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Expression of RNA encode FAMeT in mandibular organ of mud crabs *Scylla olivacea*

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Abstract: Farnesoate Acid Methyl Transferase (FAMeT) play important roles in converting farnesoate acid to methyl farnesoate (MF). The aim of the present study was to investigate expression and concentration of RNA encode FAMeT in intermolt and premolt stages. The experiment used mud crabs *Scylla olivacea*'s mandibular organ. Expression of RNA encode FAMeT showed difference of each stage. When compared with intermolt stage, the premolt stage indicated higher of RNA expression. RNA visualization showed amplicon length 450 bp. Likewise, the measuring of concentration of RNA encode FAMeT indicated excalation starting at intermolt to premolt stage.
Keywords. Crabs; Expression of RNA; FAMeT; Intermolt; Premolt.

Introduction

The development of molting in crustacean can be initiated by a variety of factors. One of the factors that lead to the emergence of early premolt is the presence of internal stimulation, thus continues to release of old carapace, known as molting. Molting is development cycle which is divided into phases namely intermolt, premolt, molting, and postmolt.

A hormone that has been widely known and has a role as a molting hormone is 20-hydroxyecdyson. Ecdisone synthesized and excreted by the Y organ and become the precursor for 20-hydroxyecdysone. Molting hormone works paracrine in reproduction and growth cells. Eyestalks, gonads, and hepatopancreas are the target organs of ecdysteroid to stimulate gonads development and growth[(1)]. Molting hormone is increased until late molting, as the spider crab (*Libinia emarginata*)[(2)], *M. rosenbergii* [(3)]. Farnesoate Acid Methyl Transferase (FAMeT) is an enzyme that plays an important role in transforming the farnesoate acid into methyl farnesoat (MF) as one of the hormones that play a role in reproduction and molting. In this study we observed expression of FAMeT in mud crab's organ based on two crucial phases.

Material and Method

Male crabs *Scylla olivacea* were obtained from South Sulawesi Province, Middle Indonesia. Intermolt and premolt phases of crabs were anasthetized in cold water (10°C). Crabs were dissected, and mandibular organ (MO) was separated from other glands.

The MO's RNA was extracted by RNeasy Mini Kit (Qiagen), amplificated by SuperScript III OneStep RT-PCR with Platinum Taq Polimerase (invitrogen) following manufacturer manual instruction. The RNA concentration was measured by spectrophotometer in 260 and 280 nm. Qualitative test of extracted RNA was performed by ß-actin.

Amplification of β-actin was conducted by β-actinF 5′-GAGCGAGAAATCGTTCGTGAC-3′ and β-actinR 5′-GGAAGGAAGGCTGGAAGAGAG-3′, as primers [(4)]. PCR condition was cDNA 45°C (30 minutes), pre-denaturation 95°C (30 sec), 50× (95°C during 10 sec, 60°C during 30 sec, 72°C during 20 sec), and 72°C (10 minutes). Then the results of RT-PCR was visualized in 2% agarose gel in TAE buffer 1×.

Amplification of FAMeT RNA was conducted by FAmeTQ1 5′-GGCACGGACGAGAACAA-3′ and FAmeTQ2 5′-GCGACGCTGAAGGAGAT-3′, as primers [(5)]. PCR condition was synthesis of cDNA 45°C (30 minutes), pre-denaturation 94°C (2 minutes), 35× (94°C 30 sec, 50°C during 1 minutes, 72°C during 30 minutes), and 72°C (10 minutes), then visualized in 1.5% agarose gel TAE buffer 1x.

Result and Discussion

Extracted RNA Quantification

Based on the results of amplification with β-actin, obtained amplicon of β-actin gene sized 202 bp, were expressly visible in all samples of intermolt and premolt phases. Extracted RNA was also tested quantitatively using a spectrophotometer at wavelengths of 260 and 280 nm. The wavelength of 260 nm as maximum absorption of nucleic acid, while 280 nm as maximum absorption of protein [(6)]. The purity of RNA determined through comparison of wavelength absorbance value.

Results of measurement of the concentration of extracted RNA with a spectrophotometer showed a quite varied in each sample. The extracted RNA concentration was highest in premolt (190 µg/µl) and lowest in intermolt (59.8 µg/µl), the comparison of wavelenght absorbance value showed best purity (more than 2 in 260/280 nm) in all samples (Table 1).

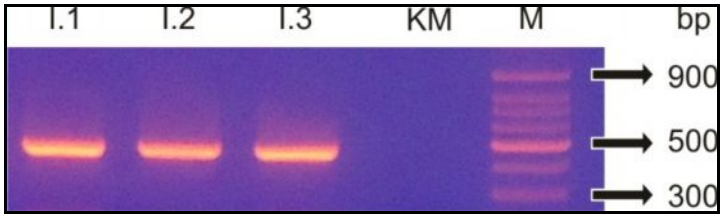
Table 1. Consentration and purity of *S. Olivacea*'s extracted RNA with spectrophotometer.

Sample	RNA concentration (µg/µl)	Purity (260/280 nm)
I.1	122.1	2.12
I.2	59.8	2.16
I.3	100.5	2.12
P.1	61.7	2.16
P.2	190.0	2.11
P.3	104.0	2.13

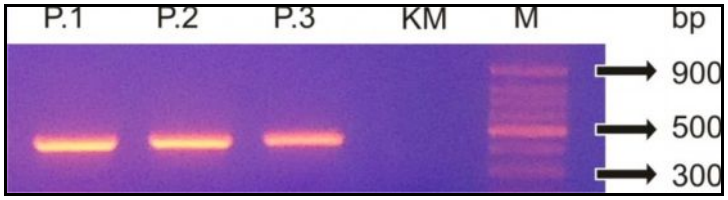
Intermolt= I.1, I.2, I.3; Premolt= P.1, P.2, P.3.

Expression of FAMeT RNA

The visualization of PCR product of FAMeT RNA showed amplicon length at 450 bp (Figure 1). The measurement of concentration showed the difference of concentration of PCR product between intermolt and premolt. The concentration of FAMeT RNA during intermolt were 774.0; 759.6; and 755.6, while in premolt were 800.2; 791.8; and 772.9. The disparity of concentration showed the excalation of expression from intermolt to premolt phase.



(a)



(b)

Figure 1. Amplification of FAMeT RNA of *S. olivacea*. (a) intermolt phase, FAMeTQ1 and FAMeTQ2 primers; M= marker 1000 bp; KM= control mixture; I.1, I.2, I.3= MO during intermolt. (b) premolt phase, FAMeTQ1 and FAMeTQ2 primers; M= marker 1000 bp; KM= control mixture; P.1, P.2, P.3= MO during premolt.

Discussion

The success of detection of FAMeT RNA was become access to the step of further study in MO. The result described physiological process in crabs during intermolt and premolt phases. Intermolt and premolt are phases which are describe a gradual changing process untill ecdysis become all ready. The last long deposit of energy happened during intermolt phase, henceforth the body prepares for unshell. In present study, phases observation performed by observing the morphology and paddle legs. These skills are needed if the observation made in the field. In this study the readiness of physiology for molting process could be observed by the expression of FAMeT RNA from hormone-producing organs.

FAMeT known as an enzyme, which has an important role as a catalyst for change farnesoate acid (FA) into MF, making s-adenosyl methionine as a cofactor in the synthesis end [(7)]. This study found that FAMeT RNA of MO has increased to the molting phases. Sequentially in intermolt towards premolt showed change in expression of FAMeT RNA, which indicated enzyme activity related to the preparation of molting.

FAMeT expression illustrates the synthesis of MF that takes place in the MO. Observations found an increased expression of these enzymes in line with the development of molting. Increased expression of enzyme in intermolt towards premolt phase clarify the roles of FAMeT as a converter of FA be MF. The increased expression were allegedly closely associated with the presence of a number of enzymes that works as a converter of FA into sesquisterpenoid products in MO. The work of these enzymes earn the products known as MF, which is a specific product which is produced only in MO[(8)].

The sesquisterpenoid MF in MO synthesized and secreted into haemolymph towards target organs. The MF released out from the organ with the assistance of binding protein in haemolymph towards target organ. One of many MF target organs is Y organs, which regulated reproduction and growth through the ecdysteroid production. The release of MF head for Y organs stimulates the synthesis of ecdysteroid, with the result that crabs on to the premolt phase. The increse of ecdysteroid level closely related with molting progress, such in crab *L. emarginata*[(2)].

The observation through spectrophotometer found difference concentration of FAMeT RNA between phases of crabs. In premolt phase presented higher concentration than intermolt. Nonetheless, this approach may not become postulate for the success of MO in synthezised and secreted the sesquisterpenoid MF. In present study we assumed that the expression of FAMeT RNA in MO indeed in low level, when compared with other organs. Because of other organs also found expression of FAMeT, but it was unclear about the role of this

enzyme. Some studies have reported FAMeT distribution in other organs, such as in species of *Metapenaeus ensis* [9], *Cancer pagurus* [10], *Nilaparvata lugens*[7], and *S. paramamosain*[5].

The MF presence at reproductive and molting phases [9], although the increase was not significance. In shrimp, FAMeT expression of MO was lower than other organs [9] so it is assumed that the possibility of the end of MF biosynthesis is catalyzed by an enzyme of FAMeT present in several tissues. If the assumption is proven, it will provide opportunities in study of FAMeT besides the MO in *S. olivacea*.

The role of the presence of FAMeT in other organs remains unclear, so that suspects that FAMeT also involved in the process of methylation in several bioactive molecule and not only catalyze the biosynthesis of MF during developmental and reproduction of crustacean[5]. The presence of various forms of FAMeT, may be related to the control mechanism to regulate synthesis of MF through regulating of enzyme forms activation expression which catalyzed the last step of FA conversion in MF [11].

This study was limited to MO and have not been able to reveal more about interacting regulations of FAMeT between MO and other organs, which may produce a different response. Therefore, there is an opportunity to explore the existance and role of the enzyme.

Conclusion

In mud crab *Scylla olivacea*,the expression of FAMeT RNA showed difference between intermolt and premolt phases. The premolt indicated higher of RNA expression than intermolt phase. We found an increased expression of these enzymes in line with the molting progress. Increased expression of enzyme in intermolt towards premolt phase clarify the roles of FAMeT as a converter of FA into MF. The RNA visualization showed amplicon 450 bp. Likewise, the measuring of concentration of FAMeT RNA indicated the excalation starting at intermolt to premolt phase. The expression of FAMeT also found in other organs, but it was unclear about the role of this enzyme and interaction. Therefore, further study is needed regarding the presence, interaction, and its role.

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