A Midgut Digestive Phospholipase A\textsubscript{2} in Larval Mosquitoes, \textit{Aedes albopictus} and \textit{Culex quinquefasciatus}

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Phospholipase A\textsubscript{2} (PLA\textsubscript{2}) is a secretory digestive enzyme that hydrolyzes ester bond at sn-2 position of dietary phospholipids, creating free fatty acid and lysosphospholipid. The free fatty acids (arachidonic acid) are absorbed into midgut cells. \textit{Aedes albopictus} and \textit{Culex quinquefasciatus} digestive PLA\textsubscript{2} was characterized using a microplate PLA\textsubscript{2} assay. The enzyme showed substantial activities at 6 and 8 \(\mu\)g/\(\mu\)l of protein concentration with optimal activity at 20 and 25 \(\mu\)g/\(\mu\)l of substrate concentration in \textit{Aedes albopictus} and \textit{Culex quinquefasciatus}, respectively. PLA\textsubscript{2} activity from both mosquitoes increased in a linear function up to 1 hour of the reaction time. Both enzymes were sensitive to pH and temperature. PLA\textsubscript{2} showed higher enzyme activities in pH 8.0 and pH 9.0 from \textit{Aedes albopictus} and \textit{Culex quinquefasciatus}, respectively, at 40°C of incubation. The PLA\textsubscript{2} activity decreased in the presence of 5 mM (\textit{Aedes albopictus}) and 0.5 mM (\textit{Culex quinquefasciatus}) site specific PLA\textsubscript{2} inhibitor, oleyloxyethylphosphorylcholine. Based on the migration pattern of the partially purified PLA\textsubscript{2} on SDS-PAGE, the protein mass of PLA\textsubscript{2} is approximately 20–25 kDa for both mosquitoes. The information on PLA\textsubscript{2} properties derived from this study may facilitate in devising mosquitoes control strategies especially in the development of inhibitors targeting the enzyme active site.

1. Introduction

Phospholipase A\textsubscript{2} (PLA\textsubscript{2}) hydrolyzes the sn-2 ester bond in phospholipids (PLs) [1]. These enzymes make up a large superfamily of proteins that act in a very wide variety of physiological and pathophysiological actions. PLA\textsubscript{2} actions include digestion of dietary lipids, remodelling cellular membranes, host immune defenses, signal transduction via production of various lipid mediators, and, in the case of platelet activating factor, inactivation of a lipid mediator. Research into noncatalytic PLA\textsubscript{2}s and into PLA\textsubscript{2} receptors and binding proteins reveals entirely new biological actions in which PLA\textsubscript{2} acts as a ligand rather than as a catalytic enzyme [2, 3]. Here, we focus attention on PLA\textsubscript{2} associated with digestion.

Lipid digestion and absorption take place in the insect midguts. Midgut cells produce and secrete lipases that digest dietary neutral lipids, such as triacylglycerols. PLA\textsubscript{2}s are responsible for two separate actions in insect physiology. For one, PLA\textsubscript{2}s hydrolyze a fatty acid from the sn-2 position of dietary PLs. Typically, the fatty acids esterified to the sn-2 positions are C18 and C20 PUFAs. These fatty acids include linoleic acid, 18:2n-6, and linolenic acid, 18:3n-3, one or the other of which is strictly essential nutritional requirements for most insects and nearly all vertebrates. Hence, midgut PLA\textsubscript{2}s are necessary for insects to meet one of their essential nutritional needs. A few insect and invertebrate species express a \(\Delta^{12}\) desaturase that inserts a double bond into oleic acid (18:1n-9), yielding 18:2n-6 and obviating the nutritional requirement [4–6]. The desaturation and elongation pathways necessary to convert C18 PUFAs to their C20 counterparts have been documented in several insect species [7, 8], from which we infer insects are able to meet all fatty
acid requirements via dietary linoleic and linolenic acids, coupled to the desaturase/elongation pathways. The second important PLA₂ activity contributes to digestion of dietary neutral lipids. Vertebrates, but not insects, produce bile salts that facilitate lipid digestion by solubilizing neutral lipids. In each PLA₂ reaction, hydrolysis of the sn-2 fatty acid from PL leads to a free fatty acid and to a lysoPLs and these lipids act as the necessary solubilizers that aid lipase digestion of neutral lipids.

PLA₂ is the key enzyme responsible in the hydrolysis of arachidonic acid which acts as precursors to lipid mediators such as prostaglandins [9]. PLA₂S occur abundantly in venoms [10], in pancreatic juices of mammals, and in synovial fluids [11]. While PLA₂S are well characterized in terms of protein and gene structures in mammalian physiology, there is relatively little information on the characteristics of insect digestive PLA₂. Nonetheless, these enzymes are very important in insect biology and they may become functional targets in some pest management programs. Seen in this light, there is a real need for new knowledge on insect digestive PLAs. In this paper, we begin to address that need. Here, we report on the presence and the characteristics of a midgut PLA₂ in larvae of the mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*.

2. Materials and Methods

2.1. Insect. The larvae of *Aedes albopictus* and *Culex quinquefasciatus* were collected from Kampung Semerah Padi, Petra Jaya, Kuching (1°34’59.3”N, 110°19’48.2”E). The larvae were collected with ovitraps filled with a cow-grass infusion solution following methods described by Tang et al. [12].

Ten ovitraps were placed near housing areas at Kampung Semerah Padi, Petra Jaya. The ovitraps were collected and replaced with new ones every week. The larvae collected were pooled together and fed with fish food until the 4th instar. The larvae were identified as *Aedes albopictus* and *Culex quinquefasciatus* [13] and midguts were isolated. A larva to be dissected was placed on a glass plate and the water surrounding the larva was blotted to dry. The midgut was removed by using a pair of forceps. By holding the thorax surrounding the larvae was blotted to dry. The midgut was dissected and contents were removed by pinching with the forceps. The a nal papillae, siphon, and Malpighian tubule attaching to the midgut were removed by pinching with the forceps.

2.2. PLA₂ Source Preparation. Midgut samples were homogenized in 200 µl buffer (0.1 M Tris[hydroxymethyl]aminoethane, pH 8; Sigma) mixed with 2 mM phenylthiourea (PTU, Sigma) by using a Bio Masher (Optima, Inc., USA). The homogenates were centrifuged at 735g for 3 minutes, then at 11,750g for 10 minutes. The supernatants were collected and used as the enzyme preparation.

2.3. Phospholipase A₂ Assay. PLA₂ substrate, 4-nitro-3-(octanoyloxy)benzoic acid (NOB; Enzo Life Sciences, Switzerland), was prepared following Nenad et al. [14] and Beghini et al. [15] with several modifications. The substrate was diluted with chloroform to 50 mg/ml. 20 µl (1 mg) aliquots were distributed into Eppendorf tubes and all of the moisture was evaporated to dryness. The dry residue was stored at −20°C. Immediately before the assay, the substrate was resuspended in 1 ml of acetonitrile. The suspension was vortexed until all the substrate dissolved.

A standard PLA₂ enzyme assay using 96-well plates was conducted following methods by Beghini et al. [15]. The standard assay mixture contains substrate (NOB), enzyme source, and buffer (0.1 M Tris buffer, pH 8) made up of a 200 µl of mixture in each well. After the addition of the enzyme source, the microplate was incubated (Asys Thermostar) for 40 minutes at 40°C. The NOB, through hydrolysis of an ester bond, will convert into a chromophore (4-nitro-3-hydroxybenzoic acid). The absorbance of chromophore concentration produced was quantified using a microplate reader at 405 nm. The effects of Ca²⁺, substrate and protein concentration, incubation time, pH, and temperature were investigated by varying each parameter.

2.4. Localizing the PLA₂ in Larvae. The homogenates were prepared from three different sections of the individual larvae. Three groups, A, B, and C, consisted of gut-free larval bodies, guts and contents, and isolated gut contents. Alimentary canals were removed by pulling out the eighth abdominal segment of the larvae with forceps, while holding its thorax with second forceps. The individual gut was quickly removed and placed into an Eppendorf tube containing 0.1 M Tris buffer and 2 mM PTU (Group B). The remaining bodies were collected into different tubes containing the same buffer (Group A). Isolated gut contents were obtained by separating the gut contents (Group C) from forty individual guts. All samples were prepared for enzyme assay as described in Table 1.

2.5. The Influence of Ca²⁺ on PLA₂ Activity. We conducted reactions in the presence of three different buffers: (1) Tris buffer with no additions; (2) Tris buffer amended with 5 mM CaCl₂; (3) Tris buffer amended with the Ca²⁺ chelator 5 mM EGTA (ethylene-glycol-bis (β-aminoethyl ether)-N,N,N',N’-tetraacetic acid). Each buffer was used during larvae dissection, homogenization, and enzyme activity assay.

2.6. Characterizing the Mosquito Digestive PLA₂. All experiments used midguts plus contents as the enzyme preparations. The influence of substrate and protein concentration, incubation time, pH, temperature, and the effect of site specific inhibitor for PLA₂ and OOPC on PLA₂ activity were assessed by varying each of the parameters.

2.7. Gel Electrophoresis. Estimation of this digestive PLA₂ from *Aedes albopictus* and *Culex quinquefasciatus* was performed according to the tricine-polyacrylamide gel electrophoresis (Tricine-SDS-PAGE) method by Schägger and von Jagow [16]. Migration of digestive PLA₂ was compared to the standard protein markers (Sigma) in a range of 26.6 kDa to 1.06 kDa. The protein marker consisted of triosephosphate isomerase from rabbit muscle (26.6 kDa), myoglobin from
horse heart (17.0 kDa), α-lactalbumin from bovine milk (14.2 kDa), aprotinin from bovine lung (6.5 kDa), insulin chain B, oxidized, bovine (3.496 kDa), and bradykinin (1.06 kDa). The protein bands on the electrophoresis gel were directly visualized by using silver staining according to the method by Gromova and Celis [17].

2.8. Statistical Analysis. Data were reported as means ± SEM of n experiments as appropriate. The significance of difference between groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test to determine the significant group. The confidence limit for significance was p ≤ 0.05.

3. Results

3.1. Localizing the PLA₂ Enzyme in Larvae. To determine the appropriate preparation for characterization of PLA₂, three samples were prepared. Substantially high PLA₂ enzyme activity from Aedes albopictus was recorded in gut plus content preparation (M = 0.328, SEM = 0.00) and gut content preparation (M = 0.3067, SEM = 0.01). There were no significant differences (F(2, 6) = 5.161, p = 0.05) in PLA₂ activity between all the preparations. In contrast, significantly higher PLA₂ activity was observed in Culex quinquefasciatus’ gut content preparation (M = 0.627, SEM = 0.01) (Figure 1). Similarly, lower PLA₂ activity was recorded in gut-free bodies of Aedes albopictus (M = 0.2733, SEM = 0.01) and Culex quinquefasciatus (M = 0.220, SD = 0.02). The gut plus content was used as an enzyme source in all subsequent experiments.

3.2. Calcium Ion (Ca²⁺) Dependency. Aedes albopictus PLA₂ activity was significantly higher in the buffer containing EGTA (M = 0.063, SEM = 0.01) and Tris buffer (M = 0.065, SEM = 0.00), while, for Culex quinquefasciatus, the PLA₂ activity was significantly high in Tris buffer (M = 0.089, SEM = 0.01) compared to enzyme activity in Tris buffer with additional calcium (M = 0.0087, SEM = 0.01) (Figure 2). These results suggest that the mosquito larval preparation is independent of Ca²⁺. Subsequent experiments were conducted in Tris buffer with no added Ca²⁺.

3.3. Characterization of PLA₂ Enzyme. The PLA₂ activities from Aedes albopictus and Culex quinquefasciatus showed a similar trend where the optimum enzyme activities were recorded at 6–8 μg/μL of protein (Figure 3). Aedes albopictus PLA₂ activity was fairly low at 2 and 4 μg/μL and then increased to a high level at 6 μg/μL (M = 0.090, SEM = 0.01) and 8 μg/μL (M = 0.091, SEM = 0.01) before it slightly declined at 10 μg/μL (M = 0.087, SEM = 0.00). Similarly, the Culex quinquefasciatus PLA₂ activity increased from 2 μg/μL.
(M = 0.0243, SEM = 0.01) of protein until it reached the highest activity at 8 μg/μL (M = 0.0713, SEM = 0.01). For subsequent experiment, 6 μg/μL of protein was used as an enzyme source for PLA2 assay in Aedes albopictus while 8 μg/μL was used as an enzyme source for PLA2 assay in Culex quinquefasciatus.

The PLA2 activities from mosquitoes, Aedes albopictus and Culex quinquefasciatus, were increased with increasing concentration of substrate until the enzyme concentration becomes a limiting factor in the reaction. The Aedes albopictus PLA2 activity increased in a linear manner with increasing substrate concentrations, up to 20 μg/μL (M = 0.121, SEM = 0.01) (Figure 4(a)).

PLA2 activity of Culex quinquefasciatus also increased from lower concentration, 5 μg/μL (M = 0.103, SEM = 0.00), until it reached its optimal activity at 25 μg/μL (M = 0.290, SEM = 0.01) (Figure 4(b)). There was no significant increase of enzyme activity at 30 μg/μL for both mosquitoes PLA2. Our standard concentration of substrate was 10 μg/μL for all subsequent experiments.

The PLA2 of Aedes albopictus was fairly low up to 20-minute incubations (M = 0.042, SEM = 0.01) and then increased substantially up to 50-minute incubation time (M = 0.161, SEM = 0.02). There was still another increase at 50 minutes (M = 0.161, SEM = 0.02) (Figure 5(a)). The enzyme activity remained constant at 60 minutes of incubation time.

On the other hand, there is no PLA2 activity of Culex quinquefasciatus recorded in the first 20 minutes of incubation time. Then, the enzyme activity continued to increase steadily even after an hour (M = 0.033, SD = 0.00) (Figure 5(b)). We used 40-minute incubations in all experiments.

The digestive PLA2 was sensitive to temperature. The reaction mixtures were incubated in a range of 24°C (room temperature) to 70°C. The Aedes albopictus and Culex quinquefasciatus PLA2 activity increased in a linear way from room temperature to a peak at 40°C (M = 0.116, SEM = 0.01; M = 0.097, SEM = 0.01) (Figures 6(a) and 6(b)). At higher temperatures (60–70°C) the enzyme activity declined. Our standard incubation temperature was set at 40°C.

pH of the reaction mixtures influenced PLA2 (Figures 7(a) and 7(b)). The PLA2 activity from Aedes albopictus and Culex quinquefasciatus increased from acidic condition to a mild alkaline condition. Aedes albopictus PLA2 increased gradually from pH 5.0 (M = 0.067, SEM = 0.01) until it reached a maximum PLA2 activity at pH 8.0 (M = 0.130, SEM = 0.01). At pH 9.0, the enzyme activity slightly decreased, but it is not statistically significant. Similarly, Culex quinquefasciatus reached its highest PLA2 activity at pH 9.0 (M = 0.1093, SEM = 0.01) before it dropped drastically at pH 10.0 (M = 0.035, SEM = 0.02). Post hoc multiple comparison (Tukey) analysis showed there was no significant difference in the enzyme activity from pH 6.0 to 9.0. Therefore, pH 8 was selected as a reaction pH for all subsequent reactions.
(a) The influence of incubation time on Aedes albopictus PLA₂ activity. 6 μg/μl of protein concentration was reacted with 10 μg/μl of substrate concentration in each different incubation time. Each point represents the mean ± SEM of triplicates from a single experiment representative of at least two experiments.

(b) The influence of incubation time on Culex quinquefasciatus PLA₂ activity. 8 μg/μl of protein concentration was reacted with 10 μg/μl of substrate concentration in each different incubation time. Each point represents the mean ± SEM of triplicates from a single experiment representative of at least two experiments.

**Figure 5**

(a) The influence of incubation temperature on Aedes albopictus PLA₂ activity. 6 μg/μl of protein concentration was reacted with 10 μg/μl of substrate concentration in different incubation temperature, respectively. Each point represents the mean ± SEM of triplicates from a single experiment representative of at least two experiments.

(b) The influence of incubation temperature on Culex quinquefasciatus PLA₂ activity. 8 μg/μl of protein concentration was reacted with 10 μg/μl of substrate concentration in different incubation temperature, respectively. Each point represents the mean ± SEM of triplicates from a single experiment representative of at least two experiments.

**Figure 6**

(a) The influence of reaction pH on Aedes albopictus PLA₂ activity. 6 μg/μl of protein concentration was reacted with 10 μg/μl of substrate concentration in different pH condition, respectively. Each point represents the mean ± SEM of triplicates from a single experiment representative of at least two experiments.

(b) The influence of reaction pH on Culex quinquefasciatus PLA₂ activity. 8 μg/μl of protein concentration was reacted with 10 μg/μl of substrate concentration in different pH condition, respectively. Each point represents the mean ± SEM of triplicates from a single experiment representative of at least two experiments.

**Figure 7**
3.4. The Influence of OOPC on PLA₂ Activity. Reactions in the presence of 50 μM to 500 μM of OOPC led to a dose-related decline in PLA₂ activity (Figures 8(a) and 8(b)). The PLA₂ of \textit{Aedes albopictus} was significantly inhibited in the presence of 5000 μM of OOPC \(F(3, 8) = 5.886, p = 0.020\). However, the PLA₂ of \textit{Culex quinquefasciatus} was not statistically inhibited in the presence of PLA₂ inhibitor, OOPC \(F(2, 6) = 3.651, p = 0.092\).

3.5. Protein Mass Determination of Partially Purified Digestive PLA₂. The protein electrophoretic profile of partially purified PLA₂ from \textit{Aedes albopictus} showed bands in different sizes which range from 14.6 to 20.3 kDa (Figure 9(a)) while for partially purified PLA₂ from \textit{Culex quinquefasciatus} it showed only one band which was at 25 kDa (Figure 9(b)). The presence of band at a similar size (25 kDa) suggests the presence of similar protein, which is the PLA₂.

4. Discussion

In this paper, we report on the characterization of a digestive PLA₂ in \textit{Aedes albopictus} and \textit{Culex quinquefasciatus} larvae. During our initial experiment, we compared the PLA₂ activities in selected fractions of the alimentary canal. Higher PLA₂ enzyme activity was recorded in the midgut plus content and isolated gut content preparations. Similar findings were
reported for a related mosquito species, *Aedes aegypti*, where higher PLA₂ enzyme activity was recorded in midgut plus content preparation. These findings suggested that midgut cells secrete more PLA₂ than they store [18].

Secretory PLA₃ is characterized as a low molecular weight molecule (13–55 kDa) [10, 19] that catalyzes substrate in full activity in the presence of calcium. sPLA₃ differs from other PLA₂ as it acts extracellularly [20]. The enzyme was secreted from the cells before catalyzing the substrate which usually occurs in the lumen of the insects’ midgut [21].

The characterization of PLA₂ in insects was assayed with radioactive substrate in the past. Here, we used a microplate assay using the chromogenic substrate, NOB. NOB is widely used in characterizing PLA₂ from snake venom [22–24] and human serum [14]. Our PLA₂ assays were performed on mosquito samples that have been partially enriched using Heparin column chromatography (1 ml HiTrap Heparin, from the cells before catalyzing the substrate which usually occurs in the lumen of the insects’ midgut [21].

In primary screwworm preparations [25]. For this work, our simple microplate assay used only a small amount of protein and substrate to obtain an optimal PLA₂ activity. This is an advantage for an investigation with limited amounts of protein sample. Since we collected our larval mosquito from the field, the number of individual larvae in each collection varied depending on their local environmental condition. The microplate assay is a practical but effective method to conduct our experiments with limited enzyme source.

Ca²⁺ is essential for both catalysis and binding of some enzymes to the substrate [26]. A study of Ca²⁺ requirement in primary screwworm PLA₂ preparations showed that the enzyme activity was almost abolished in the presence of calcium chelator, EGTA. The PLA₂ dependency on Ca²⁺ varied across species [21]. Previous studies on PLA₂ from primary screwworm, *C. hominivorax* [25], robber flies, *Asilis* sp. [27], and adult tiger beetles, *Cicindela circumpicta* [28], showed strict Ca²⁺ requirement for catalysis. Several studies on PLA₂ from venom sources such as rattlesnake, *Crotalus durissus cascavella*, venom [15] and sea anemone, *Aiptasia pallida*, nematocyst venom [29] also showed strict Ca²⁺ requirement [30].

PLA₂ activity in the *Aedes albopictus* and *Culex quinquefasciatus* preparations revealed slightly higher enzyme activity in Tris buffer without additional calcium. This is in agreement with PLA₂ from the midgut of tobacco hornworm [31] and *Aedes aegypti* larvae midgut [18].

Generally, enzyme activities are influenced by biophysical parameters, protein and substrate concentrations, pH, temperature, and reaction time. Increasing the amount of either enzyme or substrate generally will increase reaction rates because more active sites are available for reaction and more substrate molecules can bind with the active sites.

The *Aedes albopictus* and *Culex quinquefasciatus* preparations responded to the usual biophysical parameters in a way fairly similar to the *Aedes aegypti* preparations [18] except for its sensitivity to OOPC. PLA₂ activity in *Aedes aegypti* [18] was less sensitive to OOPC than the *Aedes albopictus* and *Culex quinquefasciatus* preparations. *Aedes albopictus* PLA₂ was inhibited at 5000 μM of OOPC as compared to *Culex quinquefasciatus* PLA₂, which was inhibited at a lower concentration of OOPC (500 μM). In contrast, the PLA₂ activity from *Aedes aegypti* [18] did not show any significant decrease in the presence of OOPC (5–5000 μM).

Similar finding was also recorded for tobacco hornworm, *Manduca sexta* [31], digestive PLA₁ where there was no inhibition in its enzyme activity when exposed to different concentrations of OOPC in a range of 5–500 μM. On the other hand, the primary screwworm PLA₂ preparation was more sensitive to OOPC, 50 μM [25].

The *Aedes albopictus* and *Culex quinquefasciatus* PLA₂ activity increased with time in reactions up to 1 hour, in agreement with PLA₂ enzyme from other insects, *Aedes aegypti* [18] and *C. hominivorax* [25], and PLA₂ from venoms, *Bothrops jararacussu* [22].

PLA₂ from *Aedes albopictus* and *Culex quinquefasciatus* shares some similarity and differences with a related species, *Aedes aegypti*, where the optimal enzyme activity was at 40–50°C. However, the optimum pH condition differs. *Aedes aegypti* PLA₂ activity was optimal at pH 9.0, which is similar with *Culex quinquefasciatus* PLA₂, while for *Aedes albopictus* the enzyme activity declined at pH 9. Although the maximum enzyme activity differs, all mosquitoes PLA₂ reaction studied slowed down in acidic conditions. This is in broad agreement with the pH conditions of insect midguts, which can be very high in lepidoptera and more acidic in mosquitoes [25].

This study has estimated the size of PLA₂ from *Aedes albopictus* and *Culex quinquefasciatus* and provided information on partially purified PLA₂ from crude homogenate of mosquito larval midgut by using Heparin column. In this study, both *Aedes albopictus* and *Culex quinquefasciatus* larval midgut PLA₂ estimated molecular weights were found in a range of secretory insect PLA₂. The molecular weights were estimated at 14.6–25 kDa and 25 kDa for *Aedes albopictus* and *Culex quinquefasciatus*, respectively. Distinct band at 25 kDa was shown in both PLA₂ preparations of *Aedes albopictus* and *Culex quinquefasciatus* which are more likely the protein of interest in this study.

The estimated sizes agreed with the classic characteristic of sPLA₂, which consists of small MW enzyme ranging from 13 to 15 kDa which were obtained from various organisms such as snake venoms, porcine pancreas, fungus, and bacteria [1] and for tiger beetle and human PLA₂, which were reported to be 22 kDa and 55 kDa, respectively [19].

However, other investigations such as amino acid sequence and X-ray crystal structures of *Aedes albopictus* and *Culex quinquefasciatus* need to be conducted in order to classify the type of the digestive PLA₂.

5. Conclusions

*Aedes albopictus* PLA₂ from different sample preparations showed no significant difference in their activity, while for *Culex quinquefasciatus* significantly higher PLA₂ in gut contents was shown if compared to other preparations. PLA₂ from both enzymes did not require calcium (Ca²⁺) for full enzyme activity and both showed increasing of enzyme activity with increasing concentration of substrate. The PLA₂...
enzymatic assay from both mosquitoes showed accumulation of chromogenic substance up to 60 minutes of incubation time at 40°C. Both enzymes reacted in full catalytic activity in alkaline condition. *Aedes albopictus* PLA2 was significantly inhibited by site specific PLA2 inhibitor, OOPC. However, *Culex quinquefasciatus* PLA2 was not significantly inhibited by the same inhibitor. Based on the electrophoretic pattern of the enzyme samples, protein band at 20–25 kDa was observed in both mosquitoes. To conclude, there were no differences between the characteristic of PLA2 from *Aedes albopictus* and *Culex quinquefasciatus* except for its inhibition toward site specific inhibitor PLA2, OOPC, where the inhibitor does not affect the *Culex quinquefasciatus* PLA2 activity.

**Data Availability**

The data for the findings of this study will be available upon request to any of this article’s authors.

**Disclosure**

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

**Conflicts of Interest**

There are no conflicts of interest regarding the publication of this article.

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