CHAPTER VI

Summary

1. Monoclonal antibodies (MAbs) specific to yolk proteins, vitellin (in ovary) and vitellogenin (in haemolymph), of the giant tiger prawn *Penaeus monodon* were generated using ovarian extract from gravid ovaries either native or denatured forms as immunogens.

1.1 The hybridoma clones derived from mouse immunized with native antigen mostly produce antibodies that can bind to native form of vitellin and vitellogenin (PMV-11, 15 and 22), but there is only one clone (PMV-64) produces antibody that can bind to both native and denatured forms of vitellin and vitellogenin.

1.2 All hybridoma clones derived from mouse immunized with denatured vitellin produce antibodies that can bind to both native and denatured forms of vitellin and vitellogenin. Therefore, MAbs specific to five subunits of vitellin (PMVS-93, 109, 140 and 158) including MAbs specific to an oocyte specific protein (PMVS-106) and haemocyanin (PMVS-22) were generated.

This study suggests that production of MAbs by immunization with denatured protein increases the probability to obtain MAbs that can bind to both native and denature proteins which tend to have broader applications than those obtained from immunization with native protein.

2. These MAbs were used for characterization of the molecular nature of vitellin and vitellogenin. Vitellin derived from gravid ovary exhibits five subunits, molecular mass of 104, 83, 74, 58 and 45 kD, and vitellogenin in the female haemolymph consists of four subunits, molecular mass of 200, 104, 83 and 74 kD. Vitellin in ovary first appears as 74 and 200 kD proteins. During maturation

processes, the 200 kD protein is cleaved to generate 104 and 83 kD proteins then the 104 kD is further cleaved into 58 and 45 kD proteins while the modification of the 74 kD protein was not observed. In haemolymph, vitellogenin appears as 200, 104, 83, and 74 kD proteins, whereas the 58 and 45 kD proteins were not detected

3. Indirect immunoperoxidase competitive ELISA using MAbs specific to vitellin and vitellogenin was applied for determination of vitellogenin levels in haemolymph. Specificity, sensitivity and fidelity of measurements were similar when either each MAb or combination of four MAbs were used. Therefore, combination of four MAbs specific to each subunit were used to determine the fluctuation of vitellogenin levels in the haemolymph during ovarian development and for determination of gonad inhibiting hormone activity.

4. During ovarian development of prawns induced by bilateral eye-ablation, the correlation between haemolymph vitellogenin and gonado-somatic index (ovarian index = OI) was difficult to elucidate, due to the high variations of ovarian index and haemolymph vitellogenin levels at each stage of ovarian development among individual prawn. However, the relationship between haemolymph vitellogenin levels and stage of ovarian development was highly correlated especially when individual prawn was used throughout the process of ovarian development. The vitellogenin levels in haemolymph was undetectable during the resting stage, then sharply elevated when ovary began to develop, remained at high levels during developing into ripe stage, and finally, fallen down to low levels before spawning and spent stages.

5. The results from injection of eyestalk extract into prawns with developing ovary induced by bilateral eye-ablation revealed that haemolymph vitellogenin levels elevated sharply within 2 hr, reached the maximal levels and remained unchange during 4-10 hr, and slightly declined at 24 hr. This response directly depended on the

amount of injected eyestalk extract. The elevation of haemolymph vitellogenin levels after application of eyestalk extract may be the result of desorption of vitellin from the oocyte during the degeneration of oocyte induced by gonad inhibiting hormone (GIH) which is similar to that occur in intact prawns after they were reared in captivity. Therefore it is possible to determine the GIH activity by determining the alteration of vitellogenin levels after the application of the tested samples.

These studies demonstrated the potential applications of MAbs specific to vitellin and vitellogenin to the study of reproductive biology of the prawns. Further studies using these antibodies to localize vitellin and vitellogenin in various organs will help to identify the sites of vitellogenin synthesis, the target of GIH. Development of this new GIH assay will pave way to better understanding of the role of GIH and other hormones involved in the reproduction of *P. monodon*.