all possible mutual configurations of the S3b–S4 helix pair, theoretically scanning the surface of the S4 by sliding and rotating S4 against the negative E107 of S3b. This opens the way for examining the effect of applied

voltage on the conformation, and thus to the resolution between the inspiring models.

#### 4.2. Testable hypothesis

According to our theory the charged side chains of S3b–S4 helices and the terminal dipole charges should interact in a specific way during translational and rotational motion. Thus neutralization of all the side chain charges should leave a residual dipole–dipole interaction to stabilize the helix pair. This should be experimentally testable through mutagenesis of all the relevant residues followed by estimation of the stability of the helix pair.

#### 5. Conclusion

The presence of all dipole terminal charges lowers the potential energy of the S3b–S4  $\alpha$ -helix pair, hence stabilizing the aggregation. The extracellular terminals C3 and N4 add stability to the system while N3 destabilizes. The contribution of potential energy by the dipole charges is comparable to that of the side chain charged residues. These terminal dipole charges favor different dielectric media to maintain stability of the S3b–S4 pair, with the S4 macrodipole favoring the lipid bilayer. Hence the dipole charges play a significant role in the aggregation of two helices and they cannot be ignored. The theoretical approach adopted here is not restricted to ion channels but is of general applicability to any pair of  $\alpha$ -helices interacting in different dielectric media.

The pending question, yet to be answered, is the changing conformation of the helices and hence the motion of the helices under the influence of the varying transmembrane potential. Our present work is at zero transmembrane voltage ( $V = 0$ ). But our electrostatic theory can be applied at different voltages ( $-70$  mV to  $+30$  mV) with alpha helices exposed to different dielectric media, to estimate the changing conformation of the  $K^+$  ion channel. Hence an attempt can be made to configure the conformation of the VSD at the resting potential ( $V = -70$  mV).

There is a lack of consensus between the existing models; the translational model, the helical model and the paddle model of the  $K^+$  ion channel, which are based on the motion of the S4 helix. Under the influence of the varying transmembrane potential, each motion is possible to the S4 helix and it can be explained individually by our electrostatic theory. With the help of this theory an attempt can also be made to develop a unified model combining all the three types of movement of the S4 helix.

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