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Characterizing the functionality of transmembrane ion channels using planar bilayer membranes device and stopped flow spectrometer

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Characterizing the functionality of Transmembrane Ion Channels using Planar Bilayer Membranes Device and Stopped Flow Spectrometer

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INTRODUCTION Transmembrane ion channels can be used in biomaterials as a way to selectively allow only certain molecules to pass through a given membrane. To produce these types of membranes predictably and reliably for commercial activity, its characterization is crucial. We have characterized the conductivity and ion selectivity of NaChBac, a voltage-gated sodium channel from *Bacillus haloduran,* using a planar bilayer membrane device. Planar bilayer membrane is a common technique for ion channels function characterization. By applying a controllable potential across the protein-embedded bilayer and measuring the current, the conductivity and permeability of the protein can be studied. Furthermore, we have devised a dye-free flux assay to measure the channel function using stopped flow spectrometer.

Conductance

Conductance of NaChBac was measured using a planar bilayer membrane device, where purified protein is incorporated into a planar lipid bilayer and ionic current through the channel recorded (Figure 1A). The electric potential across the NaChBac-incorporating membrane is controlled by a potentiostat, and current jumps can be observed to characterize the protein's behavior (Figure 1B). These peak current events are averaged and plotted against its holding potential (Figure 1C) to determine the conductance (i.e. the steepest slope): 16.2 ± 4.2 pS (mean ± SEM; n = 2 samples). This result agrees with 12 ± 1 pS that was reported by Ren *et al*., (Ren 2001) using patch clamp, but conflicts with 120 pS shown by Studer *et al.* (Studer 2011), who also used planar lipid bilayers to study NaChBac.

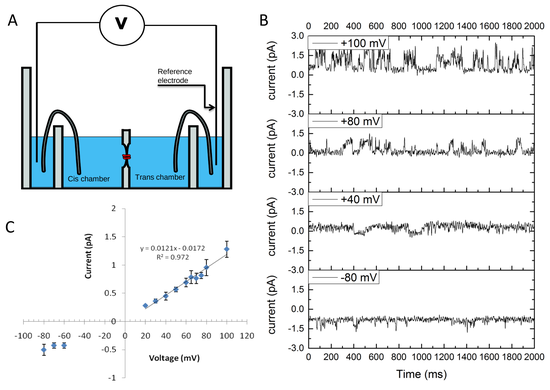

Figure 1. (A) A schematic representation of the planar lipid bilayer device used to study NaChBac. (B) Single channel recordings at different holding potentials. (C) Average peak current (I-V) plots.

$$\frac{P_{Na^+}}{P_{K^+}} = \frac{[Na^+]_i}{[K^+]_o \exp\left(\frac{-E_{rev}F}{RT}\right)} \qquad (1)$$

Relative Permeability

The experiments done to obtain the conductance curves shown in Figure 1 were performed in biionic conditions, which means the intracellular side (cis chamber) contained 150 mM NaCl and 10 mM Tris-Cl at pH 7.4, while the extracellular side (trans) contained 150 mM KCl or 150 mM $CaCl_2$ and 10 mM Tris-Cl at pH 7.4. The x-intercept reveals the reversal potentials for KCl and $CaCl_2$ and Equations (1) and (2) can then be used to find NaChBac's relative permeability for K^+ and Ca^{2+}, respectively. For KCl biionic condition, E_{rev} = 5.29 ± 1.22 mV (n = 2) which gives P_{Na+}/P_{K+} to be 1.23 ± 0.06. NaChBac in $CaCl_2$ biionic condition, E_{rev} = 8.08 ± 0.38 mV (n = 2) which gives P_{Na+}/P_{Ca2+} to be 1.23 ± 0.03 (n = 2).

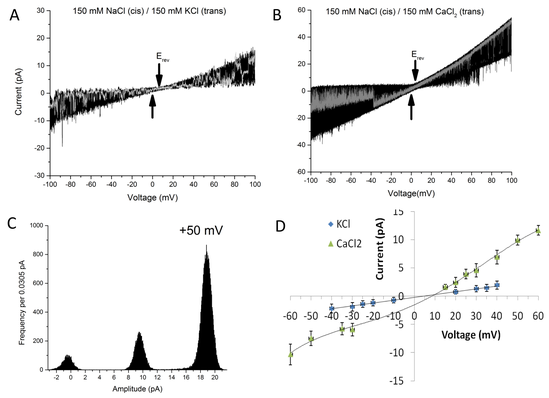

Figure 2. Relative permeability of NaChBac. 100 voltage ramp sweeps at 100mV/s are recorded and for (A) KCL and (B) $CaCl_2$ biionic conditions. 1 of the 100 sweeps is highlighted in gray for ease of viewing. (C) All point current histogram for $CaCl_2$ biionic conditions. (D) Conductance curves for KCl and $CaCl_2$ biionic conditions.

$$\frac{P_{Na^+}}{P_{Ca^{2+}}} = \left[\frac{[Na^+]_i}{4[Ca^{2+}]_o}\exp\left(\frac{E_{rev}F}{RT}\right)\left\{\exp\left(\frac{E_{rev}F}{RT}\right)+1\right\}\right]^{-1} \qquad (2)$$

Activation Voltage

Current ensembles were created by averaging the currents produced from the voltage sweeps (Figure 3A). From these ensembles, one current measurement for each test voltage were taken at 150 ms after the test voltage switches to the deactivation voltage of -120 mV. These currents were normalized and were plotted against its test voltages (Figure 3B), and then fitted using Equation (3). $V_{1/2}$, the midpoint activation voltage was found to be -28 mV and is close to the value found by Ren *et al.* (Ren 2001). Its apparent valence charge, that is the Z value, was found to be 16 which suggests that NaChBac appears to have a steep activation voltage. To make sure the NaChBac were all in the same orientation, n-ethyl lidicaine was added to the trans chamber before starting the experiment.

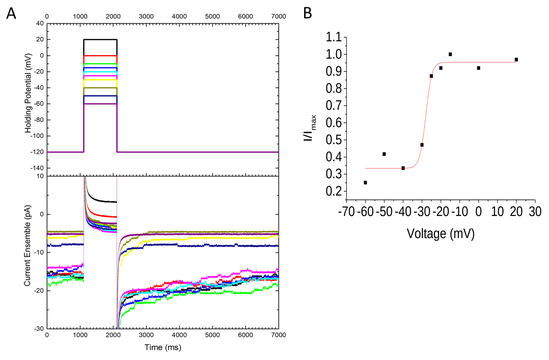

Figure 3. (A) Current ensembles for various voltage-clamp protocols. (B) Normalized activation curve and fitted using the Boltzmann equation which is shown in Equation (3).

$$\frac{I}{I_{max}} = A_2 + \frac{A_1 - A_2}{1 + \exp\left(-\frac{ZF}{RT}(V - V_{1/2})\right)} \qquad (3)$$

Functionality

Stopped flow spectrophotometer is used to show that the functionality of ion channels can be shown through the proteoliposome swelling which can be detected using mie scattering at 90 degrees from 400 nm incident light (Figure 4A). The bigger the vesicle diameter, the lower the light scattering intensity detected. NaChBac vesicles in 500mM glycine, 20 mM Tris were diluted 2X into 250 mM NaCl, 20 mM Tris buffer in the mixing chamber. This will cause the vesicles to swell only if the they are permeable to Na^+ as shown in Figure 4B. Figure 4C shows that the control has little changes in light scattering while Figure 4D shows that light intensity decreases for proteoliposomes.

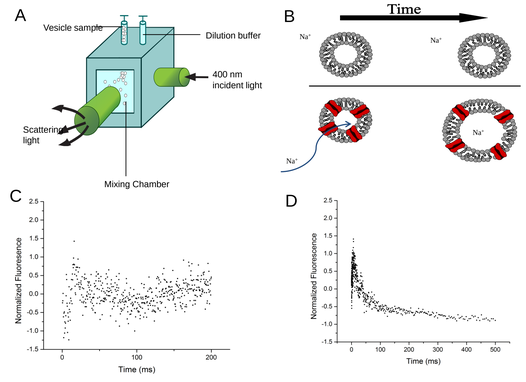

Figure 4. (A) Schematic representation of stopped flow spectrophotometer. (B) Comparing vesicles in the mixing chamber with respect to time. NaChBac vesicles swells once Na^+ enters the vesicles and causing its buffer to become hypotonic. (C) The scattering light detected with respect to time when 100 nm diameter liposomes enter NaCl buffer. (D) The scattering light detected with respect to time when 100 nm diameter NaChBac vesicles mixes with the NaCl buffer.

REFERENCES

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2. Ren et al., Science **294**, 5550 (2001).

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