

Variation of Calcium, *N*-acetylglucosamine, Glucosamine and Glucose Content during Molting Cycle of Mud Crab (*Scylla serrata* Forskål 1775)

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ABSTRACT

Calcium, *N*-acetylglucosamine, glucosamine and glucose content in the tissues of mud crab (*Scylla serrata* Forskål 1775) during molting cycle were determined. The results showed that calcium in gill decreased gradually from intermolt (C) to the lowest level of 6,527 ppm at 2-day premolt (D3) and increased again from 6-h postmolt (A1) to the highest level of 24,058.50 ppm at 7-day postmolt (B2.3) which was similar in pattern to calcium in the integument but of which at lower range (1,360.10-8,044.30 ppm). The highest content in haemolymph was found at 2-day premolt (D3). Variation of *N*-acetylglucosamine, a major component of chitin, showed similar pattern to calcium. Their content in gill and integument decreased from intermolt (C) to premolt (D1-D3) and increased at postmolt stages (A1-B2.3) while the pattern was opposite to that found in haemolymph which increased at premolt (D1-D3) and decreased at postmolt stages (A1-B2.3). For glucosamine, however, the content was quite low in both gill and integument but was moderately high at late postmolt in haemolymph. Glucose, on the other hand, seemed to be quite stable throughout the molting cycle (averaged 0.8973 ± 0.1489 % mg) in integument, but the profiles in hepatopancreas and gill were similar having the highest level at 5-day postmolt (B2.2). Glucose in muscle was at its peak of 0.7265 ± 0.1016 % mg at 2-day premolt (D3) followed by the ten times high level in haemolymph of 12.2020 ± 0.4807 % mg at late postmolt (B2.1). Variations of calcium, *N*-acetylglucosamine, glucosamine and glucose content corresponded to the changing of chemical composition of the tissues during chitin degradation of the old shed, chitin biosynthesis, mineralization of the new structure and the transportation of these substances between tissues.

Key words: calcium, *N*-acetylglucosamine, glucosamine, glucose, mud crab, molting

INTRODUCTION

Shedding in crustaceans occurs automatically during molting cycle which directly affects the structures and morphology of the shells.

There are several chemical reactions involved in this process, i.e., calcification (the formation of calcium carbonate from calcium and bicarbonate), decalcification, absorption and transport mechanisms in several organs (Cheng *et al.*, 2001).

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The changes of epithelial cell size and shape during molting are also believed to depend on the net movement of calcium ion into and out of the carapace where the reactions of chitin degradation and chitin biosynthesis occur (Greenaway, 1985).

Chitin in crustacean presents itself as mucopolysaccharides in the forms of *N*-acetylglucosamine and glucosamine (Urich, 1994). Carbohydrates, especially glucose, contribute to the precursor of chitin synthesis which is utilized for the construction of cuticle. Glucose also serves for other metabolisms in cells, i.e., synthesis of glycogen (Chang and O' Connor, 1983). Prior to molting, a large part of old chitin is hydrolysed by chitinolytic enzymes of the cuticula. The release of *N*-acetylglucosamine satisfies the energy requirements and contributes to a new shell during this period (Regnault, 1996).

The previous work (Salaenoi, 2004; Salaenoi *et al.*, 2006) confirmed the important roles of several enzymes, i.e., chitinase, chitobiase, carbonic anhydrase, alkaline phosphatase, Ca^{2+} ATPase, and proteinase, as well as some trace elements and glycogen to morphological changes of mud crab during molting cycle. Data from this study on the variations content of calcium, *N*-acetylglucosamine, glucosamine and glucose in different tissues during the molting cycle would make the whole picture complete which aided in recognizing the mechanism of molting in mud crab and might also be useful to synchronize the molting cycle or produce soft shell crabs which were easily consumed and were highly demanded at international market level.

MATERIALS AND METHODS

Animal preparation

Mud crabs of 65-90 mm in carapace width were collected from a soft-shell farm at Klung District, Chanthaburi Province. After checking the appearance of dactylopodite and propodus which were the sign of molting, crabs

were anaesthetized in cold water of 4 °C for 1 min, then sacrificed. Gills and integumental tissues were dissected and weighed right away. Haemolymph was drawn from the sinus at the base of the pereopods and put into 10% trisodium citrate at the ratio of 5:1. All samples were kept in ice. Gills and integumental tissues were ground in liquid nitrogen then freeze-dried. The dried powdered tissues were ground again and passed through a screen of 200 mesh sieve size. Haemolymph samples were kept at -20 °C for further analysis.

Morphological observation

The dactylopodite and propodus were examined to identify the stages in molting cycle according to the criteria described by Warner (1977). The molting cycle was divided into 10 stages : A1 (6-h postmolt), A2.1 (12-h postmolt), A2.2 (24-h postmolt), B2.1 (3-day postmolt), B2.2 (5-day postmolt), B2.3 (7-day postmolt), C (intermolt), D1 (2-week premolt), D2 (1-week premolt) and D3 (2-day premolt).

Calcium determination

Calcium was 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 appeared. 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 to occur. The supernatant was collected and kept in a 100 ml polyethylene bottle and tightly covered. Calcium content was determined using atomic absorption spectrophotometer (AA-680 ShiMADZU, Atomic Absorption/Flame Emission Spectrophotometer, flame: $\text{AIR/C}_2\text{H}_2$).

Determination of glucosamine and *N*-acetylglucosamine

To extract glucosamine (GlcN), 50 mg of lyophilized powder tissue sample and 500 μ l of haemolymph were hydrolyzed in tightly stoppered tubes with 2 ml of acid solution set at two different conditions (1M HCl at 60 °C for 1 h, and 1 M H₂SO₄ at 100 °C for 2.5 h). After cooling, hydrolysates were centrifuged at 3,500 g for 15 min. Supernatant was collected and diluted with water to lower acid molarity to 0.25 M and directly applied to the cation exchange resin as described by Regnault (1996).

For GlcN separation, a set of 7 cation exchange resin (Dowex 50W-X4, 100-200 mesh, H⁺ form) columns was used for simultaneous treatment of 52 hydrolysates. Unhydrolysed GlcN standard (5 mg in 2 ml of 0.25 M HCl) was used as a control. Samples were applied to the column and washed twice with 5 ml distilled water. The washing water was discarded. GlcN was eluted with 2M HCl and the collected fraction (12 ml) was kept for further analysis. The amount of GlcN was determined using Morgan-Elson procedure as modified by Hancock (2001). Sample aliquots of 1.8 ml each were neutralized with 0.5 ml Na₂CO₃ (pH 7 with the slight excess of Na₂CO₃). After gentle shaking, 0.5 ml (freshly prepared) of 2% acetyl acetone in 1.5M Na₂CO₃ was added. The tubes were tightly stoppered and heated in boiling water for 20 min. After cooling, 1 ml of absolute ethanol was added. The solution was then mixed with 0.5 ml Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in 15 ml of absolute ethanol and 15 ml of conc. HCl). The tubes were shaken vigorously to eliminate excess CO₂. Maximum color development occurred in 5 min and the chromophore was stable for 1 to 2 h. The absorbance was measured at 530 nm. The standard curve of GlcN (from 5 to 35 μ g) was made from stock solution of 1.5 mg/ml GlcN to determine the amount of GlcN in the sample mixtures. The results were expressed as μ g GlcN/mg tissue⁻¹ (dry

weight) (gill and integument) or mg GlcN ml⁻¹ (haemolymph). *N*-acetylglucosamine (GlcNAc) content was obtained by the calculated difference between total tissue GlcN and tissue GlcN.

Glucose determination

Glucose content was determined using a modified method of Folin and Wu (1920). Haemolymph samples (100 μ l) were added with 100 μ l of 10 % sodium tungstate and 800 μ l of 0.05 M H₂SO₄ and centrifuged at 4,500 g for 5 min. As for the tissue samples (gill, integument, hepatopancreas and muscle), 1 g wet weight of each tissue was homogenized in 3 ml of 5 % trichloroacetic acid and centrifuged at 4,500 g for 5 min. The supernatant was filtered three times using Whatman # 4 filter paper. Finally, 1.5 ml of supernatant was mixed with 1 ml of alkali copper (II) tartrate (4 % sodium carbonate, 0.76 % tartaric acid and 0.45 % copper sulfate) and placed in boiling water for 5 min. After cooling, the mixture was added with 1 ml of phosphomolybdic acid solution (5.68 % sodium molybdate, 1 % sodium tartrate and 3.8 M H₃PO₄) and distilled water to make the final volume of 5 ml. Glucose solution at the concentration of 0.05 mg/ml was used as standard. Sample absorbency was measured at 580 nm.

RESULTS AND DISCUSSION

Calcium deposition in gill, integument and haemolymph

The amount of calcium during the molting cycle varied in the ranges of 6,527.50 to 24,058.50 ppm, 1,360.10 to 8,044.3 ppm and only 230.78 to 353.97 ppm in gill, integument and haemolymph, respectively (Figure 1). The calcium profiles in gill and integument were similar but different from that of haemolymph. During intermolt (C), calcium content was found at high level in gill (15,032.00 ppm) of approximately four and fifty times the amounts found in integument

(3,538.05 ppm) and in haemolymph (304.16 ppm), respectively.

In gill, changing of calcium accumulation before and after molting were opposite in pattern, i.e., calcium content decreased gradually from 15,032.00 ppm at intermolt (C) to the lowest level of 6,527.50 ppm at 2-day premolt (D3) and started increasing from 11,109.50 ppm at 6-h postmolt (A1) to the highest level of 24,058.50 ppm at 7-day postmolt (B2.3) (Figure 1A). Similar pattern was also observed in integument where the calcium content slowly decreased from intermolt (C) (3,538.05 ppm) to 1-week premolt (D2) (3,230.65 ppm) and abruptly dropped at 2-day premolt (D3) to the minimum level of 1,360.10 ppm at 6-h postmolt (A1) (Figure

1B). Within 24-h after molting, calcium content in integument increased again and reached the highest level of 8,044.30 ppm at 7-day postmolt (B2.3). However, the pattern of calcium accumulation in haemolymph during molting cycle of mud crab was opposite to those found in gill and integument (Figure 1C). The content increased slightly from 304.16 ppm at intermolt (C) to the highest level of 353.97 ppm at 2-day premolt (D3) before abruptly dropped to 272.08 ppm at 6-h postmolt (A1) thereafter slightly but steadily decreased from 265.83 ppm at 12-h postmolt (A2.1) to the lowest level of 230.78 ppm at 7-day postmolt (B2.3). Quantitatively, the majority of calcium was found accumulated in gill and integument, the variation of which conformed

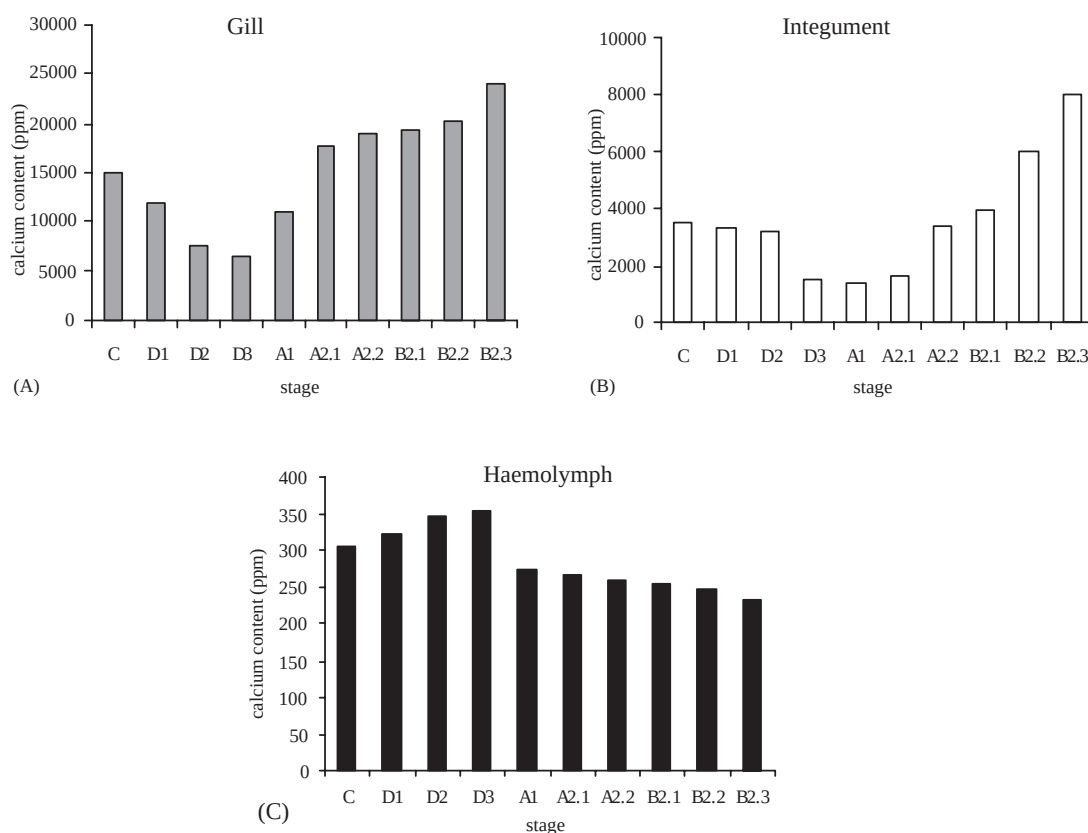


Figure 1 Calcium content in gill (A), integument (B) and haemolymph (C) of *Scylla serrata* at different stages of molting.

to similar pattern. The low level of calcium content in haemolymph with opposite pattern of accumulation may purely reflect the passage of calcium ions from gill to integument where calcification taken place.

During intermolt of mud crab, the exoskeleton was usually fully calcified and the calcium in animal was at homeostasis which corresponded to the moderate deposition of calcium in gill, integument and even in haemolymph of mud crab at 15,032.00, 3,538.05 and 304.16 ppm, respectively. However, at the premolt stages, accumulated calcium in gill and integument was supposed to reduce due to the reabsorption from the skeleton to store within the hepatopancreas via haemolymph or might be lost to the environment. So, calcium content in haemolymph should be increased as also seen in the temporary high level of 353.97 ppm at 2-day premolt (D3). Typically, losses should be high but the storage was small in aquatic species, because calcium in soluble form must be outwardly transported across the gills (Greenaway, 1985). At ecdysis, however, the skeleton was shed and the calcium remaining in the shell was lost from the body. Recalcification began immediately or shortly after as noticed from the increasing level of calcium in gill and integument (Figure 1 A-B). Storage calcium was used for calcification and therefore calcium in haemolymph was reduced (Figure 1C). It was speculated that the level of calcium in other storage organelles also decreased at recalcification period. It has been reported that the calcium content of hepatopancreas in the shore crab *Carcinus maenus* doubles from intermolt to premolt and then decreases again in early postmolt (Bondgaard and Norum, 2000). The relative and absolute amounts of calcium, magnesium, chitin, water-soluble protein, HCl-soluble protein, and sclerotin in the carapace of the Atlantic blue crab, *Callinectes sapidus* Rathbun, all increase during the first 10 days of postmolt, with calcium and HCl-soluble protein fluctuated the most (Vigh and

Dendinger, 1982).

Roer (1980) also reported that the changing of epithelial cell size and shape were responded to the net movement of Ca^{2+} either into or out of the carapace. During premolt stage, the epithelial cells separate themselves from the old cuticle and become columnar in shape with the pore canals cutting off from the apical surface. This results in a huge loss of the total surface area of the cell causing Ca^{2+} transport away from the integument and into the haemolymph. At postmolt, the situation is reversed and a large portion of Ca^{2+} transport is now towards the integument. Wheatly (1997) explained this uptake of calcium ion into the haemolymph involving three independent processes to be based on the reactions of ATP, Ca^+ ATPase and electronic balance of Ca^{2+} .

N-acetylglucosamine content in gill, integument and haemolymph

N-acetylglucosamine (GlcNAc) deposited in mud crab during the molting cycle was at the low range of 2.01 to 4.45 $\mu\text{g}/\text{mg}$ dry weight in gill, at rather high range of 39.57 to 90.99 $\mu\text{g}/\text{mg}$ dry weight in integument and at moderate range of 5.18 to 24.39 $\mu\text{g}/\text{ml}$ in haemolymph (Figure 2). GlcNAc profiles in gill and integument, however, were similar but different from that of haemolymph. In both gill and integument, GlcNAc content rapidly decreased from intermolt (C) to the minimum levels at 6-h postmolt (A1) (Figure 2 A-B). After molting was completed, GlcNAc was climbing back to reach the maximum levels of 4.45 $\mu\text{g}/\text{mg}$ at 7-day postmolt (B2.3) in gill and to 90.99 $\mu\text{g}/\text{mg}$ at intermolt (C) in integument. On the other hand, GlcNAc content in haemolymph steadily increased from 5.28 $\mu\text{g}/\text{ml}$ at intermolt (C) to 24.40 $\mu\text{g}/\text{ml}$ at early postmolt (A1), then decreased to form a stable stage (~ 20 $\mu\text{g}/\text{ml}$) until 5-day postmolt (B2.2) and finally decreased to 12.73 $\mu\text{g}/\text{ml}$ at 7-day postmolt (B2.3).

It was clearly seen that variation of N-acetylglucosamine and calcium content in the

molting cycle of mud crab showed similar pattern, i.e., the content in gill and integument decreased at premolt and increased at postmolt stages. The pattern was opposite to that found in haemolymph which increased at premolt and decreased at postmolt stages. These variations corresponded to the changing of chemical composition of the tissues during chitin degradation of the old shed, chitin biosynthesis, mineralization of the new structure and the transportation of these substances between tissues. During premolt, when the inner layers of the cuticle are digested, large quantities of *N*-acetylglucosamine are released from chitin

breakdown. Some of this is utilized immediately for new chitin synthesis. Gwinn and Stevenson (1973) found that more than half of *N*-acetylglucosamine was incorporated during postmolt. Chitin was reutilized in new chitin synthesis during the succeeding postmolt but some of the *N*-acetylglucosamine released might be stored for later chitin synthesis which was also seen in its changing pattern in this study.

The level of GlcNAc content in gill of mud crab throughout the molting cycle (Figure 2A) was quite low (~ 3-4 ug/mg dry wt.) comparing to the other tissues. This was possibly due to the fact

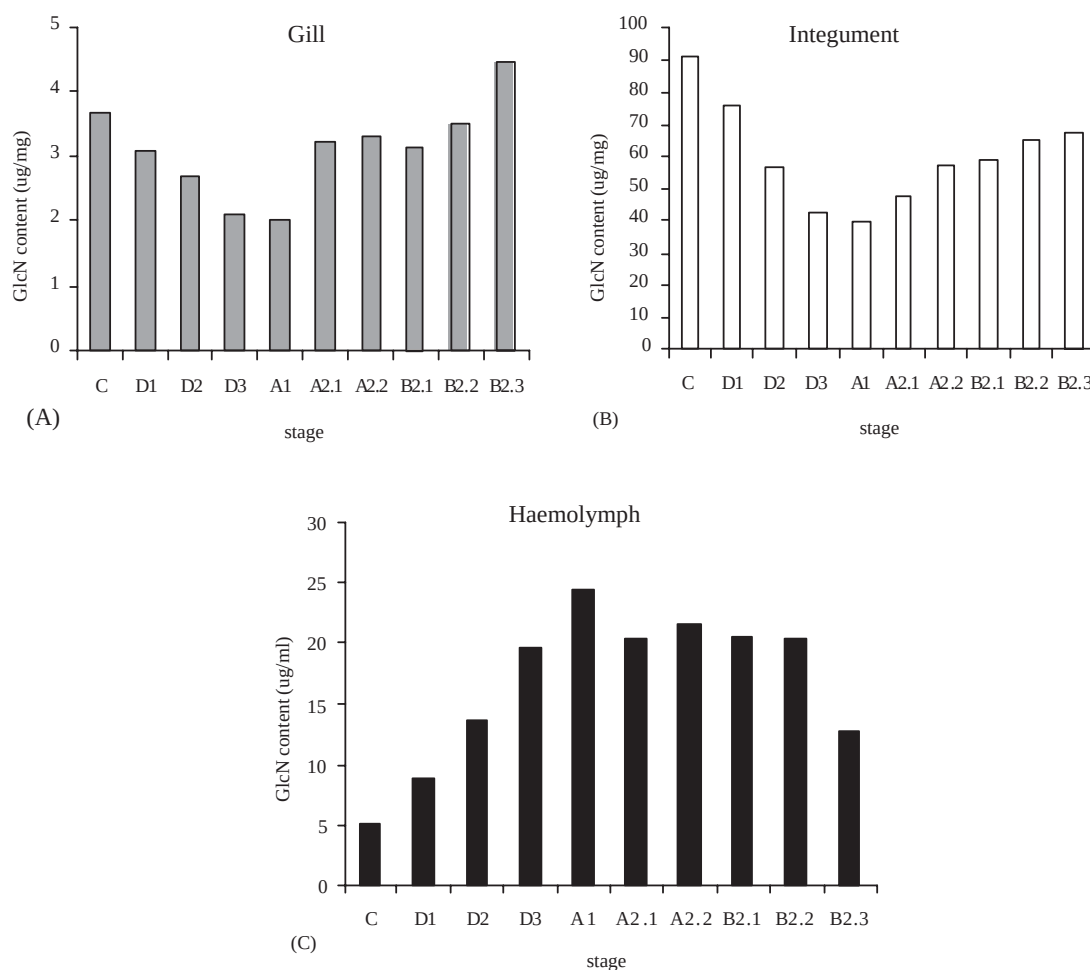


Figure 2 *N*-acetylglucosamine content in gill (A), integument (B) and haemolymph (C) of *Scylla serrata* at different stages of molting.

that gill was not a storage organ for chitin (or GlcNAc polymers) but more of a transportation passage of GlcNAc via haemolymph to integument where the usage of GlcNAc for chitin synthesis actually taken place.

Glucosamine content in gill, integument and haemolymph

Glucosamine (GlcN) content in mud crab during the molting cycle was in the low range of 0.0036 to 0.0278 ug/mg dry weight and 0.0039 to 0.1196 ug/mg dry weight in gill and integument but was in the higher range of 0 to 2.3108 ug/ml in haemolymph (Figure 3).

Not only glucosamine quantity in mud crab was quite low, its variation in all three tissues was also quite different. In gill, GlcN content steadily increased from intermolt (C) (0.0182 ug/mg dry weight) to 1-week premolt (D2) (0.0278 ug/mg dry weight) before dropping to the lowest level right after ecdysis at 6-h postmolt (A1) (0.0036 ug/mg dry weight) (Figure 3A). GlcN then gradually increased to the higher level of 0.0228 ug/mg dry weight at 7-day postmolt (B2.3). In integument, the increase of GlcN content from intermolt (C) to 1-week premolt (D2) was similar in pattern to that of gill (Figure 3B), i.e., the content increased from 0.0089 ug/mg dry weight (C) to

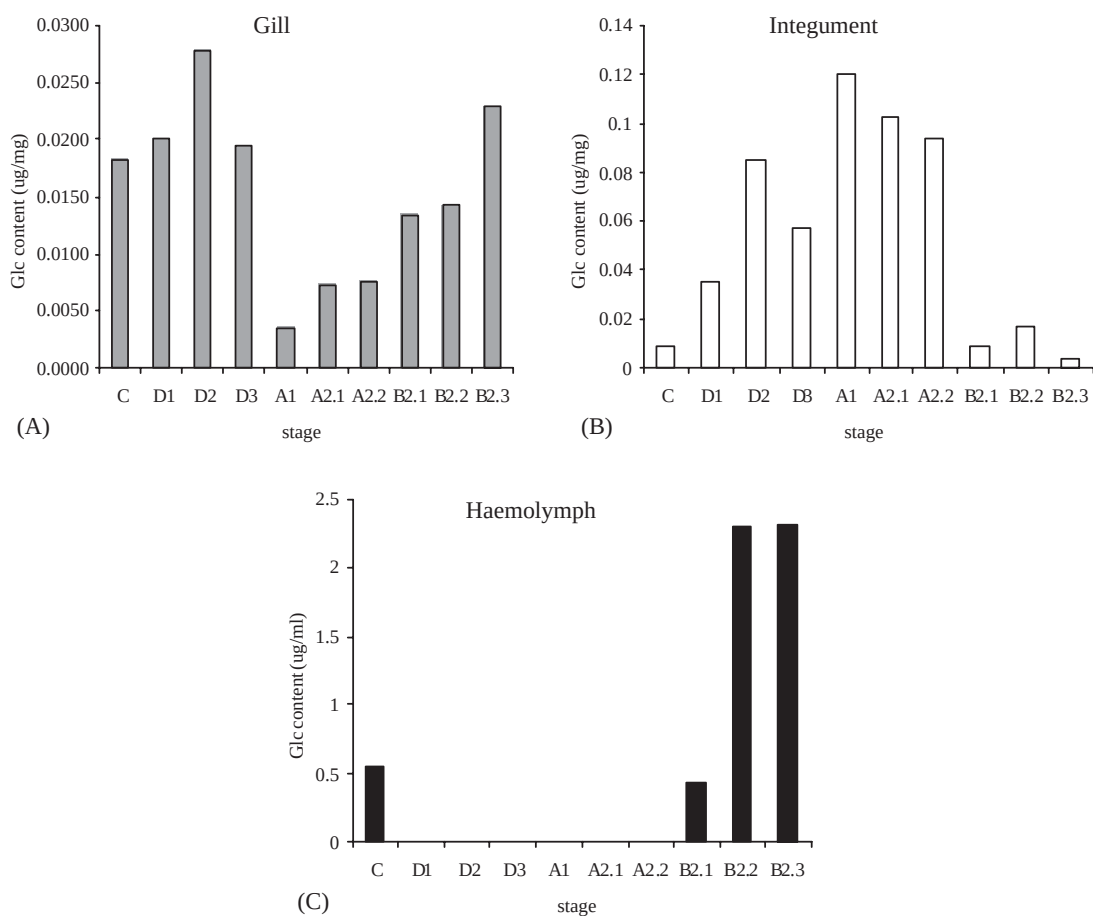


Figure 3 Glucosamine content in gill (A), integument (B) and haemolymph (C) of *Scylla serrata* at different stages of molting.

0.0854 ug/mg dry weight (D2) before dropping to 0.0578 ug/mg dry weight at 2-day premolt (D3). After molting, the changing pattern was opposite to that in gill, the high level GlcN level gradually decreased from the maximum level of 0.1196 ug/mg dry weight at 6-h postmolt (A1) to 24-h postmolt (A2.2), then drastically dropped to the low levels in the remaining three last stages of postmolt.

Similar to *N*-acetylglucosamine, glucosamine in gill of mud crab was also found at low level indicating the non-chitosan storage function of this organ. After ecdysis, however, glucosamine from all tissues was drawn to get accumulated in the integument making it disappeared from gill as well.

In haemolymph, however, glucosamine content was detectable only at four stages of molting cycle; intermolt (C)(0.5574 ug/ml), 3-day postmolt (B2.1)(0.4324 ug/ml), 5-day postmolt (B2.2)(2.2972 ug/ml) and 7-day postmolt (B2.3)(2.3108 ug/ml) (Figure 3C). The complete disappearance of glucosamine from haemolymph was observed from 2-week premolt (D1) until 24-h postmolt (A2.2). This could be due to the transferring of glucosamine from haemolymph at premolt (D1-D3) to integument for new carapace formation. After ecdysis was completed, glucosamine was then returned to haemolymph to serve as precursors of chitin in the blood of mud crab.

Glucose content in gill, integument, muscle and haemolymph

Glucose deposited in mud crab during the molting cycle was found in the ranges of 0.1477 ± 0.0133 to 0.6458 ± 0.0627 , 0.7622 ± 0.0564 to 1.0212 ± 0.6205 , 0.7434 ± 0.1393 to 3.2370 ± 0.0301 , 0.0257 ± 0.0057 to 0.7265 ± 0.1016 and 0.9668 ± 0.0860 to $12.2020 \pm 0.4807\%$ mg in gill, integument, hepatopancreas, muscle, and haemolymph, respectively (Figure 4).

Glucose profiles in hepatopancreas and

gill were similar having the maximum level in both tissues at the same 5-day postmolt (B2.2) (Figure 4 A, C). For integument, the content was quite stable throughout the molting cycle, which was different from other tissues (Figure 4 B). Although the glucose profile in muscle was not much different from that of hepatopancreas, but the range was closer to that found in gill (~ 0.2 - 0.7% mg). Glucose content in muscle was high only at the stage of 2-day postmolt (D3) ($0.7265 \pm 0.1016\%$ mg) while the remaining stages of the cycle were kept at constant level of 0.4% mg (Figure 4 D). In haemolymph, it should be noted here that glucose content of mud crab was found at rather high range and fluctuated a lot over the molting cycle. The highest level of $12.2020 \pm 0.4807\%$ mg was observed at 2-day postmolt (B1), while the lowest was found at 6-h postmolt (A1)($0.9668 \pm 0.0860\%$ mg) (Figure 4E).

It is generally known that hepatopancreas and muscle are the storage organs for glycogen. Glycogen would be released via haemolymph to the target organs where energy is required and change its form to readily available glucose resulting in the highest level of glucose in the molting cycle compared to other tissues (Figure 4E). Before molting, mud crab would increase food uptake to attain high reserve of glycogen (or glucose polymer) which is needed for molting process as well as formation of new chitin (Sedlmeier, 1995). These results showed the increase in glucose content in all types of tissues of mud crab in this aspect. After molting, however, mud crab now needed to use energy for muscle movement and digestive process causing the decrease of glucose in hepatopancreas, muscle and haemolymph. As also seen at 5-7 day postmolt, glucose leveled off and slowly decreased due to its use for chitin synthesis which was confirmed by the decrease of glycogen content in hepatopancreas and in muscle of mud crab at this same stage (Salaenoi *et al.*, 2006).

In crustaceans, *N*-acetylglucosamine and

glucosamine are basic components of chitin and their incorporation in epidermis and cuticle has been extensively studied with regard to chitin synthesis and exoskeleton renewal (Regnault, 1996). Stevenson (1972) indicated that the synthesis of chitin precursors was controlled separately from the synthesis of chitin itself. Rapid

synthesis of the precursors seems to begin when chitin synthesis is slow, allowing concentrations of the precursors to rise. Later, the rate of chitin synthesis rises and the concentrations of the precursors fall. Urich (1994) discussed the chitin synthesis in the sense that an amino group was transferred from an amino donor to fructose-6-

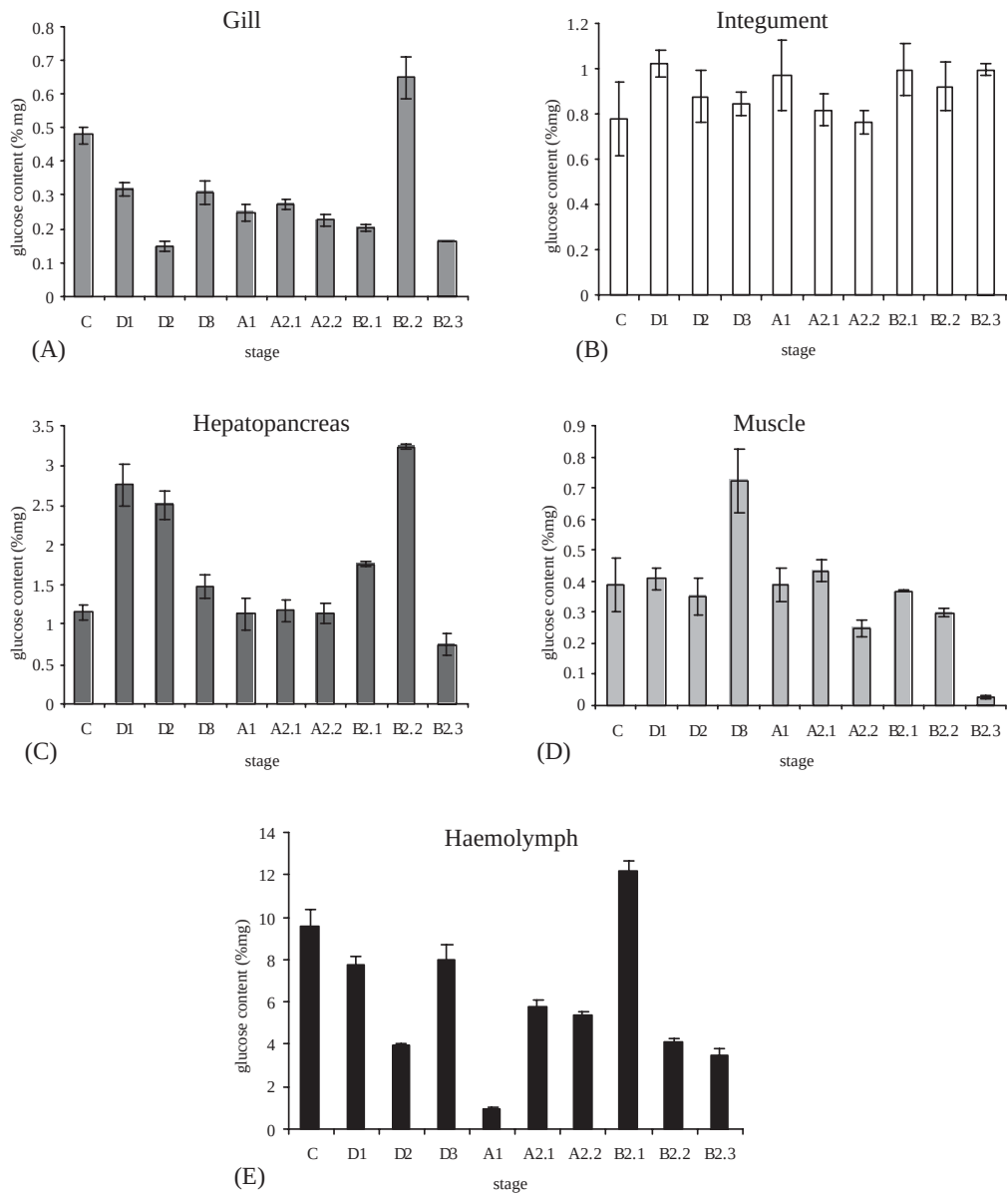


Figure 4 Glucose content profiles in gill (A), integument (B), hepatopancreas (C), muscle (D) and haemolymph (E) of *Scylla serrata* at different stages of molting.

phosphate via glucose, forming glucosamine-6-phosphate. After that, glucosamine-6-phosphate is acetylated, *N*-acetylglucosamine-6-phosphate is isomerised to 1-phosphate ester, which in turn reacts with uridine triphosphate to form UDP- *N*-acetylglucosamine. Finally, the *N*-acetylglucosamine moiety of UDP-*N*-acetylglucosamine is added to the end of a growing chitin chain. Besides beginning from glucose, the pathway could also begin from glucosamine or *N*-acetylglucosamine, which is phosphorylated directly to glucosamine-6-phosphate or *N*-acetylglucosamine-6-phosphate. This speculation is parallel to this result which showed that the content of *N*-acetylglucosamine, glucosamine and glucose in gill, integument and haemolymph accumulated in the opposite manner. When *N*-acetylglucosamine increased, glucosamine decreased and when glucosamine increased, *N*-acetylglucosamine decreased. Johnston and Davies (1972) detected glucose and glucosamine in the blood of the shore crab *Carcinus maenus* and suggested that both glycogen and chitin were present in the blood and synthesized by blood cells. They also found more polysaccharide in the blood than in the hepatopancreas and proposed that the blood was an important storage reservoir for these aquatic organisms.

These results clearly indicated that *N*-acetylglucosamine, glucosamine and glucose were closely interrelated since all these three substances are the precursors for chitin synthesis. Glucose could changed itself into glucosamine which in turn changed into *N*-acetylglucosamine to enter chitin biosynthesis pathway (Figure 5). Aside from chitin synthesis, glucose was also needed as energy source for locomotion both during ecdysis and at postmolt stage (B2.2) as confirmed by the increase level of glucose in gill and hepatopancreas at these specific periods.

CONCLUSION

Calcium was proven to have a crucial role in the molting cycle of mud crab *Scylla serrata* especially, at postmolt. This was clearly seen from the changing pattern of calcium by the absorption from seawater into gill and transported via haemolymph to get accumulated in the integument where calcification and mineralization processes taken place. *N*-acetylglucosamine and glucosamine, on the other hand, played an important role in chitin degradation and chitin biosynthesis which also took place in the integument as well. The changes in glucose, however, was found to correspond well with the physical alteration of mud crab. The accumulation of glucose at premolt and immediately after molting was as anticipated since glucose was necessary for both chitin degradation and chitin synthesis. Glucose level in the integument was

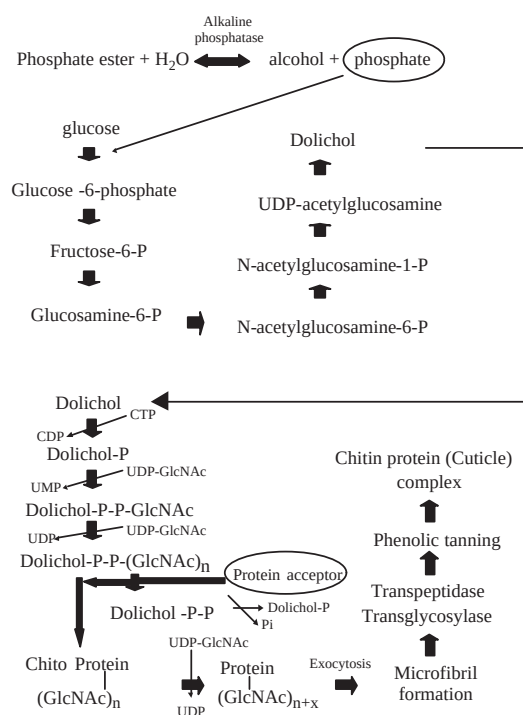


Figure 5 Chitin synthesis (Horst, 1990; Urlich, 1994).

stable compared to other mud crab tissues. Unlike gill and muscle, the integument of mud crab did not need high energy for moving, therefore, glucose level did not fluctuate as seen in other locomotive organs.

Changes in the calcium and carbohydrate contents, especially those of *N*-acetylglucosamine, glucosamine and glucose in different tissues during the molting cycle enabling in following the sequential order of calcification and energy usage as well as chitin synthesis in mud crab. Their metabolic and biosynthesis pathways can be used and planned for synchronizing the molting time of crabs which is further applied for soft-shell crab farming.

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LITERATURE CITED

- Association of Official Analytical Chemists (AOAC). 1980. **Official Method of Analysis**. 13th ed. Washington D.C., Association of Official Analytical Chemists.
- Bondgaard, M.U. and B.P. Norum. 2000. Cadmium accumulation in the female shore crab *Carcinus maenas* during the molting cycle and ovarian maturation. **Mar. Biol.** 137: 995-1004.
- Chang, E.S. and J.D. O'Connor. 1983. Metabolism and transport of carbohydrates and lipids, pp. 263-287. In L.H. Mantel (ed). **The Biology of Crustacea**. vol. 5. Academic Press, New York.
- Cheng, W., C.H. Liu, C.H. Cheng and J.C. Chen. 2001. Hemolymph oxyhemocyanin, protein, osmolality and electrolyte levels of *Macrobrachium rosenbergii* in relation to size and molt stage. **Aquaculture** 198: 387-400.
- Folin, O. and H. Wu. 1920. A simplified and improved method for determination of sugar. **J. Biol. Chem.** 41: 367-374.
- Greenaway, P. 1985. Calcium balance and molting in the crustacea. **Biol. Rev.** 60: 425-454.
- Gwinn, J.F. and J. R. Stevenson. 1973. Role of acetylglucosamine in chitin synthesis in crayfish-I. Correlation of ¹⁴C-acetylglucosamine incorporation with stages of the molting cycle. **Comp. Biochem. Physiol.** 45(B): 769-776.
- Hancock, R.E.W. 2001. Lab method. Department of Microbiology and Immunology, University of British Columbia, Canada. Available Source: <http://www.cmdr.ubc.ca/bobh/showmethod.php?methodid=26>.
- Horst, M.N. 1990. Concurrent protein synthesis is required for *in vivo* chitin synthesis in postmolt blue crabs. **J. Exp. Zool.** 256: 242-254.
- Johnston, M.A. and P.S. Davies. 1972. Carbohydrates of the hepatopancreas and blood tissues of *Carinus* sp. **Comp. Biochem. Physiol.** 41(B): 433-443.
- Regnault, M. 1996. Air exposure-induced increase in acetylglucosamine content of some soft tissues and haemolymph of a crab (*Cancer pagurus*). **J. Exp. Zool.** 275: 421-430.
- Roer, R.D. 1980. Mechanism of resorption and deposition of calcium in the carapace of the crab *Carcinus maenas*. **J. Exp. Biol.** 88: 205-218.
- Salaenoi, J. 2004. **Changes of enzymes and epidermal components during molting stages of mud crab (*Scylla serrata* Forskal 1775)**. Ph.D. Thesis. Kasetsart University, Bangkok.
- _____, J. Butpugdeethum, A. Thongpan and M. Mingmuang. 2006. Chitobiase, proteinase,

- glycogen and some trace elements during molting cycle of mud crab (*Scylla serrata* Forskål 1775). **Kasetsart J. (Nat. Sci.)** inpress.
- Sedlmeier, D. 1995. Mode of action of CHH. **Amer. Zool.** 25: 223-232.
- Stevenson, J.R. 1972. Changing activities of the crustacean epidermis during the molting cycle. **Am. Zool.** 12: 373-380.
- Urich, K. 1994. **Comparative Animals Biochemistry**. Springer-Verlag, Berlin Heidelberg. 652 pp.
- Vigh, D.A. and J.E. Dendinger. 1982. Temporal relationships of postmolt deposition of calcium, magnesium, chitin and protein in the cuticle of the Atlantic blue crab, *Callinectes sapidus* Rathbun. **Comp. Biochem. Physiol.** 72(A): 365-369.
- Warner, G.F. 1977. **The Biology of Crabs**. Paul Elek (Scientific Books) Ltd., London. 437 p.
- Wheatly, M.G. 1997. Crustacean models for studying calcium transport: the journey from whole organisms to molecular mechanisms. **J. Mar. Biol. Assoc. UK.** 77: 107-125.