Abstract

Application of molecular modeling methods to describe the control mechanisms of matrix metalloproteinase 9 activity

Matrix metalloproteinase 9 (MMP-9) is one of the most studied metalloproteinases and one of the most studied enzymatic proteins related to carcinogenesis. As an extracellular matrix structure-modifying enzyme in animal tissues, it is directly involved in a number of physiological and pathological processes. Additionally, the regulatory properties of MMP-9 in the immune response, and apoptotic or angiogenetic processes cause the comprehension of its action, and activity control mechanisms extremally important. While reading the scientific articles on MMP-9, one can easily notice, that biological research is focused primarily on the enzymatic and physiological aspects. Structural aspects, however, of both MMP-9 and its complexes, are clearly outlined, but not investigated in sufficient detail, which is a result of the high complexity of the system. For instance, MMP-9 is secreted from the cell as a mixture of monomers and homotrimers. Additionally, some cells like neutrophils, can secret MMP-9 as a covalently stabilized complex with lipocalin 2 (NGAL) protein. Each form can bind the primary MMP-9's inhibitor – the tissue inhibitor of metalloproteinases-1 (TIMP-1). Due to the significant differences between MMP-9 and other MMPs, the MMP-9-TIMP-1 interaction is much more complex. Also, it can be formed in an inhibitory or non-inhibitory mode. Such systems can imply a different pathway of monomeric proMMP-9 and the proMMP-9-NGAL complex. The characterization of interactions in those systems is a valuable aspect of the inhibition of MMP-9 activity studies. The structural research is even more complicated, considering the fact that the MMP-9 mobility and flexibility are the greatest in the whole MMPs family. Relatively recently confirmed homotrimeric proMMP-9 form is only slightly investigated. Its description is based on molecular imaging techniques, but the precise arrangement of its subunits remains unknown.

The dissertation will present the results of molecular modeling of MMP-9 structures and their complexes, which are being formed on the course of MMP-9 enzymatic activity. For the modelling of such systems, the structures of MMP-9, TIMP-1, NGAL, or their fragments, available in the Protein Data Bank, were utilized. Applied techniques include the classical molecular mechanics and dynamics, both full-atom and coarse-grained, protein-protein docking, as well as the most recent machine learning methods, like widely intriguing AlphaFold 2.

The first developed structure is a complex of the full inhibitory model of the MMP-9-TIMP-1 complex. This model explains the MMP-9 interaction with its primary inhibitor on the atomistic level. The presented research is the first such detailed description of such a complex inhibitory system in a whole MMPs family. The results indicate not only the involvement of the MMP-9 fibronectin domain in TIMP-1 interaction, but also the mutual interaction between MMP-9 fibronectin and hemopexin domains in the inactivated structure.

The second structure presented is the homotrimer of proMMP-9. This is an important step towards the understanding of the mechanics and the properties of such MMP-9 form in regard to TIMP-1 binding, but also the process of its proteolytic activation. One of the most interesting aspects is the cooperative binding of a collagen triple helix by two MMP-9 subunits.

The third model represents the structure of the MMP-9-NGAL complex. The characterization of systems containing this arrangement is the first, such detailed structural description of this MMP-9 form. As the physiological function of the MMP-9-NGAL system is still to be established, the presented results might allow for a better, more precise analysis of the experimental observations and contribute to the understanding of the functional relevance of MMP-9-NGAL complexation.

All structures, determined with molecular modelling methods, in a direct or indirect way, modify the MMP-9 activity. Theoretical and calculational research of those systems allowed to analyze and understand some of the mechanisms, which distinguish MMP-9 from every other MMPs and to widen the knowledge of MMP-9 structure, and its regulatory complexes. The understanding of the molecular basis of these regulations might contribute to the understanding of the development and escalation mechanisms of medical conditions, involving the elevated MMP-9 activity.