Contents

| | Page | |
|-----------------------------------|------|--|
| Contents | х | |
| List of Tables | xi | |
| List of Figures | xii | |
| List of Abbreviations and Symbols | xvi | |
| Chapter | | |
| 1. Introduction | | |
| Review of Literatures | 4 | |
| Objectives | 46 | |
| 2. Materials and Methods | 47 | |
| Materials | 47 | |
| Methods | 56 | |
| 3. Results | 80 | |
| 4. Discussion | 143 | |
| 5. Conclusions | 163 | |
| References | 167 | |
| Appendix | 187 | |
| Vitae | 192 | |

List of Tables

| Tabl | e | Page |
|------|---|------|
| 1 | Old and new scientific names in genus Penaeus. | 9 |
| 2 | Characteristics of ovaries and oocyte physiology at various stages of ovarian | 17 |
| | development. | |
| 3 | Sites of decapod crustacean Vg synthesis as determined by immunological | 26 |
| | methods, immunohistochemistry or radioactive incorporation technique. | |
| 4 | Similarity of the three segments in Litopenaeus vannamei Vg sequence to all | 33 |
| | known decapod Vg sequence. | |
| 5 | Nucleotide sequence of oligonucleotide primers used in this study. | 61 |
| 6 | List of Vgs from decapod crustaceans used to construct the phylogenetic tree | 70 |
| | of full- length sequence. | |
| 7 | Details of sequences used to construct phylogenetic trees of N-terminal and | 71 |
| | C-terminal regions are shown. | |
| 8 | Amino acid composition of purified Vt from Penaeus merguiensis and other | 85 |
| | shrimps. | |
| 9 | The percentage ratios of the overall amino acid identity between the full- | 110 |
| | length Vg amino acid sequences including signal peptide sequences of 11 | |
| | decapod crustacean species (13 sequences). | |
| 10 | Relative Vg levels, in ovary and hepatopancreas, and GSI at different stages | 129 |
| | of ovarian development in naturally maturing female shrimps. | |
| 11 | Proteins identified from 2-DE at differing GSI values of Penaeus merguiensis. | 138 |
| 12 | The N-terminal amino acid sequence of the purified Vt/Vg subunits in | 144 |
| | shrimps and crab. | |
| 13 | Sites of Vg synthesis in decapod crustaceans. | 154 |

List of Figures

| FigurePag | | | | |
|-----------|--|----|--|--|
| 1 | Species distribution of Penaeus merguiensis, banana shrimp, is shown as | 5 | | |
| | dark areas. | | | |
| 2 | Global wild capture production and global aquaculture production for | 6 | | |
| | Penaeus merguiensis is shown in Fig. 2A and 2B, respectively. | | | |
| 3 | Penaeus merguiensis or banana shrimp. | 8 | | |
| 4 | Lateral view of the external morphology of penaeid. | 10 | | |
| 5 | Lateral view of the internal anatomy of a female penaeid. | 11 | | |
| 6 | The top view of the internal anatomy of a female Penaeus merguiensis. | 12 | | |
| 7 | The view observed by hatchery operators when female broodstock are graded | 14 | | |
| | for ovarian development by torchlight. | | | |
| 8 | Photomicrographs showing ovarian development. | 16 | | |
| 9 | Maturation, spawning and moulting of Penaeus merguiensis in the Gulf of | 19 | | |
| | Carpentaria. | | | |
| 10 | The life history of penaeid shrimps. | 21 | | |
| 11 | Schematic interpretation of the endocytotic pathway and subsequent routing | 24 | | |
| | of Vg and Vg receptor (VgR) during Vg internalization by mosquito oocytes. | | | |
| 12 | Schematic representation of proposed model for vitellogenesis in penaeid | 29 | | |
| | species. | | | |
| 13 | Partially alignment of the deduced amino acid sequence of decapod | 30 | | |
| | crustacean Vgs. | | | |
| 14 | Representation of Vg primary sequence in significant shrimp and prawn | 34 | | |
| | species. | | | |
| 15 | Amino acid sequence of lamprey Vg and the crystal structure components. | 39 | | |
| 16 | Stereoview ribbon diagrams of the lamprey LV monomer. | 41 | | |
| 17 | Overall structure of the lamprey LV. | 42 | | |

List of Figures (Continued)

| Figu | re | Page |
|------|---|------|
| 18 | The physical map of pDrive cloning vector. | 51 |
| 19 | The physical map of pCR 4-TOPO vector. | 52 |
| 20 | 4-10% Nondenaturing PAGE of Vt at various steps of purification. | 81 |
| 21 | 5-15% SDS-PAGE of purified Vt. | 82 |
| 22 | Isoelectric focusing of purified Vt. | 83 |
| 23 | Elution profiles of standard (A) and purified Vt (B) when determining the | 84 |
| | amino acid composition. | |
| 24 | Determination of protease activity of purified Vt. | 86 |
| 25 | RT-PCR cloning strategy and schematic view of Vg cDNA. | 88 |
| 26 | Amplification of cDNA encoding the Vg cDNA fragments. | 89 |
| 27 | Vg cDNA electrophoresis patterns of 5' RACE and 3' RACE fragments. | 90 |
| 28 | Vg cDNA electrophoresis pattern of Vg open reading frame. | 91 |
| 29 | Full length Vg cDNA nucleotide sequence from Penaeus merguiensis was | 93 |
| | deposited in GenBank with accession number AY49962. | |
| 30 | 5' Ends (A) and 3' ends (B) of nucleotide sequence of Penaeus merguiensis | 97 |
| | Vg cDNA. | |
| 31 | Schematic view of Peneaus merguiensis Vg cleavage sites and N-terminal | 99 |
| | amino acid sequence positions of Vt subunits. | |
| 32 | Deduced amino acid sequence of the Penaeus merguiensis Vg with secondary | 100 |
| | structure and possible post-translation sites. | |
| 33 | Topology of deduced Vg in Penaeus merguiensis shows the secondary | 105 |
| | structure from N-terminal to C-terminal end. | |
| 34 | BLAST analysis of deduced Vg. | 107 |
| 35 | Partial alignment of the deduced amino acid sequences of Penaeus | 109 |
| | merguiensis Vg with other decapod crustaceans. | |

List of Figures (Continued)

| Figu | re | Page |
|------|--|------|
| 36 | Phylogenetic tree analysis of Vg from different crustacean. | 111 |
| 37A | Phylogenetic tree analysis of lipoprotein N-terminal region family. | 114 |
| 37B | Phylogenetic tree analysis of lipoprotein N-terminal region family. | 115 |
| 38 | Phylogenetic tree analysis of the von Willebrand factor (vWD) type D domain | 117 |
| | at the C-terminal region of Vg and other correspond proteins. | |
| 39 | Stereoview ribbon or cartoon diagrams of lamprey LV (Protein Data Bank | 120 |
| | entry 1LSH). | |
| 40 | Side views of three models of Vg and the 1LSH_A template. | 121 |
| 41 | Down views of three models of Vg and the 1LSH_A template. | 122 |
| 42 | Superimposition of all presented models. | 123 |
| 43 | RT-PCR analysis of the expression of Vg mRNA in different Penaeus | 124 |
| | merguiensis tissues. | |
| 44 | Real-time PCR standard curve of Penaeus merguiensis Vg. | 126 |
| 45 | Real-time PCR standard curve of 18s rRNA. | 127 |
| 46 | Real-time PCR analysis of Vg cDNA. | 128 |
| 47 | (A) Schematic view of <i>Penaeus merguiensis</i> Vg cDNA (AY499620) from the | 131 |
| | ovary, the lower line represents the Vg cDNA encoding the 3'end region of | |
| | hepatopancreas used in this study. (B) RT-PCR analysis showing the mRNA | |
| | encoding the 3'end of hepatopancreas Vg. | |
| 48 | Pairwise alignment of Vg cDNA from the hepatopancreas and ovary by | 132 |
| | BioEdit version 7.0.1 showing the 3' end fragments have 18 nucleotide | |
| | differences in Penaeus merguiensis. | |
| 49 | The pairwise alignment of amino acid sequences between Vg from the ovary | 133 |
| | (Genbank AY499620) and Vg from hepatopancreas in the fragment amplified | |
| | using FVg6539 as forward and RVg7666 as reverse primer, aligned by | |

List of Figures (Continued)

| Figure | | |
|--------|--|-----|
| | BioEdit version 7.0.1. | |
| 50 | The distribution of the number of amino acid differences between the ovarian | 134 |
| | sequence and the randomly mutated pseudo-sequences, when there are 18 | |
| | random nucleotide differences. | |
| 51 | Protein patterns in 2-DE gel at various stages of ovarian development in | 136 |
| | Penaeus merguiensis. | |
| 52 | Schematic view of Peneaus merguiensis Vg potential cleavage sites. | 147 |
| 53 | Alignment of the partially amino acid sequence of decapod crustacean Vgs at | 148 |
| | region that possible to be second cleavage site. | |
| 54 | Phylogenetic tree analysis of crustacean Vgs. | 150 |
| | | |

List of Abbreviations and Symbols

| A° | = | angstrom |
|-----------------|---|---|
| Аро | = | apolipophorin |
| АроВ | = | apolipoprotein |
| Вр | = | base pair |
| BSA | = | bovine serum albumin |
| CAPS | = | 3-(cyclohexylamino)-1-propane-sulfonic acid |
| °C | = | degree Celsius |
| cDNA | = | complementary deoxyrobonucleic acid |
| CHAPS | = | 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate |
| CIP | = | calf intestinal phosphatase |
| Clot | = | clottable protein |
| Cm | = | centimeter |
| СМ | = | carboxymethyl |
| cm ³ | = | cubic centimeter |
| CSD | = | cold shock domain |
| Da | = | Dalton |
| DA | = | dopamine |
| DEAE | = | diethylaminoethyl |
| DEPC | = | diethyl pyrocarbonate |
| dNTP | = | deoxyribonucleotide triphosphate |
| DMSO | = | dimethylsulfoxide |
| DTT | = | dithiothreitol |
| EDTA | = | ethylamine diamine tetraacetic acid |
| ER | = | endoplasmic reticulum |
| EST | = | expressed sequence taqs |

List of Abbreviations and Symbols (Continued)

| g | = | gram |
|-----------|---|--|
| x g | = | gravitation acceleration |
| GIH | = | gonad-inhibiting hormone |
| GlcNAc | = | N-acetylglucosamine |
| GRP | = | glucose regulated protein |
| GSH | = | gonad-stimulating hormone |
| GSI | = | gonadosomatic index |
| Н | = | hour |
| HSP | = | heat shock protein |
| IPG | = | immobilized pH gradients |
| IPTG | = | isopropylthio galactoside |
| kb | = | kilobase |
| kDa | = | kilodalton |
| LB | = | Luria Bertani |
| LC/MS/MS | = | liquid chromatography tandem mass spectrometry |
| LV | = | lipovitellin |
| М | = | molar |
| mA | = | milliampere |
| MALDI-TOF | = | matrix-assisted laser desorption ionization time-of-flight |
| min | = | minute |
| ml | = | milliliter |
| mm | = | millimeter |
| mM | = | millimolar |
| mRNA | = | messenger ribonucleic acid |
| MTP | = | microsomal triglyceride transfer protein |

List of Abbreviations and Symbols (Continued)

| NL | = | non-linear |
|--------|---|---|
| nM | = | nanomolar |
| ORF | = | open reading frame |
| O.D. | = | Optical density |
| PAGE | = | polyacrylamide gel electrophoresis |
| PCR | = | polymerase chain reaction |
| РН | = | pleckstrin homology |
| pI | = | Isoelectric pH |
| PMSF | = | phenylmethylsulfonylfluoride |
| PVDF | = | polyvinyl difluoride |
| Q-TOF | = | quadrupole- time-of-flight |
| RACE | = | Rapid amplification of cDNA ends |
| Retin | = | retinoid and fatty acid-binding glycoprotein |
| RNA | = | ribonucleic acid |
| RT-PCR | = | reverse transcription-polymerase chain reaction |
| rRNA | = | ribosomal ribonucleic acid |
| sec | = | second |
| SDS | = | sodium dodecyl sulfate |
| SOD | = | superoxide dismutase |
| TAP | = | tobacco acid pyrophosphatase |
| TEMED | = | N,N,N',N'Tetramethylethylenediamine |
| U | = | unit |
| μl | = | microliter |
| μg | = | microgram |
| v | = | volume |
| V | = | voltage |

List of Abbreviations and Symbols (Continued)

| Vg | = | vitellogenin |
|-------|---|---|
| Vgs | = | vitellogenins |
| Vt | = | vitellin |
| vWF | = | von Willebrand factor |
| W | = | weight |
| X-gal | = | 5-bromo-4-chloro-3-indolyl-beta-D-galactopyraniside |
| 2-D | = | two-dimensional |
| 3-D | = | three-dimensional |
| 2-DE | = | two-dimension gel electrophoresis |
| % | = | percentage |