Chapter 5

Conclusion

One intact form of purified lectin with a M_r of 316.2 kDa was obtained from the purification of the *P. merguiensis* hemolymph by affinity chromatography and following by gel filtration chromatography or preparative PAGE. It was an oligomeric protein made up from two distinct subunits of 32.3 kDa and 30.9 kDa, without any covalent disulfide linkages between the subunits. Overall amino acid composition and pI value of purified lectin were similar to that found for lectins from *M. rosenbergii*, *L. setiferus* and the crab *L. depurator*.

Purified lectin was composed of N-acetyl neuraminic acid (Neu5Ac), mannose, glucose and glucosamine. It was consistent that the lectin was a glycosylated protein containing potential N-glycosylation sites mainly for mannose and GlcNAc observing by affinity blot assay with various biotinylated lectins and by deglycosylation of lectin with PNGaseF and TFMS.

Purified lectin has the highest specificity for Neu5Ac, porcine stomach mucin and fetuin. Three other N-acetyl aminosugars, GalNAc, GlcNAc and ManNAc could inhibit the lectin HA but with a lower efficiency. However, asialofetuin, showed no capacity to inhibit purified lectin.

Purified lectin selectively agglutinated the major infective bacteria, *V. harveyi* and *V. parahemolyticus* and also *V. vulnificus* to a lesser degree. It did not show agglutinating activity against the shrimp non-pathogenic strains *E. coli, S. typhi* and *V. cholerae*. The specificity of purified lectin to agglutinate *V. harveyi* was completely inhibited by 6.25 mM Neu5Ac, 1 mg/ml mucin and 86 ng/ml anti-lectin antibody. The recognition of vibrios was also observed in the agglutination of bacteria by the hemolymph of the Eastern oyster, *C. virginica*, the blue crab, *C. sapidus*, penaeid shrimps *P. monodon* and *P. indicus,* and the freshwater prawn *M. rosenbergii.*

Anti-lectin antibody raised against purified lectin of *P. merguiensis* bound specifically to purified lectin and *P. merguiensis* hemolymph. It also showed cross reactivity with hemolymph lectin of *P. monodon* and *P. vannamei* but not with that of *M. rosenbergii* in dot blot analysis.

The reliable anti-lectin antibody was used to develop ELISA for quantifying lectin levels in the hemolymph of *P. merguiensis* at various stages of ovarian development and those of infected shrimps. Standardization of ELISA was achieved by using purified lectin. The standard curve for the ELISA had a linear range of 12.5-350 ng and the regression coefficient (R^2) was 0.988. The sensitivity of the assay is capable to detect lectin concentrations as low as 83.3 ng/ml. A dilution curve of the hemolymph was linear in a range of 1:50 to 1:400 dilutions.

Changes in lectin levels in the hemolymph of infected *P. merguiensis* were determined by ELISA and hemagglutination assay. Both the specific lectin contents and the specific HA in the hemolymph of *V. harveyi* injected shrimps increased significantly in parallel as the longer time of post-injection. Otherwise, those of the uninfected shrimps showed no differences at any time of post saline-injection. The similar results were reported in *P. monodon* infected with *V. vulnificus* and *P. chinensis* challenged by WSSV. Hemolymph lectin may play a role as a defense protein with simultaneous agglutinating and anti-bacterial properties.

Vitellogenic females (at stages 2-4 of ovarian development) had higher the specific lectin contents than those of non-vitellogenic females (at stage 1) and male shrimps. In comparison, the specific HA of hemolymph lectin of *P. merguiensis* at stage 1 and 2 were similar while it inreased gradually when shrimps developed to stages 3 and 4. It is possible that the hemolymph lectin may involve in ovarian maturation in this species of shrimp as in the acorn barnacle *M. rosa*.

Quantification of lectin in the hemolymph of shrimps is essential to assess the resistance power of the organism against bacterial pathogens. The assay was specific, sensitive and suitable for quantifying lectin levels in the shrimp hemolymph and it will be useful in the investigation of lectin against potential bacteria.