Characterization of molecular

and biomineralization properties of functional fragments of the human DMP1 protein

Aleksandra Porębska

# SUMMARY

Processes in which bones, teeth, eggshells or shells are formed is called biomineralization. In nature, these processes are widespread and allow living organisms to produce inorganic crystals, which are strong and durable building materials. Biominerals can be divided into several groups, due to the material from which they are made. However, the most common are calcium phosphate and calcium carbonate. Biomineralization is complex and under precise control by proteins, glycoproteins and proteoglycans. These macromolecules perform many functions in biomineralization process, associated with both the growth and creation of scaffold for mineralized tissues. It seems that acidic proteins with a disordered structure, belonging to the group of intrinsically disordered proteins (IDPs), perform a very important function. Because of them resulting biominerals have a characteristic shape, size and polymorph.

Otoliths and otoconia are biomaterials consisting mainly of calcium carbonate and an organic matrix. Otoliths are found in the inner ear of bony fish. Otoconia are found in the membranous labyrinth of mammals. Both otolith and otoconia perform important functions in the process of hearing and sensing of gravity. Thanks to them it is possible to orient the body in space, feel acceleration and balance.

DMP1 (dentin matrix protein 1) is involved in the biomineralization of calcium phosphate forming bone and dentin. Literature reports referring to the presence of DMP1 in the surrounding areas prompted the study of the role of DMP1 in the biomineralization of not only phosphate, but also calcium carbonate. The study analyzes the molecular properties of the DMP1 protein, in particular its functional 44K and 56K fragments in terms of the characteristics of inherently disordered proteins and investigates the effect of both fragments on the morphology of calcium carbonate crystals obtained *in vitro*.

For this work, a bacterial system was developed that allows for efficient production and purification of recombinant 44K and 56K fragments. An analysis of their molecular properties was carried out. Biochemical and biophysical analysis of recombinant 44K and 56K, supplemented with *in silico* examination showed that both proteins appear in solution as monomers with a characteristic, elongated shape. Structural experiments carried out in the presence of Ca2+ ions or 2,2,2-trifluoroethanol (TFE), as well as at elevated temperature showed that both molecules are characterized by high flexibility characteristic for IDPs, making them susceptible to conformational changes, including changes in the content of secondary structures also in the presence of Ca2+ ions. Based on the *in vitro* biomineralization activity test, the effect of 44K and 56K proteins on the morphology of the formed calcium carbonate crystals was demonstrated. It was noted that both proteins are involved in the nucleation of the crystal, by increasing the number of nucleating sites of crystals formed in the presence of proteins. The inhibitory effect of both proteins on crystal growth was observed. Fluorescence experiments showed that 44K and 56K proteins are components of the crystals formed in their presence, and their location in the middle of the crystal suggests that the proteins are involved in their nucleation. The obtained results have been discussed in terms of the role that the studied proteins can play in the process of biomineralization of otoconia.