Investigation of selectivity and conduction mechanism in non-selective cation NaK channel using a combination of solid-state NMR spectroscopy and molecular dynamics simulations

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In Short

- NaK is a non-selective monovalent cation channel of which it is debated how it conducts Na^+ and K^+ with equally high efficiency.
- In contrast to previous crystallographic results, solid-state NMR revealed that the selectivity filter of NaK in native-like membranes adopts two distinct conformations at extremely low ionic concentration.
- Potassium and sodium ions unequivocally stabilize a different conformational state.
- MD-based computational electrophysiology simulations suggested the functional importance of the conformational preferences induced by Na^+ or K^+ binding.
- In the second phase of the project, we aim to build a comprehensive picture of Ca^{2+} permeation in the non-selective NaK channel.

Most of biological processes require the participation of specific cations, such as Na^+, K^+, H^+, Ca^{2+} and Mg^{2+}. Discrimination of different ions through the cell with high fidelity is mainly controlled by ion channels that are embedded in membranes. Until now, best understood ion channels are bacterial mono-cation channels, e.g. KcsA and NaK. They have been used as model systems to illustrate and clarify fundamental physical principles governing ion selectivity in ion channels [1]. Interestingly, although NaK and KcsA share high sequence similarity in the selectivity filter region, they yet exhibit drastically different ion selectivity. KcsA is a highly selective potassium channel, while NaK conducts potassium and sodium with equal efficiency [2,3].

Previous X-ray crystallographic studies on KcsA, NaK and its mutants provide a valuable basis for understanding ion selectivity in potassium and non-selective cation channels. Stabilization of four ion binding sites S1-S4 in the selectivity filter was suggested to be essential for selective K^+ permeation. In non-selective cation channels, such as NaK, chimeric NaK-CNG, and HCN, the latter one recently elucidated structurally by cryo electron microscopy, one of or both binding sites S1 and S2 located at the extracellular site are absent [4–6]. Based on these observations, it was proposed that reduction of binding sites is responsible for ion non-selectivity of the channels. Despite that this model may explain many aspects of the selectivity difference in K^+ selective and non-selective channels, the finer details of the ion selectivity mechanism are not yet fully understood.

In this study, we use a combination of solid-state NMR spectroscopy (ssNMR) and advanced molecular dynamics (MD) simulations to investigate the detailed mechanism of ion non-selectivity in the NaK channel. Distinct from other methods in structural biology, ssNMR makes it possible to study membrane proteins in native-like lipid bilayers at room temperature and under physiological buffer conditions. The MD simulations allow us to determine the conductive conformation of NaK for Na^+ and K^+, and further enable us to investigate the detailed permeation mechanism of these ions [7,8]. Intriguingly, we identify two conformations of the selectivity filter in NaK, one of which is preferred by Na^+ and the other by K^+. We further underline the functional importance of the conformational preferences induced by Na^+ or K^+ binding by using MD based permeation simulations. Besides the canonical K^+ permeation pathway, we identify a side entry ion-conduction pathway for Na^+ permeation unique to NaK. We propose that structural plasticity within the selectivity filter and the selection of these conformations by different ions are key molecular determinants for highly efficient conduction of different ions in non-selective cation channels. Our results not only provide valuable insights into the conduction mechanism of NaK, but also have important implications for our understanding of the selectivity difference between the K^+ selective and non-selective channels [9].

Experimental studies revealed that NaK is conductive for divalent cations such as Ca^{2+} as well. As Ca^{2+} flux controlled by a number of non-selective channels plays an essential role in diverse biological processes, our aim in the second phase of the project is to build a comprehensive picture of Ca^{2+} permeation in the non-selective NaK channel.

WWW

http://www.leibniz-fmp.de/de/lange.html

More Information

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Project Partners

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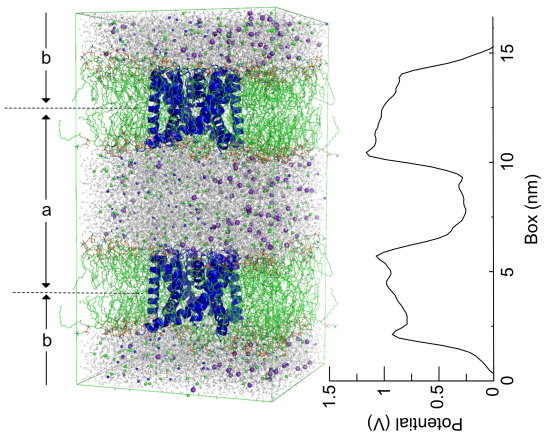

Figure 1: *Computational Electrophysiology: The system used in the computational electrophysiology simulations, consisting of two membranes (lipid in green), each including an NaK channel (blue cartoon: PDB ID: 3e83), surrounded by water, K^+ ions (purple balls) and Cl- ions (green balls). Periodic boundary conditions create two compartments, a & b, with distinct ion concentrations. Thus, a transmembrane voltage gradient is established across each membrane.*