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Monitoring of conformational changes in transmembrane proteins using HDX and FFAP radical labeling

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The chloride channel family is divided into two groups: channels and antiporters. Channels are known for their large conformational changes of subunits during ion transport. In contrast, antiporters have only tiny movements near the transport pathway. Transporters are involved in many cellular processes, and their mutation can cause serious diseases [1]. The CLC-ec1 is a Cl-/H+ antiporter of a single proton for two chloride ions from Escherichia coli. Ion transport is supposed to be accompanied by a structural change between inward and outward-facing conformation, but this transition has not been captured so far. During the transport cycle, the protonation of key Glu residues should induce an outward-facing state. This was mimicked through a mutation of three Glu residues for Gln[3]. We used this mutation to reveal the role of protonation in the ion transport mechanism of CLC-ec1. CLC-ec1 and CLC-QQQ mutant were expressed in the E. coli system and purified by IMAC chromatography in η-decyl maltoside as a solubilization reagent[1]. Solubilized protein was transferred to the saposin nanodisc by size exclusion chromatography. We then used hydrogen/deuterium exchange and radical labeling with Togni reagent (FFAP)[4] coupled to high-resolution mass spectrometry to capture the structural changes alongside the backbone as well as on the side chains of aromatic residues The optimization of the digest workflow for both methods provided full sequence coverage with reasonable lengths of the peptides. As the best conditions for the HDX we determined online digest on the column of co-immobilized pepsin-nepentesin 2 whereas for FFAP we got better sequence coverage by offline digest via cyanogen bromide and trypsin. HDX and FFAP were subsequently used for the study of the structural differences between CLC-ec1 and the CLC-QQQ mutant at pH 7.4. To prove the role of protonation, a comparison experiment at four different pH levels, from 4.4 to pH 7.4, was performed for both proteins. Results showed a critical connection between protonation and structural changes in the ion transport path and conformational change in the transmembrane helixes.

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