Modulation of Lysenin's Memory by Cu^{2+} Ions

BOISE STATE UNIVERSITY

Emmanuel A. Vergelin, Andrea Robles, Conor Neupert, Aviana R. Smith, Pangaea W. Finn, Ilinca M. Flacau

Mentor: Daniel Fologea

Correspondence: emmanuelvergelin@u.boisestate.edu

Motivation

Lysenin is a pore-forming protein extracted from the red earthworm E. fetida, which forms voltage-gated channels in artificial and natural lipid membranes. A prominent feature of the channels is their memory, originating in the conductance hysteresis that occurs during the application of slow oscillatory voltages. In this work, we showed this innate memory was strongly influenced by the addition of small amounts of Cu^{2+} ions. After Cu^{2+} addition, the lysenin channels previously closed by an applied voltage showed a stronger preference for the closed state, indicative of major changes in kinetics and equilibrium. However, the physiology behind this shift is still obscure. To fill this gap in our knowledge, we employed electrophysiology measurements to identify the changes in the closing and opening rates of lysenin channels exposed to Cu^{2+} ions and step voltages. We found Cu^{2+} simultaneously reduced the closing rates and increased the reopening rates, leading to a more prominent hysteretic behavior and improved memory. These findings may constitute the starting point on investigations of the memory of brainless microorganisms, and potential applications to bioelectronics and development of smart biological switches and nano-valves.

Materials and Methods

Our experimental setup comprised a vertical planar bilayer membrane setup (Figure 1). The membrane was produced from a mixture of Asolectin, Sphingomyelin, and Cholesterol in a small hole in the PTFE film separating the reservoirs. We used 50 mM KCl buffered with 20 mM Hepes (pH 7.4) as support electrolyte solutions. The electrical circuit was completed with salt bridges and Ag/AgCl electrodes wired to an electrophysiology amplifier. The membrane's integrity was verified using capacitance and conductance measurements, after which small amounts of lysenin were added to the grounded reservoir. Channel insertion was monitored from the ionic currents measured at -60 mV transmembrane voltage. After insertion, the response of the channels to oscillatory voltages was measured from the ionic currents recorded in response to ascending and descending voltages (triangle-shaped ramps) ranging from -20 mV to +60 mV. The relaxation times for voltage-induced closing and reopening of channels were determined from the exponential fit of the currents measured in response to step voltages. The IV plots and responses to step voltages were recorded with and without the addition of Cu^{2+} ions from a stock solution. The experiments were carried out at room temperature, and the solutions in the reservoirs were continuously mixed with magnetic stir bars.

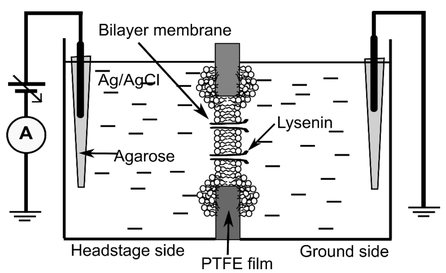

Figure 1. The experimental setup for investigating the response of lysenin channels to transmembrane voltages. The currents through lysenin channels were measured in response to fixed and variable voltages.

Results and Discussion

Lysenin inserts channels in lipid membranes

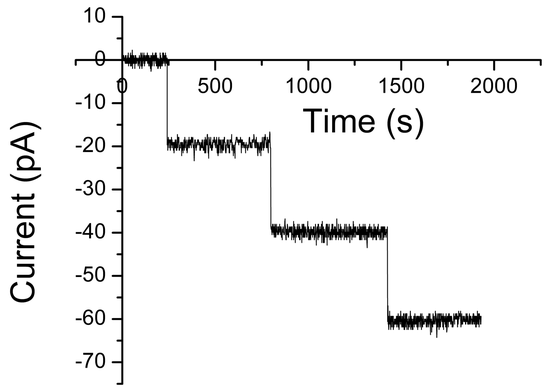

Figure 2. Lysenin channel insertion into lipid bilayer membranes. Channel insertion was monitored from the step-wise variation of the ionic currents recorded at -60 mV potential. The negative potential was used to prevent the gating that manifests at positive voltages. The trace indicates singular insertions of uniform channels (three channels are shown). The insertion is completed in around one hour, after which we assessed its responses to voltages from IV plots.

Lysenin channels show voltage gating, hysteresis, memory, and modulation by Cu^{2+} ions

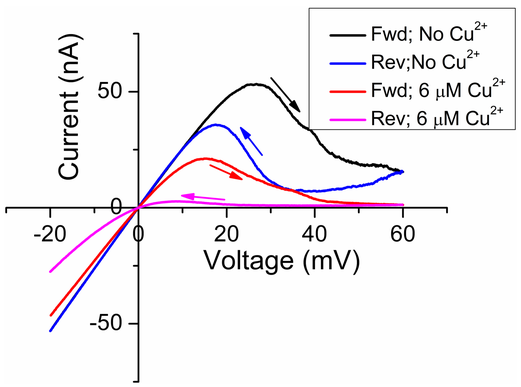

Figure 3. Lysenin channels presented hysteresis in conductance and this was modulated by Cu^{2+} ions. The IV plots were recorded in response to ramp voltages; the direction of the ramps is shown by the arrows. In the absence of Cu^{2+}, lysenin channels are open at negative voltages and start closing at voltages greater than +20 mV (black line). However, the channels closed by voltage are resistant to re-opening during descending voltage ramps (blue line) and fully reopen at lower voltages. After the addition of 6 μM Cu^{2+} ions, the channels close earlier during ascending voltage ramps (red line) and present negligible reopening during descending voltage ramps. Both plots show a strong hysteresis in conductance, indicative of memory (the channels "remember" the last state). However, the presence of Cu^{2+} shows a more significant influence on reopening, especially in the negative voltage range.

Cu^{2+} ions significantly adjust the equilibration for lysenin channels' opening and closing in response to voltages

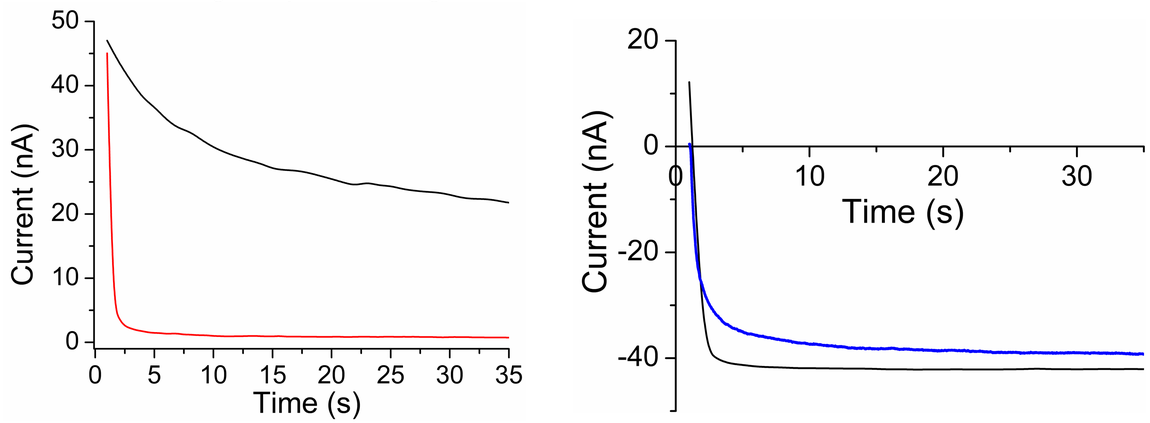

Figure 4. The effects of Cu^{2+} on channel closing and reopening are assessed from the response to voltage steps (+ and – 72 mV).
Left graph: In the absence of Cu^{2+}, the lysenin channels closed very slowly upon application of a +72 mV step voltage (black line). However, the 8 μM Cu^{2+} ions additions lead to a much faster closing in response to the positive voltage step (red line).
Right graph: In contrast, the reopening of channels upon application of -72 mV step voltage was very fast in the absence of Cu^{2+} ions (black line), but the reopening was observed to be much slower in the presence of Cu^{2+} ions. These results suggest that Cu^{2+} ions affect the functionality of lysenin channels by speeding up the closing in response to positive voltages and delaying the reopening of closed channels upon application of negative voltages.

Cu^{2+} ions adjust in a concentration-dependent manner the relaxation time for opening and closing in response to step voltages

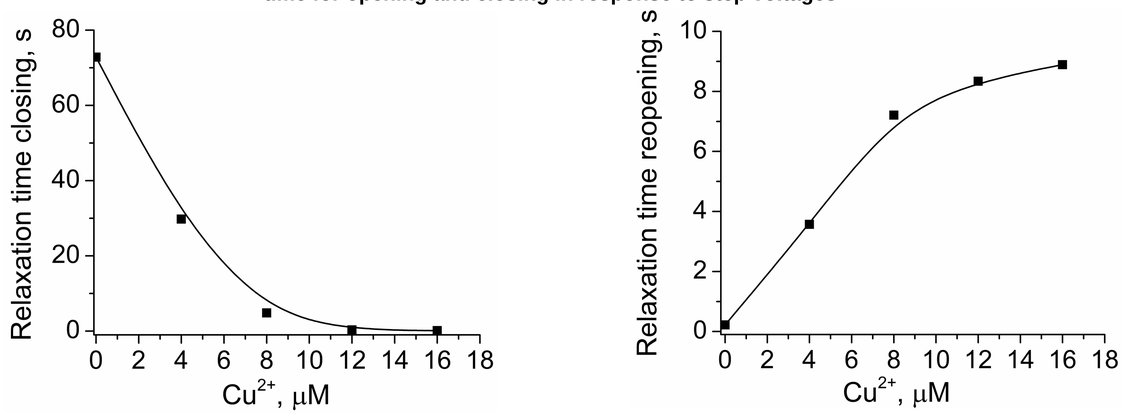

Figure 5. The effects of Cu^{2+} on channel relaxation time in response to voltage steps (± 72 mV) as a function of Cu^{2+} concentration.
Left: Cu^{2+} addition lead to a concentration-dependent decrease of the relaxation time (Tau) in the channels (closing at positive voltages).
Right: The relaxation time of reopening significantly increased as more Cu^{2+} was added to the solutions.

Conclusion

Therefore, we conclude that Cu^{2+} influences the gating, hysteresis, and memory of lysenin channels by speeding up its closing and slowing down its reopening. These changes in kinetics and equilibrium are anticipated to lead to major changes in the energy landscape, which will be investigated in future work.

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