THREE SUGAR-ACTING PROTEINS WORTH OF CRYSTALLIZATION AND STRUCTURE SOLVING

Tiina Alamäe\textsuperscript{1}, Katrin Viigand\textsuperscript{1}, Karin Mardo\textsuperscript{1}, Triinu Visnapuu\textsuperscript{1}

Institute of Molecular and Cell Biology, University of Tartu, Estonia

Crystallization of proteins in complex with ligands and following 3D structure solving are of key importance to disclose contacts between the protein and the substrate. It helps to interpret catalytic behavior of mutated proteins and design new protein variants with advanced biotechnological properties. We have isolated and mutated levansucrase Lsc3 of \textit{Pseudomonas syringae} pv. tomato to disclose catalysis-related positions and obtain biotechnologically feasible variants for the synthesis of prebiotic fructans from cheap sugars such as sucrose and molasses. We are interested in structure solving of the catalytically inactive variant of Lsc3 in complex with the substrates - sucrose and raffinose. The second enzyme to be crystallized is maltase-isomaltase MAL1 from an early diverged yeast \textit{Ogataea polymorpha}. Wide substrate specificity of MAL1 makes it strikingly similar to in silico predicted and resurrected ancestor ancMALS of maltases and isomaltases of \textit{Saccharomyces} yeasts. No yeast maltases have been crystallized yet, just one isomaltase structure (of IMA1) is available. We have designed catalytically inactive mutants of the MAL1 protein that were tested for substrate binding in a Thermofluor assay. The third enzyme worth of crystallization is a polyfructan-degrading enzyme of a potential new probiotic colon bacterium. We have isolated and purified the enzyme and studied its biochemical properties. Data on biochemical properties of these three proteins will be presented.

Acknowledgments: Financed by grants GLOMR9072 and GLTMR1050P from Estonian Research Council