The role of sterol binding and surface charge in elicitin-induced resistance

Jan Lochman[1], Michal Obořil[1], Veronika Plešková[1], Kateřina Dadáková[1], Nikola Ptáčková[1], Zbyněk Zdráhal[2], Tomáš Kašparovský[1]

[1]*Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic*

[2]*Core Facility – Proteomics, Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5, 62500 Brno, Czech Republic*

Abstract

Elicitins are family of small proteins secreted by species of the pathogenic fungus *Phytophthora* inducing a defence reaction in plants. On the basis of their pI they are classified as either alpha elicitins or more necrotizing beta elicitins. Cryptogein is beta elicitin secreted by *Phytophthora cryptogea* that can induce resistance to *P. parasitica* in tobacco plants. It contains a hydrophobic cavity capable of binding sterols and fatty acids and highly conserved omega-loop. On the basis of previous computer modelling experiments, by site-directed mutagenesis a series of cryptogein variants was prepared with altered abilities to bind lipids and with influenced surface charge. The sterol binding and phospholipids transfer activities corresponded well with the previously reported structural data. Induction of the synthesis of reactive oxygen species (ROS) in tobacco cells in suspension and proteomic analysis of intercellular fluid changes in tobacco leaves triggered by these mutant proteins were not proportional to their ability to bind or transfer sterols and phospholipids. However importance of omega-loop for interaction of the protein with the high affinity binding site on the plasma membrane was proved. On the other hand determined results sustained a crucial role of positive lysine residues on the surface of basic elicitins and suggested their significant role in correct protein-membrane interaction as well as on their ability to induce effective resistance in tobacco plants. From determined results could be suggested that possible model for the signal transduction mechanism in elicitins would be similar to those proposed for the AVR9/Cf-9 interaction in tomato or the NIP1/Rrs1 interaction in barley.