

Variations of Catalase and Glutathione Activities in Molting Cycle of Mud Crab (*Scylla serrata*)

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ABSTRACT

The activities of the antioxidative enzyme, catalase (CAT), and the non-enzyme, glutathione (GSH) in 12 stages over the molting of mud crab (*Scylla serrata*) collected from Chanthaburi Province were determined. The stages were defined as: A1 (6-hour postmolt), A2.1 (12-hour postmolt), A2.2 (24-hour postmolt), B1 (2-day postmolt), B 2.1 (3-day postmolt), B2.2 (5-day postmolt), B2.3 (7-day postmolt), B2.4 (10 day postmolt), C (intermolt), D1 (2-week premolt), D2 (1- week premolt) and D3 (2-day premolt). The results revealed that CAT which controlled and eliminated excessive H₂O₂, increased in premolt (D1–D3) then dropped in the early postmolt stages (A1–A2.2) and increased again in the late postmolt stages (B1–B2.4). GSH, a reducing substance, which is also involved in the detoxification of H₂O₂ showed the same pattern in most tissues except in the hepatopancreas where it increased from the early postmolt stage (A1) and continued to increase through the postmolt stages. The variation in activity of CAT and GSH corresponded well with the metabolic activity through the molt cycle.

Keywords: catalase, glutathione, antioxidant defense, molting cycle, *Scylla serrata*

INTRODUCTION

Crab increases its body size through molting. The interval of molting depends on the crab age and size. For growth to occur, crabs must undergo periodic of postmolt, intermolt, premolt and ecdysis. All events occur automatically with various factors known to affect the changing structures and morphology and several biological changes have been reported during the molting cycle, such as in glucose, *N*-acetylglucosamine, glucosamine (Salaenoi *et al.*, 2006a), glycogen, trace elements, proteinase (Salaenoi *et al.*, 2006b) organic and inorganic compounds in hemolymph, epidermal tissue and cuticle (Pratoomchat *et al.*, 2002), total free sugar in hemolymph,

hepatopancreas glycogen and cuticular protein (Sugumar *et al.*, 2013), hepatopancreas chitobiase (Zou and Fingerman, 1999; Salaenoi *et al.*, 2006b), alkaline phosphatase and Ca²⁺ATPase (Salaenoi *et al.*, 2012).

Oxidation, a chemical reaction that transfers electrons from a substance to an oxidizing agent, can produce free radicals that initiate chain reactions that can cause damage or cell death (Sies, 1997). Under unusual conditions, an antioxidant will be synthesized within the animal in order to prevent cell stress; antioxidants have an important role in the protection of cells during the critical time of molting (Arun and Subramanian, 1998). Studies of antioxidants in crustaceans are widely used as biomarkers for environmental stress

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produced by seasonal changes (Pital and Chainy, 2013), pollutants (Pan and Zhang, 2006), salinity changes (Pital and Chainy, 2010) and in multiple life stages (Hoguet and Key, 2007). Daily variation of catalase (CAT) activity was found in the lithodid crab *Lithodes santolla* (Schvezov *et al.*, 2013) and in the mouse (Sani *et al.*, 2006).

During normal cell function, a variety of biochemical reactions in aerobic organisms induce reactive oxygen species (ROS). The major types of ROS are hydrogen peroxide (H_2O_2), superoxide anion radicals ($O^{\cdot -}$) and the hydroxyl radical ($\cdot OH$) which are highly cytotoxic and produce deleterious effects on biomolecules (Pital and Chainy, 2010). The mechanisms of defense against these ROS types utilize both enzymatic and non-enzymatic molecules. The antioxidant enzymes are superoxide dismutase (SOD), CAT and glutathione peroxidase (GPX) and the non-enzymatic antioxidants are glutathione, ascorbic acid and carotenoids (Halliwell and Gutteridge, 2007). SOD catalyzes the conversion of superoxide anion to hydrogen peroxide. The hydrogen peroxide is converted to water and oxygen by CAT. GPX decomposes peroxides using peptide GSH as the co-substrate (Halliwell and Gutteridge, 2007). The tripeptide, GSH (γ -glutamyl-cysteine-glycine) is one of the most important non-protein thiols which serves as a cellular homeostatic modulator and GSH aids in cell protection through the detoxification of the oxyradical, metals and xenobiotics (Hoguet and Key, 2007). The depletion of GSH can result in a reduction in a cell's antioxidant defenses (Canesi *et al.*, 1999). The baseline levels of GSH which may be useful as the cellular biomarker of contaminant exposure was investigated in multiple life stages of *Palaemonetes pugio* (Hoguet and Key, 2007).

Since the oxidative metabolism of an animal is directly related to the O_2 uptake, the hypothesis of this study was that changing the molting stage may alter the antioxidant defense of *Scylla serrata*. Information on the responses of antioxidant defense in the molting cycle of *Scylla serrata* has not been reported previously.

The study of the antioxidant enzyme CAT and non-enzyme GSH can help in the understanding of the relationship of these antioxidant reactions in the molt cycle of crab *Scylla serrata*.

MATERIALS AND METHODS

Animal preparation

Mud crabs of 96–106 mm carapace width were collected from a soft-shell crab farm in Chantaburi province, Thailand. Molting stages of *Scylla serrata* were identified according to Warner (1977) and Salaenoi *et al.* (2004) as: A1 (6-hour postmolt), A2.1 (12-hour postmolt), A2.2 (24-hour postmolt), B1 (2-day postmolt), B2.1 (3-day postmolt), B2.2 (5-day postmolt), B2.3 (7-day postmolt), B2.4 (10-day postmolt), C (intermolt), D1 (2-week premolt), D2 (1-week premolt) and D3 (2-day premolt). After checking the appearance of the dactylopodite and propodus for the molting stages, crabs were anaesthetized in cold water at 4°C for 1 min, and sacrificed. Hemolymph samples were withdrawn from the sinus at the base of the pereopods and 10% trisodium citrate was used as an anticoagulant at the ratio of 5:1. The hepatopancreas, integument, gill and muscle were ground in liquid nitrogen then kept at -20°C for further analysis.

Determination of catalase

CAT activity was determined according to the method of Takahara *et al.* (1960). The reaction mixture was composed of 50 mM glycine buffer, pH 8, crude extract and 3 mM H_2O_2 . The mixture was incubated at 25 °C for 5 min before measuring the absorbance at 240 nm. The unit of enzyme activity was calculated as the ability which made the optical density at 240 nm of H_2O_2 decrease 0.01 in 1 min and was expressed as units per milligram protein.

Determination of glutathione

GSH was analyzed according to the method of Anderson (1985). Crude extract of

crab tissue was added with 5 % trichloroacetic acid before being centrifuged at $6,000\times g$ for 10 min. The clear supernatant was incubated with 5, 5'-dithiobis-(2-nitrobenzoic) acid (DTNB) in 100 mM phosphate buffer, pH 7 for 30 min at 25 °C. The absorbency of the solution was measured at 412 nm. The result was expressed as micromoles of 2-nitro-5-mercaptobenzoic acid (TNB) per minute per milligram protein. One unit of TNB was calculated from the increase in optical density by 0.001 unit within 1 min.

Statistical analysis

Results were expressed as mean \pm SD. Means were compared and analyzed by one way analysis of variance followed by a least significant difference test. Differences among the means were considered significant at the $P < 0.05$ level.

RESULTS

Antioxidant responses were evaluated using the CAT and GSH activity levels during the molting cycle of *Scylla serrata*. Samples were collected at the 12 stages of the molting cycle from the haemolymph, hepatopancreas, gill, integument and muscle. In the intermolt stage (C), CAT activities were 29.62 ± 1.09 , 20.47 ± 0.33 , 10.59 ± 0.13 , 2.01 ± 0.09 and 0.56 ± 0.01 units per milligram protein in the hepatopancreas, muscle, integument, hemolymph and gill, respectively (Figure 1). The activities of GSH were 12.28 ± 0.32 , 9.64 ± 0.44 , 1.38 ± 0.14 , 0.47 ± 0.04 , 0.11 ± 0.020 micromoles TNB per minute per milligram protein in the muscle, integument, hepatopancreas, gill and hemolymph, respectively (Figure 2).

CAT activities of the hepatopancreas, muscle and integument were highest in stage B2.1 (56.58 ± 0.31 , 32.88 ± 0.99 and 22.91 ± 1.11 units per milligram protein, respectively). The gill and hemolymph samples showed the highest activity at stage B2.2 and B2.4 (8.42 ± 0.36 and 3.30 ± 0.47 units per milligram protein, respectively). The highest CAT activities in each tissue showed

significant increases when compared to the intermolt stage.

The GSH levels of the integument and hemolymph were highest in stage D3 (17.88 ± 1.05 and 0.22 ± 0.06 micromoles TNB per minute per milligram protein, respectively). On the other hand, in the muscle, hepatopancreas and gill, the glutathione levels were highest in stage B1 (17.54 ± 0.19 , 12.69 ± 0.40 and 4.54 ± 0.0808 micromoles TNB per minute per milligram protein, respectively). The highest GSH levels in each tissue showed a significant increase when compared to the intermolt stage.

DISCUSSION

In the present study, the CAT and GSH activity showed the same pattern in all tissues where they increased in the premolt (D1–D3) then dropped in the early postmolt stages (A1–A2.2) and increased again in the late postmolt stages (B1–B2.4), with the exception of GSH in the hepatopancreas where it increased from the early postmolt stages (A1). GSH and CAT are commonly used as antioxidant biomarkers (Kusano and Ferrari, 2008). The oxidants and antioxidants may result from pollutant exposure as well as high metabolic activity (Filho *et al.*, 2001). Physiological and biological changes have been found in the molt stages such as an increase in the metabolic rate and oxygen consumption (Chang, 1995), variation of free sugar, glycogen and chitinase activity (Zou and Fingerman, 1999; Sugumar *et al.*, 2013) and of hyperglycemic hormone (Chung *et al.*, 1999). During the embryonic development of *Macrobrachium malcomsonii*, the oxygen uptake was increased and was followed by increased CAT activity and glutathione transferase (GST) (Arun and Subramanian, 1998). However, Hotard and Zou (2008) reported that GST which plays an important role in the biotransformation of xenobiotic compounds, is not involved in the molting cycle of fiddler crab. The increasing

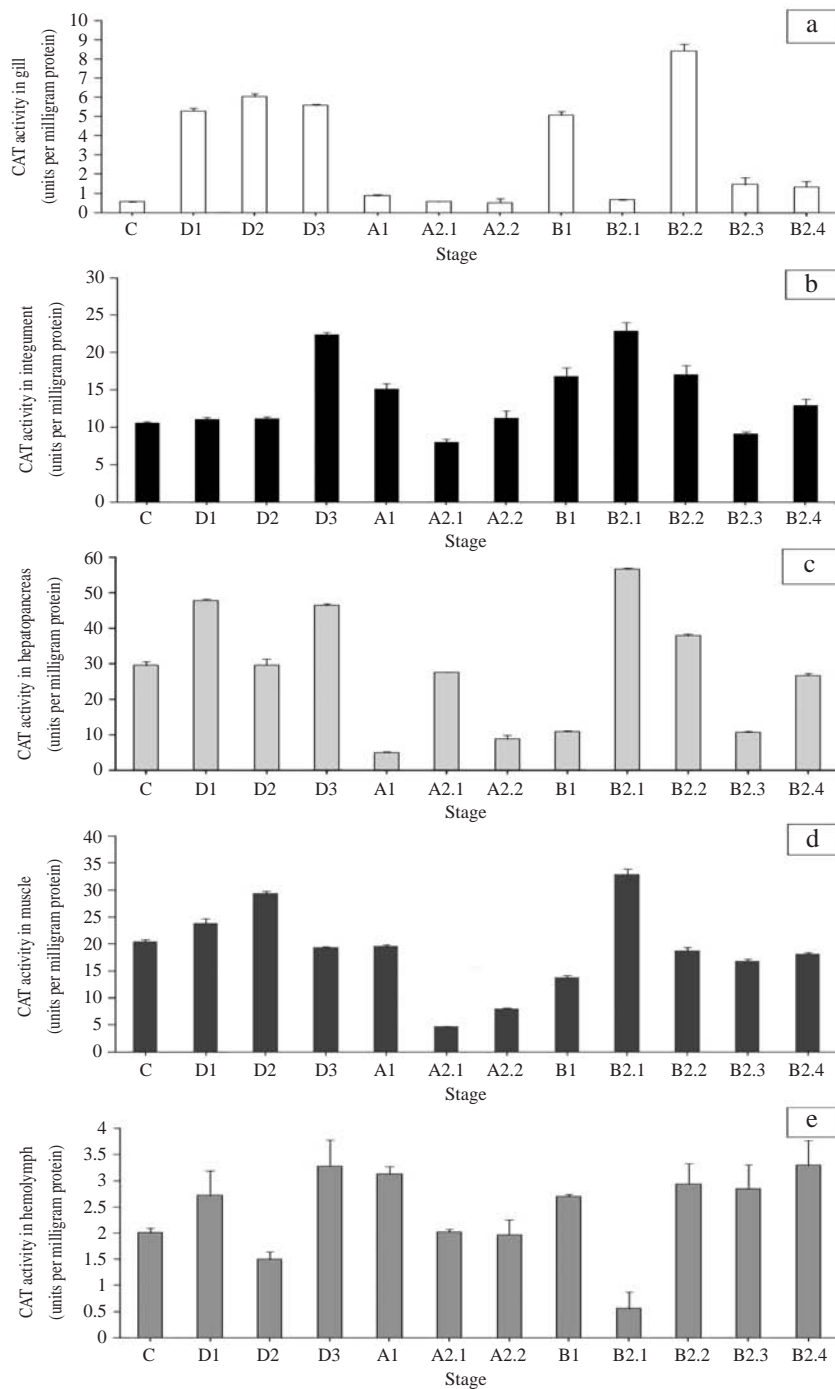


Figure 1 Catalase (CAT) activity during the molting cycle of *Scylla serrata* in different organs: (a) Gill; (b) Integument; (c) Hepatopancreas; (d) Muscle; and (e) Hemolymph. Molting cycle stages: A1 (6-hour postmolt), A2.1 (12-hour postmolt), A2.2 (24-hour postmolt), B1 (2-day postmolt), B2.1 (3-day postmolt), B2.2 (5-day postmolt), B2.3 (7-day postmolt), B2.4 (10 day postmolt), C (intermolt), D1 (2-week premolt), D2 (1-week premolt) and D3 (2-day premolt). (τ indicates the SD.)

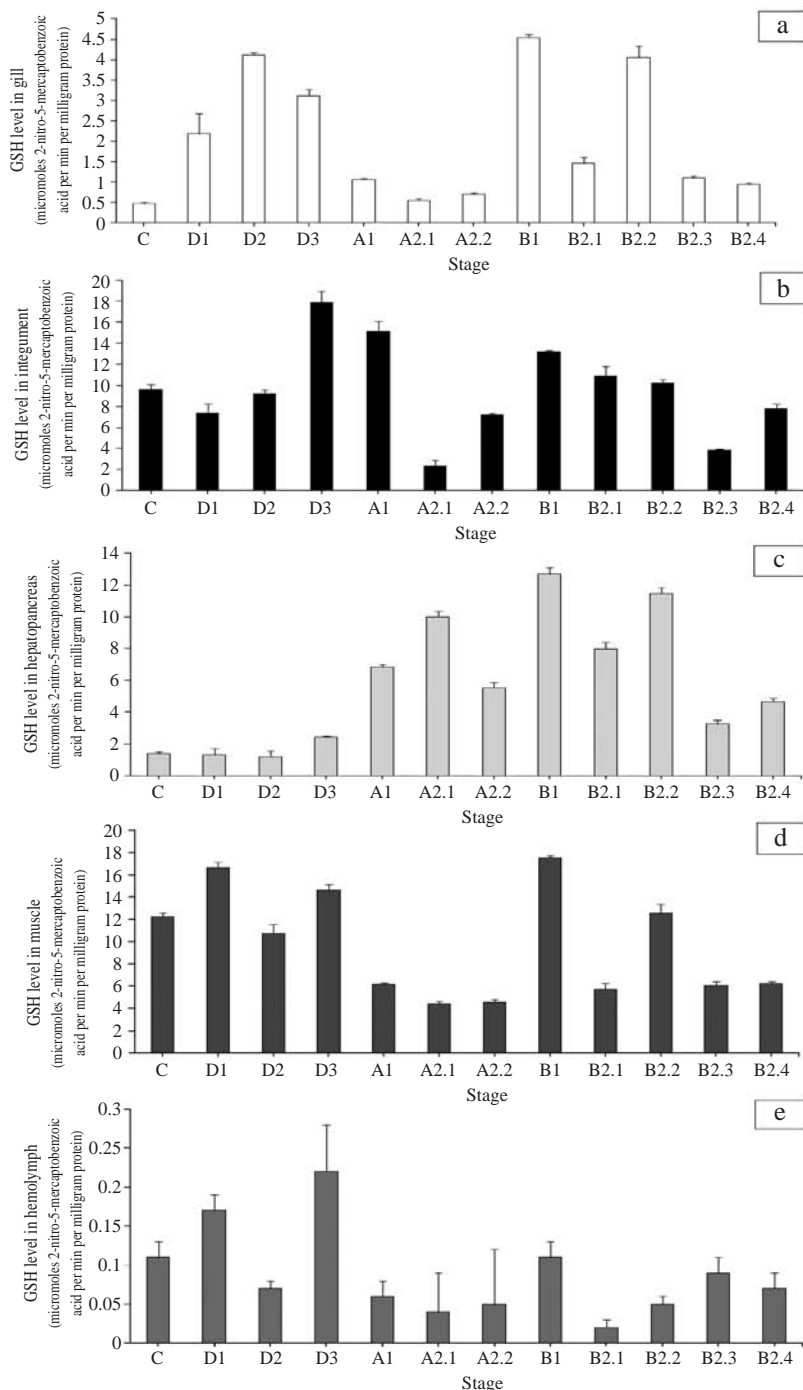


Figure 2 Glutathione (GSH) level during the molting cycle of *Scylla serrate* in different organs: (a) Gill; (b) Integument; (c) Hepatopancreas; (d) Muscle; and (e) Hemolymph. Molting cycle stages: A1 (6-hour postmolt), A2.1 (12-hour postmolt), A2.2 (24-hour postmolt), B1 (2-day postmolt), B2.1 (3-day postmolt), B2.2 (5-day postmolt), B2.3 (7-day postmolt), B2.4 (10 day postmolt), C (intermolt), D1 (2-week premolt), D2 (1-week premolt) and D3 (2-day premolt). (τ indicates the SD.)

activities of CAT and GSH in this study reflect an increase in the synthesis of H_2O_2 from the high metabolic activity in the premolt and late postmolt stages which coincides with a previous finding, that metabolic activity and oxygen consumption in decapod crustaceans were increased during premolt and declined rapidly following ecdysis (Chang, 1995). Sugumar *et al.* (2013) found that the total free sugar content in the hemolymph increased during the premolt stages and gradually declined in postmolt before sharply increasing in intermolt as well, while glycogen in the hepatopancreas decreased during postmolt A and B then increased during intermolt and premolt. During intermolt, crabs feed actively and the rate of feeding declines prior to molting and stops completely during molting; feeding begins again when the exoskeleton hardens in the postmolt stages (Phlippen *et al.*, 2000). To provide enough energy for ecdysis, the hepatopancreas plays an important role in the temporary storage of lipids and glycogen, and the accumulation of lipids was reported during late premolt D3–4 and then depleted during the postmolt A–B (Tian *et al.*, 2012). In the current study, GSH in the hepatopancreas increased from the early postmolt stage (A1) which was not the same pattern as in the other tissues. This may have been due to the multiple oxidative reactions that take place in the hepatopancreas which is a major site to generate free radicals and the reducing ability of GSH aids in the detoxification of oxyradicals (Houget and Key, 2007). It was also found that non-enzymatic antioxidants mainly acted in the hepatopancreas of *Paralomis granulosa* after air exposure (Romero *et al.*, 2007).

The highest activities of GSH in the muscle, hepatopancreas and gill were observed in stage B1 while the highest activities of CAT in the muscle, hepatopancreas and integument were in stage B2.1, which indicates that both enzyme and non-enzymatic antioxidants have complementary roles in hydrogen peroxide detoxification. The information derived from the current study

provides a pattern of antioxidant accumulation which corresponded with the metabolic activity through the molt cycle. Furthermore, future study on the correlation of antioxidant enzymes and lipid peroxidation should be undertaken, to gain better understanding of the redox status during the molt cycle.

CONCLUSION

Activities of CAT and GSH prominently showed in the hepatopancreas, integument and muscle. Expressions of CAT and GSH activity showed the same pattern which was significantly higher prior to molting and postmolt compared to intermolt. The variation in CAT and GSH activity corresponded well with the metabolic activity through the molt cycle.

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