BMBGG Ph.D. DEFENSE

Friday, October 4, 2019

Miao Li

Johnson Cancer Research Center
Conference Room
36 Chalmers Hall, 9:00 a.m.
Refreshments available

Study of Serpins and Antifungal Peptides in *Manduca sexta*

Insects initiate innate immune responses after microbial infection. Extracellular proteolytic cascades lead to melanization of pathogens and parasites present in the hemocoel, as well as production of antimicrobial peptides to combat foreign microbes. Serpins are a superfamily of serine protease inhibitors, which regulate the proteolytic cascades by blocking activity of proteases. An antifungal peptide, diapausin, is among the induced antimicrobial peptides in *M. sexta* hemolymph, helping to protect this insect from fungal infection.

Synthetic peptides with the sequences derived from part of the *M. sexta* serpin-3 reactive center loop (RCL) were used to test the hypothesis that they may inactivate serpin-3 by mimicking the insertion of the RCL that occurs during the protease inhibition reaction. Among four RCL-derived peptides with sizes of 5-9 amino acid residues, the shortest peptide, Ac-SVAFS-NH_2, demonstrated the greatest effect on blocking the inhibitory activity of recombinant serpin-3 by enhancing the conversion of serpin-3 from inhibitor to substrate for prophenoloxidase-activating protease-3. Ac-SVAFS-COO^- with improved solubility showed the same effects on serpin-3 as Ac-SVAFS-NH_2. Addition of Ac-SVAFS-COO^--treated recombinant serpin-3 to plasma led to elevated levels of prophenoloxidase activation, suggesting that the serpin in complex with the peptide could not regulate prophenoloxidase-activating proteases. Similar results were observed when the RCL-derived peptide was mixed with plasma, to allow it to interact with endogenous serpin-3. Circular dichroism analysis showed that serpin-3 complexed with Ac-SVAFS-COO^- has a deeper trough at 215 nm compared with the free serpin-3, consistent with a slight difference in secondary structure upon interaction with Ac-SVAFS-COO^-. Furthermore, the complex of serpin-3 and Ac-SVAFS-COO^- displayed greater heat stability than free serpin-3, which is consistent with the predicted peptide insertion into β-sheet A.

Recombinant diapausin-1 and diapausin-2 blocked growth of *Saccharomyces cerevisiae*, with IC_{50} of 30 μM and 60 μM, respectively. Observation of the cell morphology revealed the disruption of daughter cell separation after cell division, resulting in cell clusters in the presence of diapausin. Morphological analysis revealed that diapausin-1 causes appearance of an elongated mother cell with a small, flattened bud and wider neck, as well as less elliptical shape. The growth of *Candida albicans* and *Candida krusei* was inhibited by diapausin-1, with IC_{50} of 60 μM and 20 μM respectively. Furthermore, diapausin-1 impaired the germination of conidia and hyphal growth of *Beauveria bassiana*, an insect pathogenic fungus. FITC-labeled diapausin-1 bound to the surface of *S. cerevisiae*, and pull-down experiments showed that diapausin-1 binds to β-1,3-glucan, a primary component of fungal cell walls. This study advances our understanding of an insect immune response to fungal infections and may contribute to the identification of new targets for the development of antifungal drugs.

Major Professor: Dr. Michael Kanost