Abstract

Project Code : MRG6180012

Project Title : Characterization of a ficolin-like lectin from the black tiger shrimp *Penaeus monodon*

Investigator : Dr. Kittikhun Wangkanont

E-mail Address : kittikhun.w@chula.ac.th

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Abstract:

Fibrinogen-related lectins are carbohydrate-binding proteins of the innate immune system that recognize glycan structures on microbial surfaces. These innate immune lectins are crucial for invertebrates as they do not rely on adaptive immunity for pathogen clearance. Here we characterize a recombinant fibrinogen-related lectin PmFREP from the black tiger shrimp *Penaeus monodon* expressed in insect cells. PmFREP is a disulfide-linked dimer of pentamers that binds N-acetyl glucosamine in a $Ca2^{+}$ ion-independent manner. PmFREP recognized and agglutinated *Pseudomonas aeruginosa*. Weak binding was detected with other bacteria, including *Vibrio parahaemolyticus*, but no agglutination activity was observed towards these bacteria. The biologically active PmFREP will not only be a crucial tool to elucidate the innate immune signaling in *P. monodon* and other economically important species, but will also aid in detection and prevention of shrimp bacterial infectious diseases.

Keywords : Lectins, immune system, shrimp

Characterization of a ficolin-like lectin from the black tiger shrimp *Penaeus monodon*

1. ความสำคัญและที่มาของปัญหา

With more than 300,000 metric tons of shrimp being exported each year, shrimp aquaculture has been one of the most profitable agricultural products of Thailand. However, the industry has been periodically affected by various infectious diseases. In addition to viral diseases such as the white spot syndrome virus or the yellow head virus, Early Mortality Syndrome (EMS) caused by the bacterium *Vibrio parahaemolyticus* has severely hampered the shrimp industry in recent years. With the continuous emergence of new infectious diseases, it is therefore crucial to understand the shrimp immune system so that a cure or prevention could be developed for future outbreaks.

The innate immune system is the predominant defense mechanism in invertebrates. Microbes are usually recognized by pattern recognition receptors that will then activate downstream immune signaling pathways. One type of such receptors is lectins, or carbohydrate-binding proteins. Several families of immune lectins are found in animals. For example, C-type lectins, such as mannose binding lectins, can bind invading microbes and activate the lectin complement pathway that lead to pathogen destruction and clearance. Some fibrinogen-like domain (FBD)-containing lectins are also known to activate the lectin complement pathway as well. These immune signaling pathways are well-characterized in mammalian systems. However, it is unclear whether immune lectins in shrimp possess the same molecular properties. Therefore, characterization of immune lectins in shrimp will contribute to the fundamental knowledge of the shrimp immune system.

In addition to the immune function of lectins, scientists have long been using lectins as carbohydrate recognition tools for various applications. For example, concanavalin A from jack bean that can recognize mannose and glucose residues is widely used to characterize glycoproteins and also to activate T cells by receptor cross-linking. Wheat germ agglutinin can bind sialic acid and N-acetyl-D-glucosamine (GlcNAc) residues, and has been employed in animal tissue staining. Intelectins in chrodates can recognize microbe-specific carbohydrate residue such as galactofuranose and could be used to differentiate various serotypes of *Streptococcus pneumoniae*. Virtually all organism contains lectins, and there are many more lectins still to be discovered. Therefore, characterization of novel lectins will likely yield new tools to detect and identify various cell types with a wide spectrum of application in biotechnology.

Given the importance of understanding the lectin structure and function, this proposal aims to characterize PmFREP, a FBD-containing lectin, from *Penaeus monodon*. PmFREP was initially isolated as a peptidoglycan binding protein in the hemolymph. PmFREP has a carbohydrate-binding domain (CRD) that is homologous to other FBD-containing lectins, such as ficolins. However, the N-terminal domain of PmFREP is distinct. This project aims to express and characterize PmFREP. The results will provide insights into the lectin-mediated immune system of shrimp and may also yield new tools for molecular recognition.

2. วัตถุประสงค์

1. To identify the optimal expression and purification strategy for PmFREP
2. To determine preliminary ligand specificity of PmFREP
3. To identify bacteria that can be recognized by PmFREP