Abstract

Transmembrane channels and transporters of the ClC protein family are present across all living organisms. They are found on the cytoplasmic and lysosomal membranes of the cells, where they participate in maintaining ion homeostasis. When dysfuncional, they lead to serious health complications. To develop treatment for these diseases, it is essential to describe transport mechanism of ClC proteins. The antiporter ClC-*ec1* from *E.coli* is used as a model protein for the entire ClC protein family. This homodimeric protein, which transports one proton against two chloride ions, has a separate transport path in each monomer. Based on the crystal structure, it is believed that during transport the protein alternates between outward and inward-facing conformations. Conversion to the outward-facing conformation of the protein is accompanied by the protonation of three glutamates located in the transport path. To study these conditions, a QQQ mutant was designed that has these glutamates replaced by glutamines. Until now, the study of the transport mechanism of ClC-*ec1* has mainly relied on studies based on X-ray crystallography. Crystallography provided static images, which did not contain sufficient information about protein dynamics. Therefore, to study transport mechanism of ClC-*ec1*, we chose a dynamic method – hydrogen-deuterium exchange mass spectrometry. In this thesis, ClC-*ec1* and its QQQ mutant were over-expressed in bacteria, isolated *via* detergent solubilization and purified by affinity chromatography and gel filtration. Next, the proteolytic step of the analysis was optimized to obtain sufficient spatial resolution and sequence coverage. Finally, the HDX-MS experiments were performed. In the first experiment, the conformations of ClC-*ec1* and its QQQ mutant were compared in the pH range 4.4 to 7.4. Since ClC-*ec1* was not fully protonated at pH 4.4, another experiment was performed at an even lower pH – 3.0. The last HDX-MS experiment followed changes in conformation during stepwise protonation within the pH range of 3.0-4.5 and compared them with pH 6.5.

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Keywords: H/D exchange, mass spectrometry, protein structure, membrane proteins, proton-chloride antiporter

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