Biophysical Chemistry 184 (2013) 126–130

Contents lists available at ScienceDirect

Biophysical Chemistry

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

Letter to the Editor

A model for the hysteresis observed in gating of lysenin channels

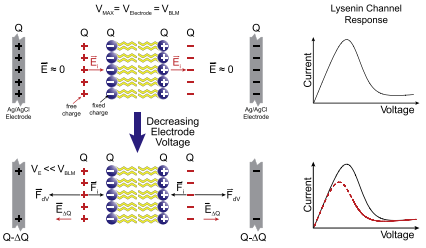
Eric Krueger [a,*], Radwan Al Faouri [a], Daniel Fologea [b,*], Ralph Henry [c], David Straub [d,e], Greg Salamo [a]

[a] Department of Physics, University of Arkansas, Fayetteville, AR 72701, USA
[b] Department of Physics, Boise State University, Boise, ID 83725, USA
[c] Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA
[d] Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA
[e] Department of Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

HIGHLIGHTS

- Lysenin channels exhibit both dynamic and static hysteresis elements.
- Static hysteresis arises from the collection of free charge on a polarized BLM.
- Experimental data supports model as a shift in I–V curve.
- Temperature dependent hysteresis also supports mechanism suggested by model.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form 28 August 2013
Accepted 3 September 2013
Available online 12 September 2013

Keywords:
Lysenin
Voltage-gated channels
Hysteresis

ABSTRACT

The pore-forming toxin lysenin self-inserts to form conductance channels in natural and artificial lipid membranes containing sphingomyelin. The inserted channels exhibit voltage regulation and hysteresis of the macroscopic current during the application of positive periodic voltage stimuli. We explored the bi-stable behavior of lysenin channels and present a theoretical approach for the mechanism of the hysteresis to explain its static and dynamic components. This investigation develops a model to incorporate the role of charge accumulation on the bilayer lipid membrane in influencing the channel conduction state. Our model is supported by experimental results and also provides insight into the temperature dependence of lysenin channel hysteresis. Through this work we gain perspective into the mechanism of how the response of a channel protein is determined by previous stimuli.

1. Introduction

Hysteresis in biological systems has important implications in protecting cells from random perturbations in stimuli and provides a mechanism to promote sequential cellular processes [1–5]. It is also present in the activity of ion channels and porins which serve to regulate transport across a cell membrane [6–10]. Their regulatory behavior has been extensively studied both experimentally and theoretically and is the result of an interaction between a voltage-sensitive domain on the protein channel and an applied electric field [11–16]. The inactivation and reactivation of the channel have been shown to exhibit a hysteresis in the channel conductivity [6–10]. The dynamic hysteresis demonstrated by these membrane transporters has been observed as the result of the competing time scales between the slow equilibrium and the period of the stimulus [8,9,17–19].

Recently, the pore-forming protein lysenin has shown promise for characterizing voltage-induced gating and hysteresis due to its transport and regulation characteristics similar to ion channels [20,21]. Lysenin is a 297 amino acid pore-forming protein that self-assembles into ~3 nm diameter oligomeric channels in bilayer lipid membranes (BLMs) containing sphingomyelin [20,22–24]. Experimental investigations with lysenin channels inserted into a vertical planar BLM have shown that the channels responded to an applied voltage by blocking the ionic current when the electric field was oriented opposite to the direction of protein insertion [20]. Recently, the structure of the lysenin monomer has been uncovered and has provided some insight into the

* Corresponding authors at: Department of Physics, Boise State University, Boise, ID 83725-1570, USA. Tel.: +1 208 426 3717; fax: +1 208 426 4330.
E-mail addresses: erickrueger879@boisestate.edu (E. Krueger), radwanaf@uark.edu (R. Al Faouri), danielfologea@boisestate.edu (D. Fologea), rahenry@uark.edu (R. Henry), straubkarld@uams.edu (D. Straub), salamo@uark.edu (G. Salamo).

0301-4622/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bpc.2013.09.001

charged regions of the protein which we believe to be responsible for the gating [25]. Using this information, we assembled a model that describes a mechanism for the origins of lysenin gating and provided an explanation for the dynamic and static hysteresis.

Several theoretical models have explored the dynamic hysteresis observed in ion channels [17,26–28]. However, a persistent static hysteresis has not been sufficiently modeled even though hysteresis is thought to be an intrinsic behavior of ion channels [6,27]. In this paper we investigate the mechanisms responsible for the voltage-induced gating and consequential hysteresis exhibited by lysenin channels. We present a model that addresses the static and dynamic nature of lysenin channel hysteresis and is able to recognize the mechanisms involved that contribute to the observed phenomena. Our experimental observations suggest an interaction between the protein channel and the bilayer membrane which are supported by our model.

2. Channel gating and hysteresis

The voltage-induced gating observed by lysenin channels is thought to be the result of a change in the channel conformation due to the interaction between a voltage-sensitive element on the protein and the applied electric field. The channel inactivation is observed as a decrease in macroscopic ionic current as the channel transitions from a conducting state to a non-conducting state as shown in black in Fig. 1a. The current increases linearly with increasing voltage until the channels begin to transition into the closed state where the I–V curve breaks its linearity. A more detailed explanation of the channel gating has been previously published [20]. The bistable system was measured to be a dynamic equilibrium between the two states of the protein which gives rise to the voltage-dependent probability of finding the channel in the open (conducting) state, which can be described by Boltzmann's statistics as:

$$P_{open} = \frac{1}{1 + e^{\frac{-\Delta E + qV}{kT}}} \quad (1)$$

where ΔE is the difference in the energies between the open and closed states, q is the gate charge on the protein, V is the applied voltage, k is the Boltzmann constant and T is the temperature. Further details concerning the dynamic equilibrium can be found in the Supplementary data. The observed current in Fig. 1a produced by the applied voltage across the BLM yields an ionic current given by Ohm's law as:

$$I = VgNP_{open} \quad (2)$$

where g is the conductance of a single channel, N is the total number of channels. It follows that the observed current for increasing voltages can therefore be described as:

$$I = \frac{gVN}{1 + e^{\frac{-\Delta E + qV}{kT}}} + G_L V \quad (3)$$

where G_L is added as the leakage conductance observed when the open probability approaches zero. Eq. (3) produces an excellent fit ($R^2 = 0.999$) to the lysenin channel inactivation data for a population of channels shown in green in Fig. 1a.

Channel reactivation was observed upon decreasing the applied voltage and measuring the macroscopic current as shown in red in Fig. 1a. However, the reactivation curve takes a different reaction pathway than inactivation until all of the channels return to the open state. The two distinct I–V curves result in an observed hysteresis in the macroscopic current as previously reported [21].

This hysteresis behavior was observed when the voltage sweep rate exceeded the characteristic time of the channels and is not observed or predicted for ion channels [17,19]. Initial speculation into the mechanism of "static" hysteresis pointed towards an analogous mechanism

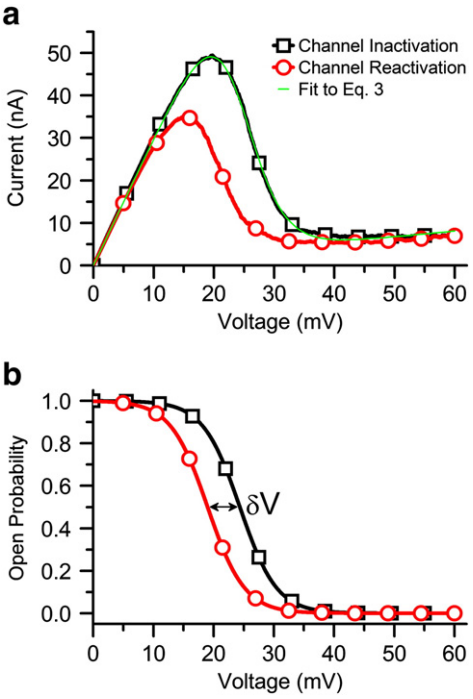


Fig. 1. Lysenin channel inactivation (black) and reactivation (red) due to applied voltages. (a) Decreasing voltages initiate channel reactivation along a different pathway than inactivation which produces a hysteresis in the current. A fit of Eq. (3) (green) to the experimental inactivation data where ΔE = 0.20 ± 0.001 eV, q = 8.35 ± 0.02e, G_L = 134.3 ± 0.3 nS. (b) The open probabilities from the inactivation and reactivation I–V curves are separated by a difference in voltage δV. Sampling rate = 5 Hz, symbols are shown every 250 data points.

to that of a ferroelectric in which dipoles flip in direction and are locked into a polarization state by dipole–dipole interactions that must be overcome in order to flip the dipoles back. However, protein–protein interactions were ruled out because hysteresis was observed in single channel measurements indicating that the hysteresis is not due to neighbor–neighbor interactions [21]. Another possibility is that the hysteresis is due to a conformational change. However, given the clear evidence that the channel opening and closing is a probability process, reopening of the channel would follow the same equilibrium probability distribution and closing the channel for a given applied electric field.

Deviating from the hypothesis that changes in the protein occur during the state transition, we focused on the BLM itself for origins of the hysteresis. To examine this possibility, the open probability given by Eq. (1) can be plotted for the inactivation and reactivation I–V curves shown in Fig. 1b.

Analysis of the channel open probability for increasing and decreasing voltage ramps shows that the open probability of the decreasing voltage follows the same profile as the increasing voltage ramp, but it is shifted to lower voltages by δV. This suggests that the channels do not respond immediately to the decrease in voltage. To investigate this behavior, we turned our attention to the electric fields present in the BLM.

The BLM is bathed in an electrolyte solution, so the voltage applied by the electrodes is transferred to the membrane by the ions in the solution. The voltage drop from the electrolyte solution was measured to be approximately 10,000 times less than that of a single channel. Therefore, the electrode voltage was approximately equal to the voltage across the membrane. The ions in the solution provide the free charge which produces an electric field through the dielectric BLM as shown in Fig. 2.

The electric field external to the BLM from the ions is $\mathbf{E}_{ext} = (\sigma_f / \varepsilon)$, where σ_f is the surface charge density of the ions surrounding the BLM. The external field results in a polarization of the BLM given by

$\mathbf{P} = \varepsilon_0 \chi \mathbf{E}_{ext}$, where ε_0 is the permittivity and χ is the electrical susceptibility of the BLM. The induced electric field therefore is $\mathbf{E}_{BLM} = -(\sigma_b / \varepsilon)$ where σ_b is the bound surface charge density of the induced dipoles in the membrane. The bound surface charge shown on the lipids in Fig. 2 does not signify the net charge of the lipid itself, but rather is indicating the polarization of the membrane responsible for $\mathbf{E}_{BLM}$. The electric field inside the BLM can be given as the sum of the external and dipole electric fields which can be written as a function of the external field.

$$\mathbf{E}_{int} = \mathbf{E}_{ext}/(1+\chi) \tag{4}$$

According to Eq. (4), the external electric field is greater than the internal field by a factor of the dielectric constant of the BLM. The internal electric field is the net field that interacts with a charged component on the protein to close the channels. Reversal of the external field produces a reversed internal field, but does not induce gating as shown for the negative voltages [20]. The induced dipoles inside the membrane provide the fixed charge in the BLM that interacts with the free charge in solution. It is the coulombic interaction between the free charge and fixed charge that we hypothesize to be the origin of the observed hysteresis behavior. Additionally, since the value of δV was found to be independent of the number of channels we rule out the possibility that the polarization of the protein plays a significant role in the hysteresis.

Starting at low voltages, the increasing voltage applied across the electrodes is equal to the voltage across the BLM. The free charge in solution collected along the electrolyte/membrane interface, while the electric field polarized the BLM. An electric field arises at the interface between the free charge and fixed charge, $\mathbf{E}_i$, as shown in Fig. 3a. The total charge on the electrode, Q, is equal to the total charge built up on the BLM surface. Since the electrolyte resistance was measured to be negligible, the voltage across the electrodes is nearly equal to the voltage across the BLM and the electric field in the bulk solution is 0.

Upon decreasing the voltage across the electrodes, the charge in the electrodes is reduced by ΔQ. The free charge, however, is not decreased due to the coulombic interaction with the fixed charge. An electric field is produced due to the difference in voltages, $\mathbf{E}(\Delta Q)$, as shown in Fig. 3b. The magnitude of $\mathbf{E}(\Delta Q)$ is less than $\mathbf{E}_i$, so the subsequent force pulling the free charge away from the membrane is too small to separate the charges. Consequently, the internal field acting on the channels remains constant.

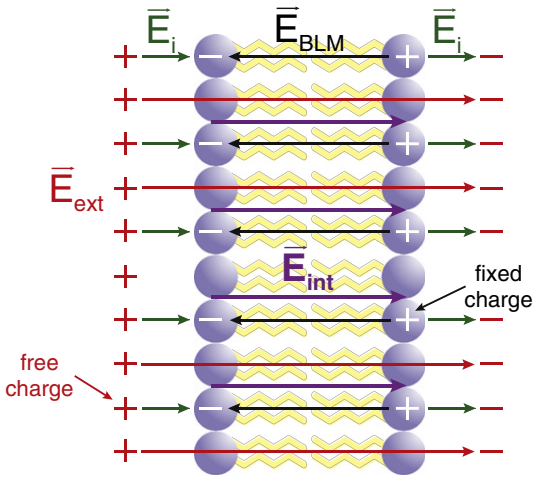


Fig. 2. The internal electric field in a BLM is the result of an external field caused by the free charge and the field caused by the polarization of the BLM. The bound charge in the BLM and free charge in solution create an electric field at the solution/BLM interface, $\mathbf{E}_i$. The bound charges shown on the lipid headgroups indicate membrane polarization and not the net charge of the lipid.

The reduction in the electrode voltage is observed in the I–V curve shown in Fig. 3c. The decreasing voltage curve (red) deviates from the increasing voltage curve (black) where the leakage current term dominates in Eq. (3). The channels have not started to reopen because the internal electric field has not decreased. This is the beginning of the lag between the applied electrode voltage and the voltage producing the internal field. Upon further reduction in the electrode voltage, shown in Fig. 3d, the charge in the electrode is reduced by some factor $2\Delta Q$. The electric field in the bulk solution becomes large enough that the force produced can overcome the force between the free charge and the fixed charge. Reducing the number of free charges at the interface reduces the internal electric field acting on the lysenin channel. Consequently, the reduction in $\mathbf{E}_{int}$ allows the reactivation of channels which is observed as an increase in the ionic current shown in Fig. 3e. Continuing to decrease the electrode voltage increases the open probability, shown in Fig. 1b, resulting in two values of voltage which produce the same current. The true voltage, V_T, shown in Fig. 3e is the voltage across the BLM which is responsible for the change in the open probability indicated in Eq. (3). The apparent voltage, V_A, in Fig. 3e is the voltage across the electrodes that was recorded in the I–V curve. As the voltage decreases further, channel reactivation causes the red dashed current response shown in Fig. 3e. Reversal of the current to increasing once again will cause the I–V curve to retrace the inactivation (black) pathway.

The model used to describe the inactivation given by Eq. (3) does not describe the observed hysteresis. Eq. (3) assumes that the applied electrode voltage and the BLM voltage are equal throughout the experiment. Since the electrode voltage and the BLM voltage lag one another resulting in a delayed reactivation response, the model should reflect that behavior. The voltage lag, δV, causes a delay in the open probability of the lysenin channels, not the current measured in the I–V curve. Therefore, the open probability given in Eq. (1) becomes:

$$P_{open} = \frac{1}{1 + e^{\frac{-\Delta E + q(V+\delta V)}{kT}}}. \tag{5}$$

The voltage dependent term has been adjusted for the lag in voltage. Likewise, the current described by Eq. (3) changes to:

$$I = \frac{gVN}{1 + e^{\frac{-\Delta E + q(V+\delta V)}{kT}}} + G_L V. \tag{6}$$

Eq. (6) now describes the reactivation current of the hysteresis accounting for the shift in the open probability due to the voltage lag.

The model represented by Eq. (3) was shown in Fig. 1a to provide an excellent fit to the inactivation data. Using the same parameters for open channel conductance, ΔE, and q, the reactivation data shown in red in Fig. 1a can be fit with Eq. (6). As shown in Fig. 4, Eq. (6) provides an excellent fit ($R^2 = 0.996$) to the reactivation data provided earlier.

This model identifies a shift in the I–V curves resultant from the persistent electric field inside the BLM after the reduction in electrode voltage. The data shown in Figs. 1 and 4 are representative of hysteresis curves from the voltage-gating behavior of lysenin channels inserted in asolectin-based BLMs [20,21]. Without asolectin as a component of the bilayer, lysenin channels have not been shown to exhibit voltage-induced gating [29,30]. Consequently, hysteresis is also not observed with these conditions.

3. Temperature

As further verification of our model, we examined the temperature dependence of the hysteresis. Previous work has demonstrated that the voltage gating behavior is temperature dependent, as would be expected in a dynamic equilibrium [21]. Our model predicts that the δV should decrease at higher temperatures, and at a critical temperature (T_c), the hysteresis should disappear altogether ($\delta V = 0$). Using the

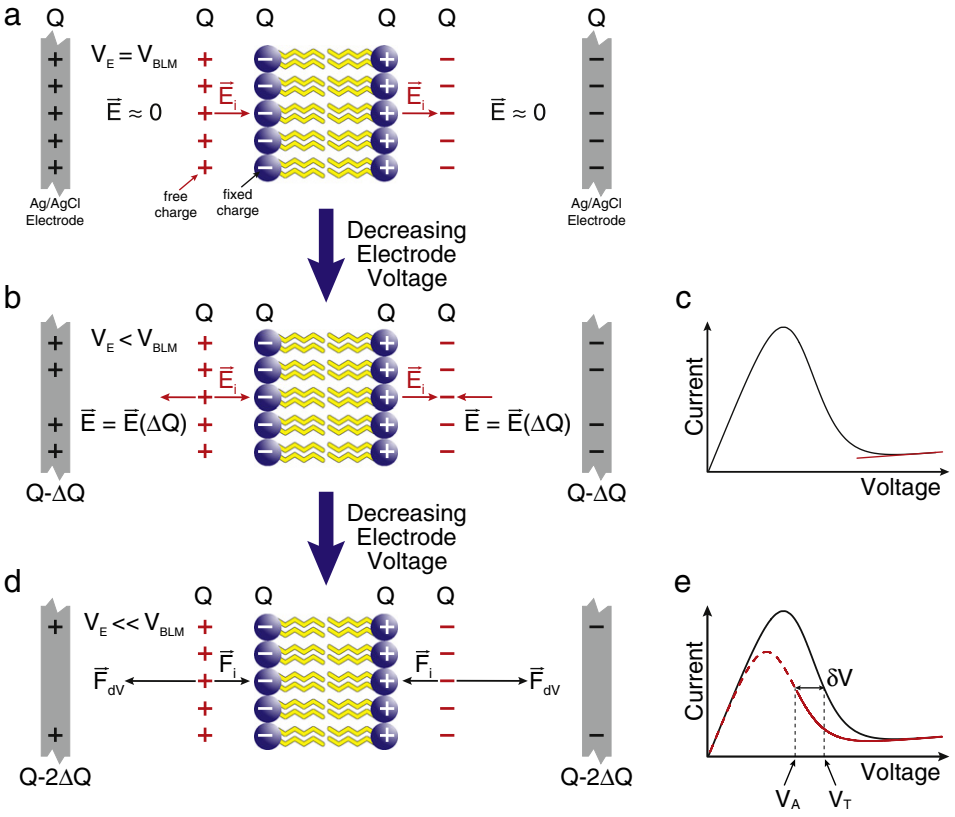


Fig. 3. (a,b,d) Schematic of the charge interactions from the electrodes and BLM resulting in the observed hysteresis. (c,e) The theoretically observed I–V curve due to the coulomb interaction between free charges in the electrolyte solution and the fixed charges in a polarized BLM. The charges shown on the lipid headgroups indicate polarization not the net charge of the lipid.

experimental procedures described elsewhere, the temperature was varied over the range of 10 °C to 40 °C after BLM formation and lysenin channel insertion [21].

As described, the free charge in solution is held at the BLM/electrolyte interface by the coulombic interaction with the fixed charge. Taking into account the free charge's thermal energy, we assume that there is a temperature at which the thermal energy of the free charge is high enough to overcome the coulombic interaction without help from the electrodes. Hence, no force from the electrodes ($\mathbf{F}_{\delta V}$) would be required for the free charge to escape from the interface. Assuming a spring-like restoring force proportional to the average motion due to temperature, we find that the force required to pull the free charge away from the BLM is directly proportional to δV. The thermal energy of the free charge allowing it to escape the BLM interface is related to δV by the square root of the temperature. Therefore, a plot of δV versus $T^{1/2}$ is predicted to be linear, with T_c being the point where $\delta V = 0$, as shown in Fig. 5.

As expected, the difference between the inactivation and reactivation decreases with increasing temperature. Using this model, we expect the hysteresis to completely vanish at 321.61 ± 0.001 K as indicated in Fig. 5. The complete disappearance of the hysteresis was not experimentally observed since it is outside the range of temperatures examined.

Our model is in agreement with experimental observations made with lysenin channels and provides an explanation as to the origins of channel gating and hysteresis. Further research will be required to corroborate our findings with measurements of the membrane polarization and by pinpointing specific regions within the channels that are responsible for channel gating. In the meantime, it is hoped that this work will bring about new discussions as to the origins and mechanisms

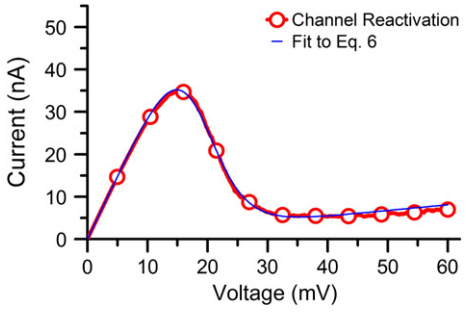


Fig. 4. The fit using Eq. (6) (blue) provides an excellent fit ($R^2 = 0.996$) for the experimental data for channel reactivation (red) where $\delta V = 5.64 \pm 0.01$ mV. Sampling rate = 5 Hz, symbols are shown every 250 data points.

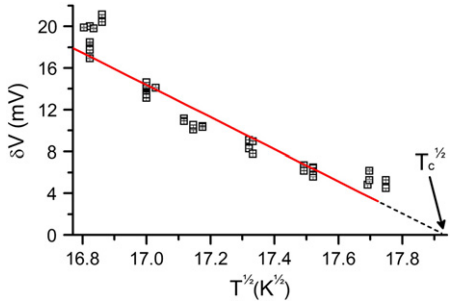


Fig. 5. A plot of δV for different temperatures reveals a linear relationship between δV and $T^{1/2}$. The linear fit (red) determined that $T_c = 321.61 \pm 0.001$ K. The error bars represent the propagated standard errors from fitting the hysteresis curves with Eqs. (3) and (6).

of cellular memory to further our understanding of the basic functioning of biological systems.

Acknowledgment

The authors would like to acknowledge financial support from the Howard Hughes Medical Institute (Grant 52005890) and the NSF (Grant DMR-0520550).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bpc.2013.09.001.

References

[1] W. Sha, J. Moore, K. Chen, A.D. Lassaletta, C.-S. Yi, J.J. Tyson, J.C. Sible, Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts, PNAS 100 (2003) 975–980.
[2] M.J. Solomon, Hysteresis meets the cell cycle, PNAS 100 (2003) 771–772.
[3] X. Tan, R.V. Iyer, Modeling and control of hysteresis, IEEE Control. Syst. Mag. (2009) 26–29.
[4] D. Angeli, J.E. Ferrell, E.D. Sontag, Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems, PNAS 101 (2004) 1822–1827.
[5] J. Hu, K.R. Qin, C. Xiang, T.H. Lee, Modeling of hysteresis in gene regulatory networks, Bull. Math. Biol. 74 (2012) 1727–1753.
[6] L. Kaestner, P. Christophersen, I. Bernhardt, P. Bennekou, The non-selective voltage-activated cation channel in the human red blood cell membrane: reconciliation between two conflicting reports and further characterization, Bioelectrochemistry 52 (2000) 117–125.
[7] R. Mannikko, S. Pandey, H.P. Larsson, F. Elinder, Hysteresis in the voltage dependence of HCN channels: conversion between two modes affects pacemaker properties, J. Gen. Physiol. 125 (2005) 305–326.
[8] A. Mathes, H. Engelhardt, Voltage-dependent closing of porin channels: analysis of relaxation kinetics, J. Membr. Biol. 165 (1998) 11–18.
[9] M.C. Menconi, M. Pellegrini, M. Pellegrino, D. Petracchi, Periodic forcing of a single ion channel: dynamic aspects of the open-closed switching, Eur. Biophys. J. 27 (1998) 299–304.
[10] P.S. Pennefather, W. Zhou, T.E. DeCoursey, Idiosyncratic gating of HERG-like K^+ channels in microglia, J. Gen. Physiol. 111 (1998) 795–805.
[11] F. Bezanilla, How membrane proteins sense voltage, Nat. Rev. Mol. Cell Biol. 9 (2008) 323–331.
[12] C. Miller, A charged view of voltage-gated ion channels, Nat. Struct. Biol. 10 (2003) 422–424.
[13] K.M. Robertson, D.P. Tieleman, Molecular basis of voltage gating of OmpF porin, Biochem. Cell Biol. 80 (2002) 517–523.
[14] K.J. Swartz, Sensing voltage across lipid membranes, Nature 456 (2008) 891–897.
[15] X. Tao, A. Lee, W. Limapichat, D.A. Dougherty, R. MacKinnon, A gating charge transfer center in voltage sensors, Science 328 (2010) 67–73.
[16] B. Hille, Ion Channels of Excitable Membranes, 3rd ed. Sinauer Associates, Sunderland, 2001.
[17] T. Andersson, Exploring voltage-dependent ion channels *in silico* by hysteretic conductance, Math. Biosci. 226 (2010) 16–27.
[18] L.M. Nowak, J.M. Wright, Slow voltage-dependent changes in channel open-state probability underlie hysteresis of NMDA responses in Mg^{2+}-free solutions, Neuron 8 (1992) 181–187.
[19] M.A. Pustovoit, A.M. Berezhkovskii, S.M. Bezrukov, Analytical theory of hysteresis in ion channels: two-state model, J. Chem. Phys. 125 (2006).
[20] D. Fologea, E. Krueger, R. Lee, M. Naglak, Y.I. Mazur, R. Henry, G.J. Salamo, Controlled gating of lysenin pores, Biophys. Chem. 146 (2010) 25–29.
[21] D. Fologea, E. Krueger, Y.I. Mazur, C. Stith, O. Yui, R. Henry, G.J. Salamo, Bi-stability, hysteresis, and memory of voltage-gated lysenin channels, Biochim. Biophys. Acta 1808 (2011) 2933–2939.
[22] T. Ide, T. Aoki, Y. Takeuchi, T. Yanagida, Lysenin forms a voltage-dependent channel in artificial lipid bilayer membranes, Biochem. Biophys. Res. Commun. 346 (2006) 288–292.
[23] R. Ishitsuka, T. Kobayashi, Lysenin: a new tool for investigating membrane lipid organization, Anat. Sci. Int. 79 (2004) 184–190.
[24] A. Yamaji-Hasegawa, A. Makino, T. Baba, Y. Senoh, H. Kimura-Suda, S. Sato, N. Terada, S. Ohno, E. Kiyokawa, M. Umeda, T. Kobayashi, Oligomerization and pore formation of a sphingomyelin-specific toxin, lysenin, J. Biol. Chem. 278 (2003) 22762–22770.
[25] L. De Colibus, A.F.-P. Sonnen, K. Morris, C.A. Siebert, P. Abrusci, J. Plitzko, V. Hodnik, M. Leippe, E. Volpi, G. Anderluh, R.J.C. Gilbert, Structures of lysenin reveal a shared evolutionary origin for pore-forming proteins and its mode of sphingomyelin recognition, Structure 20 (2012) 1498–1507.
[26] B. Das, K. Banerjee, G. Gangopadhyay, Entropy hysteresis and nonequilibrium thermodynamic efficiency of ion conduction in a voltage-gated potassium ion channel, Phys. Rev. E 86 (2012) 061915.
[27] H. Flyvbjerg, E. Gudowska-Nowak, P. Christophersen, P. Bennekou, Modeling hysteresis observed in the human erythrocyte voltage-dependent cation channel, Acta Phys. Polon. B 43 (2012) 2117–2140.
[28] E. Gudowska-Nowak, H. Flyvbjerg, P. Bennekou, P. Christophersen, Hysteresis in channel gating, Unsolved Problems of Noise and Fluctuations: UPoN 2002: Third Annual Conference, American Institute of Physics, 2002.
[29] T. Aoki, M. Hirano, Y. Takeuchi, T. Kobayashi, T. Yanagida, T. Ide, Single channel properties of lysenin measured in artificial lipid bilayers and their applications to biomolecule detection, Proc. Jpn. Acad. Ser. B 86 (2010) 920–925.
[30] D. Fologea, R. Al Faori, E. Krueger, Y.I. Mazur, M. Kern, M. Williams, A. Mortazavi, R. Henry, G.J. Salamo, Potential analytical applications of lysenin channels for detection of multivalent ions, Anal. Bioanal. Chem. 401 (2011) 1871–1879.