DESCRIPTION FOR THE GENERAL PUBLIC

Biominerals are composite structures created by living organisms from inorganic and organic matter. This gives them properties unavailable for purely inorganic and purely organic materials such as resistance to compression, stretching and piercing with subsequent elasticity and resistance to breaking. Examples are otoliths and otoconia, calcium carbonate biominerals from inner ear of fish and land vertebrates, respectively. In fish, otoliths are biominerals resembling small stones with complicated shapes, which grow through the whole life of a fish. In contrast, humans have otoconia, which are microscopic crystals embedded in a gelatinous membrane, which do not grow after birth. Relatively high density of these biominerals allows to sense linear accelerations and keep the balance. Detachment and displacement of otoconia to semicircular canals, which are also engaged in keeping balance, cause benign paroxysmal positional vertigo (BPPV). The disease is especially frequent among the elderly, because the otoconia slowly degrade with aging and thus may detach more easily. Organic matrix of otoliths and otoconia, which accounts for approximately 4% of their mass, is crucial for proper formation of these biominerals. Despite that, structure and function of the matrix components are poorly understood, as is the exact mechanism of biomineralization. This encouraged us to undertake extensive research on the biomineralization of otoliths and otoconia and the role of proteins in the process. In the proposed project, we will focus on otolin-1, a collagen like protein from the organic matrix of otoliths and otoconia, which influences the appearance of calcium carbonate crystals formed *in vitro*. A part of this protein known as the C1q-like fragment is especially interesting, because it may be responsible for cross linking of otolin-1, which allows to form a special scaffold for biominerals. Together with the collagenous fragment it may interact with other organic components of the matrix. Our latest research showed that the C1q-like fragment of otolin-1 from human and zebrafish is strongly stabilized by the calcium ions. Especially, they allow to form stable trimers of the C1q-like fragment. This indicates the key role of C1q-like fragment in biomineralization, since the calcium ions are directly involved in formation of otoliths and otoconia. A human isoform becomes stabilized at lower calcium ion concentrations than the zebrafish isoform, which is especially interesting, because the concentration of these ions in human inner ear is much lower than in the fish inner ear. The aim of the project is to elucidate a molecular mechanism of function of the C1q-like fragment of otolin-1 from human and zebrafish. Planned research is based on complementary experimental techniques, which allow to obtain detailed structural information about the protein in aqueous solution. In the beginning, we will look for the genetic differences in otolin-1 genes occurring among human and fish populations. Subsequently thanks to molecular modeling we will check, how these differences may affect the investigated proteins. After selecting the variants, which could exert the biggest changes on the structure of the C1q-like fragments, we will prepare *Escherichia coli* cells transformed with plasmid DNA, which will allow to obtain the material for experimental work. Finally, we will analyze the effect of mutations on structure and function of the C1q-like fragment of otolin-1 from human and zebrafish. To achieve this goal, we will use advanced instrumental techniques such as spectropolarimetry, analytical ultracentrifugation, differential scanning fluorimetry, spectrofluorimetry and others. Obtained data will allow to propose a mechanism of adopting proper structure and binding of calcium ions by the C1q-like fragment of otolin-1 and explain the differences, which we had observed for human and zebrafish proteins. We will also be able to predict effects of genetic differences in the otolin-1 genes, which are yet to be discovered thanks to genetic research continuously conducted all over the world. We will be able to explain the mechanisms underlying balance organ disorders, especially benign paroxysmal positional vertigo (BPPV).