Determining the interaction site of a novel Coprinopsis cinerea lectin to Listera innocua peptidoglycan

Sandeep Misra (skmisra@olemiss.edu), University of Mississippi, **Sandeep Misra**, University of Mississippi, **Sushil Mishra**, University of Mississippi, **Nika Janez**, Jožef Stefan Institute, **Robert Doerksen**, University of Mississippi, **Jerica Sabotic**, Jožef Stefan Institute, **Joshua Sharp**, University of Mississippi

Lectins are proteins that reversibly bind to carbohydrates and glycoconjugates, often with relatively low affinity. Understanding how they recognize carbohydrates selectively is important for fully understanding the importance of lectin-carbohydrate interactions, which play many important roles in a wide range of biological processes. A novel protein purified from Coprinopsis cinerea showed sequence similarity to known lectins suggesting that it may be a lectin. Initial NMR studies indicate that it binds to peptidoglycan from L. innocua, however the binding site is unclear. To verify that the protein is a lectin and to identify the peptidoglycan binding site of this protein, as well as any conformational changes to this protein upon binding, we employed hydroxyl radical protein footprinting (HRPF) and molecular docking.

The samples, containing 5 µM of the protein, 1 mM adenine in 50 µM phosphate buffer, pH 7.8, with or without 15 µM peptidoglycan, and 100 mM hydrogen peroxide, were irradiated using a Fox Protein Footprinting System (GenNext Technologies) using inline dosimetry to monitor the effective free radical dose. The samples were collected into a quench solution containing 35 mM methionine amide and 0.3 mg/mL catalase. After quench, the samples were heat denatured, divided into two equal parts and digested with trypsin and chymotrypsin separately for 16 hr. 0.1% formic acid was added to the samples and the samples were run on an Orbitrap Exploris mass spectrometer coupled with a Dionex Ultimate 3000 nanoLC system (Thermo Fisher, CA).

The dosimetry of the samples with or without peptidoglycan was comparable, indicating no need for compensation for radical scavenging. Both trypsin and chymotrypsin were used, achieving more than 83% sequence coverage of the protein. We detected a total of nine peptides oxidized and one peptide was detected with no oxidation by the hydroxyl radicals. Three peptides (corresponding to protein residues 39-48, 49-58, and 82-104) were significantly protected from oxidation modification ($p \leq 0.05$) in the presence of peptidoglycan, with the other peptides showing no significant changes in oxidation upon peptidoglycan binding. This result validates that the protein is binding to the peptidoglycan, and suggests that these protected regions are either directly involved in binding to peptidoglycan or are affected due to allosteric changes upon binding to peptidoglycan. No regions of exposure upon peptidoglycan binding were detected. Molecular dynamics simulation studies identified three amino acids in the protected regions (F42, W50, W95) that may play a direct role in the binding of this protein to peptidoglycan. Current mutagenesis studies are underway to validate the importance of these residues in protein-peptidoglycan interactions.