Biophysical Chemistry 252 (2019) 106196

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

The dynamics of K^+ channel gates as a biological transistor

Yuval Ben-Abu*

Department of Physics and Project Unit, Sapir Academic College, Sderot, Hof Ashkelon, 79165, Israel

ARTICLE INFO

Keywords:
Potassium channels
Coupled system
Gate linker
Mutation
Harmonic oscillators coupling

ABSTRACT

Potassium channels are pore-forming membrane proteins that open and close in response to changes in a chemical or electrical potential, thereby regulating the flow of potassium ions across biological membranes. Two regions of the same channels are acting in tandem and enable ion flow through the channel pore. I refer to this coupled action as a "gate linker". To closely examine the role of the gate linker in the channel function, I mutated the amino acids in the cDNA of this region, and used from knowen mutaion, either alone or together with the amino acids of adjacent regions.

I have emphasized the importance of the linker between these two gates - mutations in this region may cause conformational changes that play a fundamental role in mediating the coupling between the voltage sensor, activation gate and selectivity filter elements of Kv channels. I observe that free energy considerations show the significance of the coupling between the activation and inactivation gates. Moreover, a symmetry between the coupling and sensor spring strength leads to the destruction of ion conductivity. I present a thermodynamic framework for the possible study of multiple channel blocks. The arising physical perspective of the gating process gives rise to new research avenues of the coupling mode of potassium channels and may assist in explaining the centrality of the "gate linker" to the channel function.

1. Introduction

Potassium channels are pore-forming membrane proteins that open and close in response to changes in a chemical or electrical potential, thereby regulating the flow of potassium ions across biological membranes. These channels are homotetramers that, by selectively transferring potassium ions across the cell membrane, hyperpolarize the cell to form the energetic basis for neuronal and muscular activity [1]. Four identical subunits of the channel are embedded within the cell membrane and are arranged symmetrically around an ion-conducting pore, which is identical in all known potassium channels [1]. A potassium ion traversing the membrane through the pore domain of a K^+ channel encounters two structural gates along the ion conduction pathway: an activation gate at the intracellular entrance to the channel and a distant, slow inactivation gate at the extracellular entryway of the channel at the selectivity filter (Fig. 1). The activation–inactivation gate coupling for the two channels differs in function. The coupling is bidirectional in K_v channels; i.e., the activation gate induces the closure of the inactivation gate [2,3] and vice versa [4,5]. However, the coupling is nondirectional in K_{2P} channels, in which case the activation gate induces the closure of the inactivation.

This communication between distant functional elements is a fundamental property of many allosteric proteins. Information transfer between such elements may be achieved by the propagation of conformational changes through a protein structure, induced by changes in a chemical or electrical potential. Conformational changes are central to the function of voltage-activated potassium channels (Kv), pore-forming proteins that open and close in response to changes in a membrane potential. Such conformational transitions regulate the flow of potassium ions across the membrane [6–9], a process underlying many fundamental biological processes, in particular the generation of nerve and muscle action potentials [10]. Moreover, these conformational changes play a fundamental role in mediating the coupling between the voltage sensor, activation gate and selectivity filter elements of Kv channels [6–9].

Recent systematic energy perturbation analysis of the pore domain of the archetypal Shaker Kv channel [11] revealed that gating-sensitive positions—i.e., those positions where a mutation dramatically affects the closed-open equilibrium of the channel—cluster between the activation and inactivation gates.

In a previous study, which presented an antiphase model of potassium channel coupling [12], I observed how the gap between the activation and inactivation gates controls the energy between the two gates and the coupling process. This antiphase movement of the gate was controlled by the 'coupling spring' $\bar{k}$, which connects between the

* Corresponding author.
E-mail address: yuvalb@sapir.ac.il.

https://doi.org/10.1016/j.bpc.2019.106196
Received 7 April 2019; Received in revised form 2 June 2019; Accepted 2 June 2019
Available online 04 June 2019

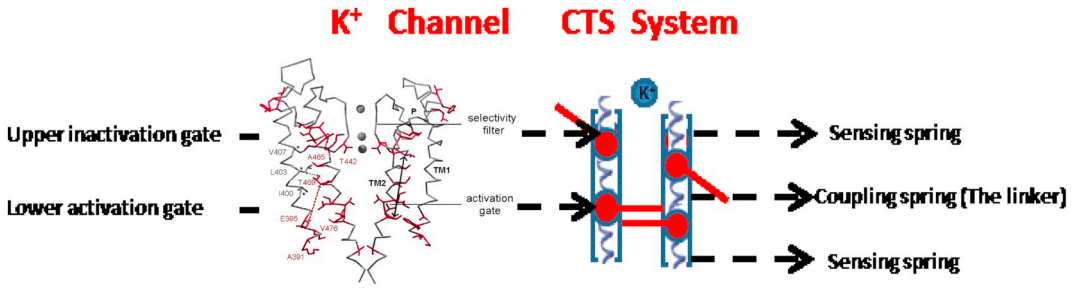

Fig. 1. The structure of a potassium channel (left) and its spring-system analogue (right). The sensing springs are analogous to the upper and lower gates of the channel, and the coupling spring is analogous to the gate linker, which connects the two gates.

two gates. A weakness or changes of this spring dramatically affect the gating process. This spring is a model for the amino acids that connect the activation and inactivation gates in actual channels. The spring is also affected by its external surroundings (e.g., by the two external springs). Mechanical changes in the coupling spring are analogous to mutations in the "gate linker" region of the actual potassium channel.

Here, as a continuation of the antiphase model, I demonstrate the functionality of the antiphase model by mutating the gap between the activation and inactivation gates. Our findings may also offer insight into the physical mechanism underlying the communication between distant channel elements.

2. Materials and methods

2.1. Molecular biology and electrophysiology

I used the Shaker-IR system [11] to introduce (Hoshi et al., 1990) a cDNA mutation in residues 6–46 by using the QuickChange method (Stratagene) and confirmed the mutation by sequencing the entire cDNA. I prepared mRNA through T7 polymerase transcription and injected the mRNA into *Xenopus laevis* oocytes. One or two days after injecting the mRNA, I recorded potassium ionic currents through the pore by using a two-electrode voltage clamp setup (OC725B, Warner Instrument Corp.). To form the electrodes, I pulled borosilicate capillaries (World Precision Instruments Inc.) to a resistance of ~0.5 MΩ and filled the capillaries with 3 M KCl. The bath solution contained (in mM): 58 NaCl, 40 RbCl, 0.3 CaCl2, 1 MgCl2, and 5 Hepes (pH 7.6). I typically held the oocytes at a voltage of between −100 mV and − 80 mV and then applied 50–200 ms steps of different test voltages, followed by repolarization, usually to the holding voltage. I typically measured the tail current amplitude 2–4 ms after repolarization. Analog data from the amplifier were low-pass filtered (2 kHz, −3 dB) with an 8-pole Bessel filter (Frequency Devices), digitized at 10 kHz, and stored on a PC hard drive. All experiments were conducted at room temperature (22 °C).

2.2. Data analysis

I used the measured tail currents to generate voltage activation curves. Then, I used Origin (version 5, Microcal Software Inc.) to fit these curves to a two-state Boltzmann equation:

$$I/I_{max} = (1 + e^{-ZF(V-V1/2)/RT})^{-1}$$

where I/Imax is the normalized tail current amplitude, Z is the effective charge, V1/2 is the half activation voltage, and T, F, and R are constants.

3. Results and discussion

I first studied the role of the gate linker region by mutating residues in an actual Kv channel. This residues which found at the specific region between the two gates, were proven as critical for the pore Kv channel stability [13–17,19]. Specifically, I tested whether these mutations would stabilize the channel in the open conformation (in which case the channel opens for negatives voltages) or in the closed conformation (in which case the channel opens for positives voltages). Some of the mutations in this region stabilized the channel in the open conformation, others stabilized it in the closed conformation, and some halted the transmission of current through the channel altogether as described elsewhere [12–15].

Next, I tested whether the gate linker region is solely responsible for the channel gating or, alternatively, whether amino acids outside this region can also affect the gating process. To this end, I performed single- and double-mutations in amino acids that were either within or near the gate linker region (Fig. 2). Similar to the findings of previous studies [13-18], mutating amino acids either within or near the gate linker region altered the stability of the channel conformation (Fig. 3), indicating that an intact gate linker region is not sufficient for proper channel gating; rather, it appears to be supported by nearby amino acid residues. Moreover, as long as the mutations far from the ion conduction pathway (gate linker), the close conformation is more stable more energy is needed to open it. To exam this, the mutation was composed from two residues: one from the S5 and the another from the ion conduction pathway site, means S6. In both cases, these mutation cause the channel to be in the open conformation. The meaning, mutation that found "too far" from the gate linker site, for example S5 subunit, together with mutation in the gate linker can reduce the effect of the S6

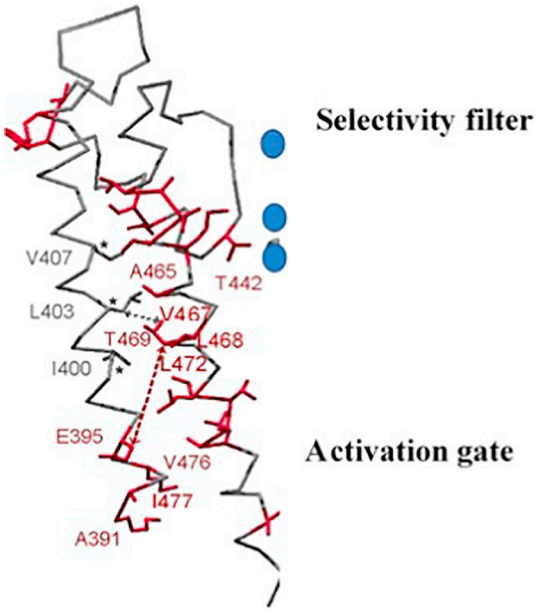

Fig. 2. The structure of S_5 and S_6 K^+ channel subunits that represent the mutations residues. These mutations preformed in two main sites between the two gates: S5 and S6 that located between the activation and inactivation gates.

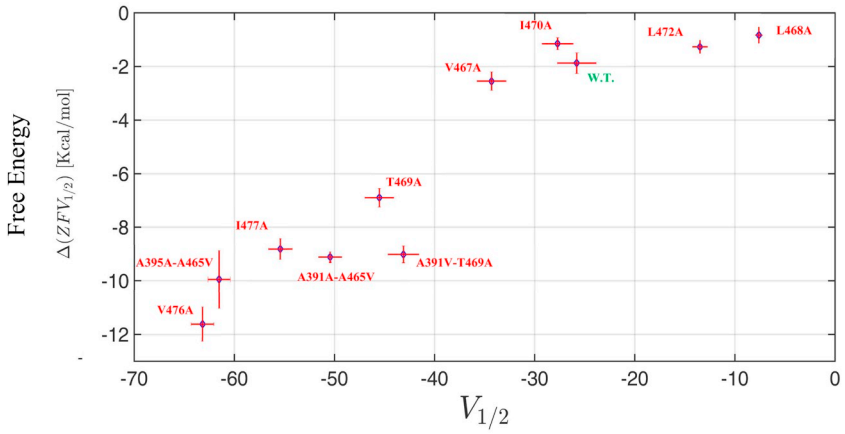

Fig. 3. The correlation between $V_{1/2}$ and the free energy. Each point represents a different mutant, as described in Fig. 1. As long as the channel opens for a negative voltage, the open conformation is more stable, and the free energy is high. This means that the channel does not exert work to open the channel.

mutation alone. As it seems, the open conformation is more stable, may as S5 residues mutations effect.

To gain further insights into the coupling mechanism between the gates, I modeled the channel gating as a coupled spring system and studied its behavior when the coupling spring, which models the gate linker, is either weak or absent. In this model, stretching the inactivation gate to the 'open' state at the rate of X1 shrinks the activation gate at the rate of X2 and the coupling spring $\bar{k}$ by the difference X2–X1. Thus, the movement equilibrium of the two gates, which describes how the movement of one gate is affected by the movement of the other, can be expressed as:

$$\begin{cases} m\ddot{x}_1 = -kx_1 + \bar{k}(x_2 - x_1) = -(k + \bar{k})x_1 + \bar{k}x_2 \\ m\ddot{x}_2 = -kx_2 + \bar{k}(x_1 - x_2) = -(k + \bar{k})x_2 + \bar{k}x_1 \end{cases} \quad (1)$$

First, I subtract or add the coupled equations in 1. After dividing by the mass of the gate, I obtain:

$$\begin{cases} (\ddot{x}_1 + \ddot{x}_2) = -\left(\frac{k}{m}\right)(x_1 + x_2) \\ (\ddot{x}_1 - \ddot{x}_2) = -\left(\frac{k + 2\bar{k}}{m}\right)(x_1 - x_2) \end{cases} \quad (2)$$

where:

$$\begin{cases} y_- \equiv x_1 - x_2 \\ y_+ \equiv x_1 + x_2 \end{cases} \quad (3)$$

$$\begin{cases} \omega_0^2 \equiv \frac{k}{m} \\ \bar{\omega}^2 = \frac{k + 2\bar{k}}{m} \end{cases} \quad (4)$$

Combining the equations in (2), (3), and (4) yields:

$$\begin{cases} \ddot{y}_- = -\bar{\omega}^2 y_- \\ \ddot{y}_+ = -\omega_0^2 y_+ \end{cases} \quad (5)$$

These equations describe the new coordinates for the sum and difference that are not conjugated and, therefore, act harmoniously as:

$$\begin{cases} y_- = A\cos(\bar{\omega}t + \alpha) \\ y_+ = B\cos(\omega_0 t + \beta) \end{cases} \quad (6)$$

where A, B, α, and β are determined by the initial conditions.

The physical model suggests that the strength of the gate linker (the coupling spring) is the main parameter that affects the coupling between the gates. Thus, the movement of both gates (springs) in the same direction with the same velocity weakens the coupling spring. Both gates move with the same angular frequency:

$$\omega_0 = \sqrt{\frac{k}{m}} \quad (7)$$

In the scenario described above, the middle spring is weak. However, the velocity of the ion current is so low that it cannot control the gate opening or closing. If I consider the centrality of the gate linker region (modeled as a coupling spring) for the channel gating, this scenario should not be physical. On the other hand, if both gates move in opposite directions, under the assumption of equal and opposite gate opening and closing velocities, the coupling spring is actuated and exerts a force on both gates. Such a spring should thus move with a higher angular frequency of:

$$\bar{\omega} = \sqrt{\frac{k + 2\bar{K}}{m}} \quad (8)$$

Mutations in the gate linker region of the modeled potassium channels are analogous to a weakening of the coupling spring in the mechanical model, as a result of activation reduction, such that:

$$k \gg \bar{k} \quad (9)$$

The explanation of this scenario requires engaging the system no symmetrically. At $t = 0$, the upper inactivation gate (Fig. 1) moves to the right at a rate of A1. In this case, I return to the original variables:

$$\begin{cases} x_1(t) = \frac{(y_- + y_+)}{2} = \frac{(A\cos(\omega_0 t + \alpha) + B\cos(\bar{\omega}t + \beta))}{2} \\ x_2(t) = \frac{(y_+ - y_-)}{2} = \frac{(B\cos(\bar{\omega}t + \beta) - A\cos(\omega_0 t + \alpha))}{2} \end{cases} \quad (10)$$

while the initial conditions are:

$$\begin{cases} x_1(0) = A_1 > 1 \\ \dot{x}_1 = \dot{x}_2(0) = 0 \\ x_2(0) = 0 \end{cases} \quad (11)$$

By substituting these values into eqs. 10, I obtain $\alpha = \beta = 0$ and $A = B$. Inserting these values into eqs. 10 gives:

$$x_1(t) = A_1 \cos\left(\frac{\bar{\omega} + \omega_0}{2}t\right)\cos\left(\frac{\bar{\omega} - \omega_0}{2}t\right) \quad (12)$$

Eq. (12) shows that energy is transferred from one gate to the other, and the movement amplitude of one gate is increased, while that of the other is decreased. The gates are then poorly coordinated, and in the real-world analogue, ions would not pass through the pore. In the opposite scenario, the two gates are coordinated and are represented by the equation:

$$x_2(t) = A_1 \sin\left(\frac{\overline{\omega} + \omega_0}{2}t\right)\sin\left(\frac{\overline{\omega} - \omega_0}{2}t\right) \tag{13}$$

In this scenario, the two gates work in tandem, albeit in opposite directions. This is enabled by the very strong spring.

In the specific cases where the coupling spring is much weaker than the spring k, I obtain:

$$x_1(t) \approx \cos(\omega_0 t)\cos(\Delta\omega t) \equiv A_{mod}(t)\cos(\omega_0 t) \tag{14}$$

where

$$A_{mod} = A_1 \cos\Delta\omega t \tag{15}$$

and

$$x_2(t) \approx \mathrm{A}_1 \sin(\omega_0 t)\sin(\Delta\omega t) \equiv B_{mod}(t)\sin(\omega_0 t) \tag{16}$$

where

$$B_{mod} = A_1 \sin\Delta\omega t \tag{17}$$

Fig. 4 summarize the equations mention above and some mention elsewhere [11–13]. K+ channels represented by schematic cycle connecting the four possible K+ channel pore states (Fig. 4). In the case $\leq \bar{k}$, where the lower activation (A) and upper slow inactivation (I) pore gates are either closed (C) or open (O). The four A_CI_O, A_OI_O, A_OI_C and A_CI_C states, positioned at the corners of the cycle, are composite states each representing the many conformational states usually associated with that state [11–13]. Horizontal transitions represent activation gate opening (or closing), once with the inactivation gate open (A_CI_O-A_OI_O transition) and once with it closed (A_CI_C-A_OI_C transition). Similarly, vertical transitions represent closure (or opening) of the upper inactivation gate when the other gate is either closed (A_CI_O-A_CI_C) or open (A_OI_O-A_OI_C). When the middle spring is very weak, means $k \gg \gg \bar{k}$, the four state are uncontrolled and the channel is closed. In the real channel the meaning is K+ can't move through the channel. This case could represent by some solvent, lipids, materials, or stretch that has some effect on outside and inside cell- where found outer and inside springs. When they act, the change the operation of mid spring, and of course by this on the gating process. Effect outside and inside channel. Effect on the

These are simply two products of harmonic functions. One harmonic function has a low frequency ($\Delta\omega$), while the other function rapidly changes with frequency (ω_0). The product yields an amplitude-modulated wave, with an "envelope" of the slowly changing wave and a "carrier" of the rapidly changing wave. The movement is antiphased, much like the synchronous movement of the mammalian heart's atria and ventricles. When ions are being "pumped" in by the first gate, the gate stops its function, and the second gate moves the ions further. To examine the linker strength and coupling, I consider the entropy of the potassium channel.

4. The thermodynamic point of view from a single-channel coupling gate

To further analyze the roles of the sensor springs and the linker importance, I have chosen to look at this system within the framework of classical thermodynamics. This might have the added value of yielding insights into an aggregate of such mechanisms, such as the human central nervous system. In Fig. 1, the two sensor springs have rest lengths of l, the linker spring has a rest length of D, and x_1 and x_2 are the coordinates of the masses m_1 and m_2, respectively.

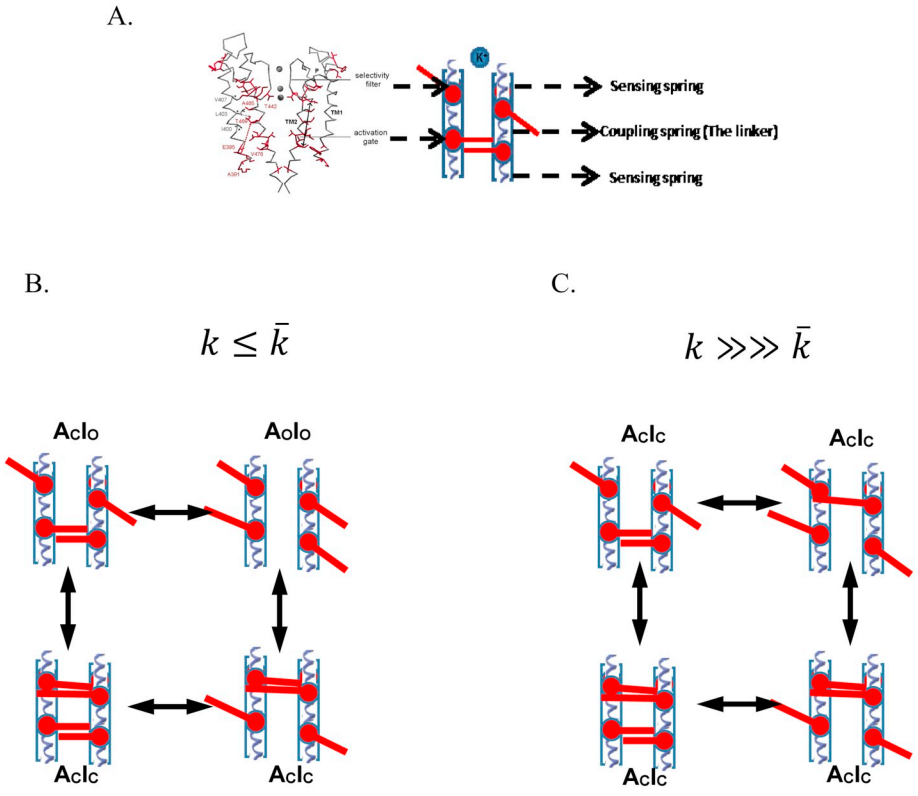

Fig. 4. Potassium channel model as reparented in eqs. 8–17: A. Structure of Potassium channel and its spring system analogue. B. Simplified schematic cycle connecting the four possible K+ channel pore states in the case $k \leq \bar{k}$, where the lower activation (A) and upper slow inactivation (I) pore gates are either closed (C) or open (O). The four A_CI_O, A_OI_O, A_OI_C and A_CI_C states (see main text), positioned at the corners of the cycle, are composite states each representing the many conformational states usually associated with that state [11–13]. Horizontal transitions represent activation gate opening (or closing), once with the inactivation gate open (A_CI_O-A_OI_O transition) and once with it closed (A_CI_C-A_OI_C transition). Similarly, vertical transitions represent closure (or opening) of the upper inactivation gate when the other gate is either closed (A_CI_O-A_CI_C) or open (A_OI_O-A_OI_C). C. Simplified schematic cycle connecting the four possible K^+ channel pore states in the case $k \gg \gg \bar{k}$.

I start by defining the Lagrangian and Hamiltonian of the system. I then find the partition function, and using the canonical ensemble framework, I extract the entropy. The Lagrangian is given by:

$$\mathscr{L} = \frac{1}{2}[m_1 \dot{x_1}^2 + m_2 \dot{x_2}^2 - k(l - x_1)^2 - k(l + D - x_2)^2 - \bar{k}(x_2 - x_1 - D)^2]$$

I make the following transformation: $\xi_1 = x_1 - l$; $\xi_2 = x_2 - (l + D)$;thus, I arrive at the following Hamiltonian:

$$\mathscr{H} = \frac{p_1^2}{2m_1} + \frac{p_2^2}{2m_2} + \frac{k}{2}(\xi_1^2 + \xi_2^2) + \frac{\bar{k}}{2}(\xi_1 - \xi_2)^2$$

In turn, I find the partition function for this system by using the following definition:

$$Z = \sum e^{-\beta\varepsilon}$$

I use the Hamiltonian to average the energy of the system, so I obtain:

$$Z = \int_{p_1=-\infty}^{\infty} \int_{p_2=-\infty}^{\infty} \int_{\xi_1=-l}^{l+D} \int_{\xi_2=\xi_1}^{l+D} e^{-\beta\mathscr{H}}\, dp_1 dp_2 d\xi_1 d\xi_2$$

The momentum degrees of freedom integrate neatly to yield $\sqrt{\frac{4\pi^2 m_1 m_2}{\beta^2}}$; however, the coordinate integral is slightly more complicated. If I make the assumption of small amplitude movement, the probability of, for instance, ξ_1 to go all the way to $-l$ is so small that there is no effective difference between $-l$ and $-\infty$ in terms of the contribution to the partition function. Thus, I can effectively set

$$z = \sqrt{\frac{4\pi^2 m_1 m_2}{\beta^2}} \cdot \int_{\xi_1=-\infty}^{\infty} \int_{\xi_2=-\infty}^{\infty} e^{-\beta\mathscr{H}}\, d\xi_1 d\xi_2$$

In turn, this yields:

$$Z = \frac{4\pi^2}{\beta^2}\sqrt{\frac{m_1 m_2}{(k + 2\bar{k})k}}$$

I immediately find that when $k \gg \bar{k}$, I return to the uncoupled mass and spring system.

The single-channel free energy is given by $= -\tau \ln Z$, and using the relation $\sigma = -\frac{\partial F}{\partial \tau}\Big|_V$,

I obtain:

$$\sigma = 2 + \ln\left(4\pi^2\tau^2\sqrt{\frac{m_1 m_2}{k(2\bar{k} + k)}}\right)$$

Let us observe that when $\bar{k} \gg k$, the approximate entropy is symmetric with respect to the interchange $k \leftrightarrow \bar{k}$, which indicates a two mass and spring system: one with $\omega = \sqrt{\frac{k}{m_i}}$ and the other with $\omega = \sqrt{\frac{\bar{k}}{m_{i\neq j}}}$.In turn, this hints at the need to fine tune the linker strength. As shown here, the linker has an appreciable effect on the gating process of the potassium channel.

To study the free energy of the potassium channel, I estimate the energy change of the gating caused by a mutation [11]. To examine these changes, I parametrize the activation process by fitting data to a two-state Boltzmann function, which has two independent variables: $V_{1/2}$ (the voltage at half activation) and Z (proportional to the slope at half activation) [11]. If a channel had only two states, then the free energy difference between closed and opened states at zero voltage would be equal to $-ZFV_{1/2}$, and the energy change caused by a mutation would be $-F(Z_{wt}V_{1/2.wt} - Z_{mut}V_{1/2\ mut}) = \Delta(ZFV_{1/2})$. The first property of gating insensitivity at many sites is fortunate because it means there is a subset of sensitive positions that stand out above the background. The second property of a correlation between $V_{1/2}$ and Z is interesting because it has important implications for the possible mechanisms of gating and for the transitions altered by a mutation. To see why this is the case, consider first a two-state gating mechanism, the model used to evaluate $V_{1/2}$ and Z. In this simple model, by affecting the equilibrium between the two states (but not the gating charge), mutations would alter $V_{1/2}$ without changing Z; that is, they would shift the activation curve midpoint but not the slope. The same is true for a gating mechanism in which four subunits undergo independent closed to open transitions, allowing conduction once all the subunits reach the open state. To explain the kind of correlation between $V_{1/2}$ and Z that is actually observed in Fig. 2, multiple transitions between the closed and open states must be invoked, and mutations must alter the ratio of equilibrium constants connecting the states. As long as the channel opens for a negative voltage, the open conformation is more stable, and the free energy is high. This means that the channel does not exert work to open the channel.

It is interesting to note that the majority of mutations at the sensitive positions of the “linker” caused the activation curve to shift leftward along the voltage axis (Fig. 1). In other words, mutations most often shift the gating equilibrium toward the open or close state. This could occur in one of two ways: mutations could either stabilize the open state relative to closed state, or they could destabilize the closed state relative to the open state. However, there are more ways to disrupt protein packing than to stabilize it, and extensive experience tells us that mutations most often are destabilizing to a protein's structure. Therefore, I interpret the tendency of mutations to shift the gating equilibrium toward the open state as evidence that it is easier to destabilize the closed conformation of the channel with mutations.

Why might mutations be more destabilizing to the closed conformation? I think that the answer to this question might have to do with the stability of the closed versus open pore structures of the wild-type channel. I know that KcsA with its C terminus deleted (which is essentially a K+ channel without a gating domain) has such a stable closed conformation that it is very difficult to open under any circumstance. The more stable closed state might be explicable on the basis of the identifiable structural differences in KcsA and MthK in closed and open K+ channels. In the closed conformation, the inner helices are nearly straight, packed against adjacent outer helices in a canonical two-helix packing pattern and packed against each other at the bundle. In the open conformation, the inner helices bend at the expense of broken hydrogen bonds within the membrane, and many of the helix packing interactions of the closed conformation are lost [20-23]. A comparison of these structures gives the qualitative impression that the open pore is under strain and that the closed conformation is a more stable structure due to an increased number of favorable protein contacts. Thus, I suggest that mutations are more perturbing to the closed channel because there are more ways to disrupt a more optimally packed protein. In conclusion, I have presented a general strategy for the direct analysis of cooperativity between activation and inactivation gates [20-25]. Moreover, I have emphasized the importance of the linker between these two gates - mutations in this region may cause conformational changes that play a fundamental role in mediating the coupling between the voltage sensor, activation gate and selectivity filter elements of Kv channels. To summarize, I find that free energy considerations show the significance of the coupling between the activation and inactivation gates. Moreover, I observe that a symmetry between the coupling and sensor spring strength leads to the destruction of ion conductivity. I present the thermodynamic framework for the possible study of multiple channel blocks.

Funding

Not applicable.

Availability of data and materials

All data are not explicitly included in the article. However, authors can share the data upon request.

Ethics approval and consent to participate

No ethical approval was needed in these experiments.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

I would like to thank Prof. Steven Vogel, Duke University.

References

[1] R. MacKinnon, Potassium channels, FEBS Lett. 555 (1) (2003) 62–65.
[2] T. Baukrowitz, G. Yellen, Modulation of K+ current by frequency and external [K+]: a tale of two inactivation mechanisms, Neuron 15 (4) (1995) 951–960.
[3] T. Baukrowitz, G. Yellen, Two functionally distinct subsites for the binding of internal blockers to the pore of voltage-activated K+ channels, Proc. Natl. Acad. Sci. U. S. A. 93 (23) (1996) 13357–13361.
[4] G. Panyi, C. Deutsch, Cross talk between activation and slow inactivation gates of Shaker potassium channels, J Gen Physiol 128 (5) (2006) 547–559.
[5] G. Panyi, C. Deutsch, Probing the cavity of the slow inactivated conformation of shaker potassium channels, J Gen Physiol 129 (5) (2007) 403–418.
[6] F.J. Sigworth, Voltage gating of ion channels, Q Rev Biophys 27 (1994) 1–40.
[7] W.N. Zagotta, T. Hoshi, R.W. Aldrich, Computing transient gating charge movement of voltage-dependent ion channels, J Gen Physiol 103 (1994) 321–362.
[8] G. Yellen, The moving parts of voltage-gated ion channels, Q. Rev. Biophys. 31 (1998) 239–295.
[9] F. Bezanilla, The voltage sensor in voltage-dependent ion channels, Physiol. Rev. 80 (2000) 555–592.
[10] B. Hille, Ion Channels of Excitable Membranes, Sinauer, Sunderland, MA, 2001.
[11] O. Yifrach, R. MacKinnon, Energetics of pore opening in a voltage-gated K(+) channel, Cell 111 (2) (2002) 231–239.
[12] Y. Ben-Abu, Gate antiphase of potassium channel, Symmetry 9 (8) (2017) 150, https://doi.org/10.3390/sym9080150.
[13] Y. Ben-Abu, et al., Inverse coupling in leak and voltage-activated K+ channel gates underlies distinct roles in electrical signaling, Nat. Struct. Mol. Biol. 16 (1) (2009) 71–79.
[14] Y. Li-Smerin, K.J. Swartz, Helical structure of the COOH terminus of S3 and its contribution to the gating modifier toxin receptor in voltage-gated ion channels, J Gen Physiol 117 (3) (2001) 205–218.
[15] D.H. Hackos, T.H. Chang, K.J. Swartz, Scanning the intracellular S6 activation gate in the shaker K+ channel, J Gen Physiol 119 (6) (2002) 521–532.
[16] Y. Li-Smerin, K.J. Swartz, Localization and molecular determinants of the Hanatoxin receptors on the voltage-sensing domains of a K(+) channel, J Gen Physiol 115 (6) (2000) 673–684.
[17] Y. Li-Smerin, D.H. Hackos, K.J. Swartz, Alpha-helical structural elements within the voltage-sensing domains of a K(+) channel, J Gen Physiol 115 (1) (2000) 33–50.
[18] T. Hoshi, W.N. Zagotta, R.W. Aldrich, Biophysical and molecular mechanisms of shaker potassium channel inactivation, Science 250 (1990) 533–538.
[19] E. Sadovsky, O. Yifrach, Principles underlying energetic coupling along an allosteric communication trajectory of a voltage-activated K+ channel, Proc. Natl. Acad. Sci. U. S. A. 104 (50) (2007) 19813–19818.
[20] N. Zandany, L. Lewin, V. Nirenberg, I. Orr, O. Yifrach, Entropic clocks in the service of electrical signaling: 'Ball and chain' mechanisms for ion channel inactivation and clustering, FEBS Lett. 589 (2015) Sep 14.
[21] N. Zandany, O. Yifrach, A mechanistic framework for studying Kv channel clustering, Channels (Austin) 9 (4) (2015) 163–165.
[22] N. Zandany, S. Marciano, E. Magidovich, T. Frimerman, R. Yehezkel, T. Shem-Ad, L. Lewin, U. Abdu, I. Orr, O. Yifrach, Alternative splicing modulates Kv channel clustering through a molecular ball and chain mechanism, Nat. Commun. 6 (2015) 6488, https://doi.org/10.1038/ncomms7488 Mar 27.
[23] W.N. Zagotta, T. Hoshi, R.W. Aldrich, Shaker potassium channel gating. III: evaluation of kinetic models for activation, J. Gen. Physiol. 103 (1994) 321–362.
[24] E. Schoppa, F.J. Sigworth, Activation of shaker potassium channels. III. An activation gating model for wild-type and V2 mutant channels, J. Gen. Physiol. 111 (1998) 313–342.
[25] W.N. Zagotta, T. Hoshi, R.W. Aldrich, Shaker potassium channel gating. III: evaluation of kinetic models for activation, J. Gen. Physiol. 103 (1994) 321–362.