Original Article

Electrostatic interaction between dipoles and side chains in the voltage sensor domain of K^+ channel

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Abstract

Background: It is well known that α-helices of protein, possessing equal and opposite charged ends, behaves like a macrodipole, but the relative importance of such macrodipoles to the aggregation of a pair of helix in the voltage sensor domain (VSD) of K+ ion channel, has not been assessed. In the VSD, importance has been given primarily to the helically arranged Arginine residues of helix, but the role of the charged residues of S3b is less focused. **Method and Objective:** Applying electrostatic theory, we have studied the interaction between the charges of S3b-S4 α-helix pair of KvAP through virtual mutagenesis. **Result and Conclusion:** We have shown that the terminal charges arising from the inherent dipolar property of α-helices play an important role in affecting the stability of the S3b-S4 pair, and in determining its spatial position at zero transmembrane potential. Moreover, the negatively charged side chain of S3b was found to be the primary stabilizing factor in holding S3b-S4 pair together as a "paddle". Comparison of sequences of S3b helix of K+ channels from different species showed a previously unreported positional conservation of negative residues, highlighting their functional importance. These charges may contribute to the energetic of α-helix movements in an electric field.

Key words: Local interaction force, macrodipole, paddle, potential energy, terminal charges, α-helix

INTRODUCTION

An α-helix possesses a dipole moment by virtue of the alignment of its peptide bonds having half-positive and negative charges at their ends. For this fractional charge separation, a single peptide unit behaves like a microdipole.[1] When these microdipoles align along the axis of the α-helix, making hydrogen bonds with the neighboring peptide units, the α-helix behaves like a macrodipole with positive C-terminal and negative N-terminal on either end. The length[1] and the dipole moment $(\vec{p})$ of an α-helix macrodipole is 1.5N Å and 3.5N Debye, respectively, where N is the number of residues of the alpha helix.[2] Several groups have suggested that the α-helix macrodipole can stabilize certain conformations of the protein.[3,4]

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The structure and function of different voltage-gated potassium (K^+) ion channels have been reviewed.[5] The transmembrane subunits of the channel comprise six α-helices. The structure of voltage-gated K^+ ion channel of *Aeropyrum pernix* (KvAP) obtained by different methods[6-8] shows that, in a single subunit, the α-helices are not ideally antiparallel but organized in a pairwise fashion, i.e., S1-S2, S3-S4, and S5-S6. The first four helices (S1, S2, S3, and S4) collectively are called the voltage sensor domain (VSD), the remaining (S5, S6) being pore domain (PD). The S3 helix has a kink at Proline P99, dividing it into two distinct helices S3a and S3b. S3b pairs with S4, forming a tightly bound "paddle" unit shuttling in and out of the membrane.[9]

S3b and S4 form a somewhat antiparallel macrodipole pair.[6-8] Taking S3b and S4 α-helices of the full-length K^+ ion channel

of KvAP (PDB 1ORQ)[6], the 14-residue (A100-L113) S3b α-helix is 21 Å, while the 17-residue (R117- R133) S4 α-helix is 25.5 Å long. The terminal dipole charges of S3b and S4 macrodipoles are N3 (positive), C3 (negative) and N4 (positive), C4 (negative), respectively. Besides these charges, the α-helices have a number of charged residues on their surfaces. The negative Glutamic acid (E-107) and positive Histidine (H-109) of S3b are apart by a translational distance of x = 3 Å along the helix axis and by rotational angle of ϕ =200° about the same axis. The S4 α-helix has five positive Arginine residues: R117, R120, R123, and R126 (designated as R1-R4, respectively), which lie periodically in a helical path of pitch 4.5 Å and angular separation of 60°; and R133 (R5), which is 10.5 Å below R4 and separated by 20° [Figure 1]. All residues have unit electronic charge, except Histidine, which has half. The R117 residue is near the N-terminal and R133 is near the C-terminal of the S4 macrodipole.

Various experimental and theoretical studies have shown the importance of the positive Arginine residues of S4[10,11] and their interactions with the negative charged residues of S1-S3a.[12,13] However, there is no specific information on the role of the negatively charged acidic side chain of S3b and the dipolar charges (N3, C3, N4, and C4) on the stability of the S3b-S4 pair in KvAP. Since the gating process is an electrical activity, all charges in the system of the VSD, including the dipolar charges, are expected to have some role in the process. Here, the electrostatic theory was used to understand (i) the effect of dipolar charges on the antiparallel arrangement of the two α-helix macrodipoles and the proximity of the extracellular and intracellular terminals of the S3b-S4 pair; (ii) the role of charged residues and the dipolar terminal charges affecting the stabilization of the S3b-S4 aggregation; and (iii) the contribution of energy of E107 of S3b in the "paddle" structure.

ELECTROSTATIC THEORY

According to the electrostatic theory,[14] two electric dipoles interact to give the mutual electrostatic potential energy, which depends on their dipole moments $\vec{p}_1$ and $\vec{p}_2$, and their angular separation (θ). Hence, the potential energy value (U) varies as

$$U = \frac{1}{4\pi\varepsilon_0\varepsilon_p r^3}\left[\vec{p}_1.\vec{p}_2 - \frac{3(\vec{p}_1.\vec{r})(\vec{p}_2.\vec{r})}{r^2}\right]; \quad (1)$$

where $\vec{p}_1, \vec{p}_2$ are dipole moment vectors and $\vec{r}$ is the position vector of $\vec{p}_2$ with respect to $\vec{p}_1$, while r is the center-to-center distance between the two dipoles.

When the distance between two dipoles is less than the length of individual dipoles, the interaction of the individual charges predominates. Hence, the electrostatic coulombic potential energy of the system of charges of macrodipoles is calculated as

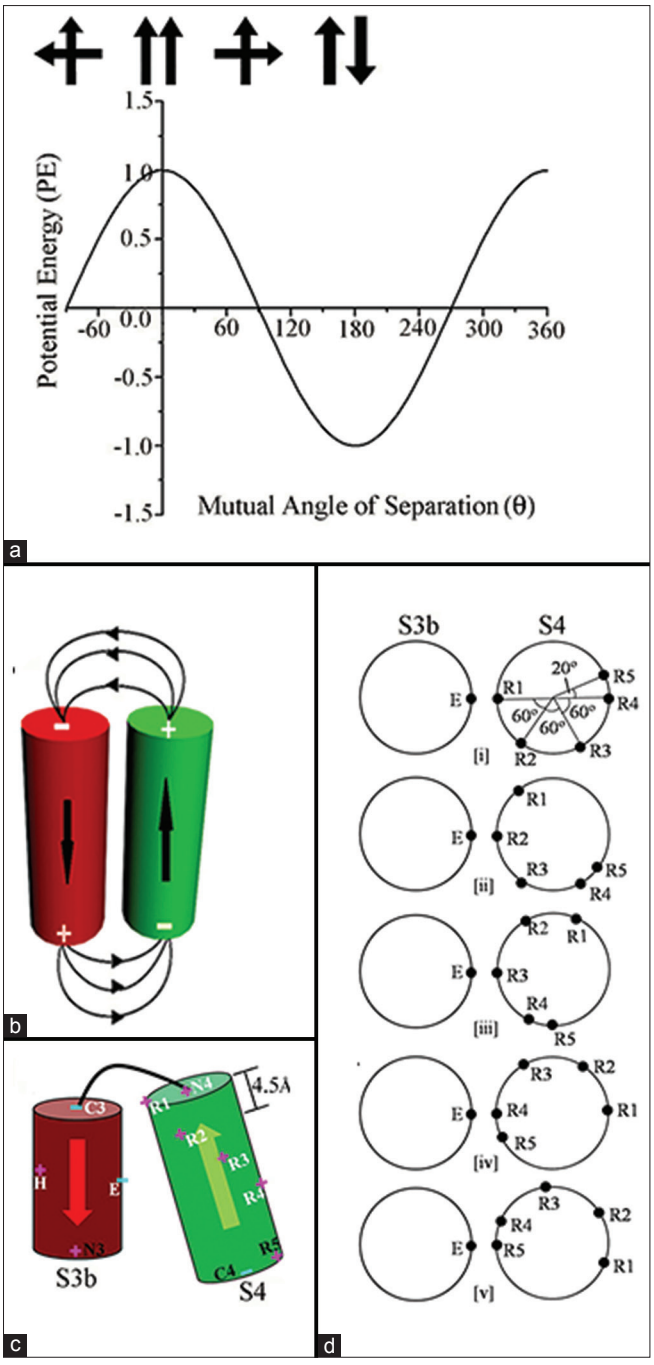

Figure 1: Interaction between α-helical macrodipoles. (a) Potential energy vs. angular separation (θ) of two macrodipoles. The mutual orientations at different θ values are shown at the top (arrow head, positive end). (b) Lines of force following the shortest path from positive to negative pole. (c) The charge distribution on S3b-S4 helix pair of full length ion channel. The extracellular end is at the top. (d) Schematic diagram of the relative angular position of the charged residues on S3b-S4 pair of KvAP from the extracellular end (E-E107, R1-R117, R2-R120, R3-R123, R4-R126, R5-R133)

$$PE_{coulomb} = \frac{1}{4\pi\varepsilon_0\varepsilon_p}\sum_{i=1}^{m}\sum_{j=1}^{n}\frac{q_i q_j}{|\vec{r}_{ij}|}; \quad i \neq j, \tag{2}$$

The electrostatic force $\vec{F}$ between charges q_i, q_j of the two adjacent macrodipoles is

$$\vec{F} = \frac{1}{4\pi\varepsilon_0\varepsilon_p}\sum_{i=1}^{m}\sum_{j=1}^{n}\frac{q_i q_j}{|\vec{r}_{ij}|^2}; \quad i \neq j, \tag{3}$$

where, $\vec{r}_{ij}$ is the distance between the charges q_i and q_j, ε_0 is the permittivity of the vacuum, and ε_p is dielectric constant of the medium (protein) in which the macrodipoles are embedded. The force or potential energy is negative or positive depending upon whether the interaction is attractive or repulsive.

RESULTS AND DISCUSSION

Pair-wise arrangement of α-helices

The potential energy (PE) profile of any two dipoles (equation 1) at different angular separation (θ) shows that when a pair of dipoles are in parallel position (θ = 0°), the energy is maximum; when perpendicular (θ = 90°), energy is zero; and when in antiparallel orientation (θ = 180°), the energy is minimum [Figure 1a] and is maximally stabilized. A pair of α-helix macrodipoles tend to stabilize in an antiparallel position also because the lines of force always travel through the shortest path (14) from positive to negative terminals, bringing the opposite poles of two adjacent macrodipoles as close as possible [Figure 1b]. In keeping with this theory, the structure of the KvAP channel shows the helix pairs S1-S2, S3b-S4, and S5-S6 with the C-termini of S1, S2, S3, S4, and S5 being close to the N-termini of S2, S3, S4, S5, and S6, respectively. However, these pairs are not ideally antiparallel.[6] This can be explained by the help of the local force between the two terminals of the S3b-S4 macrodipole pair as an example.

According to the different structures (full-length[6] and isolated VSD[8,9]) of KvAP ion channel, the 17 residue S4 helix (R117-R133) in the full-length ion channel is shorter than the 31-residue S4 helix (R117-L148) in the isolated VSD. The PDB structure (1ORQ) of the full-length ion channel [Figure 1c] shows that at the intracellular end of the S3b-S4 pair, the dipolar charge N3 (+0.5e) of S3b helix is at the vicinity of the dipolar charge C4 (-0.5e) and R133 (+1.0e) of S4 helix, while at the extracellular end, the dipolar charge C3 (-0.5e) of S3b is near the dipolar charge N4 (+0.5e) and R117 (+1.0e) of S4. In isolated VSD, the S4 helix is longer, with C4 terminal 15 residues farther away from R133; hence, in the vicinity of N3 of S3b only the R133 of S4 remains.

In the full-length ion channel, the charges at the intracellular end of S3b (+N3) and S4 (-C4 and +R133) helix pair experience a net positive (repulsive) local force (Figure 2a, solid symbol) due to the interaction between N3-C4 and N3-R133, keeping the N3 terminal of S3b away from S4. The charges at the extracellular termini of S3b (-C3) and S4 (+N4 and + R117) experience a net negative (attractive) local force [Figure 2b], pulling the C3 pole of S3b closer to S4. The other, more remote charged residues add to the repulsive or attractive force towards N3 or C3 pole, respectively, but the magnitude of these forces are weaker due to greater interatomic distances. This unequal spacing between two poles [Figure 1c] of the S3b-S4 pair at two cellular ends is quite evident from the PDB 1ORQ structure,[6] of KvAP ion channel protein. The force varies between the two termini with the rotation of S4 about its own axis. As the angle of rotation (ϕ) increases, the attractive force at the extracellular end decreases, but the repulsive force at the intracellular end increases, thus maintaining the unequal spacing between the two termini at two cellular ends. The S4 helix is assumed to be more mobile than S3b; hence, in all orientations, the intracellular termini will always remain separated due to repulsive force and the extracellular termini will remain closer.

In comparison with the full-length ion channel, in the isolated VSD, according to the structure (PDB 2KYH and 1ORS)[8, 9], the extracellular termini experience a net attractive force but at the intracellular end, the net repulsive force (between N3 and R133) (Figure 2a, open symbol) is stronger in nature. This is because the S4 helix in isolated VSD is longer (R117-L148) with C4 terminal 15 residues below R133 and farther away from N3; hence, the attractive force between N3 and C4 becomes weaker and the repulsive force between N3 and R133 predominates, making the bifurcation larger. In keeping with this finding, the observed distance between the N3 of S3b and R133 of S4 is approximately 26% and 63% greater in the structures of PDB 1ORS and 2KYH, respectively, than in PDB 1ORQ, hence justifying the bifurcation at the intracellular side between the two antiparallel S3b-S4 helices, which is explained by the electrostatic theory.

At the termini of S3b-S4 pair of the full-length ion channel, the effect of the primary charges (N3, C4, R133, C3, N4, and R117) involved in these local forces was studied by virtual mutagenesis. (1) On neutralizing all the dipolar charges (N3, C3, N4, and C4), the local force was found to be almost negligible between the termini of the α-helix pair at both the intracellular end [Figure 2c] and extracellular end [Figure 2d]. (2) When the charged residues (R117 and R133) were mutated, both terminals experienced a comparable local attractive force similar to a typical antiparallel macrodipole pair. Thus, in both cases, the intracellular bifurcation collapsed in absence

of the repulsive force. Therefore, the coordinated role of the charged residues and the dipolar charges at the two termini of S3b-S4 pair has a significant effect on the unequal spacing of the mutual spatial position of S3b-S4, i.e., holding the extracellular poles (C3 and N4) closer and the intracellular N3 end far apart from the S4 helix.

Role of the charges on the stability of aggregation of S3b-S4 pair

The total potential energy (equation 2), of the system of charges of the S3b-S4 pair was computed as a function of the translational motion of S4 along its helical axis and at different angles of rotation (θ = 0°, 60°, 120°, 180°, 200°) [Figure 1d] about its axis. The energy profiles [Figure 3a] showed minimum energy when the positive residues R117, R120, R123, R126, and R133 face

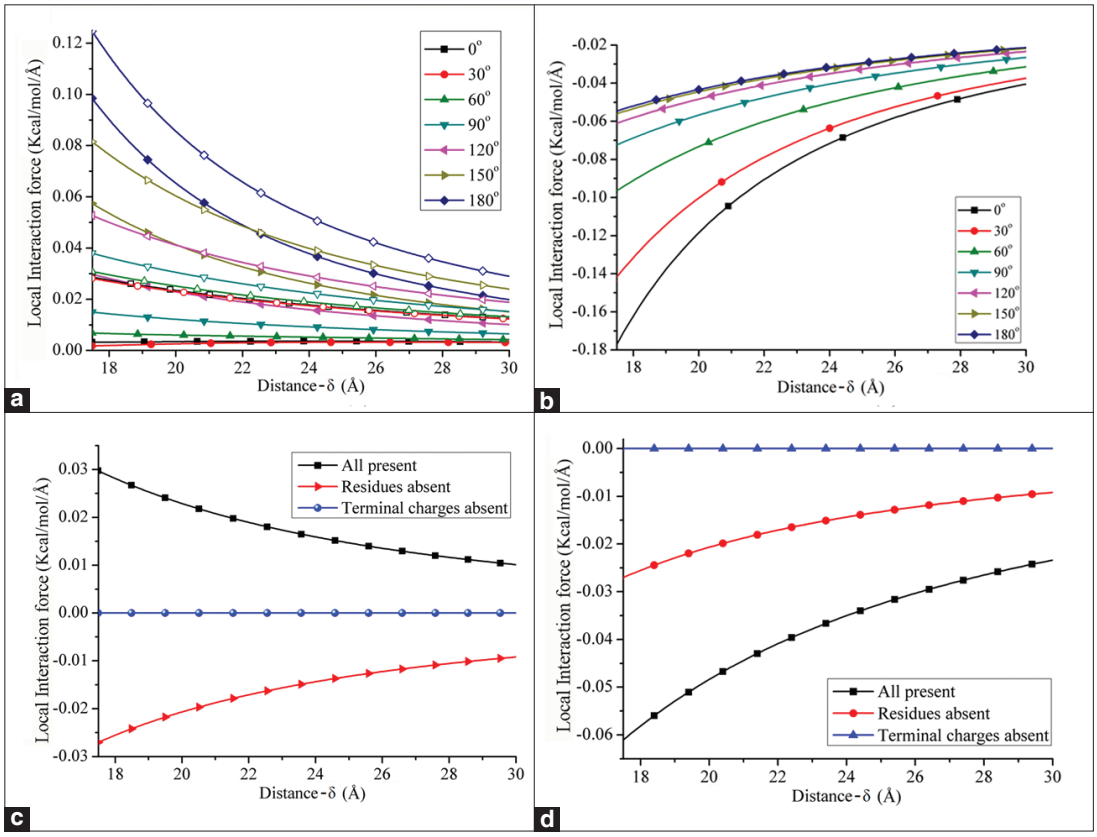

Figure 2: Local interaction force between the adjacent termini of S3b-S4 α-helix pair vs. the distance (δ) between the two helices at different angle of rotation (ϕ) of S4. (a) At the intracellular side, distance (δ) is between N3 of S3b and R133 of S4 for full length ion channel (solid symbol) and for isolated VSD (open symbol). (b) At the extracellular side, distance (δ) is between the dipolar ends C3 of S3b and N4 of S4. With S4 rotated by ϕ =120°, the local force in presence of all charges and in absence of charged residues/dipolar charges (c) at ïntracellular end and (d) at extracellular end

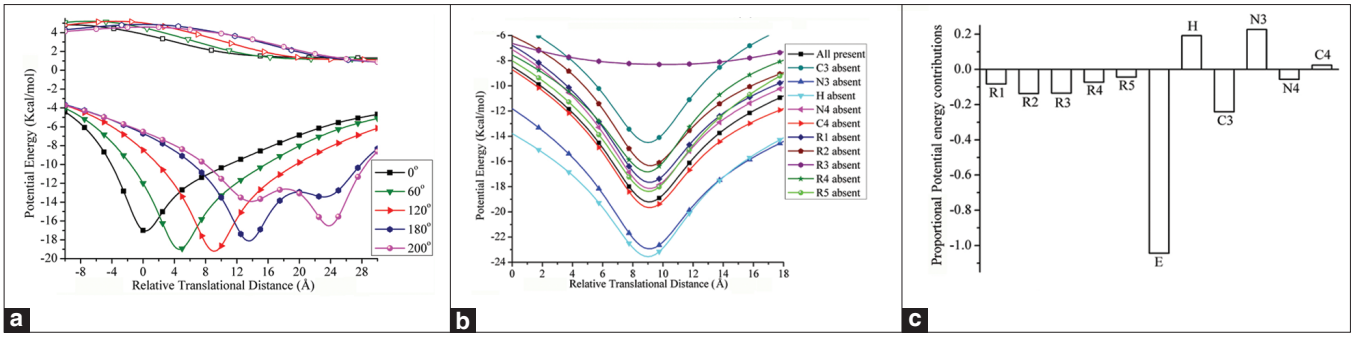

Figure 3: Role of dipolar and side chain charges in the stability of the S3b-S4 pairs. (a) Potential energy profile of the system of charges on S3b-S4 vs. the relative translational distance between S3b and S4 along the helix axis, in presence (solid symbol) and absence (open symbol) of E107 on S3b at different angles of rotation (ϕ) of S4. (b) With S4 rotated by 120° potential energy in the presence of all charges and in the absence of each charge. (c) Proportional potential energy contribution of the individual charges in stabilization of the macrodipole pair

the negative E107 at the translational positions of x = 0, 4.5, 9.0, 13.5, and 24 Å and rotational positions of ϕ = 0°, 60°, 120°, 180°, and 200°, respectively. When S4 was rotated by ϕ =120° and translated by x = 9.0 Å, i.e., with the positive R123 residue in close proximity to negative E107, the system attained the minimum energy of 19.25 kcal/mol. This can be one of the possible conformations of the S3b-S4 pair when there is no transmembrane potential (the zero-potential state). Indeed, the crystal structures show the presence of E107 of S3b facing S4 at the vicinity of R123 and H109 is diametrically on the other side.[6-8]

On neutralizing E107 of S3b for all possible rotational positions of S4, the total energy becomes positive and the profile almost flattens out [Figure 3a], i.e., the net energy of aggregation of S3b-S4 changes from attractive to repulsive, while [Figure 3b] on neutralizing the other charges, the negative (attractive) energy varied. Hence, E107 is the prime candidate in keeping S3b-S4 α-helices together behaving like a single "paddle" unit.[9]

Considering the minimum energy profile position at x = 9.0 Á and ϕ =120°, the effect of different charges on the stabilization of S3b-S4 pair was studied. Through virtual mutagenesis, on neutralizing each charge one at a time, the energy profile changed [Figure 3b], implying that each charge (dipolar charge or charged residue) has a significant role in stabilization of the S3b-S4 pair. In the absence of the charged residues (R117, R120, R123, R126, and R133), the total energy has increased, and hence the system lost stability, while in the absence of H109, the energy stability was increased. On neutralization of the C3 and N4 terminal charges, the potential energy profile was raised, while the absence of N3 and C4 charges lowered the minimum energy value. Therefore, the dipolar end charges, in spite of being weaker, have a substantial role in the stability of S3b-S4 helix pair, which is still unexplored experimentally.

Figure 3c shows a comparative study of the potential energy contribution of individual charges in stabilizing the S3b-S4 α-helix pair. Of all the charges, the contribution of the potential energy of Glutamic acid E107 of S3b is maximum, but all other charges also contribute to the stability. In particular, contribution of the dipolar terminal charges C3 is much greater than that of any of the positive Arginine residues of S4, while contribution of N4 is comparable to some of the Arginine residues of S4 α-helix. Experimental evidences favor a significant role played by the Arginine residues of S4 in the gating process[5], but the role of the dipolar charges and the Glutamic acid of S3b in KvAP, so far have not been taken into consideration. Our calculations show, however, that in reference to the stability of the S3b-S4 pair, the dipolar charges and the negative Glutamic acid cannot be ignored.

The alignment of the S3b-S4 sequence of different members of the K^+ ion channel sub-family[15] showed a previously unreported positional conservation of negatively charged Glutamic acid/Aspartic acid residues in the S3b α-helical region [Table 1]. The extracellular half of the S3b helix in 11 out of 15 members contain at least one negative residue between the ninth and fourteenth positions from the Proline, that bends the S3 helix into two parts—S3a and S3b. This conservation indicates a biological function of the negative charge in the S3b helix and the existence of the "paddle" unit of the S3b-S4 α-helix pair. Four members have an additional negative residue while the Shaker channel, exceptionally, contains four contiguous negative residues that would form a negatively charged spiral in three dimensions. It is expected that the negative charge distribution on the S3b helix would significantly impact its interaction with the S4 helix in the absence or presence of an electric field.

The Shaker ion channel contains this cluster of four acidic residues at its N-terminal end of S3b. Deletion of either one or three of these residues (as triplet) in S3b have been found not to affect the voltage gating.[16,17] However, in both the cases, there is at least one remaining acidic residue, which may be sufficient for maintaining stability as shown in our work with KvAP [Figure 3a and c]. Moreover, by virtue of deletion, no matter how small the α-helices S3b and S4 are, the terminal dipolar charges are always present to take part in the stability and will also have substantial role in the gating process.

CONCLUSION

The electrostatic theory explains various aspects of the stability of the S3b-S4 α-helix pair of the VSD of KvAP

Table 1: Alignment of the sequence of S3b helix of K^+ ion channel subfamilies of different species. Negatively charged residues (red) and positively charged residues (green)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
P	F	Y	I	T	L	L	V	E	S	L	S	G	S	Q	T			KV8.1
P	S	F	V	S	I	Y	L	D	R	T	W							BK
P	A	G	L	L	A	L	I	E	G	H	L	A	G	L				KVAP
P	Y	F	I	T	L	G	T	E	L	A	E	K						KV1.2
P	Y	Y	V	T	I	F	L	T	E	S	N	K	S					KV2.1
P	V	D	Y	I	F	L	I	V	E	T	R							HCN4
P	F	D	L	L	I	F	G	S	G	S	E							HERG
P	Y	Y	I	G	L	V	M	T	N	N	E	D	V	S	C			KV4.3
P	Y	F	I	T	L	A	T	V	V	A	E	E	E	D	T	L	N	L SHAKER
P	F	Y	L	T	L	L	A	G	A	A	L	G	D	Q	R	G	A	KV9.1
P	Y	Y	I	S	V	L	M	T	V	F	T	G	E	N	S			KV10.1
P	F	Y	L	E	I	G	L	S	G	L	S	S	K	A				KV3.1
P	F	Y	V	S	L	T	L	T	H	L	G	A	R	M	M			KV5.1
P	F	Y	V	S	L	L	A	G	L	A	A	G	P	T	G			KV6.2
A	S	I	A	V	V	S	A	K	T	Q	G	N	I	F	A	T		KV7.5

ion channel at zero potential. (i) The α-helix pair arrange themselves in a dipole pair-wise fashion like two macrodipoles obeying the electrostatic laws; (ii) the unequal spacing of the extracellular and intracellular poles of S3b-S4 is due to the local attractive and repulsive forces, respectively, which makes the S3b-S4 pair not ideally antiparallel. The presence of the dipolar charges along with the terminal charged residues is the cause of this spacing; (iii) in the absence of any transmembrane voltage (zero potential), positive R123 of S4 is in the closest proximity of the negative Glutamic acid E107 of S3b; (iv) E107 of S3b has a dominant role in stabilizing the S3b-S4 "paddle"; and (v) the extracellular terminal dipolar charges C3 of S3b and N4 of S4 contribute substantially to the stability of S3b-S4 α-helix pair.

This report on the role of the dipolar charges in stabilization is important because in the ion channel, all α-helices will behave like macrodipoles and their terminal charges will not only add impetus to the stabilization of other helix pairs, but also may have contribution in the gating process in presence of the transmembrane voltage. The Glutamic acid, which is found to be playing a dominant role in holding the S3b and S4 together as a "paddle", can give an insight to the paddle model as to why the S4 helix carry the S3b as a cargo during the gating process. Our subsequent work will be to untangle some controversy that arose between different models with our dipole electrostatic theory.

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