A SURPRISING CLARIFICATION OF THE MECHANISM OF ION-CHANNEL VOLTAGE-GATING

AR. PL. Ashok Palaniappan*

An intense controversy has surrounded the mechanism of voltage-gating in ion channels. We interpreted the two leading models of voltage-gating with respect to the thermodynamic energetics of membrane insertion of the voltage-sensing ‘module’ from a comprehensive set of potassium channels. KvAP is an archaeal voltage-gated potassium channel whose x-ray structure was the basis for determining the general mechanism of voltage-gating. The free energy of membrane insertion of the KvAP voltage sensor was revealed to be a single outlier. This was due to its unusual sequence that facilitated large gating movements in its native lipid membrane. This degree of free energy was the least typical of the other voltage sensors, including the Shaker potassium channel. We inferred that the two leading models of voltage-gating referred to alternative mechanisms of voltage-gating: each is applicable to an independent set of ion channels. The large motion of the voltage-sensor during gating proposed by the KvAP-paddle model of gating is unlikely to be mirrored by the majority of ion channels whose voltage sensors are not located at the membrane-cytoplasm interface in the channel closed state.

*Department of Research, Chettinad Hospital and Research Institute, Chettinad University, Kelambakkam, Tamilnadu 603013, India.
E-mail: plnppnashok@yahoo.com
Ph: +91 44 47429045
1 INTRODUCTION

With the determination of the structure of a voltage-gated K⁺ channel¹, a controversy took shape regarding a contradiction in the principle of voltage-gating in ion channels. Two competing models of voltage-gating had been put forward, and the accumulation of evidence had done little to resolve the controversy². The voltage-sensing S4 helix in all voltage-gated ion channels consists of arginine residues at intervals of third position, and the movement of these gating charges ‘activates’ the channel. The canonical model of voltage-gating posits that the S4, in the channel closed state, is surrounded by protein which provide an aqueous crevice to shield it from the lipid bilayer. Upon depolarisation, the S4 undergoes small vertical displacements of ~2-3Å to initiate conformational changes associated with channel opening³,⁴. The competing model of voltage-gating, called the ‘paddle model,’ stipulates that the S4, in the channel closed state, is located at the membrane-cytoplasm interface, and upon depolarization, undergoes a large transverse movement of ~20Å to translocate to the extracellular side and gate the channel⁵,⁶,⁷.

2 METHODS

The paddle model is supported primarily by studies with the KvAP channel in its native lipid membrane. Experimental studies with other voltage-gated K⁺ channels, especially the eukaryotic Shaker channel, have unambiguously supported the alternative ‘canonical’ model. Shrivastava et al considered whether the KvAP channel is ‘different’ from other voltage-gated K⁺ channels⁸ and concluded otherwise based on the conservation of the essential S4 motif of basic residues.

Hessa et al developed an amino acid hydrophobicity scale⁹. Using this hydrophobicity scale, the free energy (ΔG) of membrane insertion of the KvAP voltage sensor was determined to be ~0 kcal/mol¹⁰. This provided the necessary thermodynamic basis for the location of the KvAP voltage sensor at the membrane-cytoplasm interface in the channel closed state. Here, we extend the investigation of thermodynamic stability of the voltage sensor to more contentious sequences of voltage sensors. We constructed a comprehensive dataset of 147 90% non-redundant voltage-gated K⁺ channels representing various subfamilies including KCNA (Shaker), KCNB (Shab), KCNC (Shaw), KCND (Shal), KCNF, KCNG, and KCNS. The ΔG of membrane insertion of the voltage sensor of each channel was scored using the amino acid hydrophobicity scale.

3 RESULTS AND DISCUSSION

Fig. 1 shows the ΔG of membrane insertion for the voltage sensors in the comprehensive dataset. It was seen that all the S4’s possessed a significant positive free energy, which would in turn function to impede all large motions in the lipid environment of the membrane. Therefore it is unlikely that the voltage sensors were located at the membrane-cytoplasm interface in the channel closed state, since gating would entail large movements of the voltage sensor in the membrane for such a location. This result provided support for the ‘canonical’ model of gating for the majority of voltage sensors.
On the other hand, the KvAP S4 voltage sensor was the clear outlier in the plot, with its \( \Delta G \) of membrane insertion more than 3\( \sigma \) from the mean \( \Delta G \). This finding complicated the extension of conclusions about voltage-gating drawn from studies of the KvAP channel. The paddle model of gating applied to only those channels with an ‘interfacial’ S4 in the closed state, which is implausible for the majority of channels. It is worth noting that the \( \Delta G \) of membrane insertion of the voltage sensors of channels homologous to the KCND subfamily was as high as 16 kcal/mol.

![Figure 1](image-url)  

**Figure 1** \( \Delta G \) of membrane insertion of voltage sensors of a comprehensive (prokaryotic and eukaryotic) set of 147 90% non-redundant \( \text{K}^+ \)-channels, grouped by homology to the closest eukaryotic subfamily. KvAP' has the lowest insertional \( \Delta G \). After correction for position-dependence, the KvAP value is close to zero\(^{10}\).

The voltage-gating properties of the prokaryotic one-domain sodium channel NaChBac might be similar to the KvAP channel\(^{11}\). Figure 1 indicates the \( \Delta G \) of membrane insertion of the NaChBac voltage sensor, which is midway between the KvAP voltage sensor and the rest of the voltage sensors.

Fig. 2 shows the residue frequencies in the same comprehensive set of channels compared with the KvAP S4. It was seen that seven of the 18 residues in the KvAP S4 were leucines, whereas the comprehensive set had about three leucines on average. A variety of less hydrophobic residues such as M, A, T, H, and K were seen in the universal group but absent in the KvAP sequence. Also shown in fig. 2 is the alignment of the consensus sequence of the comprehensive set with the KvAP and
S4Consensus  ILRVLRVRIFRIFRLSRL
KvAP          LFRLVLRLRFLRILLIIS
NaChBac       VIRILRVLRLRAISVVP

Figure 2 Residue frequencies of the comprehensive set vs KvAP. There is a mismatch in the propensity for hydrophobicity between the comprehensive set and KvAP. Below, alignment of the consensus S4 sequence of the comprehensive set with the KvAP and NaChBac S4 sequences. Arginines are highlighted.

NaChBac S4 sequences. The consensus sequence differed from the KvAP sequence in a total of 12 out of the 18 positions. On the contrary, the consensus sequence differed from the Shaker S4 sequence in only one position (position #10: I↔V). (See fig. 3 for comprehensive alignment.)

4 CONCLUSION

The ΔG of membrane insertion of the voltage sensor constrains the magnitude of its translocation in the phospholipid membrane during gating. The higher the ΔG, the greater is the constraint for movement. The KvAP voltage sensor has the lowest ΔG, and is therefore capable of maximum translocation. As ΔG increases, there is a continuum of possible translocations of decreasing magnitude. For the majority of voltage sensors, free interaction with lipid is energetically unfavourable. Hence we would expect that they undergo minimal translocation during gating, whereas the paddle model of gating required the unimpeded interaction between the phospholipid and ion channel in both the channel open and closed states. Later results from Dr. R. MacKinnon’s laboratory showed that the phospholipid membrane played a role in KvAP gating. This implied
that there is co-evolution between the voltage sensor of the ion channel and the phospholipid membrane. Co-evolution is emerging as an important player in biology; there is substantial evidence for co-evolution between catalysis and regulation in potassium channels in particular\textsuperscript{13}. More work is required to ascertain the chemical interactions between ion-channel and membrane, and their correspondence with the exhibited mechanism of gating. It would be interesting to explore if the existence of multiple mechanisms for gating is an exceptional observation in the study of protein function.

Figure 3 Alignment of 90\% non-redundant S4 sequences from the comprehensive dataset. Accession codes refer to the Genbank Gene Products database. At the top is KvAP, and the percent values denote homology to the KvAP sequence in terms of sequence identity. The degree of homology between the KvAP S4 and all the other S4 sequences is <40\% in every case, with only 17\% homologies also observed.
REFERENCES

1. Jiang Y., Lee, A., Chen, J., Ruta, V., Cadene, M., Chait, B. T. & MacKinnon, R. (2003) X-ray structure of a voltage-dependent K⁺ channel. *Nature* 423, 33-41.
2. Blaustein, R. O. & Miller, C. (2004) Ion channels: shake, rattle or roll? *Nature* 427, 499-500.
3. Posson, D.J., Ge, P., Miller, C., Bezanilla, F. & Selvin, P.R. (2005) Small vertical movement of a K⁺ channel voltage sensor measured with luminescence energy transfer. *Nature* 436, 848-851.
4. Chanda, B., Asamoah, O.K., Blunck, R., Roux, B. & Bezanilla, F. (2005) Gating charge displacement in voltage-gated ion channels involves limited transmembrane movement. *Nature* 436, 852-856.
5. Jiang, Y., Ruta, V., Chen, J., Lee, A. & MacKinnon, R. (2003) The principle of gating charge movement in a voltage-dependent K⁺ channel. *Nature* 423, 42-48.
6. Long, S.B., Campbell, E.B. & MacKinnon, R. (2005) Voltage-sensor of Kv1.2: Structural basis of electromechanical coupling. *Science* 309, 903-908.
7. Ruta, V., Chen, J. & MacKinnon, R. (2005) Calibrated measurement of gating-charge arginine displacement in the KvAP voltage-dependent K⁺ channel. *Cell* 123, 463-475.
8. Shrivastava, I.H., Durell S.R. & Guy, H.R. (2004) Model of voltage gating developed using the KvAP channel crystal structure. *Biophysical Journal* 87, 2255-2270.
9. Hessa, T. et al. (2005) Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature* 433, 377-381.
10. Hessa, T., White, S. H. & von Heijne, G. (2005) Membrane insertion of a potassium-channel voltage sensor. *Science* 307, 1427.
11. Blunck, R., Starace, D.M., Correa, A.M. & Bezanilla, F. (2004) Detecting rearrangements of Shaker and NaChBac in real-time with fluorescence spectroscopy in patch-clamped mammalian cells. *Biophysical Journal* 86, 3966-3980.
12. Schmidt, D., Jiang, Q.X., MacKinnon, R. (2006) Phospholipids and the origin of cationic gating charges in voltage sensors. *Nature* 444, 775-9.
13. Palaniappan, A. (2005) Theory-based investigations of the potassium-selective ion channel protein family. PhD thesis, University of Illinois.