**Abstract**

Sodium/calcium exchangers (NCXs) are membrane transporters which play an important role in Ca\(^{2+}\) homeostasis and Ca\(^{2+}\) signaling. These proteins have been implicated in various Ca\(^{2+}\)-dependent physiological processes such as neurotransmission, skeletal and smooth muscle contraction, cardiac contractility and apoptosis. Here, we use solid supported membrane (SSM) technology to performed functional analysis of NCX-Mj, an archaeal NCX isoform which has recently been crystallized. Using this approach we characterized the substrate affinity, ion specificity and inhibition by divalent cations of this archaeal exchange protein. In conclusion we found a high functional similarity of NCX-Mj and eukaryotic NCX isoforms, although the prokaryotic protein lacks a large regulatory domain. Furthermore our results demonstrate that SSM based technology is well suited for state-of-the-art functional characterization of transporter proteins.

**Method**

The NCX-Mj protein was reconstituted in liposomes, which were immobilized on the SSM sensor. The NCX current is generated by the exchange of Na\(^{+}\) versus Ca\(^{2+}\), usually with an electrogenic stoichiometry of 3 Na\(^{+}\) to 1 Ca\(^{2+}\), leading to a net cation flux. To induce an outward flowing Na\(^{+}\), liposomes were loaded with 140 mM Na\(^{+}\) and Ca\(^{2+}\). Alternatively liposomes were loaded with 20 mM Ca\(^{2+}\) and transport activated with 10 mM Na\(^{+}\).

**SSM Technique**

**Inhibition**

Inhibition of NCX-Mj by divalent cations. (A) Na\(^{+}\) and Ca\(^{2+}\)-activated NCX-Mj currents are inhibited by 100 \(\mu\)M Ca\(^{2+}\). (B) Concentration response curves of Ca\(^{2+}\), Mg\(^{2+}\) and Mn\(^{2+}\). (C) Reversibility of Ca\(^{2+}\)-inhibition. After activation of \(I_{\text{NCX}}\) by Ca\(^{2+}\) and subsequent block by Ca\(^{2+}\) only partial recovery of \(I_{\text{NCX}}\) upon washout of Ca\(^{2+}\) was observed. (D) Ca\(^{2+}\) affinity of NCX in the absence and presence of 100 \(\mu\)M or 4 mM Mg\(^{2+}\). In the presence of 4 mM Mg\(^{2+}\), Ca\(^{2+}\) induced NCX-Mj activity is shifted towards higher Ca\(^{2+}\) concentrations indicating competition between Mg\(^{2+}\) and Ca\(^{2+}\).

**Apparent Affinity**

Apparent Ca\(^{2+}\) and Na\(^{+}\) affinity of NCX-Mj (black circles) and human NCX1 (grey circles). Control experiments (triangles) were performed with empty liposomes/parental HIK cell membranes (A) Apparent Ca\(^{2+}\) affinity was measured using vesicles loaded with 140 mM Na\(^{+}\). Outward \(I_{\text{NCX}}\) was activated by application of solution containing differing free Ca\(^{2+}\) concentration as indicated (mM). (B) Apparent Na\(^{+}\) affinity was determined using vesicles loaded with 20 mM Ca\(^{2+}\). Inward \(I_{\text{NCX}}\) was activated by application of varying Na\(^{+}\) concentrations as indicated. (C-D) Representative current traces of outward/inward NCX-Mj \(I_{\text{NCX}}\) evoked by different Ca\(^{2+}\)/Na\(^{+}\) concentrations, respectively.

**Selectivity**

Positive control (Na\(^{+}\)/Ca\(^{2+}\)) | Negative control (empty liposomes) | NCX-Mj with different substrates
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**Platform**

The experiments were performed on the SURFE2R N1, a system for SSM based transporter recordings. It’s including a specialized perfusion system and performs amplification and data recording.