

## CHAPTER VII

### CONCLUSION

The full-length of DAR\_Pem cDNA is 1,990 nucleotides long containing a 1,359 nucleotides ORF that can be translated into a putative DAR\_Pem of 452 amino acids. Amino acid sequence analysis of DAR\_Pem showed high homology to DAR type 1 of *Panulirus interruptus* (D<sub>1β</sub>\_Pan), *Bombyx mori* (BmDOP1), *Drosophila melanogaster*, and *Apis mellifera* at 70%, 58%, 46.6% and 46.1%, respectively. Characteristic of DAR\_Pem was corresponded to seven transmembrane domains G-protein coupled receptors (GPCRs). It shared general consensus sequences of GPCR structure such as DRY sequence, ligand binding residues such as aspartic acid (D) in TM III, serine (S) in TM V and phenylalanine (F) in TM VI domains, and two cysteins that may form disulfide bond between e1 and e2. In addition, N-linked glycosylation site was predicted in the N-terminal extracellular loop and also several phosphorylation sites were located in i3 and intracellular C-terminal tail regions. A more diverse sequence between DAR\_Pem and DAR type 1 in other species appeared in the C-terminal tail region. Therefore, C-tail region (15 kDa) was selected for polyclonal antibody production in rabbit. DAR\_Pem mRNA was ubiquitously expressed in shrimp tissues with highest level in the brain, whereas low expression was found in the ovary. The high expression of DAR\_Pem in the brain of shrimp at ovarian developmental stage I to III before declining in stage IV suggested that DAR\_Pem may be involved in the inhibition of the release of GSH in the brain. Although DAR\_Pem mRNA was expressed in diverse tissues, it could not be detected at protein level in these tissues by western blot with anti C-tail DAR antibody so far. To investigate functional activity of DAR\_Pem in mediating cAMP level, recombinant DAR\_Pem was expressed as a membrane protein in COS-1 cells. Activation by DA showed that DAR\_Pem mediated an increase in cellular cAMP levels, which was in agreement with the functions of DAR\_type1.