SJSU | DEPARTMENT OF CHEMISTRY

CHEMISTRY Departmental Seminar

Spring 2022
CHEM 285/191 Schedule
Tuesday at 4:30-5:45PM
Duncan Hall 250
May 3rd, 2022

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Mutagenesis Studies of JHA15, a Midgut Protease from the Aedes aegypti Mosquito

The female *Aedes aegypti* mosquito is one of the most effective vectors and carriers of viruses that lead to disease. The mosquito is a carrier of the Zika, Dengue, Chikungunya, and Yellow Fever viruses.[1] These viruses are effectively transmitted through the female *A. aegypti* mosquito during the uptake of a blood meal. The blood meal contains proteins which are degraded by midgut proteases to the necessary nutrients needed to enable the development and production of eggs.[2,3] Although recent studies have suggested that JHA15 may be involved in the blood meal digestion process, direct evidence is lacking. [4] Therefore, the protease was isolated and studied *in vitro*. *In vitro* studies include the recombinant expression of the *A. aegypti* mosquito JHA15 gene in T7 SHuffle express *E. coli* cells. Initial expression and purification of different JHA15 constructs seem to have auto-catalytic activity. The focus here is to recombinantly express and biochemically understand the auto-activation of JHA15. This project focuses on mutating the pro-peptide region of different JHA15 constructs to prevent auto-activation and to determine if the protease constructs do indeed auto-activate at this region. JHA15 mutants were recombinantly and solubly expressed in T7 SHuffle express *E. coli* cells, purified, and found that the auto-activation activity was absent as they did not cleave the substrate Bz-Phe-Val-Arg-pNA.

Studying the overall *in vitro* activity of the protease will help with midgut protease inhibitor development. Blood meal protein digestion is necessary for egg production in the mosquito but studying JHA15 and other midgut proteases could help in reducing the number of mosquito eggs produced and minimize pathogen transmission. Specificity of the inhibitor will ensure that ecologically beneficial pollinators are not affected.

1. Centers for Disease control and Prevention. https://www.cdc.gov/
2. Rascon, A.A.; Gearin, J.; Isoe, J.; Miesfeld, R.L. *BMC Biochemistry.* **2011,** *12,* 1–10.
3. Attardo, G.M.; Hansen, I.A.; Raikhel, A.S. *Insect Biochem Mol Biol.* **2005**, *35*, 661–675.
4. Bian, G.; Raikhel, A.S.; Zhu, J. *Insect Biochem Mol Biol*. **2008**, *38*, 190–200.

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