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STRUCTURAL BIOLOGY OF THE HISTIDINE BIOSYNTHETIC PATHWAY IN PLANTS

In this doctoral dissertation, I present my results from the studies of the HISN2, HISN3, HISN5, and HISN6 enzymes catalyzing the histidine biosynthetic pathway (HBP) in plants. The studies were conducted using X-ray crystallography and cryogenic electron microscopy (cryoEM), enriched by bioinformatic and phylogenetic analyses, and enzymatic characterization. The main goals of this research were to (i) determine three-dimensional structures of the HBP enzymes, (ii) characterize their enzymatic activity, (iii) develop protocols for an *in vitro* enzymatic synthesis of substrates, (iv) design scaffolds for future inhibitors, and (v) understand the phylogenetic origin of the enzymes. The structures for HISN2, HISN3, and HISN6 are the first structures of those enzymes from plants.

The histidine biosynthetic pathway in plants comprises eleven reactions catalyzed by eight enzymes, named HISN1-8, regarding their consecutive action. The pathway is an interesting object for studying plant metabolism, due to its interconnection with purine *de novo* biosynthesis and nitrogen metabolism. The alarming need for sustainable sources of food products and soil safety became the foundation for this project. The understanding of the structural peculiarities of enzymes involved in histidine biosynthesis in plants enables development of small molecules, acting as inhibitors or activators, to modulate the efficiency of histidine production. Histidine, due to the properties of its imidazole-containing side chain, is able to chelate metal cations from soil and water, contributing to the process of phytoremediation. This can increase the safety of soil and related food products, which should be free of metal contamination that causes, for instance, prevalent nickel allergies. The food safety might also be assured by the mitigation of the herbicide resistance, which impairs the yield of food production. The approaches presented in this dissertation broad our understanding of the HBP in plants, because such studies in eukaryotes have been neglected for decades, although the histidine biosynthesis was quite well understood in prokaryotes.

Building upon the research conducted by my supervisor, Prof. M. Ruszkowski, who characterized structurally and functionally the HISN1, HISN7, and HISN8 enzymes, I was able to study the structures, activity, and evolution of the HISN2, HISN3, HISN5, and HISN6

enzymes. As a source of the coding sequences for those enzymes, I chose a genome-sequenced barrel medic, *Medicago truncatula* (*Mt*), which is a model for legumes that are environmentally and economically important plants.

The structural studies provided insights into the interactions of *Mt*HISN2 with AMP, which allowed to update the catalytic mechanism. AMP turned out to be an effective inhibitor of *Mt*HISN2 in physiologically relevant concentrations, therefore, suggesting the existence of a second-tier regulatory mechanism of the pathway flux. The crystal structures of *Mt*HISN3 with its substrate and product contributed to the understanding of differences between plant and bacterial homologs, which may account for the development of kingdom-specific herbicides. Molecular dynamics (MD) simulations of a plant-specific fragment suggested that it facilitates products release, thereby contributing to a high catalytic efficiency. A set of high-resolution crystal and cryoEM structures of *Mt*HISN5 was utilized to identify ligand-binding hot-spots. These results combined with the results of virtual screening (VS) campaigns, served for the proposition of candidate molecules and linkers for the development of future herbicides. The process of a stereospecific enzymatic synthesis of *Mt*HISN5's substrate, resulted in the novel protocol for studying the activity of those enzymes. The crystal structures of *Mt*HISN6 revealed changes of its dynamics, based on its interactions with ligands. The kinetic studies revealed *Mt*HISN6's high selectivity towards the substrate, compared to its bacterial homologs. Structural differences between these homologs stimulated VS campaigns in the regions of the highest possibility for development of the kingdom-specific herbicides.

The phylogenetic studies of the HBP enzymes conducted using sequence similarity networks (SSN) and phylogenetic trees, revealed interesting results about their origin. In this group of enzymes, only HISN5 seems to origin from Cyanobacteria, which is consistent with the endosymbiotic theory. The genes encoding other enzymes, i.e., HISN2, HISN3, and HISN6, were likely acquired early in the evolution through a horizontal gene transfer from the Myxococcota, Bacillota, and Chloroflexota, respectively.