Kasetsart J. (Nat. Sci.) 40 : 517 - 528 (2006)

Chitobiase, Proteinase, Glycogen and some Trace Elements during Molting Cycle of Mud Crab (*Scylla serrata* Forskål 1775)

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ABSTRACT

Activity profiles of chitobiase, proteinase and the content of glycogen and trace elements in gill, integument and haemolymph of mud crab (*Scylla serrata*) during molting cycle were determined. Chitobiase activity was at the highest level of 0.5467±0.0136 μmol min^{-1}mg $protein^{-1}$ at 1-week premolt (D2) in gill and at 0.1413±0.0026 μmol min^{-1} mg $protein^{-1}$ in integument but reached the maximum level of 0.0851±0.0123 μmol min^{-1}mg $protein^{-1}$ at 24-h postmolt (A2.2) in haemolymph. The high activities of proteinase, on the other hand, were found to be 0.6511 ± 0.0934 units mg $protein^{-1}$ at 7-day postmolt (B2.3), 0.1553 ± 0.0419 units mg $protein^{-1}$ at 1-week premolt (D2), and 0.1827±0.0110 units mg $protein^{-1}$ at 12-h postmolt (A2.1) in gill, integument and haemolymph, respectively. These two enzymes are needed for carapace degradation as well as for chitin synthesis in preparation for carapace formation. For glycogen content, high accumulation of glycogen in the integument was observed throughout the molting cycle except at intermolt (C). In gill, however, the level of glycogen was high only before molting began (D2) while glycogen content in hepatopancreas and muscle, energy reserve tissues, was rather fluctuated but reached the maximum levels at 24-h postmolt (A2.2). As for trace elements, copper, a major component of hemocyanin, was found at high level at intermolt stage (C) in all tissues while magnesium, involving both nerve conduction and muscle contraction, showed the highest content at 7-day postmolt (B2.3) and 1-week premolt (D2) in integument and haemolymph, respectively. On the other hand, zinc showed high content at premolt stage in haemolymph and early postmolt stage in gill supporting its function in calcification. The results confirmed the roles of chitobiase, proteinase, glycogen, and trace elements which corresponded well with molting stages and physiological changes of mud crab.

Key words: molting cycle, chitobiase, proteinase, glycogen, mud crab, trace elements

INTRODUCTION

Molting is one of the vital events occurred in decapod crustacean to increase body size. It consists of several interesting features: the slow building up of reserve substances (glycogen) at intermolt, the formation of new skeleton at premolt, the shedding of the old skin at ecdysis and the absorption of organic matter and minerals (Zn, Mg and Cu) and the hardening of the new

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Received date : 21/04/06 Accepted date : 26/06/06

cuticle at postmolt (Hickman *et al*., 2001). Mud crabs (*Scylla serrata*) are known to molt all-year-round and all-their-lives, but the time interval of molting depends largely on their ages and sizes (Warner, 1977). Crabs enter the period of ecdysis through apolysis which occur at premolt stage. Apolysis is the process of separation of epidermis from the old exoskeleton which is triggered by steroid hormones, ecdysteroids, produced from Y-organs (Fingerman, 1997). Partial digestion of the old chitinous cuticle of crabs is initiated by the molting fluid in the apolytic space. This fluid is secreted from epidermis and contains proteinases and two chitinolytic enzymes, chitinase and chitobiase (Samuels and Reynolds, 1993; Espie and Roff, 1995). Several physiological changes, biochemical compounds and enzyme activities were found fluctuated over the time of molting, i.e. osmolality (Passano, 1960), inorganic ion, free amino acid (Wheatly, 1985), organic compound (Pratoomchat *et al*., 2002), cell type composition (Zilli *et al*., 2003), carbonic anhydrase (Salaenoi, 2004), alkaline phosphatase, Ca^{2+}ATPase and glucosamine content (Salaenoi *et al*., 2004). The changes in key enzymes and biochemical compounds at different stages of molting would elucidate the important roles of these compounds. Therefore, the present study was conducted to determine the proteinase and chitobiase activities as well as the variation of glycogen and some trace elements contents in the vital organs, i.e. gill, integument, hepatopancreas, muscle and haemolymph which could enable us to understand the mechanism of morphological changes during the molting transition and provide a guideline to synchronize the molting time which ultimately lead to mass production of soft-shell mud crab for commercial purpose.

MATERIALS AND METHODS

Animal preparation

Mud crabs, *Scylla serrata* of 65-85 mm in carapace width were collected from a soft-shell crab farm at Klung District, Chanthaburi Province. Each crab was kept in an individual closed system aquarium containing 5 l seawater having 20-26 ppt salinity at 21-26 °C. The cultured seawater was changed twice a week. Crabs were fed *ad libitum* with freshly chopped fish every two days. The dactylopodite and propodus were examined to identify the stages in molting cycle according to the criteria described by Warner (1977). The molting cycle of mud crab was divided into 11 stages: C (intermolt), D1 (2-week premolt), D2 (1-week premolt), D3 (2-day premolt), A1 (6-h postmolt), A2.1 (12-h postmolt), A2.2 (24-h postmolt), B1 (2-day postmolt), B2.1(3-day postmolt), B2.2 (5-day postmolt), B2.3 (7-day postmolt).

Tissues collection

Mud crabs were anaesthetized in cold water for 1 min, then dried with soft cloth and cleaned with 70% ethanol. Haemolymph was withdrawn from the sinus at the base of fourth and fifth pairs of pereiopods and transferred into the tubes containing 10% tri-sodium citrate (5:1 v/v) and kept at -20°C until use. Gill, integument, hepatopancreas, and crab muscle were removed and transferred to cold Tris-HCl buffer, pH 8.0. The tissue samples were either homogenized or ground to fine powder in liquid nitrogen.

Enzyme assay

To find the optimum pH, chitobiase activity was assayed according to the method described by Espie and Roff (1995). The reaction mixture consisted of 50 µl crude enzyme extract, 1,150 µl of different 0.1 M buffers, and 200 µl of 2.5 mM *p*-nitrophenyl-*N*-acetyl-β -D-glucosaminide. Three different buffers used in the experiment were McIlvaine (pH 3-7), Tris-HCl (pH 7-10), and glycine-NaOH (pH 10-12). Incubation was done for 10 min at 40 °C, and 2 ml of 0.2 M Na_2CO_3 was added to stop the reaction. The reaction mixture was centrifuged at 4,500 g for 10 min. The absorbance of supernatant was

measured at 420 nm (spectrophotometer JASCO V-550). As for the optimum pH of proteinase, a modified method of Vega-Villasante *et al*. (1995) was used. The assay system contained 100 ml of crude enzyme, 650 ml of 0.1 M buffers at different pH ranges (3 to 12), and 250 ml of 2% azocasein. Incubation was done at 40 °C for 15 min, and 1.2 ml of 10 % TCA was added to stop the reaction. After centrifugation at 4,500 g for 10 min, 1.5 ml of 0.5 M NaOH was added to 1.5 ml of supernatant and the absorbance was measured at 440 nm. Optimum temperature for the activities of chitobiase and proteinase were determined at 30, 40, 50, 60, 70, and 80°C, at their respective optimal pH. The specific activity of chitobiase was expressed as μmoles of *p*-nitrophenol min^{-1}mg $protein^{-1}$ while that of proteinase was determined as unit mg $protein^{-1}$. One unit of proteinase was defined as an increase of 0.01 optical density (OD) unit at 440 nm / min. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Glycogen content

To determine glycogen content in mud crab, 1 ml of haemolymph or 1 g of tissues (integument, gill, hepatopancreas, and muscle) were thoroughly mixed with 5 ml of 5% TCA and centrifuged at 4,500 g for 15 min. The supernatant was filtered three times using Whatman # 4 filter paper and 95% ethanol was added at 5:1 volume of the sample solution. The mixed solutions were either left at room temperature overnight or incubated in the 37-40°C water bath for 3 h. Centrifugation was done at 4,500 g for 15 min and the glycogen in the supernatant was determined according to the method described by Carroll *et al*. (1995).

Trace elements content

Trace elements (Cu, Mg and Zn) were determined using the method of AOAC (1980). The analysis was done by wet ashing, acid hydrolysis under vacuum condition. Approximately 0.2 g of tissue sample was dissolved in 10 ml of acid mixture (conc. HNO_3, conc. H_2SO_4 and conc. $HClO_4$ at the ratio of 5:1:2) in a 75 ml test tube. The sample tube was set in a digesting apparatus under a fume hood and heated at 180-200°C until a clear solution was obtained. After cooling, the solution was diluted with deionized distilled water to make a total volume of 50 ml. It was thoroughly mixed and left for precipitation. The supernatant was collected and kept in a 100 ml polyethylene bottle and covered tightly. The element content was determined by using atomic absorption spectrophotometer (AA-680 ShiMADZU, Atomic Absorption/Flame Emission Spectrophotometer, flame: AIR/C_2H_2).

RESULTS AND DISCUSSION

Optimum condition for chitobiase and proteinase activities

Chitobiase activity in gill and haemolymph of *Scylla serrata* showed two optimum pH values (pH 5 and 7) while pH 8 was the best pH for chitobiase in the integument (Figure 1). As for the optimum temperature, chitobiase seemed to work best at 50 °C in the integument and haemolymph of mud crab but it preferred a lower temperature of 40 °C in the gill

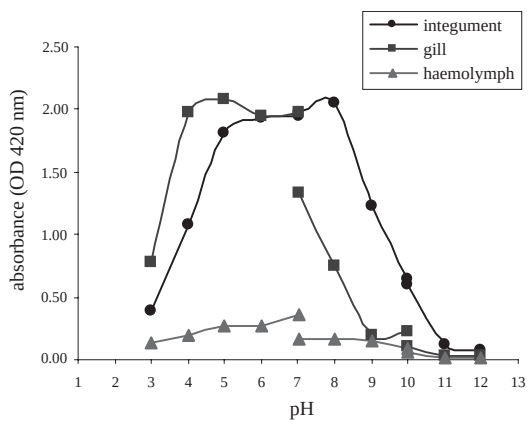

Figure 1 Optimum pH of chitobiase in gill (■), integument (●) and haemolymph (▲) of mud crab.

(Figure 2). Two optimum pH values of chitobiase at pH 4 and 5 were also found in the integument of the migratory locust *Locusta migratoria* with an optimum temperature as high as 70 °C (Zielkowski and Spindler, 1978), while chitobiase in the epidermis of the fiddler crab *Uca pugilator* showed the optimum pH at pH 5 and optimum temperature at 50 °C (Zou and Fingerman, 1999) which was quite similar to those found in mud crab (*Scylla serrata*).

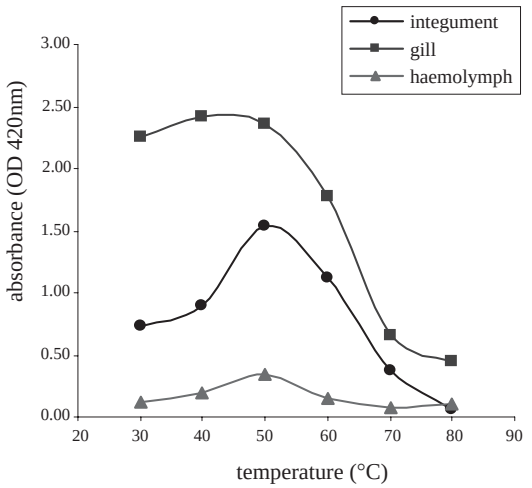

Figure 2 Optimum temperature of chitobiase in gill (■), integument (●) and haemolymph (▲) of mud crab.

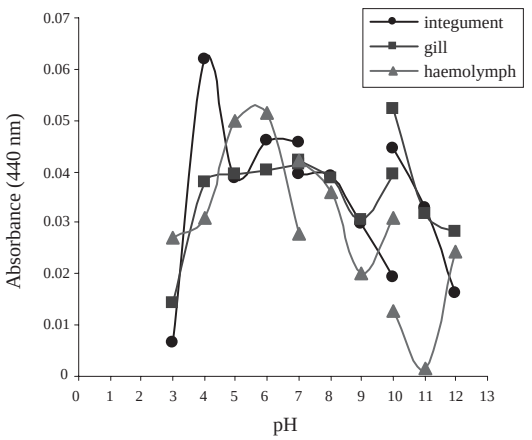

Figure 3 Optimum pH of proteinase in gill (■), integument (●) and haemolymph (▲) of mud crab.

Proteinase, on the other hand, showed its highest activity at pH 4, 10 and 6 in the integument, gill and haemolymph, respectively, while the optimum temperature for this enzyme was found at 60 °C in both gill and integument but at 40 °C in the haemolymph (Figures 3 and 4). Optimum pH and temperature of proteinase in the present study were comparable to other crustacean species; midgut gland of mud crab (pH 7.0-7.4 and 50 °C) (Pavasovic *et al*., 2004) and hepatopancreas of the red shrimp *Pleoticus muelleri* (pH 7.5-8) (Gimenez *et al*., 2001). However, Garcia-Carreno *et al.* (1994) revealed that the maximum activity of proteases in most crustaceans were generally in the ranges of pH 5.5 to 9.0. As for temperature, proteinase in the stomach of red fish *Sebaster mentella* gave highest activity at 35-40 °C (Munilla-Moran proteinase in the stomach of red fish *Sebastes mentella* showed highest activity at 35-40 °C (Munilla-Moran and Saborido-Rey, 1996), but at 50 °C in the midgut gland of mud crab (Pavasovic *et al.*, 2004).

Activity of chitobiase and proteinase during molting cycle

Chitobiase activity in the gill of mud crabs was found in the ranges of 0.0178 ± 0.0002

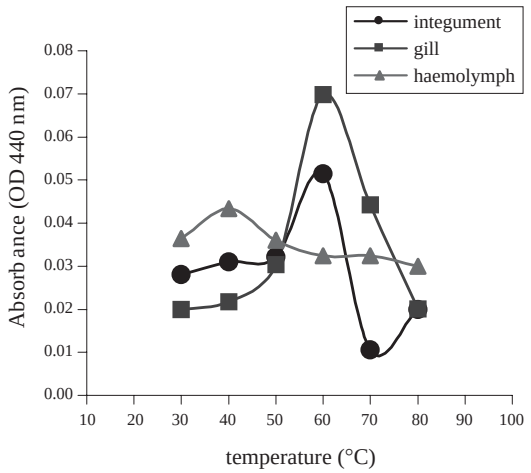

Figure 4 Optimum temperature of proteinase in gill (■), integument (●) and haemolymph (▲) of mud crab.

to 0.5467 ± 0.0136 μmol min^{-1}mg $protein^{-1}$ (Figure 5A). The activity was at maximum level of 0.5467 μmol min^{-1}mg $protein^{-1}$ at 1-week premolt (D2) and dropped quite rapidly at 2-day premolt (D3) to the lowest level of 0.0178 μmol min^{-1}mg $protein^{-1}$ at 24-h postmolt (A2.2). This pattern of chitobiase change was also similar to the pattern found in the integument (Figure 5B) although the chitobiase range was lower (0.0294 ± 0.0001 to 0.1413 ± 0.0026 μmol min^{-1}mg $protein^{-1}$). Activity profile of chitobiase in the haemolymph, on the other hand, was found to be opposite to those in gill and integument. It was stably maintained at low level throughout the molting cycle but drastically increased to 0.0851 ± 0.0123 μmol min^{-1}mg $protein^{-1}$ (Figure 5C) at 24-h postmolt (A2.2) and dropped again to only 6.5 ± 0.2 nmol min^{-1}mg $protein^{-1}$ at the next stage, i.e., 2-day postmolt (B1).

It was distinctively seen that chitobiase specific activity in gill and integument was at its highest peak just before molting began (D2 stage). At this stage crabs, discharged a large amount of molting fluid to dissolve the old endocuticle and prepared themselves for the formation of a new carapace. Two chitinolytic enzymes, chitinase and chitobiase were predominantly found in this molting fluid in epidermis (Samuels and Reynolds, 1993) to digest chitin in subsequent order. Chitinase hydrolyzed chitin into dimer, trimer and oligomers of *N*-acetylglucosamine while chitobiase proceeded them into monomer. Zou and Fingerman (1999) confirmed the function of chitobiase in the molting cycle of the fiddler crab *Uca pugilator* as seen from its high specific activity at premolt (D2) stage, while Salaenoi *et al*. (2004) showed similar evident of high specific activities of chitinase in gill and integument of mud crab *S. serrata* just before molting began. The rise in chitobiase activity in gill and integument before ecdysis as shown in our results confirmed the function of this enzyme to degrade chitin at premolt.

In haemolymph, however, chitobiase was found at high level after molting. At this stage, mud crabs had to absorb large amount of water while their movement was limited which made them vulnerable to both fungi and parasitic infection from inflow of water into the body. The chitinolytic enzymes, therefore, could play an important role in the defense mechanism for mud crabs at this critical stage as also seen in turbot (*Scophthalmus maximus*)(Manson *et al*. , 1992).

As for proteinase, the activity profile in the gill was in the ranges of 0.0493 ± 0.0106 to 0.6511 ± 0.0934 units min^{-1}mg $protein^{-1}$ (Figure 6A). There were two stages of high proteinase activity of 0.4903 ± 0.0662 and 0.6511 ± 0.0934 units min^{-1}mg $protein^{-1}$ at 1-week premolt (D2) and 7- day postmolt (B2.3) while in the rest of the

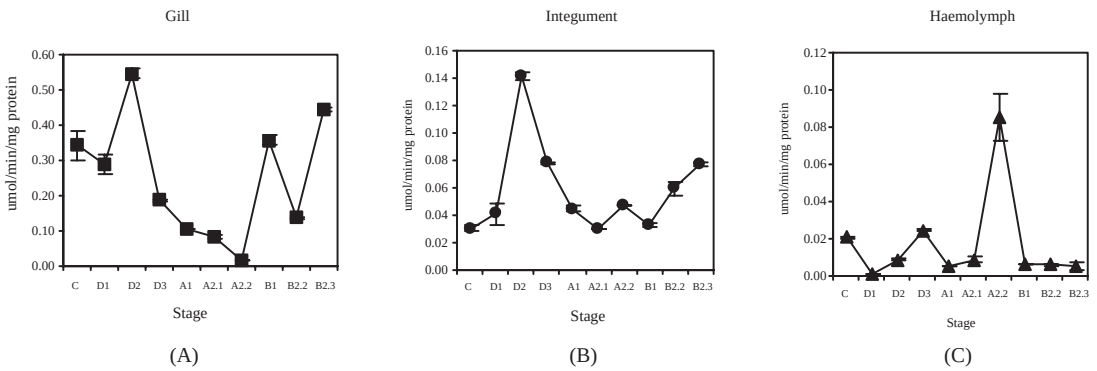

Figure 5 Chitobiase specific activity in gill (A), integument (B) and haemolymph (C) over the molting cycle of mud crab.

cycle the activity was quite low. Although proteinase in the integument of mud crab showed the maximum level also in the D2 stage at 0.1553 ± 0.0419 units min^{-1}mg $protein^{-1}$, this enzyme was highly fluctuated throughout the molting cycle (Figure 6B). Proteinase in the haemolymph of mud crab, however, showed similar pattern as that of chitobiase. The enzyme was kept at a low level throughout the cycle but increased only at 12-h postmolt (A2.1) stage (Figure 6C).

It was clearly seen that proteinase specific activity in gill and integument was high at D2 stage similar to the pattern of chitobiase activity. Beside the parallel function in chitin degradation, both chitobiase and chitinase also help proteinase in disassemble endocuticle to eliminate the old carapace which contains several components, i.e., protein, lipid, chitin, pigments and elements (Kramer and Koga, 1986). In this process, proteinase would act as a major enzyme to hydrolyze protein into small monomers which could be reabsorbed into the cells for the next round of molting. *N*-acetylglucosamine, on the other hand, as the product of chitin degradation would then be absorbed and stored in hepatopancreas to be later released as an intermediate compound for chitin biosynthesis.

The exceptionally high specific activities of both chitobiase and proteinase found in haemolymph at early postmolt stages (A2.1-A2.2) of *S. serrata* could be resulted from the high demand of water uptake to increase the body size. As mentioned earlier, the inflow of water also brings in fungi and parasites along with it making mud crabs at this stage to be easily attacked. Chitobiase and proteinase, therefore, act as a defense mechanism to protect *S. serrata* from these microorganisms. Manson *et al*. (1992) reported the similar defense mechanism based on the high specific activity of chitinolytic enzymes found in the blood of fish. In addition, high proteinase and chitobiase specific activities were also seen in gill at postmolt stage (B2.3) while the carapace was rather soft and food intake could be absorbed through gills (Salaenoi *et al*., 2004) making the mud crab more prone to pathogenic infection and, therefore, need these two enzymes for protection as well.

Glycogen content

Glycogen in the gill of mud crab during molting cycle was found in the ranges of only 8.1 ± 0.9 ug glycogen/g tissue wet weight to 0.1692 ± 0.0095 mg /g tissue wet weight, while those in the integument, hepatopancreas and muscle were in the higher ranges of 0.0989 ± 0.0107 to 0.4436 ± 0.0016, 0.0489 ± 0.0067 to 0.3809 ± 0.0035 and 0.0162 ± 0.0053 to 0.4237 ± 0.0081 mg/g tissue wet weight, respectively (Figure 7).

Changing profiles, however, were

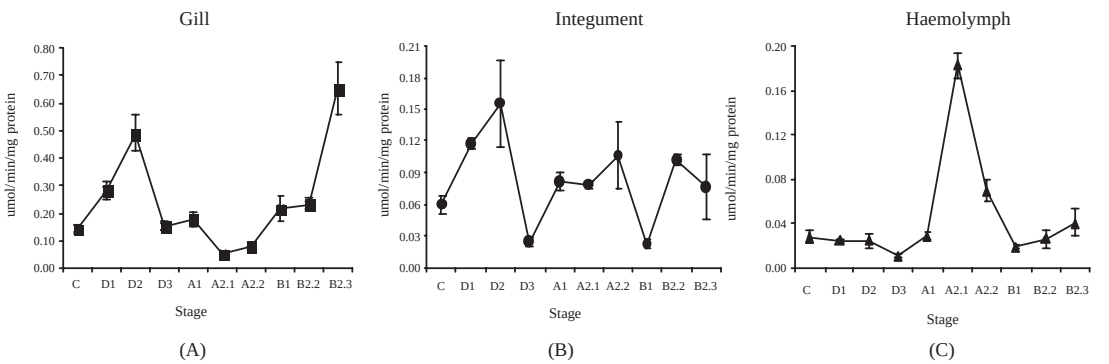

Figure 6 Proteinase specific activity in gill (A), integument (B) and haemolymph (C) over the molting cycle of mud crab.

different in each tissue. Glycogen in gill was high during intermolt (C) through 1-week premolt (D2) but was kept at low level in all the remaining stages (Figure 7A). For the integument, glycogen was found at a low level only at intermolt (C) and became stably high (0.3298 ± 0.0317 to 0.4436 ± 0.0016 mg glycogen/g tissue wet weight) all through the molting cycle. As for hepatopancreas, glycogen content was found to fluctuate a lot both before and after molting. The highest level of this substance was at 24-h postmolt (A 2.2) (0.3809± 0.0035 mg glycogen/g tissue wet weight). The glycogen content in muscle, however, was somewhat opposite to the profile found in gill as shown from the low level in premolt stages (C to D) and accumulated at a high level in A2.2 stage (24-h postmolt) (0.4237 ± 0.0081 mg glycogen/g tissue wet weight) but dropped to a low level again (0.0162 ± 0.0053 to 0.0502 ± 0.0049 mg glycogen/ g tissue wet weight) in the late postmolt stages of B1-B2.3.

The changes in glycogen content during molting cycle were found to correspond well with the physical alteration of mud crab. The accumulation of glycogen before (D1-D3) and after (A1-B1) molting was also reported by Chan *et al*. (1988) confirming that glycogen was necessary for both chitin degradation and chitin synthesis in the form of glucose (Hornung and Stevenson, 1971). Our results also showed the rapid accumulation of glycogen in gill before molting took place and became depleted in the remaining time while high glycogen content was found throughout the cycle in the integument where constant supply of glycogen was needed.

It is generally known that hepatopancreas

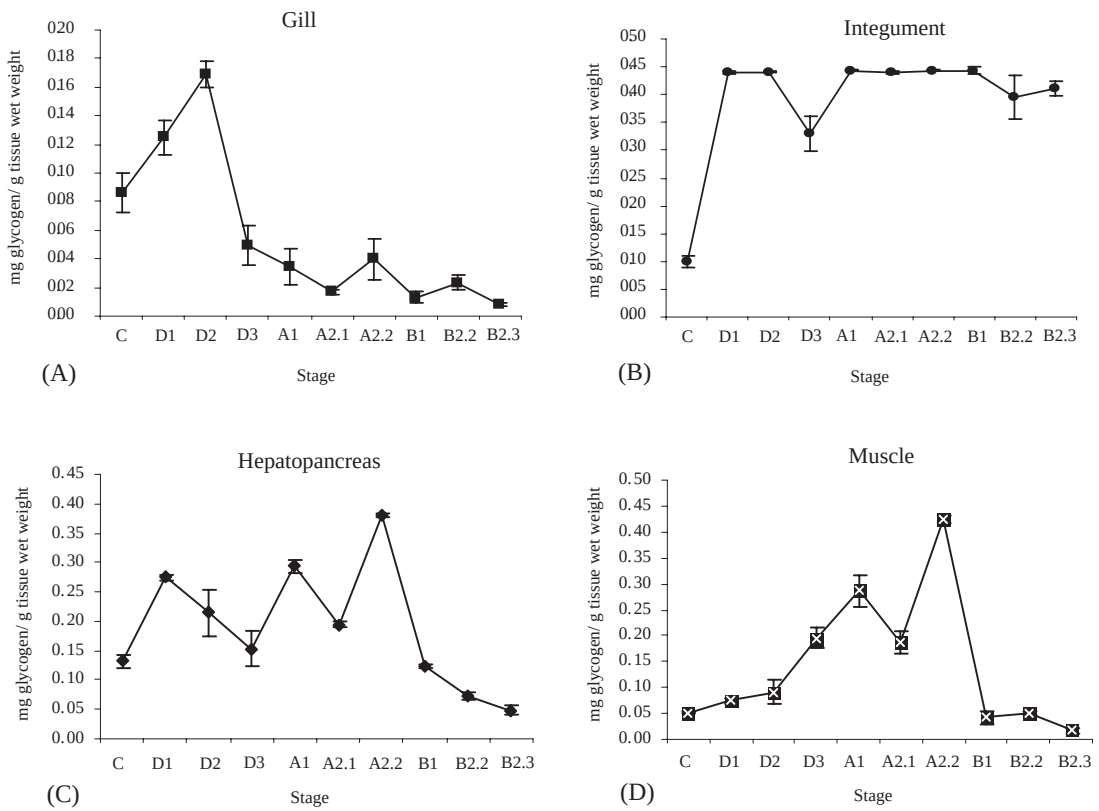

Figure 7 Glycogen content in gill (A), integument (B) hepatopancreas (C) and muscle (D) over the molting cycle of mud crab.

and muscle are the storage organs for glycogen. Glycogen would be released through haemolymph to the target organs where energy is required and its form is changed to readily usable glucose. Before molting, mud crab will increase food uptake to attain high level of glycogen reserve which is needed for shell movement and molting process as well as formation of new chitin (Sedlmeier, 1995). The increase in glycogen content was observed in all types of tissues. However, after molting (B1-B2.2) mud crab began to move and taking in some food (Warner, 1977; Salaenoi, 2004), glycogen once again became hydrolyzed to glucose providing enough energy for muscle movement and digestive process (Rosas *et al*., 1995) as also seen in the increase of glycogen in muscle and hepatopancreas of mud crab.

Trace elements

The amounts of copper (Cu) in gill, integument and haemolymph over the molting cycle of mud crabs were in the ranges of 189.89 to 244.57, 5.43 to 87.71 and 30.05 to 85.49 ppm, respectively (Figure 8A-C). Copper in gill was stably high throughout the molting cycle. On the contrary, the highest amount of copper in integument (87.71 ppm) decreased abruptly from intermolt stage (C) to the lowest content (5.43 ppm) at 2-day postmolt stage (B1) then slowly rose to a higher level of 36.90 ppm at 7-day postmolt (B2.3) (Figure 8B). Similar to the copper pattern in integument, the high content (85.49 ppm) in haemolymph was shown at intermolt (C) to 1-week premolt (D2) but the value was stably low in the remaining stages (A1-B2.3) of molting cycle

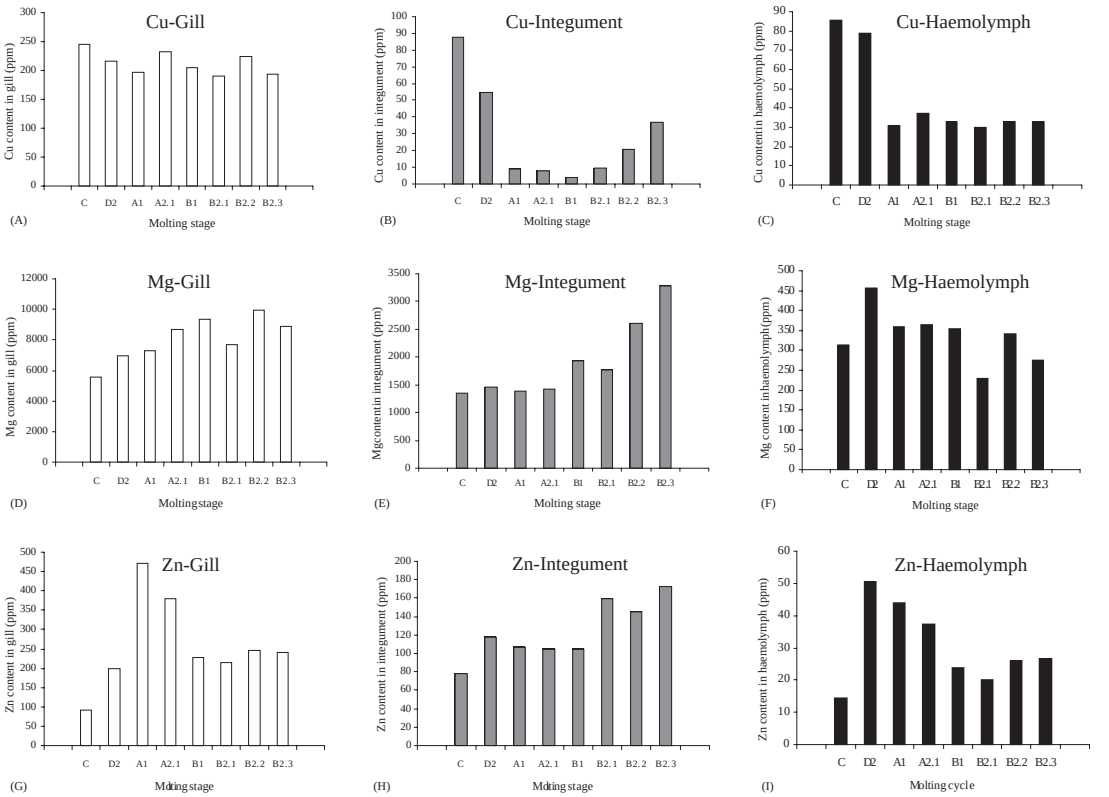

Figure 8 Trace elements content over the molting cycle of mud crab: top row; copper content in gill (A), integument (B) and haemolymph (C): middle row; magnesium content in gill (D), integument (E) and haemolymph (F): bottom row; zinc content in gill (G), integument (H) and haemolymph (I).

(Figure 8C).

As for magnesium, the content in gill, integument and haemolymph were 5,587.10 to 9,970.70, 1,346.65 to 3,274.60 and 228.50 to 457.64 ppm, respectively (Figure 8 D-F). Magnesium content in gill increased from intermolt (C) through 1-week premolt (D2) and 7-day postmolt (B2.3) (Figure 8D). The maximum content in gill was found at 5-day postmolt (B2.2) (9970.70 ppm). In integument, magnesium content which was quite stable from intermolt (C) to 12-h postmolt (A2.1), became fluctuated from 2-day postmolt (B1) to 3-day postmolt (B2.1) and reached the maximum level (3274.60 ppm) at 7-day postmolt (B2.3) (Figure 8E). Contrast to the integument, the maximum content in haemolymph was found at 1-week premolt (D2)(457.64 ppm) and the lowest content (228.50 ppm) was shown at 3-day postmolt (B2.1) while the content in the rest of the cycle was quite stable (Figure 8F).

Zinc content in gill, integument and haemolymph during molting cycle of mud crab were found at 91.66 to 472.09, 77.80 to 172.74 and 14.43 to 50.66 ppm, respectively (Figure 8G-I). Zinc in gill increased abruptly from the lowest content (91.66 ppm) at intermolt stage (C) to the highest content (472.09 ppm) at 6-h postmolt (A1) before sharply decreasing to a lower level of 228.30 ppm at 2-day postmolt (B1) and became stable in the remaining stages of molting cycle (Figure 8G). In integument, zinc content did not change much from intermolt (C) to 2-day postmolt (B1) but abruptly increased to a high level of 159.73 ppm at 3-day postmolt (B2.1) and reached its maximum level at 7-day postmolt (B2.3) (172.74 ppm) (Figure 8H). Although zinc content in haemolymph was lower than in gill and integument, its profile was similar to that found in gill showing higher levels in the premolt stages and less in the postmolt stages (Figure 8I).

It is known that trace elements are necessary for several physiological processes of animals. Copper is important for the metabolic functioning of hemocyanin (Mangum, 1992) which is a major component of crab haemolymph. The high content of copper in haemolymph at the normal stage of crab supported its vital role in keeping them in healthy form. Engel (1987) demonstrated that molting in the blue crab profoundly affected the tissue and cytosolic concentrations and partitioning of copper. At ecdysis, the concentration of hemocyanin dramatically decreased and large amount of copper was released into the cytosolic pools. Some mechanism was, therefore, needed to detoxify the copper and to assist in the excretion of this metal. Our results confirmed the previous reports as seen from the significant decrease of copper content in integument and haemolymph at ecdysis and postmolt stages to be used for new carapace formation. In gill, however, the copper level was maintained at high level due to the constant exchange of copper ions with the seawater.

As for magnesium, it was found to involve in nerve conduction, muscle contraction and blood coagulation during molting cycle in crustacean (Chen *et al*., 2000). This could explain the high content of magnesium in haemolymph of mud crab before ecdysis to help co-ordinate the muscle and nerve for carapace shedding. Meanwhile, the high maintenance of magnesium in gill could be the results of the constant movement of this organ and the exchange of ions with seawater as previously described. In addition, a suspicious drop of magnesium in all tissues at a certain stage of B2.1 (3-day postmolt) corresponded well to the high usage of magnesium for mud crab movement after ecdysis. Interestingly, the changes in magnesium content in the integument and haemolymph of *S. serrata* followed the same pattern of Ca^{2+}ATPase change as reported by Salaenoi (2004) and also supported the finding of Chen *et al*. (1974) on the co-operations of Mg and Ca^{2+}ATPase in phosphorylation and mineralization during molting cycle.

As for zinc, it is a constituent of many important enzymes, including carbonic anhydrase

and several peptidases which play important roles in CO_2 exchange in molting cycle (Bottcher and Siebers, 1993). Since carbonic anhydrase has to work with alkaline phosphatase and Ca^{2+}ATPase to initiate calcification and mineralization while zinc itself is a cofactor of carbonic anhydrase, the high zinc content found in the gill right after ecdysis (A1-A2) could very well satisfy its function in preparation for the beginning of calcification process. In integument where calcification of carapace was taking place, the amount of accumulated zinc in this tissue corresponded well with the apparent carapace formation, i.e., stable at early postmolt (A1-B1) and started to increase at 3-day postmolt (B2.1) when zinc-like carapace was seen, and distinctively high at late postmolt (B2.1-B2.3) when rigid carapace was formed. In haemolymph, the surge of zinc content after intermolt (C) and steady declining after ecdysis indicated the transferring of zinc from haemolymph to gill and later on to integument for calcification and carapace formation.

CONCLUSIONS

It could be concluded that chitobiase, proteinase, glycogen and trace elements contents corresponded well to the stage of molting especially in the period of dissolution of the old cuticle and the synthesis of new shell. Both chitobiase and proteinase played important roles in the molting cycle of mud crab *S. serrata* not only for the degradation of old carapaces to prepare the crabs for a new round of ecdysis, but they also functioned in a defense mechanism to protect the crabs from infection at this vulnerable stages. Changes of glycogen in different tissues during the molting cycle enabled us to follow the sequential order of energy usage as well as chitin synthesis in mud crab. The pattern of trace elements accumulation corresponded well to the function of calcification, mineralization and the physiological process over the molting cycle. It was clearly seen that all reactions happened in molting stage were interrelated. The enzymes and chemical compounds were needed at certain period of time during molting.

Synchronization of ecdysis, therefore, is a tedious job which needs careful balance of chemical compounds to initiate the right reaction at the right time, but it is a real challenge to further investigate for soft-shell crab production.

ACKNOWLEDGEMENTS

This work was financially supported by Kasetsart University Research and Development Institute (KURDI), project no. 04113613.

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