

Thesis Title: Monodin, a New Sialic Acid-Specific Lectin from Black Tiger Prawn
(*Penaeus monodon*)

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ABSTRACT

Although hundreds of lectins which are multivalent carbohydrate-binding are known, only few are specific for sialic acids. Sialic acid-specific lectins are mostly found among invertebrates but their roles are speculative. In this study, a new lectin, called monodin, was purified from the hemolymph of black tiger prawn (*Penaeus monodon*) by affinity chromatography using a fetuin-agarose column and fast protein liquid chromatography using Superose 12 column and Mono Q column. Its native Mr was 420,000 and its subunit Mr was 27,000. Monodin was found to be a heat-labile glycoprotein containing 3.89% neutral sugars. By agarose gel isoelectric-focusing, its relative pI value was shown to be 8.2.

The carbohydrate-binding activity of monodin was found to be specific to N-acetylneuraminic acid because it was inhibited by the sugar at 3.12 mM. Its activity was also inhibited by other N-acetyl amino sugars, GalNAc and GlcNAc, at 6.25 mM and ManNAc at 12.5 mM. The inhibition of the monodin activity by oligosaccharides and sialoglycoproteins suggested

that the binding activity of monodin required the $\alpha 2 \rightarrow 3$ and/or $\alpha 2 \rightarrow 6$ linkage of NeuNAc to subterminal sugars and also the O-glycosidic linkage to the protein. Its binding activity required 1 mM Ca^{2+} which can be substituted by Sr^{2+} at 20 mM.

Anti-monodin was raised in rabbits against the 27,000 Mr protein subunit of monodin obtained from SDS-PAGE. Using immunoblotting, anti-monodin reactive material, presumably monodin, was found in the ovary and muscle of *P. monodon*. The presence of monodin in the prawn hemocytes was detected in the hemocyte membrane, cytosol and mitochondrial fractions, suggesting the hemocyte as a possible site of monodin synthesis. In addition, immunoreactive monodin was detected in serum of other prawns of closely related species and genus, *Penaeus merguensis* and *Metapenaeus monoceros*. The monodin contents in the serum of *P. monodon* did not differ between the sexes but varied depending on age and molting cycle. Monodin level was low in the young prawn of 75 days old. During a molting cycle, a lower amount of monodin was detected in the prawn of stage A (postmolt) and a higher amount was noted in other stages with highest amount in stage B (postmolt) through stage D₀ (premolt). A possible role of monodin in molting was suggested.

The monodin contents in the hemolymph of most prawns with bacterial infection were higher than that of the uninfected prawns, suggesting its role as an antibacterial agent. The antibacterial activity of monodin was demonstrated by its ability to induce specific agglutination of *Vibrio vulnificus*, the major infective bacteria in the prawn. The binding specificity of monodin to NeuNAc of the bacterium was confirmed by the ability of other sialic acid-specific lectins, limulin, LFA and WGA in inducing the bacterial agglutination.