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Structural studies of *Pseudomonas syringae* effector protein HOPQ1 and its complex with plant 14-3-3 protein

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Plant pathogenic bacteria *Pseudomona syringae* in order break the host innate immunity barriers introduce to the plant cell tens of different molecules called effectors [1]. Effectors modify plant proteins that are important in the early steps of pathogen recognition via pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) pathway [2]. PTI relays on recognition of evolutionarily conserved molecules, like flagellin protein a part of the bacterial motility apparatus, by the membrane receptors. Effectors could modify host factors by phosphorylation, degradation, ADP-ribosylation and changing the cellular localization. Effectors could also act as transcription factors. During evolution, plants also evolved the mechanism to recognize effectors and trigger, Effector Triggered Immunity (ETI), that leads to the programed cell death, called hypersensitive response (HR) at the site of the infection in order to stop spreading of the infection [2]. HopQ1 protein is one of the effector molecules that are transported via type III secretory system (T3SS) to the plant cells during infection [3]. HOPQ1 is a putative nucleoside hydrolase protein. This protein possess two domains: the N-terminal fragment predicted to be disordered and the globular nucleoside hydrolase like (NH) domain. NH domain is conserved structurally across in members of the family. This domain binds calcium ion in the active site of the enzyme. It is interesting that although HOPQ1 protein possess NH domain its enzymatic activity was not shown experimentally. In previous studies it was shown that HOPQ1 is phosphorylated at two sites and one of them, serine 51 is located in 14-3-3 protein binding motif [4].
14-3-3 proteins forms a large family of conserved regulatory factors that are mainly responsible for protein-protein interaction [5]. The regulatory mechanisms of 14-3-3 proteins are the change of cellular localization, conformation, stabilization and binding mode of target proteins.

In this work we proposed the structure of the *Pseudomona syringae* HOPQ1 protein and its complex with 14-3-3 from tobacco (*Nicotiana benthamiana)* protein in solution using small-angle X-Ray scattering technique. Using *ab-initio* and rigid body modeling, we discovered that N-terminal fragment of HOPQ1 in solution is disordered and has dynamic structure. We also tested the effect of calcium ions depletion on the structure of HOPQ1 protein and structure and behavior of HOPQ1 protein variant (Q2A) with mutated amino-acids within putative Ca^{2+} binding site. HOPQ1 protein in the presence of EGTA exist in solution as an elongated dimer. Dimerisation of HOPQ1 protein could be reversed by the addition of excess of Ca^{2+} ions. Q2A mutant also forms elongated dimers in solution regardless of the addition of EGTA. This confirms that calcium binding may regulate oligomerization of HOPQ1 protein.
HOPQ1 protein exists as a 1:1 complex with the dimer of 14-3-3 protein. In the obtained model of the complex between HOPQ1 and 14-3-3 proteins, 14-3-3 protein binds to the disordered tail of HOPQ1 protein possibly sterically blocking access to the putative nuclear translocation signal located downstream of 14-3-3 binding motif. Such interaction may explain the ability of 14-3-3 protein to change localization of HOPQ1 protein inside plant cell.

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[1] M. Lindeberg, S. Cunnac, A. Collmer, *Trends Microbiol.* **20** (2012) 199**.**
[2] D. G. J. Jones, J. L. Dangl, *Nature* **444** (2006) 323**.**
[3] W. Li, Y. H. Chiang, G. Coaker, *PLoS One* **8** (2013) e59684.
[4] F. Giska, M. Lichocka, M. Piechocki, M. Dadlez, E. Schmelzer, J. Hennig, M. Krzymowska. *Plant Physiol.* **161** (2013) 2049.
[5] A. L. Paul, F. C. Denison, E. R. Schultz, A. K. Zupanska, R. J. Ferl, *Front Plant Sci.* **3** (2012) 190.