# Chapter 5

# Conclusion

## 1. Characterization of purified vitellin

The N-terminal amino acid sequence of 78 and 87 kDa subunits of purified Vt are identical, indicating that post-translation modification i.e. glycosylation and lipidation, happens to this Vt subunit. The N-terminal amino acid sequence of 104 kDa subunit is located after the Arg724 to Arg727 (RTRR) cleavage site which is also conserved in other shrimps. Purified Vt protein from *P. merguiensis* was acidic, the pI value is 5.3 and does not detected protease activity. The amino acid composition of purified *P. merguiensis* Vt was only slightly different from those of Vt from other penaeid shrimps.

## 2. Characterization of cDNA encoding Vg from the ovary

The Vg cDNA sequence from ovary of *P. merguiensis* was submitted to NCBI and the GenBank accession number is AY499620. Vg cDNA consisted of 7,961 nucleotides including 7,758 bp of a single large open reading frame (ORF), 33 bp of 5' untranslated region, 167 bp of 3' untranslated region, a stop codon (TAA) and polyadenylation signal (AATAAA). The ORF of this cDNA encodes 2,586 amino acid residues, including 18 residues of signal peptide. The deduced Vg contains multiple examples of the consensus cleavage site for subtilisin family of serine endoprotease but only one cleavage site, RTRR (Arg724 to Arg727) was confirmed by determining the N-terminal amino acid sequence of the 104 kDa subunit. If only the RTRR cleavage site was used, 78 and 203 kDa subunits by molecular mass prediction were expectedly present, indicating that the 203 kDa subunit may have future endoprotease processing. If the cleavage site between residues 1,731 and 1,734 is used, it will produce 110 and 93 kDa subunits, which are a slightly different molecular mass when compared with SDS-PAGE results. Vg processing in banana shrimp needs future study to clarify the cleavage site used by subtilisin endoprotease or other enzymes.

**3. Comparison of the deduced amino acid sequence of Vg from *Penaeus merguiensis* using alignment and phylogenetic trees analysis**

Amino acid comparison using BLAST indicated three putative conserved domains were found in the sequence of *P. merguiensis* Vg: lipoprotein N-terminal domain, DUF1081 and vWF type D domain. The deduced amino acid sequence of Vg from *P. merguiensis* has most similarity to Vg sequences within Penaeidea and the lowest Crustacean similarity was found to Portunoidea. The lipoprotein N-terminal domain of the Vg in banana shrimp has similarities with proteins that are involved in lipid transport such as ApoB, Apo, Retin and MTP, and shows that these proteins arise from the same common ancestor. The lipoprotein N-terminal phylogenetic tree reveals that Vg in decapod crustaceans are paralogues with Vg from other species whereas the result of tree analysis of vWF from the C-terminal region showed that this region is conserved in all Vg and located in one evolutionary cluster. There may be functional differences between decapod crustacean and other Vgs related to the evolutionary differences.

**4. Tertiary structure modelling of N-terminal region Vg**

Tertiary structure models of the N-terminal region of *P. merguiensis* Vg were constructed using 3D-JIGSAW, EasyPred and Modeller servers using lamprey LV chain A structure (1LSH_A) as a template, which shares approximately 19.4% sequence identities. All structure models have antiparallel β-sheet domains including the N-sheet, C-sheet, a partial A-sheet, and a large helical domain. The model from EasyPred gave a different orientation between the N-sheet and other domains. The lamprey LV structure does not have precise information about interaction between domains, and there are several breaks in the structure; thus our prediction models have extensive regions of loop or disordered structure.

**5. Expression of the Vg gene in different tissues.**

Levels of Vg mRNA expression in *P. merguiensis* were determined for several tissues: ovary, hepatopancreas, muscle, heart and intestine, by RT-PCR approach. Vg PCR fragments were detected only in the ovary and hepatopancreas of female shrimps, and neither in heart, muscle or intestine of vitellogenic females nor hepatopancreas of male shrimps. Thus the ovary and hepatopancreas are conclusively shown as the sites of vitellogenesis in banana shrimp.

**6. Changes in Vg mRNA expression levels during ovarian development by real-time PCR**

Changes in Vg mRNA expression at various stages of ovarian development were determined using Taqman real-time PCR in both sites for vitellogenesis, ovary and hapatopancreas, in *P. merguiensis*. The dynamics of relative Vg values are different between ovary and hepatopancreas. In the ovary, the relative values of Vg mRNA increase from the pre-vitellogenic stage (stage 1) and reach the highest level at the early vitellogenic stage (stage 2), and thereafter expression levels rapidly decrease. In contrast, the highest relative value of Vg in the hepatopancreas was reached at the vitellogenic stage (stage 3) of ovarian development, and dropped during the late vitellogenic stage. Ovary may be the major site of Vg synthesis since the relative Vg expression value in the ovary is greater than in the hepatopancreas at all stages of ovarian development.

**7. Cloning of Vg cDNA from the hepatopancreas and nucleotide-amino acid differences analysis**

Vg cDNA from the hepatopancreas was cloned at the 3' end region. The size of the PCR product is 1,128 bp. Vg cDNA and deduced amino acid sequence from the hepatopancreas and that from the same region of the ovary were compared and indicated that nucleotide and amino acid sequence identity was 98.4% and 98.7%, respectively, which appeared distributed evenly along the sequence. A program, written by Dr. Ingrid B. Jakobsen, tests whether the nucleotide-amino acid differences between the ovary and hepatopancreas are due to laboratory errors or evidence for multiple gene sequences. This test gave the probability of the observed sequence differences being due to random laboratory error as less than 0.001 based on comparison of the 3' end region sequences from both sites of Vg synthesis. However, the presence of two different Vg genes in *P. merguiensis* is still not confirmed since a full-length cDNA from the hepatopancreas is not available; thus this sequence should be investigated in subsequent studies.

**8. Proteomic analysis at differing GSI values of ovarian development**

The proteomic maps from 2-DE gels of ovarian proteins at different GSI values showed patterns of differential expression of proteins, reflecting changing protein activities in each stage of ovarian development in *P. merguiensis*. From the present results, the proteins that

are present in all stages of ovarian development could be essential proteins that might play important roles in the development of ovary or for cell viability such as cytoskeleton and oxygen transfer proteins.

Some proteins were detected at only some GSI values of ovarian development, suggesting that these proteins have specific functions in particular stages of the developing ovary. For example, vasa protein is expressed only at GSI 2.998 and it plays roles in oocyte formation and specification of posterior structures for the embryo. Neurotransmitters affect ovarian development by stimulation or inhibition of hormones involved in ovarian maturation such as GSH and GIH. Ras related protein rab3 which plays a role in neurotransmitter release, was expressed only at GSI 0.995 and 2.998, suggesting that neurohormones play an important role for regulation of ovarian maturation at this stage of ovarian development in banana shrimp. Furthermore, some proteins affected by steroid hormones, such as CG6203, were found, which might be involved in estrogen-mediated induction and stabilization of Vg mRNA. Calreticulin was expressed at all GSI values of ovarian development. It might have a specific function ensuring expression of steroid-sensitive genes such as Vg besides its chaperoning function.