ORIGINAL ARTICLE

A MATHEMATICAL MODEL FOR CHARGE DISTRIBUTIONS IN EXTERNAL MICRO-ENVIRONMENT OF ION CHANNELS

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Background: Current in ion channels has been modelled using Hodgkin-Huxley approach for past half century. There are models that are modified versions of Hodgkin-Huxley model but they only take macroscopic properties of current into consideration, thus termed large-scale models. In this paper another approach is used for modelling the immediate environment of ion channels in general and voltage-gated sodium channels in particular. **Methods:** Model of voltage-gated sodium channels is obtained using molecular dynamic analysis. Geometry of model that is obtained by molecular dynamic analysis is then mathematically approximated for electrical potential. Mathematical model of electric potential is built right from the first principle (i.e., Coulomb's law). **Results:** Results show the computational graphing of such model in 3D. R-Minimal<R1<R2<R3<R-Maximal values of radii are plotted. This gives a progressive pattern to ion distribution in the outside environment of the ion channel. The distribution breaks at R-maximal and the ion channel behaves as a point charge at R-minimal. **Conclusion:** This type of models gives in-depth insight of physiology of the ion channels. This model directly implies and explains the clustering of preferred ions just outside the ion channel.
Keywords: voltage-gated sodium channels, mathematical model

INTRODUCTION

Ion channels were first introduced by Hodgkin and Huxley in 1952.[1] Since their introduction and then the confirmation of their existence they have been proved to posses complex structure. Since the inception of ion channels, they have been studied in combination to the systemic current flow such as the Hodgkin-Huxley system of differential equation.

This paper tends to high-light individualistic biophysical properties of all ion channels in general but voltage-gated sodium channels in particular. This is to show how simple assumptions on electrodynamics of voltage-gated sodium channel's selectivity ring can alter external environment of the voltage-gated sodium channel pore. This gives rise to non-uniform potential just outside the voltage-gated sodium channel.[2] The DEKA component, which is a cylinder, is charged and has four amino acid residues, Aspartate, Glutamate, Lysine and Alanine. Lysine and Alanine are positively charged, while Aspartate and Glutamate are negatively charged.[3] This also shows that the selectivity pore is not uniformly charged. This selectivity pore is only about 0.3–0.5 ηm large and only large enough to allow Na^+ ions and not K^+ ions. This is probably how Na^+ and K^+ selectivity is conferred but until now the complete mechanism of voltage-gated sodium channel selectivity is not understood.[2] The main reason being that the structure of voltage-gated sodium channel has not been revealed through any of the original imaging techniques such as x-ray crystallography, NMR etc.[3] All models of voltage-gated sodium channels are only computational models that employ computational physics at various levels to reveal the structure of voltage-gated sodium channels.[2,3]

For theoretical models like the one discussed in this paper, it is important that it first quantifies the exact mechanism and then makes predictions that are tested. This is also a reason why voltage-gated sodium channels are chosen to study the model. Their structure is not fully revealed and they selectivity mechanism is not clear either. Secondly, the selective pore of voltage-gated sodium channel resembles a ring (see below). And thirdly, there is a competitive block on voltage-gated sodium channel by intracellular Mg^{++} ions as Lin *et al*[4] proposed. This is an important phenomenon as is discussed below.

This paper is only intended to show the potential of this method in evaluation of external models of ion channels and how they can be used to model charge distributions around the channel.

Overview of voltage-gated sodium channel physiology and ion channels related large-scale models:

Here the only concern is about a class of sodium channels called voltage-gated sodium channels. For details of the sodium channel families and channelopathies see Nadeem and Hussain[5]. Voltage-gated sodium channel are transporters of Na^+ ions inside the cell when cell is excited through the generation of action potential, i.e., when the electro-negativity in the cell falls. A relative potential of electrical field between the cell membrane activates the voltage-gated sodium channel (hence the name voltage-gated) and the rapid influx of the Na^+ ions leads to increased relative positive charge in that portion of the cell and increases the excitability of the cell and other Na^+ channels. As demonstrated by Hodgkin and Huxley[1], voltage-gated sodium channels appear to have three key features: voltage-dependent activation, rapid inactivation and

selective ion conductance. It is widely believed that the selectivity of the voltage-gated sodium channel is due to the pore which has a specific size and charge associated with it. As described above the structure of the voltage-gated sodium channel has not been revealed by the x-ray crystallography or nuclear magnetic resonance (NMR),[3] and the structure of the voltage-gated sodium channels will not be revealed in very near future because of its very small size. However some portions of voltage-gated sodium channel have been studied with x-ray crystallography and NMR.

What Hodgkin and Huxley studied in was the temporal behaviour. The series of five papers including the final conclusive paper, discussed the inflow and outflow of currents using basic circuit equation and then modifying it accordingly to match the behaviour of charge in and outside the Atlantic squid axon.[1] They studied the channels in different states that were modelled by differential equations. The model of Hodgkin and Huxley does not show individual charge distribution surrounding the neuron or how do different charges distribute themselves around the channel pore. Another such model called the FitzHugh–Nagumo model[2] is also large-scale simulation of group neurons. It is a simplified version of Hodgkin and Huxley and gives detailed mathematical insight of the dynamics of action potential.

Besides these there has been Morris-Lecar model[6] that combines the Hodgkin-Huxley model and Fitzhugh-Nagumo model. The model is another large-scale model that studies the relationships using the voltage of membrane and activation of the ions channel within it. This model also like other large scale-models does not reveal anything about the individual distribution of charges. Another such model is Hindmarsh-Rose model and is augmentation of the other such models as mentioned above.

Another way by which the behaviour of the ion-channels can be modelled is by a Markovian process.[7,8] This way the channels are treated as a population that are affected by thee independent gating variables. Each of these variables can attain a value between 1 (fully permeate to ions) and 0 (fully non-permeate). The product of these variables yields the percentage of the conducting channels. Markovian processes are conditional only on the present state of the system, its past and future is independent.

To conclude, such models cannot reveal much about the individual distribution of charges. These discrete models including the differential models above cannot reveal much about the distribution of individual charges outside the neuron or surrounding it.[9,10] They are only mentioned here for the completeness of background information and to show that these models are completely different interpretations of one system than the one this paper tries to introduce.

Structure and Subunits and Geometry of the Selectivity Filter:[11–16]

Voltage-gated sodium channels are made up of a complex α-subunit in association with a beta-subunit. Nine subunits have been functionally characterised. The primary sequence predicts that the voltage-gated sodium channel's α-subunit folds into four domains (I–IV), which are similar to one another and contain six α-helical trans-membrane segments (S1–S6). In each of the domains, the voltage sensor is located in the S4 segment, which contains a positively charged amino-acid residue in every third position. A loop between helices S5 and S6 is embedded into the trans-membrane region of the channel to form the narrow, ion selective filter at the intracellular end of the pore. This is called DEKA filter, corresponding to its amino acid sequence.

MATERIAL AND METHODS

Mathematical modelling was done in three steps. First, the exact shape of DEKA component was elucidated. Secondly, the mathematical model was approximated for the shape of DEKA component. Thirdly, it was shown how the electric potential will sum-up to give increased potential for *in vivo* ion-channels.

Molecular Dynamics Simulation of Selectivity Pore:

DEKA component using Molecular dynamics was modelled. Because of the scope of this paper MD analysis is not discussed here in detail. However, Laskowski *et al* describes it in detail and author has used their tool to model DEKA component. This facility is available at ProFunc server (http://www.ebi.ac.uk/thornton-srv/databases/profunc/).[17] Given a sequence this server models the exact template of it. The molecular dynamics analysis shows the pore of voltage-gated sodium channel's DEKA component to be roughly a circle.

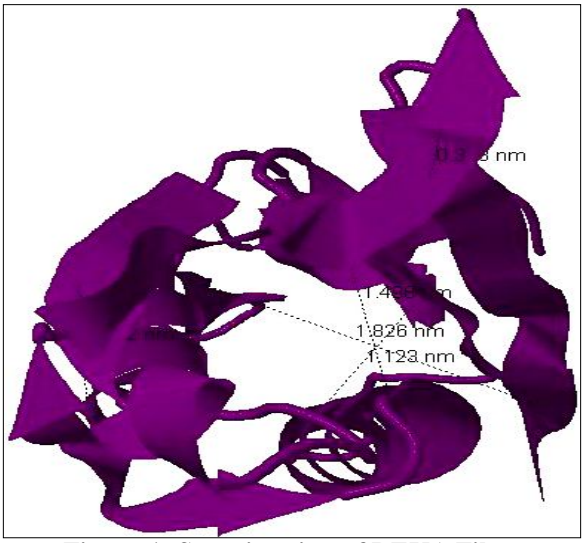

Figure-1: Superior view of DEKA Filter

Bezanilla *et al*[11] and Hille[12–14] have assumed the channel to be a rigid pore and the main selectivity mechanism is the size of the pore that only allows fully dehydrated Na^+ ions. Dehydration energy is derived by the oxides lining the pore. This would make Mg^{++} more selective because of its shorter radius and only little larger energy of hydration than Na^+.

Eisenman[16], Nonner *et al*[18] and Boda *et al*[19] predicted models that described the selectivity mechanism that are criticised by Lipkind and Fozzard[3]. They have described the role of Lysine at the specific site of the channel that is responsible for the selectivity of the sodium channel.

Until the X-ray structure is not available of the sodium channels we can only attempt to model the channel through MD analysis refining our models. So in the light of this model and role of Lysine at a specific site in the channel[3] the channel is neither rigid nor symmetric.

Mathematical model of charge conferred by selectivity pore:

As is proved with molecular dynamics in previous section that DEKA component is roughly circular so the model will be approximated on the ring of charge.

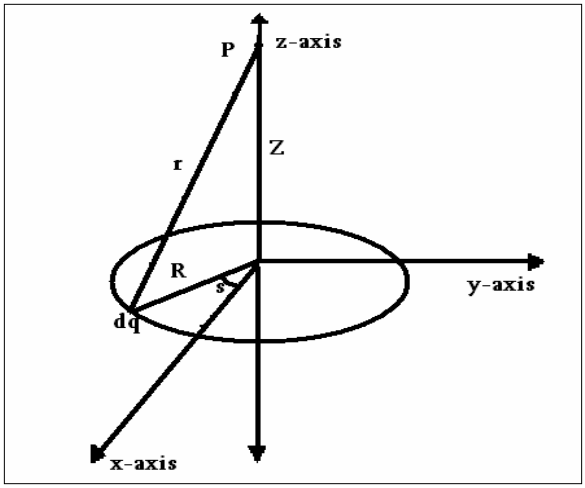

Figure-1.2: Generalised model of a channel

In Figure-1.2, R is the radius of the ring dq is the charge of the differential element DL, given in Eq. (1). λ is the charge density. Eq. (2) describes the charge that dL carries. Eq. (3) is the electric potential Vp on point P. Q in Eq. (5) is the total charge on the ring. In Eq. (6) Z>>R which is the case in external micro-environment of the ion-channel. This also is our limit to what constitutes the external micro-environment as anything significantly nearer than this will give rise to Eq. (8) on Cartesian coordinates. Hence, as shown in Eq. (6) charges will behave as point charges. In Eq. (3) and (7), Coulomb's law and electrical potential in the presence of electric field, respectively have been incorporated.[20]

$$dL = RdS; (1)$$

$$dq = \lambda dL = \lambda RdS; (2)$$

$$dV_p = \frac{1}{4\pi\epsilon^0}\frac{dq}{r} = \frac{1}{4\pi\epsilon^0}\frac{\lambda RdS}{\sqrt{Z^2+R^2}}; (3)$$

$$V_p = \int dV_p = \frac{1}{4\pi\epsilon^0}\frac{\lambda R}{\sqrt{Z^2+R^2}}\oint dS = \frac{1}{4\pi\epsilon^0}\frac{2\pi\lambda R}{\sqrt{Z^2+R^2}}; \quad (4)$$

$$\because 2\pi\lambda R = Q \ \therefore \ V_p = \frac{1}{4\pi\epsilon^0}\frac{Q}{\sqrt{Z^2+R^2}}; \ (5)$$

$$\because Z >> R \ \therefore V_p \approx \frac{1}{4\pi\epsilon^0}\frac{Q}{z}; (6)$$

$$\because dV_p = -\vec{E}.d\vec{S} \ \therefore dV_p = \left(E_x\hat{i} + E_y\hat{j} + E_z\hat{k}\right).\left(dx\hat{i} + dy\hat{j} + dz\hat{k}\right)$$

$$\Rightarrow E_x = -\frac{\partial V_p}{\partial x}, E_y = -\frac{\partial V_p}{\partial y}, E_z = -\frac{\partial V_p}{\partial z}; (7)$$

$$E_z = \frac{\partial V_p}{\partial z} = -\frac{\partial V}{\partial z}\left(\frac{1}{4\pi\epsilon^0}\frac{Q}{\sqrt{Z^2+R^2}}\right); (8)$$

Effect of potential summation over external micro-environment:

In vivo there is usually a clustering of such ions at junctions such as node of Ranvier and initial segment of the axon as Hill and Nishino[21], and Rasband and Trimmer[22] suggested. This phenomenon renders the mode of modelling more effective as it amplifies the effects of model by strengthening the electrical potential at these sites. This clustering can simply be described as sum of all individual potentials: Vt here is the total potential produced by a cluster of ions.

$$\because V_t = V_1 + V_2 + V_3 \cdots + V_n \ \therefore V_t = \sum_{i=1}^{n} V_t = \frac{1}{4\pi\epsilon^0}\sum_i \frac{Q_i}{Z_i}; (9)$$

RESULTS

Results are obtained by computationally plotting the mathematical model. Plotting is done using Java. This is shown that at R-minimal, where the radius of the channel (in this case voltage-gated sodium channel) is about zero, here the channel acts as a point charge. But still there is some symmetry to the outer environment. This symmetry progressively develops until the radius reaches R-maximal, where the symmetry breaks. The other transitional radii are given numbers R1, R2 and R3 respectively such that, R-Minimal<R1<R2<R3<R-Maximal. Channel is in the centre of the graphs and the ions are scattered according to the force of the channel protein. Main thing that is to be appreciated in this plot is the clustering of ions near the ion channel. This clustering makes our model more robust even in the presence of difference in geometry of the selectivity ring.

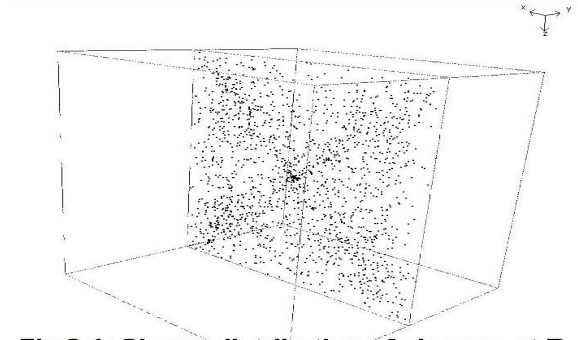

Fig 2.1: Shows distribution of charges at R minimal (channel pore is a point charge)

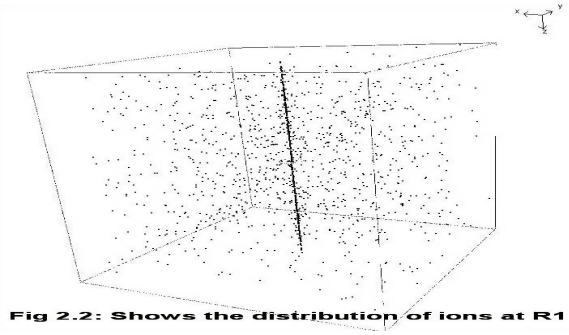

Fig 2.2: Shows the distribution of ions at R1

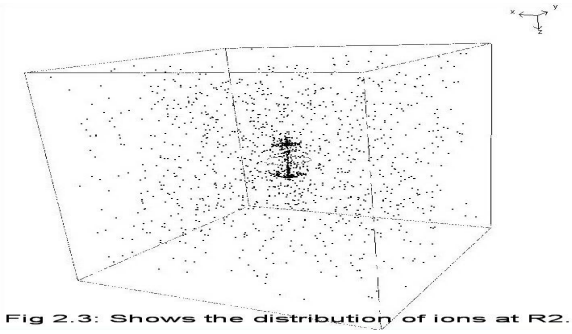

Fig 2.3: Shows the distribution of ions at R2.

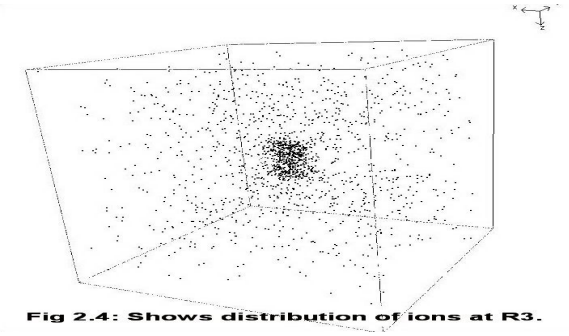

Fig 2.4: Shows distribution of ions at R3.

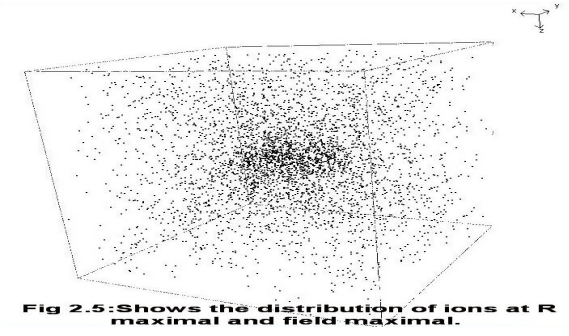

Fig 2.5:Shows the distribution of ions at R maximal and field maximal.

DISCUSSION

In the preceding section a simple mathematical model of the charge distribution on the voltage-gated sodium channel selectivity filter is proposed and it shows that the non-uniform charge distribution does not matter as the resultant of the electrostatic forces is all that is needed to model the external environment of the channel.

In this paper, main effort is to model the selectivity of the voltage-gated sodium channel as it is the subject of different theoretical methods such as molecular dynamics etc. The reason behind is that the charged channel is so small that through the passage the individual ions come close together and properties such as electro-negativity, bond-length etc. also have a major impact. However, when modelling just the external environment of voltage-gated sodium channel there is no need to model such details as they don't contribute significantly on the environment.

This model suggests that protein filter (DEKA ring) attract ions according to the field produced by them. The DEKA component which is a ringed structure was proved to behave as a point charge at a considerable distance from the ions, and nearer to the channel, ions behave differently. The clustering of ions depends, at all points in space around channel, on the resultant (sum or difference) of charges. So closer the ion to the channel the more it is affected by a unit of charge (depends on the charge's amino acid that constitutes the ring) and lesser by the other resultant forces. Thus, charge distribution starts having a wider pattern when they are nearer to the channel pore or when the channel pore is wider (as in leak channels and calcium channels). This also implies that there would be greater concentration of favoured ions at the pore than unfavourable ions. This implies that process of selection starts outside the pore. Therefore it is equally important to model outside environment of an ion channel than its inside. This observation is verified by Roth and Gillespie.[23] They suggest a clustering of positive ions just outside the pore. Also Zhou, MacKinnon[24] and Zhou, MacKinnon[25] in two separate works have shown that the clustering of selectivity in the outer environment of the K^+-channel, which is very close to voltage-gated sodium channel in structure. Once in the pore the ions are in other type of environment as explained above.

This model suggests that the ion channel would cluster those ions that have greater charge and atomic mass. For example, if there were two ions outside the pore like Na+ and Mg^{++}, Mg^{++} would be in greater concentration outside the sodium ions because both ions carry almost equal mass but Mg^{++} ions carries twice as much charge as Na^+ ions. In other words Mg^{++} ions have greater potential energy than Na^+ ions. This may produce block of Na^+ ions and this block would be competitive in nature as exactly noted by Lin *et al*.[4]

Roth and Gillespie[23] have also shown that a cylinder of protein surrounding a pore of radius R, (representing the wall of the channel) has properties similar to those of a cylinder with hard, smooth walls surrounding a pore of slightly large radius R+ΔR, when the cylinder of protein is represented as a fluid of wall particles. Just like this model except ΔR has not been modelled because the model does not involve internal

environment of the ion channel. Although this model was developed for voltage-gated sodium channels it can be generalised to vast class of ion channels that have a similar selection behaviour.[14,15]

The way ion channels are modelled in this paper is totally different from the classical approaches mentioned above. The modelling approach can be beneficial at least in the following three ways:

1. Distribution of ions as probabilities of interaction: As is shown in results, the electrostatic distributions of charges surrounding the ion channel becomes constant after some time. This could be studied as statistical distributions with their statistics depending on electrostatics. This way the probability of interaction can be modelled knowing the exact concentration gradient around the ion channel. For example tetrodotoxin is a voltage-gated sodium channel toxin. The concentration gradients of the tetrodotoxin around the voltage-gated sodium channel can be deduced using this model. This new concentration distribution can be used to deduce the exact probability of Tetrodotoxin and voltage-gated sodium channel interaction.
2. As First-Principle models they can be incorporated in the large-scale models: This can bring accuracy to the large scale models as the position of all ions and potential associated with them would become clear.
3. Enhanced pharmacodynamics and kinetics: This can give new insight into the pharmacodynamics of drugs just outside the target. This will result in more accurate drug designs and dosage adjustments.

CONCLUSION

Author has calculated the geometry of voltage-gated sodium channel and incorporated the structure and charge distribution in a mathematical model. There is a clustering of ions just outside the selectivity pore. This paper is intended to show the potential of this model in predicting the charge distribution in external micro-environment of ion channels like voltage-gated sodium channels. This has been achieved by building the model from first principles and literature review. For this model, like all mathematical models, there is further need of confirming the implications made by experimental techniques.

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