BA.09 - A solution NMR study of a prokaryotic Na+/Ca2+ exchanger incorporated in detergent micelles

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Na+/Ca2+ exchangers (NCXs) are essential for the maintenance of Ca2+ homeostasis in different cell types and are therefore considered important pharmacological targets. However, high-resolution structures for eukaryotic NCXs have not been obtained to date. NCX-Mj, an orthologue from the thermophilic archaea M. jannashii, has emerged as a structural model of the NCX transmembrane domain. While NCX-Mj kinetic properties have been extensively studied, only its outward-facing state structure has been solved and, hence, a complete model of the ion translocation mechanism is missing. This project aims to study the NCX-Mj intrinsic dynamics in DDM micelles by solution NMR spectroscopy. A synthetic gene coding for NCX-Mj was cloned in pET24 to express a protein in fusion with a 9xHis tag at the C-terminal end. *E. coli* C43 harbouring the pET24a-NCX-Mj plasmids were cultured in minimal media with 15NH4Cl as nitrogen source. Harvested cells were sonicated and membrane proteins were solubilised with 2% sarkosyl. After centrifugation, NCX-Mj was purified from the supernatant by batch Ni2+-affinity chromatography, at which point sarkosyl was exchanged for 0.3% DDM. NCX-Mj was further separated by size-exclusion chromatography, yielding 2 mg NCX-Mj per culture litre. SEC-MALS analyses revealed monodispersity of the protein detergent complex (PDC) fractions, as well as different elution times for empty micelles and PDCs, albeit with surprisingly longer retention times for the latter. These analyses suggested that monodispersity could be compromised by changes in the protein:detergent ratio. The observation of well-dispersed peaks along the 1H dimension in preliminary 1H-15N TROSY spectra is consistent with a folded protein. Analyses of NCX-Mj mutants that stabilise a given state (outward- or inward-facing) are underway. By comparing 1H-15N TROSY spectra of wild-type and mutated NCX-Mj we expect to identify sets of peaks for each state.
Keywords: Na+/Ca2+ exchanger, Solution NMR spectroscopy, Structural biology
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BA.10 - BaCopA, a Cu(I) ATPase from the Antarctic bacterium Bizionia argentinensis

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Copper ions are cofactor for several enzymes and participate in some cellular redox reactions. Intracellular excess of copper ions generates reactive radicals that cause damage to DNA, proteins and lipids. For this reason, intracellular levels must be regulated to avoid toxic concentrations. A subfamily of P-ATPases (denoted as PIB-1) are present in prokaryotic and eukaryotic organisms, and constitute one of the main transporters responsible for the elimination of excess copper ions from the cytosol. In this work we characterize a putative PIB-type ATPase belonging to Bizionia argentinensis(BaCopA), a gram-negative bacterium isolated from the superficial seawater of Potter Cove, Antarctica. BaCopA was cloned and expressed in *Saccharomyces cerevisiae* as a GFP-fusion His-tagged protein for its subsequent purification and detection. Activity assays indicate that purified BaCopA is able to catalyze ATP hydrolysis at 5°C. ATPase activity of BaCopA increases when Cu (I) and ATP are added to the reaction medium.However, an inhibitory effect of ATPase activity occurs with the addition of vanadate, a specific inhibitor of P-ATPase-type enzymes.A structural model was built by homology modeling using the resolved structure of L. pneumophilaCopA as template (PDB: 4BBJ). The structural alignment shows a high degree of similarity, with the typical topological pattern of PIB-1 ATPases. Comparison with its mesophilic and hyperthermophilic counterparts led to the identification of key residues conserved in functional domains and differences in non-covalent interactions and surface charges. The detailed analysis of this interaction network suggests greater structural flexibility in BaCopA and, therefore, a better adaptation to low temperatures.
Keywords: ion transport ATPases, psychrophilic enzymes, bioinformatics