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Voltage Gating Interactions of the Protein Lysenin with Metal Ions in an Artificial Lipid Bilayer

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1. Motivation

- Ion channels and protein pores are essential to cellular life
- Certain pores are regulated by voltage across the cell membrane
- The pore-forming toxin, lysenin, found in the coelomic fluid of the worm *E. foetida* is regulated by positive voltage[1]
- This regulation of lysenin has also been found to be sensitive to metal ions[2]
- The sensitivity of lysenin extends to distinguishing between valency of the metal ions in the surrounding solution
- Understanding how lysenin's voltage dependency reacts to differing metal ions can be used as a model for other physiologically important ion channels

2. Lysenin

- Lysenin is a pore forming toxin which creates a 3-4 nm diameter non-specific pore in cell membranes
- It self-inserts into cell membranes which contain the lipid sphingomyelin[3]
- Many mammalian cell membranes contain up to 20% of sphingomyelin, Lysenin responds to voltage in a very predictable manner
- Lysenin pores close with positive voltage with respect to the protein head
- The pores reopen consistently when the voltage is reduced or reversed
- The voltage response can be described by an open probability of the pores

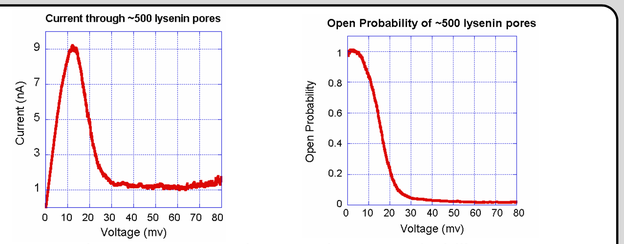


***Figure 1**. Lysenin response to voltage and open probability curve*

3. Methods

- Experiments were performed in a custom-built lipid bilayer chamber
- The chamber consists of two Teflon blocks machined to create a well when placed together
- Teflon film with a ~70 micrometer hole is placed between the blocks tor create a two chambered setup separated by the film
- A lipid mixture of asolectin, sphingomyelin, and cholesterol are painted on the hole to create an artificial lipid bilayer
- Buffer of 20 mM HEPES and 135 mM NaCl is added to each chamber
- Lysenin is added to one side and allowed to self-inject into the membrane
- Experiments are performed by adding metal salts in the stoichiometric quantity to the chambers and measuring the conductance across the bilayer at varying voltages

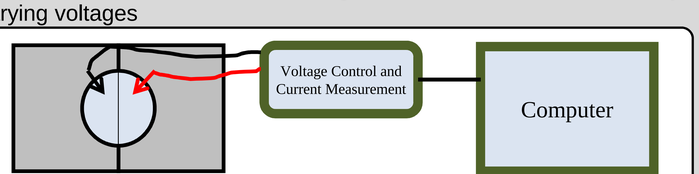
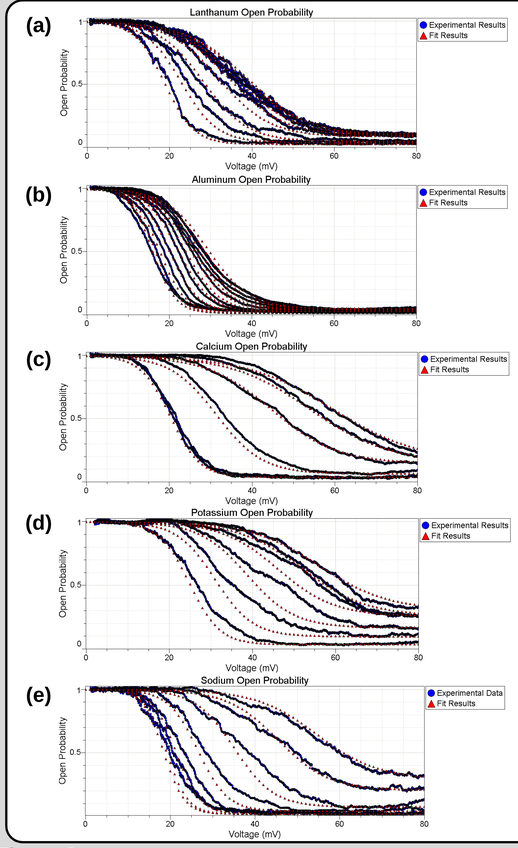


***Figure 2**. Setup to measure conductance across a lipid bilayer*

4. Experimental Results



Concentration (nM)	
0	400
100	500
200	700
300	900

Concentration (nM)	
0	200
20	240
40	320
80	480
120	880
160	

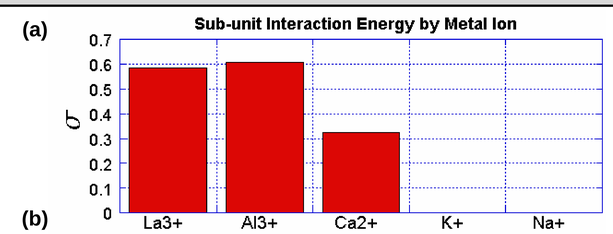
Concentration (μM)	
0	
8	
12	
16	
20	

Concentration (mM)	
130	190
150	210
170	230

Concentration (mM)	
75	175
100	200
125	225
150	

***Figure 3**. (a) Lanthanum 3+ (b) Aluminum 3+ (c) Calcium 2+ (d) Potassium (e) Sodium*
The increasing concentrations cause the probability curves to shift to the right.

5. Analytical Results



Metal Ion	ΔE	q_0	q_f	k_s (1/μM)	1/k_s (μM)	σ	R^2
La^{3+}	0.1323	7.0487	3.1144	6.3267	0.1580	0.5860	0.9953
Al^{3+}	0.1448	9.1009	4.7542	10.4075	0.0960	0.6088	0.9986
Ca^{2+}	0.1429	7.0672	1.9429	0.12353	8.0950	0.3247	0.9910
K^{+}	0.1712	9.2065	2.6777	6.959E-06	143702.5	0.0000	0.9789
Na^{+}	0.1916	10.4909	2.6484	6.303E-06	158654.9	0.0000	0.9592

***Figure 4**. (a) Chart showing the change in σ (subunit interaction parameter)*
(b) Table of analytical results

6. Analysis using Cooperativity

- The transformation of the data to open probability allows for easier comparison
- The process is simply dividing the current data by the slope of the initial linear section
- For a given concentration of metal ions, the data could be fitted by Boltzmann statistics with a leakage constant to account for membrane leak

$$P_{open} = \frac{Current}{Initial\_Slope} \qquad P_{open} = \frac{1 - Leakage}{1 + e^{\frac{qV - \Delta E}{k_b T}}} + Leakage$$

q=gating charge
V=voltage
ΔE=closing energy
T=Temperature

- The Boltzmann statistics, however, do not account for the response to metal ion concentration
- Due to the sigmoidal shape of the gating charge in response to metal ion concentration, a cooperative model was added to the Boltzmann statistics model
- The Koshland-Nemethy-Filmer (KNF) model accounts for cooperativity between subunits of proteins in response to ligand concentration
- The KNF model was modified to account for the 6 subunits of lysenin and to replace the gating charge in the Boltzmann statistics model

$$P_{open} = \frac{1 - Leakage}{1 + e^{\frac{((q_0 - q_f)(1 - KNF[\sigma, k_s, A]) + q_f)V - \Delta E}{k_b T}}} + Leakage$$

$q_{0,f}$ =initial/final gating charge
σ=sub-unit energy constant
k_s=ion binding constant
A=metal ion concentration

- The model has three parameters, σ,k_s, and A
- σ, the sub-unit interaction energy constant, measures how the individual sub-units of the lysenin pore respond to their neighboring sub-unit's conformation
- k_s, the ion binding constant, measures the inflection point of the gating charge with respect to metal ion concentration
- A, is the metal ion concentration
- This model was fitted to the individual types of metal ions to find the best fit of the variables using the software package Mathematica

7. Conclusions

- Lysenin, a 6 sub-unit pore forming toxin, was studied to see the affects of metal ions on the voltage regulation of the pore
- Normally lysenin responds to positive voltage by closing with almost certainty at +30 mV
- However increasing metal ions in the solution cause the response to be delayed to higher voltages
- This delay can be modeled using cooperativity between the sub-units of lysenin
- The very small amount of trivalent ions needed to create the voltage reaction change compared to the divalent or monovalent ions indicates a significant difference in response to ion valency
- The σ parameter of the model indicates the sub-unit interaction energy constant and from Figure 4 (a), it is clear that the valency of the metals plays a role in the determination of σ
- Future work will expand on the concept of exactly the mechanism of membrane pore's voltage regulation response to metal ions

References

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[3]. Shogomori, H. and T. Kobayashi (2008). "Lysenin: A sphingomyelin specific pore-forming toxin." Biochimica et Biophysica Acta (BBA) - General Subjects **1780(3): 612-618.**

Acknowledgements

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