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Otolin-1 – biomineralization-related protein of human otoconia and fish otoliths

Biomineralization is a physiologically regulated process in which living organisms produce mineral structures that provide a variety of functions. Otoliths and otoconia, sensors of gravity and acceleration composed of biogenic calcium carbonate and located in the inner ear - are two intriguing biomineral structures found among vertebrates. Otoliths and otoconia consist of less than 10% organic matrix, of which protein components play a key role in their formation. One of the components of the organic matrix of human otoconia and fish otoliths is otolin-1, a short collagen-like protein, a member of the C1q superfamily. Otolin-1 is an inner ear-specific otoconin whose biological role has been outlined in studies on *Danio rerio*, a model organism for human ear diseases. The molecular basis of the action of otoliths and otoconia organic matrix individual components is essential for understanding the mechanisms of their mineralization and diseases associated with the inner ear, such as benign positional vertigo.

The aim of the thesis was to perform a comparative study of otolin-1 from *Danio rerio* and *Homo sapiens*, focusing on their molecular properties in the presence of a natural ligand - calcium ions, and to recognize their role in bioinspired mineralization of calcium carbonate *in vitro*. Part of the work focused on the analysis of the proteome of *Cyprinus carpio* otoliths and *Gadiculus argenteus* fossil otoliths. The development of overexpression and purification method of recombinant otolin-1 allowed an in-depth structural and functional analysis of the proteins. Despite the high similarity of the amino acid sequences and the secondary structure of otolin-1 form *Danio rerio* and *Homo sapiens* reflecting the general tendencies of the proteins studied, the details of their molecular behavior may be crucial for their role in the mineralization of otoliths and otoconia. The presence of calcium ions was shown to thermally stabilize otolin-1 and affect the formation of its oligomers. Conducted analyses indicate, that lower concentrations of calcium ions have a high effect on otolin-1 from *Homo sapiens*, in contrast to otolin-1 from *Danio rerio* modulated by higher concentrations. Additionally, a partial, low-resolution 3D map of otolin-1 from *Danio rerio* obtained using cryo-electron microscopy was shown. As a result of a set of bioinspired calcium carbonate mineralization experiments in the presence of otolin-1, the role for the protein related to nucleation and selective inhibition of crystal walls with simultaneous induction of the formation of subsequent mineralization planes was proposed. This phenomenon is specifically evident for the *Danio rerio* homolog. Proteomic studies on *Cyprinus carpio* otoliths have identified proteins, that could be potential isoforms of otolin-1. Analyzing the proteome of the fossil otoliths from *Gadiculus argenteus* revealed the presence of two proteins or their fragments preserved in the otoliths dating to the Pliocene era.