FUNGAL BIOLOGY 121 (2017) 911–919

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio

Intrinsic disorder is a common structural characteristic of RxLR effectors in oomycete pathogens

*Danyu SHEN, Qingling LI, Peng SUN, Meixiang ZHANG, Daolong DOU**

Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, China

ARTICLE INFO

Article history:
Received 10 January 2017
Received in revised form
5 July 2017
Accepted 21 July 2017
Available online 28 July 2017
Corresponding Editor:
Simon V. Avery

Keywords:
Phytophthora
Protein structure
Plant–pathogen interaction
Effector function

ABSTRACT

Intrinsic disorder is common in nature and has been studied to play important biological roles in bacterial effectors. However, disorder in oomycete RxLR effectors has not been investigated previously and the roles are unknown. Our results of PONDR VL-XT disorder analysis showed that predicted oomycete RxLR effectors were significantly more disordered than other effectors and secretome. The distribution of disorder content presented preference that RxLR-dEER regions were enriched in disordered residues, suggesting potential role of disorder in effector translocation. In contrast, the disorder content was depleted in the C-terminal regions, especially for W/Y/L motifs. We also found that around 42 % of putative RxLR proteins were predicted to contain at least one α-helix-forming molecular recognition feature (α-MoRF), and most α-MoRFs were located in the C-terminal regions. Furthermore, both of the disorder mutants of PsAvh18 and PcAvh207 lost the cell death-inducing activity, indicating the potential important role of disordered structure in RxLR effector function. Overall, these results demonstrate that intrinsic disorder is a common characteristic of oomycete RxLR proteins, and we postulate that such structure feature may be important for effector translocation or function. This study extends our understanding of RxLR effectors in protein structures, and opens up new directions to explore novel mechanisms of oomycete RxLR effectors.

Introduction

A diverse number of plant pathogens, including bacteria, fungi, and oomycetes, secrete effector proteins into plant cells to manipulate host cellular processes and enable parasitic colonization (Chisholm *et al.* 2006; Kamoun 2007). Effectors constitute a heterogeneous group of proteins with diverse structures and cellular functions. One of the best-studied types of effectors in oomycetes is RxLR family of cytoplasmic effectors. They contribute to virulence by suppressing plant defense responses. Meanwhile, they function as avirulence proteins when plant corresponding resistant genes are present (Tyler 2009). Up to now, twenty avirulence genes encoding RxLR effectors have been cloned in oomycetes (Anderson *et al.* 2015). However, the molecular mechanisms of their virulence or avirulence functions remain unknown in many cases.

RxLR effectors have modular structures, containing a signal peptide, followed by a relatively conserved RxLR or RxLR-like (RxLR variant such as KxLR or RxLK) motif functioning in translocation into host cells, while the remaining C-terminal

* *Corresponding author*. Tel.: +86 025 84396973; fax: +86 025 84395325.
E-mail address: ddou@njau.edu.cn (D. Dou).
http://dx.doi.org/10.1016/j.funbio.2017.07.005

domains carry the effector activity (Tyler 2009; Kale & Tyler 2011). In addition, nearly half of RxLR effectors contained one or more repeating modules made up of adjacent W, Y, and L motifs (Jiang et al. 2008). In particular, most RxLR proteins show extensive sequence divergence, and lack similarity to other known proteins. Bioinformatics analyses of oomycete genome sequences lead to identification of an extremely large superfamily of RxLR effectors. There are nearly 350–600 RxLR members identified in hemibiotrophic *Phytophthora* species (Tyler et al. 2006; Jiang et al. 2008; Haas et al. 2009). Reduced number of RxLR effectors was found in the biotrophic pathogens *Plasmopara halstedii* and *Hyaloperonospora arabidopsidis* (Baxter et al. 2010; Sharma et al. 2015). However, no RxLR effector has been found in necrotrophic *Pythium* species and animal pathogen *Saprolegnia parasitica* (Levesque et al. 2010; Jiang et al. 2013).

Intrinsically disordered proteins or regions are flexible segments that lack stable secondary and/or tertiary structure under physiological conditions (Wright & Dyson 1999; Dunker et al. 2001). The presence of protein intrinsic disorder is common in nature (Uversky 2010), and eukaryotic proteomes are shown to have a largely higher occurrence of disordered proteins compared to prokaryotic proteomes. Disordered proteins have a distinct feature at the level of amino acid sequences. In fact, they own a higher content in charged residues and a lower content in hydrophobic residues than globular proteins (Xue et al. 2014). The unique nature of disordered sequences has led to the development of various algorithms designed for disorder prediction. Disordered proteins possess their respective biological functions that are typically related to signal transduction, recognition, and transcriptional regulation (Iakoucheva et al. 2002; Dunker et al. 2005; Uversky et al. 2005).

Intrinsic disorder has been studied in bacterial effectors. Bacterial pathogens secrete numerous effector proteins via the type III secretion system into host cells to manipulate the immune responses (Dean 2011). Not only the average percentage of disorder content, but also the strikingly long disordered segments are predicted to be highly enriched in bacterial effectors (Marin et al. 2013). Some effectors including AvrPto, YopE, and HopPmaL, are demonstrated to be partially disordered by NMR spectroscopy (Wulf et al. 2004; Rodgers et al. 2008; Singer et al. 2012). The disordered structures of bacterial effectors are supposed to be relevant to their effector biology. Translocation of disordered regions would be of native advantage, as it could spare active unfolding, which is required prior to insertion into the narrow channel formed by the type III secretion machinery (Stebbins & Galan 2001). Furthermore, disordered effectors generally evolve faster than ordered proteins, which results in the hypothesis that intrinsic disorder helps to avoid direct recognition by plant resistance proteins (Marin et al. 2013).

Till now, the crystal structures of several RxLR effectors, including ATR1 and ATR13 from *H. arabidopsidis*, Avr3a4 and Avr3a11 from *Phytophthora capsici*, *Phytophthora infestans* PexRD2, and *Phytophthora sojae* Avh5, have been generated (Boutemy et al. 2011; Chou et al. 2011; Leonelli et al. 2011; Yaeno et al. 2011; Sun et al. 2013). These RxLR effectors contain helical-rich C-terminal domains, whereas the N-terminal RxLR domains are highly disordered. It will be interesting to investigate the feature of disorder in oomycete RxLR family. In this study, a large-scale prediction of intrinsic disorder and further feature description were carried out.

Materials and methods

Datasets

The protein sequences of *Phytophthora sojae*, *Phytophthora ramorum*, and *Phytophthora capsici* were downloaded from Joint Genome Institute database (genome.jgi.doe.gov). *Phytophthora infestans* and *Hyaloperonospora arabidopsidis* sequences were obtained from Broad Institute database (www.broadinstitute.org) and EumicrobeDB (eumicrobedb.org). *Plasmopara halstedii* sequences were downloaded from a local server (dx.doi.org/10.12761/SGN.2015.7). RxLR effectors were obtained from previous publications (Jiang et al. 2008; Haas et al. 2009; Lamour et al. 2012; Sharma et al. 2015). The secretome for each species was predicted by combination of SignalP 3.0 (Bendtsen et al. 2004), TargetP 1.1 (Emanuelsson et al. 2000), and TMHMM 2.0 (Sonnhammer et al. 1998). The secreted proteins should satisfy the following requirements: SignalP HMM score >0.9, no TMHMM predicted transmembrane domain following signal peptide cleavage site, and localization of 'S' in TargetP.

Disorder predictions

The prediction of disordered residues was performed using a well-characterized disorder predictor PONDR VL-XT, which integrated three different neural networks and was trained using experimentally confirmed disordered protein regions (Romero et al. 2001). This predictor was shown to provide high quality predictions compared to other disorder predictors. We also used another two predictors, VSL2B (Obradovic et al. 2005) and VL3 (Obradovic et al. 2003), to predict the overall disorder content in proteins. All the three predictors use the protein sequence as the input and classify each amino acid within a sequence as either structured or disordered. We used α-MoRF-II predictor (Cheng et al. 2007) to predict α-MoRF regions in RxLR proteins. This predictor concentrates on short binding regions within long disordered regions that are possible to form helical structure upon binding.

Amino acid composition analysis

Amino acid compositional analysis was performed by Composition Profiler (www.cprofiler.org) using the PDB Select 25 as reference for ordered proteins. Enrichment or depletion for each amino acid type was calculated as $(C_x - C_{order})/C_{order}$, where C_x was the absolute composition of each amino acid in the RxLR dataset, and C_{order} was the corresponding value for the control set of ordered proteins from PDB Select 25.

Agrobacterium tumefaciens infiltration assays

Two RxLR genes including PsAvh18 and PcAvh207, were amplified using *Phytophthora sojae* cDNA, and then cloned into the PVX vector pGR107. Constructs were introduced into *A. tumefaciens* strain GV3101 by electroporation (Hellens et al.