Warangkana Narksen 2008: Purification and Characterization of Androgenic Gland Hormone from Giant Freshwater Prawn, *Macrobrachium rosenbergii* de Man. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Associate Professor Sunanta Ratanapo, Ph.D. 165 pages.

Androgenic gland (AG) in higher crustacean is responsible for the development of male characteristics. Implantation of the whole androgenic gland into giant freshwater prawn (*Macrobrachium rosenbergii* de Man) led to transformation of female recipient into male. The purposes of this research were to purify, characterize and clone the AGH from androgenic tissues of the freshwater prawn. The androgenic tissues and vas deferens were homogenized in acid-alcohol followed by acetone precipitation. AGH found specifically in the androgenic tissues was purified by the reverse phase HPLC on Sep-Pak C18 or Lichrosorb column. Analysis by MALDI-TOF MS indicated that the purified AGH was a protein with molecular mass of 10.2 kDa. No change of mass after deglycosylation by PNGase F or reduction of disulfide bridges by DTT revealed that the purified AGH had no glycosylation and disulfide bridges. Peptide mass fingerprint of the purified AGH did not show matching to any androgenic gland hormones available in the NCBI database, indicating that the purified AGH differs from the numerous crustacean androgenic gland hormones.

Partial cDNA fragment of AGH was amplified using degenerated primers, designed based on the nucleotide sequences of AGHs from other crustacean species. No nucleotide sequences of these PCR products were similar to androgenic gland hormone of isopods and insulin-like androgenic gland factor of *C. quadricarinatus*. The 766 5′ ESTs of *M. rosenbergii* androgenic gland from cDNA library were established and categorized into seven categories on the basis of general functions. The SMART domain search tool predicted the existence of a domain between 66-105 residues of F-D010 clone similar to insulin growth factor-binding protein and to insulin / insulin-like growth factor / relaxin family as same as the pro-*Cq-IAG* ,three pro-AGHs in isopods.

The biological activities of *M. rosenbergii* AGH fractions from each purification step were determined by observation of masculinization after direct injection of each sample into the post-larvae female freshwater prawns. The result was not successful because of high mortality of the prawns. However, *in vivo* biological activity assay of this protein should be further investigated to confirm the being of androgenic gland hormone of the purified protein.

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