

Production of fragments from the Na⁺/Ca²⁺ exchanger for solution NMR

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Introduction: The Na⁺/Ca²⁺ exchanger (NCX) is a membrane transporter that operates in the maintenance of intracellular Ca²⁺ homeostasis. The exchanger, which is present in several species of mammals (NCXs) and insects such as *Drosophila melanogaster* (CALX), has a transmembrane domain containing 10 transmembrane helices (TMH). The NCX transmembrane domain is responsible for the transport of Ca²⁺ and Na⁺ through the lipid bilayer. A large cytoplasmic loop that connects TMH5 to TMH6 is responsible for the exchanger regulation. The loop contains a Ca²⁺-sensor domain called CBD12. The conformations of the linker regions that connect CBD12 to TMH 5 and 6 are unknown, but the loop segments near the TMH5 and TMH6 are fairly conserved and may be involved in the ionic regulation mechanism. **Objectives:** Characterize the conformation of the CALX TMH5 and TMH6 and their juxtamembrane regions. Analyze their possible roles in the allosteric regulation mechanism. **Material and Methods:** Protein production in *E. coli*. Isolation from inclusion bodies or from the membrane fraction. Solubilization with detergents. Affinity chromatography purification with Ni²⁺ resin. **Results and Discussion:** The gene coding the fragment TMH5 was purchased already cloned in fusion with a maltose binding protein (MBP) tag to address the target protein to the inner membrane and a histidine tag for purification. The best *E. coli* strain was BL21(DE3)RIL plus-condon. The protein was obtained from inclusion bodies (IB) and from the membrane (MEM). The best detergent to solubilize the fragment from IB and MEM was Triton X-100. During the purification, we observed low interaction of the protein with the Ni²⁺ resin. **Conclusions:** The expression was well controlled and with yielded high amounts of the target protein. Purification tests were successful, however the cleavage of the MBP tag still needs to be done.

Keywords: Na⁺/Ca²⁺ exchangers, protein membrane, protein folding, NMR.

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