Journal of Research in Biology

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Original Research paper

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Fluctuation of protein level in Haemolymph, ovary and Hepatopancreas during non-reproductive and reproductive Molt Cycle of *Albunea symmista*

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ABSTRACT:

In *Albunea symmista*, an antagonistic relationship could be visualized between molting and reproduction. Proteins necessary for the formation and hardening of the exoskeleton and maturation of ovary .The biochemical link between molting and reproductive cycle of *Albunea symmista* was established through the estimation of protein in haemolymph, ovary and hepatopancreas. The haemolymph protein level during non- reproductive molt cycle gradually raised from post molt to early premolt (2.7± 0.2 to 6.3 ± 0.2 mg/ml)and it get decreased in the late premolt due to water influx through new cuticle (6.3 ±0.2 to 4.3 ± 0.1 mg/ml).However during reproductive molt cycle , the protein content of haemolymph also gets decreased due to reproductive activities (3.9 ± 0.2 to 2.1 ±0.1 mg/ml).The ovarian protein level during non-reproductive molt cycle get gradually increased due to accumulation of protein (74.8 ± 1.5 to 145.8 ± 1.7 µg/mg)During reproductive molt cycle fluctuation of protein level occurs soon after spawning , where the ovary remains in the spent condition (47 µg/mg) then after hatching, the ovary continues its maturation (47 to 74.9 ± 3.7 µg/mg).The protein content in the hepatopancreas shows fluctuation both during reproductive molt cycle (29.5 ± 1.9 to 148.2 ± 1.8 µg/mg) and non-reproductive molt cycle (144.8±1.0 to 125.1 ±5.2 µg/mg).

Keywords:
Albunea symmista, molting, reproduction, haemolymph, ovary, hepatopancreas and protein.

Web Address:
http://jresearchbiology.com/Documents/RA0019.pdf.

Article Citation:
Kanagalakshmi K.
Fluctuation of protein level in Haemolymph, ovary and Hepatopancreas during non-reproductive and reproductive Molt Cycle of *Albunea symmista.*
Journal of research in Biology (2011) 2: 68-72

Dates:
Received: 07 May 2011 /**Accepted:** 12 May 2011 /**Published:** 28 May 2011

Journal of Research in Biology

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INTORDUCTION

Proteins play vital role in both molting and reproduction as it involves in the formation of cuticle and synthesis of yolk. Variation in the amount of haemolymph protein involved in the formation of new cuticle during molting stages has been reported in the crayfish, *Astacus astacus* (Domboriceanu,1932;Crowley,1963), in the crabs, Maia squainado (Drilhon,1935;Drach and Teissier,1939) *Cancer magster*,*Calinectus sapidus* (Leone,1953) *Carcinus maenus* (Robertson,1960),lobsters *Homarus vulgaris* (Glynn,1968) and *Homarus americanus* (Barlow and Ridgway 1969).

It is also now well known that both epidermal cells and ovary depends on precursor molecule synthesized elsewhere in the somatic organs for their final sequestration to form the new cuticle and storage yolk inside the ripe oocyte respectively. The protein components transported through the haemolymph directly sequestrated into the growing oocyte for deposition as yolk. The lipid constituents from the lipogenic tissue are transported to the ovary as well as other organ including epidermis is mainly achieved by the two haemolymph lipoprotein such as lipoprotein I (LPI) and Lipoprotein II (LP II) (Chino and Kitazava, 1981; Champman, 1980; Lee and puppione, 1988) The hepatopancreas which is considered to be the chief organ for protein synthesis consist of proximal and distal segment. The hepatopancreas serves as an extra ovarian source of yolk protein for Crustacea (Byard, 1976). Developing oocyte also acquires the yolk protein by pinocytic mechanism from haemolymph (Zerbib, 1973). The protein is also synthesized by the oocyte itself (Kessel, 1968).

Apparently, an analysis of the biochemical composition of the haemolymph as well as the synthetic organs during molting and reproductive stages would reveal their precise involvement in the cuticle formation as well as vitellogenesis.

MATERIALS AND METHODS

The total protein was estimated by the method of Lowery et al., (1951) in haemolymph and in tissues such as ovary and hepato pancreas. A standard graph was plotted using the absorbance values against each concentration.

RESULT

Fluctuation of protein during Moulting and Reproduction

Molting and reproduction both are the energy demanding physiological processes. Hence, the total protein was estimated in haemolymph, ovary and hepatopancreas of *Albunea symmista* during different molt stages and ovarian stages.

Fluctuation of haemolymph protein during molting and reproductive stages

During non-reproductive molting stages, oocyte maturation occurs. The level of protein shows a gradual increase of 2.7± 0.2 mg/ml in post molt stages (A and B) to 6.3±0.2mg/ml in premolt (D2) ($P>0.05$). However, the D3 and D4 stages register a protein level of 4.3±0.1mg/ml which is slightly lower than that of D2 stage ($P>0.05$). This might be due to water influx through the soft new cuticle during ecdysis. However, the protein level was further reduce to 3.9± 0.1 mg/ml in post molt stage ($P>0.05$). The haemolymph shows further decline in its protein level during reproductive molt stage (Fig. 1).

During reproductive molt stages, the haemolymph protein level was higher in post molt stages (A and B) (3.9± 0.1 mg/ml) when compared to that of intermolt stages. Whereas, the protein level shows a significant decline during intermolt stage-C1 (2.1±0.1 mg/ml) ($P>0.05$). However, the protein level is further increased in intermolt stage-D2 (2.4±0.1 mg/ml). But, it shows a steady decline in the premolt (2.0 ± 0.1 mg/ml). This haemolymph protein shows an increase in protein level further decrease during late premolt stages D3 and D4 (2.8± 0.2 mg/ml) ($P>0.05$) (Fig. 2).

The haemolymph protein steadily increased during different ovarian stages from maturing to ripe stages (2.5 ±0.5 to 4.8±1.0 mg/ml)

Fluctuation of ovarian protein during molting and reproductive stages

The quantity of ovarian protein shows a gradual increased from (74.8 ± 1.5 to 145.8 ± 1.7 ug/mg) during non-reproductive molt. Further, a steady increase in the level of ovarian protein was also observed at the early intermoult-C1 (127.2 ± 15.7 ug/mg) as well as during premolt stages_D2, D3 and D4 (145.8 ± 1.7ug/mg) ($P>0.05$) (Table 11). After this, the protein value remains more or less constant in premolt and postmolt stages (Fig. 51, Table 6). After successful spawning, the protein level in the ovary remains more or less constant (47 ug/mg) ($P<0.05$) in the intermolt (C1 and C2) and early premolt (D0) until hatching. However, a steady increase from 47 to 74.9 ± 3.7 ug/mg ($P>0.05$) in the protein level was observed after hatching (**Fig. 1**).

The ovary shows an upward trend in the

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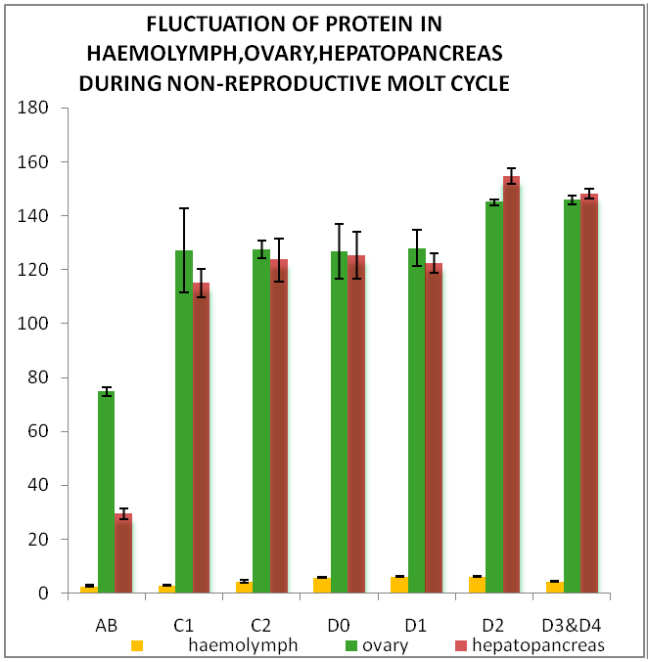

Fig. 1.

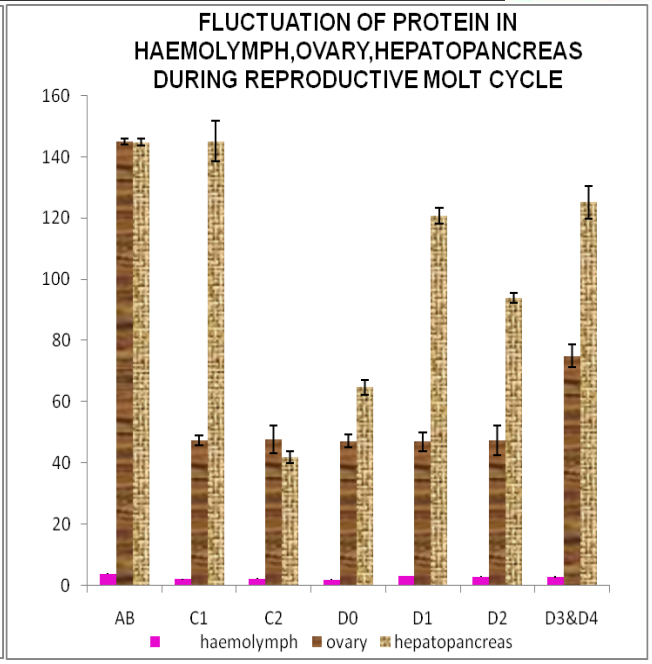

Fig. 2.

protein level from maturing to ripe stage (47.3 ± 3.5 to 145.3 ± 1.3 ug/mg) (**Fig. 2**).

Fluctuation of hepatopancreas protein during molting and reproductive stages

The hepatopancreatic protein shows an upward trend from 29.5 ± 1.9 in postmolt to 148.2 ± 1.8 ug/mg (P>0.05) in premolt during non-reproductive molt stage (Fig. 1). Non-reproductive molt stages also shows a similar fluctuation in protein level (144.8 ± 1.0 to 125.1 ± 5.2 ug/mg) (P>0.05) (Fig.2).

DISCUSSION

In Albunea symmista, an antagonistic relationship could be visualized between molting and reproduction. To investigate the role of biomolecules in the process of molting and reproduction, total protein level of haemolymph, ovary and hepatopancreas was quantified.

The haemolymph protein of Albunea symmista shows a steady increase from postmolt (2.7 +_ 0.2 mg/ml) to premolt (6.3 +_ 0.2 mg/ml) during the non-reproductive molt. This is due to the continuous supply of protein necessary for the synthesis of cuticle. Thereafter, the protein content slowly decreases in D2 and D4 (6.3 +_ 0.2 to 4.3 +_ 0.1mg/ml). This might be due to the utilization of the protein for new cuticle formation. A sharp decline in protein content of haemolymph is mainly due to water influx through the soft new cuticle which dilutes the haemolymph (Skinner, 1985; Chang, 1992). The haemolymph protein level also shows fluctuation during reproductive molt cycle. The haemolymph protein level recorded a significant decline from postmolt to intermolt (3.9 +_ 0.1 to 2.1 +_ 0.1 mg/ml). The protein shows an increasing trend in D1 stage (3.2 +_ 0.2 mg/ml). The gradual increase in the protein level from maturing to ripe stage (2.5 +_ 0.5 to 4.8 +_ 1.0 mg/ml) has suggested that the proteins in the haemolymph is necessarily sequestered into the ovary for its maturation.

The ovarian protein content shows gradual increase during the non-reproductive molt cycle (74.8 +_ 1.5 to 145.8 +_ 1.7 ug/mg). The increase in protein content shows an accumulation of yolk protein into the oocyte for its maturation. In the reproductive molt, the protein level falls sharply just after spawning, (145.8 +_ 1.7 to 47 ug/ml). From C1 to D0 the ovary development does not proceeds and the ovary remains in spent stage until hatching where the protein value remains static and the maturation of ovary does not coincides with the development of oocyte in Albunea symmista unlike that of Emerita asiatica. After hatching the protein level increases from 47 to 74.9 +_ 3.7ug/ml in the ovary. The ovary doesn't molt and the maturation in this cycle. Therefore, the animal undergoes molt and the maturation of ovary continues in the following molt cycle. The reproductive process such as spawning and hatching do not occur during this cycle and the ovary completes its maturation and remains ready for subsequent spawning. The increase in the protein level from maturing to ripe

stage (47.3 +_ 3.5 to 145.3 +_ 1.3 ug/mg) indicates that the accumulation of protein in the oocyte occurs during the ovarian developmental stages.

The protein content of the synthetic organ hepatopancreas shows a higher fluctuation both during reproductive molt cycle (29.5 +_ 1.9 to 148.2 +_ 1.8 ug/mg) and in non-reproductive molt cycle (144.8 +_ 1.0 to 125.1 +_ 5.2 ug/mg). However it doesn't show any correlation with ovary and haemolymph protein. This is mainly due to that hepatopancreas is not only involved in cuticle and vitellogenin synthesis but also involved in synthesis of other proteins which play a role in physiology of this sand crab *Albunea symmista.*

Emerita asiatica which breeds continuously and repeatedly also shows similar results (Subramoniam, 1977, 1979, Gunamalai, 2002). A steady increase of haemolymph protein from postmolt (A) (5.23 +_ 1.58 mg/ml) to premolt (D0') (22.47 +_ 5.31 mg/ml) as well as a sharp dip in the premolt stage D1 (22.47 +_ 5.31 to 16.88 +_ 4.83 mg/ml) were due to embryonic development and hatching of the larvae. The protein level rises sharply to reach a peak value in D2 stage (25.42 +_ 3.26 mg/ml). The protein level further get decreased due to ecdysis in D3 & D4 stage (9.18 +_ 0.32 mg/ml). The protein content of both ovary and ovarian index rises spontaneously from C1 to D0 stage (8.18 +_ 2.83% to 15.75 +_ 1.44%) at the time of hatching, the ovary almost completes its maturation and ready for next spawning (Gunamalai, 2002).

A similar increase in protein content of haemolymph during postmolt (AB), intermolt (C), early premolt (D0, D1 and D2) and its decrease during late premolt (D3) has been reported in Peanaeus indicus. A significant increase of protein level in ovary has also been observed from stage II to stage V (Read and Caulton, 1979).

Further investigation on the fluctuation of carbohydrate and lipid levels in the synthetic site together with their utilization during different physiological events will throw more light on the biochemical basis of the growth and development in *Albunea symmista.*

ACKNOWLEDGEMENT

I express my heartfelt gratitude to my mother Mrs.K. Vasantha and my husband Mr.R. Vivekanandan for their constant motivation and moral support. I extend my gratitude to my daughter V. Nandika

REFERENCES

Barlow J and Ridgway GJ. 1996. Changes in serum protein during the molt and reproductive cycles of the American Lobster, *Homarus americanus***.** J Fish. Res. Biol., Canada. 26:2101-2109.

Byard EH. 1976. The female specific protein and reproduction in the lobster, *Homarus americanus*. PhD. Thesis, Univ. of Western Ontario, London Ontario. 107.

Chang ES. 1992. Endocrinology. In: Marine shrimp culture: Principles and practices. (A.W. fast and L.J.Lester, Eds). Elsevier Science publishers B.V Amsterdam. 53-91.

Chapman MJ. 1980. Animal lipoproteins: chemistry, structure and comparative aspects**.** J. Lipid. Res., 21: 789-853.

Chino H and Kitazawa K. 1981. Diacyl glycerol carrying lipoprotein of hemolymph of the locust and some insects. J Lipid. Res., 22:1042-1052.

Crowley GJ. 1963. Studies in arthropod serology. I Change in haemolymph composition as related to the ecdysial cycle. Wasmann. J. Biol., 21:177-191.

Domboriceanu A. 1932. Composition chimique et physic chemiques du ziquide cavitative chez les crustace decapods (physiologic de la calcification). Archs. Roum. Path. Exp. Microbiol., 5L:239-309.

Drach P and Teissier G. 1939. Mul et protidemie chezles crabs. C.R Seanc. Soc. Bio., Paris. 131:1199-1201.

Drilhon A. 1935. Etude biochemique de la mue chenz crustaceces brachyoures (*Maia squinada*). Ann. Physiol. Physicochem. Biol., 11:301-326.

Glynn JP. 1968. Studies on the ionic, protein and phosphate changes associated with the molt cycle of *Homarus vulgaris***.** Comp. Biochem. Physiol., 26:937-946.

Gunamalai V. 2002. Synchronisation of molting and oogenic cycles in a continuously breeding population of the sand crab *Emerita asiatica* on the madras coast, south india *.J.Crust.Biol.,* 22:377-389

Gunamalai V. 2002. Synchronisation of molting

and oogenic cycles in a continuously breeding population of the sand crab, *Emerita asiatica* on the Madras coast, South India. J. Crust. Biol., 22:377-389.

Kessel MS. 1968. Mechanism of protein yolk synthesis and deposition in crustacean oocytes. Z. Zelforsch., 89:151-176.

Lee RF and Puppione DL. 1988. Lipoproteins I and II from the hemolymph of the blue crab, *Callinectus sapidus*, Lipoprotein II associated with vitellogenins. J. Exp. Zool., 248:278-289.

Leone CA. 1953. Preliminary observations on intra -specific variations of the levels of total protein in the sera of some decapods crustacean. Science, New York. 118:295-296.

Lowry DH, Rosenbrough NJ, Farr FL and Randell RJ. 1951. Protein measurement with the folin phenol reagent**.** J. Biol. Chem., 193:265-275.

Read GHL and Caulton MS. 1979. Changes in mass and chemical composition during the moult cycle and ovarian development in immature and mature *Penaeus indicus* Milne edwards. Comp. Biochem. Physiol., 66:431-437.

Robertson JD. 1960. Ionic regulation in the crab, *Carcinus maenus* (L) in relation to the moulting cycle. Comp. Biochem. Physiol., I:183:212.

Skinner DM. 1985. Molting and regeneration. In: "The Biology of Crustacea", (D.E. Bliss and L.H. Mantel, Eds), Academic press, New York. Vol. 9:43-146.

Subramoniam T. 1977. Continuous breeding in the tropical anomuran crab, Emerita *asiatica* (Milne Edwards). J.Exp. Mar. Biol. Ecol., 65:259-268.

Subramoniam, T. 1979. Some aspects of reproductive ecology of a mole crab, Emerita *asiatica* (Milne Edwards).**J**.Exp. Mar Biol. Ecol., 65:259-268.

Zerbib C. 1973. Contribution a L'efude ultrastructuale de L'ovocyte chez la crustace amphipode *Orchestria gammerella* Pallas C.R. Acad Sci. (Paris) Ser. D. Nat. Sci., 277:1209-1212.