Lysenin Channel's Selectivity to Monovalent Ions

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Anion vs Cation Selectivity of Lysenin Channels

Motivation

-Ionic selectivity is an essential function of ion channels in any cell

-This selectivity leads to the creation of transmembrane voltages, which is key for the functionality of any cell

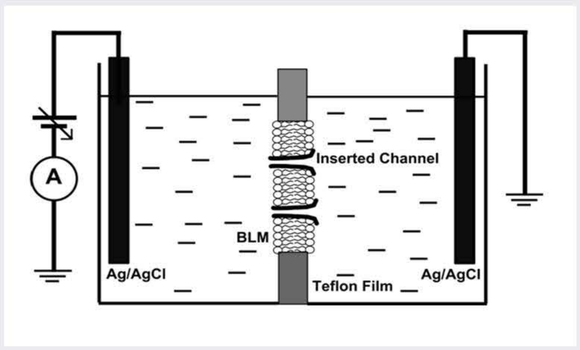
-The physiology of excitable cells such as neurons and muscle cells relies entirely on the creation and modulation of transmembrane voltages

-Replication of such functionalities by other protein channels provides insights into their biological role, as well as opening avenues for novel explorations in science and bioengineering

Overview of the Procedure

- Lysenin channels self insert into artificial membrane systems containing sphingomyelin
- Once the channels are inserted, the creation of concentration gradients leads to the development of transmembrane voltages
- The transmembrane voltages may be determined from the x-intercepts of the IV plots
- The voltage vs concentration ratio may be used in conjunction with the Goldman Hodgkin Katz (GHK) equation to provide the permeability ratio, hence inform on the selectivity

Experimental Setup

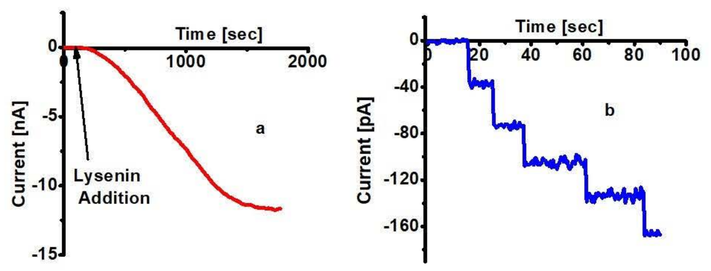


Experimental Procedure

1. A bilayer lipid membrane is produced in a small hole in a PTFE film separating two reservoirs filled with 50 mM KCl electrolyte buffered with 20 mM HEPES (pH 7.2)
2. Two Ag/AgCl electrodes assure electrical connections to the electrophysiology amplifiers
3. Channel insertion is monitored from the changes in membrane conductance upon addition of lysenin to the ground side
4. Concentration gradients are produced by addition of known amounts of KCL (from a 3 M stock solution) to the ground side
5. Voltage ramps are used to record the IV plots and determine the transmembrane potentials from the x-intercepts

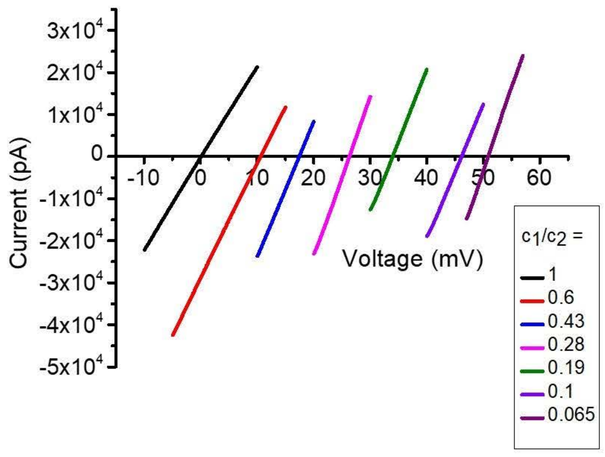
Experimental Results

Channel Insertion



Addition of lysenin monomer to the ground side of the bilayer membrane leads to the development of ionic currents (at -80 mV transmembrane voltage, panel a), indicative of channel insertion. Individual insertion of uniform channels may be observed at increased resolution (panel b). Application of a negative voltage for insertion monitorization is needed because lysenin channels gate at positive voltages.

Transmembrane voltages produced by chemical gradients



Gradual addition of KCl concentration in the ground reservoir leads to the development of transmembrane voltages owing to the lysenin channel selectivity. Consequently, the ionic currents recorded at 0 mV are non-zero; the ionic currents become zero when an equal and opposite transmembrane voltage is applied to the membrane. The IV plots show how the transmembrane voltage shifts upon successive addition of KCl to the ground reservoir. The exact value of each transmembrane voltage is determined from the linear fit of the IV plots; the transmembrane voltage is exactly the opposite of the x-intercepts.

Acknowledgement: This work was supported by the NSF CAREER grant #1554166.

Biophysical Modeling

Goldman-Hodgkin-Katz equation (GHK) calculates the transmembrane voltage V_m based on permeability of cations and anions. The equation includes permeability constants and concentrations of anions and cations; R is the universal gas constant, T is the absolute temperature, and F is the Faraday's number.

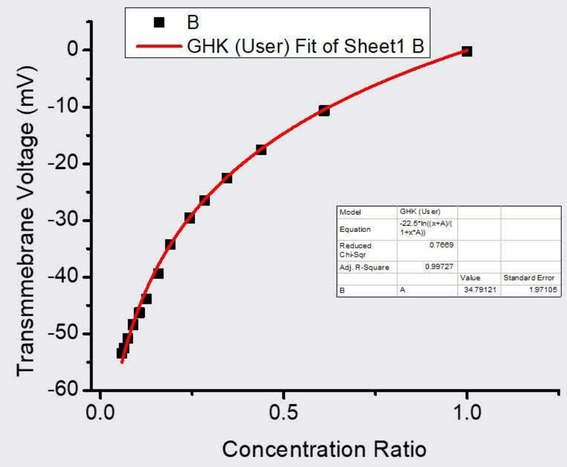
$$Vm = -\frac{RT}{F} ln\left(\frac{P_{Na^+}[Na^+]_1 + P_{K^+}[K^+]_1 + P_{Cl^-}[Cl^-]_2}{P_{Na^+}[Na^+]_2 + P_{K^+}[K^+]_2 + P_{Cl^-}[Cl^-]_1}\right)$$

The indices indicate the particular side of the membrane for which each concentration is calculated. Our experimental setup includes only K+ and Cl-, therefore Na+ ions are neglected. Moreover, since the changes in concentrations due to diffusion are negligible, the anion and cation concentrations may be considered in each reservoir.

Therefore, the above equation leads to:

$$Vm = \frac{RT}{F} \ln\left(\frac{\frac{[K^+]_1}{[K^+]_2} + \frac{P_{Cl^-}}{P_{K^+}}}{1 + \frac{[K^+]_1}{[K^+]_2} \cdot \frac{P_{Cl^-}}{P_{K^+}}}\right)$$

which may be fitted to the experimental data to determine the permeability ratio.



The graph above is the transmembrane voltage plotted against the concentration ratio of Potassium (K). Fitted with the GHK equation we can see that the permeability ratio is P_{Cl^-}/P_{K^+} = 34.8.

Conclusion

Lysenin channels present selectivity of anions over cations, which leads to the development of transmembrane voltages comparable to the ones developed by ion channels in cells. Consequently, they may be used to facilitate the creation of transmembrane voltages in artificial membrane systems or to modulate the transmembrane potential of cells.