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THIRANAN WONGSOMBAT : MOLECULAR CLONING OF A cDNA ENCODING NEUROPEPTIDE HORMONE IN THE CHH/MIH/GIH FAMILY FROM *Penaeus monodon.* THESIS ADVISORS : APINUNT UDOMKIT, Ph.D., BURACHAI SONTHAYANON, Ph.D., SAKOL PANYIM, Ph.D. 128 P. ISBN 974-663-590-5

Crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH) and gonad inhibiting hormone (GIH) are members of a novel neuropeptide family produced in the X-organ sinus gland complex in the eyestalks of crustacean. These hormones play important roles in controlling several physiological processes such as regulation of growth and maturation in crustaceans. This study was an attempt to clone and characterize the cDNA encoding neuropeptide hormone in the CHH/MIH/GIH family (Pem-CMG3) from black tiger prawn, *Penaeus monodon,* by using the technique of Rapid Amplification of cDNA Ends (RACE). Total RNA from a single pair of eyestalk was used as a template for reverse transcription-polymerase chain reaction (RT-PCR). The primer PM2, designed from the amino acid sequence that is conserved among the hormones in CHH/MIH/GIH family, was used to amplify the 3' fragment of Pem-*CMG3* cDNA. Analysis of the nucleotide sequences of several recombinant clones and their deduced amino acid sequence revealed that four of them exhibited a high degree of homology (52-99%) with the hormones in the CHH/MIH/GIH family. The nucleotide sequence of 3'-CMG#71 showed the lowest degree of homology (48%) to the consensus among the four clones but shared 72% identity to a cDNA encoding growth-related peptide of *P. monodon*, Pem-*CMG*, that was successfully cloned in our laboratory. In order to generate the 5' end of the *CMG3* cDNA, a set of specific primers (GSP1, GSP2, GSP3) were designed corresponding to the nucleotide sequences of the 3'UTR of 3'-CMG#71 clone. Nucleotide sequences analysis of the 5'-CMG3 recombinant clones revealed that three clones contained cDNA insert homologous to the cDNA of hormones in the CHH/MIH/GIH family. These three individual clones showed differences at 2 positions, one of them gave rise to change in amino acid level. The 5' and 3' fragments of CMG3 shared a 211 bp overlapping sequence. Differences in nucleotide sequences between these two fragments were found within the overlapping region, two of them caused change in one amino acid residue. The full-length cDNA of Pem-*CMG3* was obtained by PCR amplification with specific primers, CMG-F and GSP1, designed according to the sequences at the 5' and 3' ends of the RACE products. Nucleotide sequence analysis of six recombinant clones revealed two types of cDNA encoding growth-related peptide that are different from each other at one encoded amino acid position. Four out of six clones shared one type, and the rest shared another type. The full-length cDNA of 617 bp revealed a 381 bp open reading frame of the Pem-CMG3, a 74 bp 5'UTR and a 161 bp 3'UTR. The deduced amino acid was composed of 74 amino acid residues of mature peptide and 53 amino acid residues of leader peptide. The mature peptide of Pem-CMG3 shared 31-93% identity in amino acid sequence to the hormones in the CHH/MIH/GIH family from several crustaceans. Using the reverse transcription-polymerase chain reaction (RT-PCR) method, the gene encoding the Pem-CMG3 was shown to be expressed in the eyestalk, not in the muscle and hepatopancrease.

In conclusion, the cDNA encoding a new member of the CHH/MIH/GIH family of *P. monodon* (Pem-CMG3) was successfully cloned. The Pem-CMG3 peptide contains all characteristics of this hormone family and the expression of Pem-*CMG3* transcript seems to be restricted to the eyestalk.