P205

# Pathogenesis-related Protein 10 Isolated from Hot Pepper Functions as a Ribonuclease in an Antiviral Pathway

Chang-Jin Park[P], Ki-Jeong Kim[1], Yun-Chul Shin[1], Boo-Ja Lee[1], Kyung-Hee Paek[C]

*School of Lifesciences and Biotechnology, Korea University, Seoul 136-701*

Plants respond to infection of pathogens by increasing the expression of a number of genes, some ofwhich may be associated with disease resistance. These genes include those that encode pathogenesis-related (PR) proteins and antibiotic proteins. PR proteins are defined as plant proteins that are induced in pathogenical or related situations and are expressed as a component of the active plant defense repertoire more rapidly and to a greater extent in incompatible interaction in which a resistant plant is challenged with an avirulent pathogen. Although the biological and/or biochemical function of many PR proteins have been studied for several decades, only a few PR proteins such as chitinase and -1,3-glucanase were clarified for their catalytic functions and the roles of other PR proteins in the plant defense response remain to be determined. PR proteins are currently classified as 14 families and only one of which, PR-10 family, has not been examined for biological properties including any antimicrobial activity.To gain a better understanding of plant defense responses against viral pathogens, we isolated several cDNAs which were specifically expressed during an incompatible interaction between *Capsicum annuum* cv. Bugang and *Tobacco mosaic virus* (TMV) pathotype P0 (TMV-P0), using differential screening. A number of cDNAs encoding PR proteins including PR-4, PR-6 and PR-14, SAR8.2 protein, and many other putative defense-related proteins were obtained. This study deals with PR-10 that belongs to an extensive family of intracellular defense-related protein with structural homology to ribonuclease.In contrast to most other PR proteins which are extracellular, intracellular PR proteins were first described in cultured parsley cells upon elicitor treatment. These intracellular proteins have molecular masses and acidic characteristics similar to those of PR-1 proteins, but are not structurally related to the PR-1 proteins of tobacco. Parsley "PR1" has been suggested to represent the "type member"for a ubiquitous class of intracellular, defense-related proteins, the PR-10 family. PR-10 proteins have amino acid sequence similarity to the major food allergen of celery and pollen allergens of tree. Studies revealed that the PR-10 proteins also share homology to a ribonuclease isolated from phosphate-starved ginseng cells, suggesting that PR-10 proteins may possess such activity. The ribonuclease activity was demonstrated in major birch pollen allergen, Bet v1, that was known to have homology to PR-10 proteins. Recently, it was reported that a PR-10-like protein from *Lupinus albus* (white lupin) exhibits ribonuclease activity. PR-10 proteins may have functions other than ribonuclease. Warner et al. (1994) have indicated that the expression of the asparagus AoPR1 gene closely correlates with sites of phenylpropanoid biosynthesis. The identified PR-10 protein in the tapetum of lily anthers suggests a potential role in the sporopollenin pathway. Although these results about the putative enzyme activity of PR-10 protein have been reported, the biological functions of this class of proteins remain unknown. Here a hot pepper (*Capsicum annuum*) cDNA clone encoding pathogenesis-related protein 10 (CaPR-10) was induced in the incompatible interaction with TMV-P0 or *Xanthomonas campestris* pathovar *vesicatoria* but not induced in the compatible interaction. CaPR-10 gene also was induced in hot pepper plants treated with salicylic aicd (SA), methyl jasmonic acid (MeJA), ethylene, methyl viologen (MV), or NaCl. In this study, CaPR-10-fused soluble-modified green fluorescent protein (smGFP) was transiently expressed in tobacco bright yellow-2 (BY-2) suspension cultured cells by polyethylene glycol (PEG)-mediated transformation method and fluorescent signals derived from smGFP were accumulated throughout the cell interior. Characterization of enzymatic properties of CaPR-10 indicated thatthe recombinant protein exhibits a ribonucleolytic activity against TMV RNA as well as pepper total RNA and shows its putative antiviral activity in several conditions. It cleaves all types of RNA tested with a pH optimum of 4.5 to 5.5, is insensitive to EDTA and do not exhibit DNase activity. Therefore, this RNase activity may be considered an RNase I class but its molecular mass is about 18 kDa, characteristic of the RNase II class. The CaPR-10 protein existed at very low level in leaf tissue but was dramatically induced as soon as plants were inoculated with TMV-P0, and this was correlated with the increase of its ribonucleolytic activity. Immunoblot analysis and pull-down assays using proteins extracted from pepper leaves showed that TMV-P0inoculation led to the phosphorylation of CaPR-10, a modification that should affect its capacity for RNase function. To find out the relationship of RNase activity and phosphorylation of CaPR-10, in-gel RNase assay was repeated against yeast tRNA using the native proteins extracted from the roots. Quantification of the root data using Quantity One 1-D analysis Software showed that phosphorylated protein, which was in approximately 0.39-fold lower amounts, had 4.85-fold higher RNase activity compared to dephosphorylated form. We present data that the induction and subsequent phosphorylation of CaPR-10 increased its ribonucleolytic activity to cleave invading viral RNAs and this activity should be important to its antiviral pathway during viral attack *in vivo*.