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Research Article

A Midgut Digestive Phospholipase A_2 in Larval Mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*

Nor Aliza Abdul Rahim,[1] **Marlini Othman**,[1] **Muna Sabri**,[2] **and David W. Stanley**[3]

[1]*Department of Paraclinical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia*
[2]*Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia*
[3]*United States Department of Agriculture, Agricultural Research Service, Biological Control of Insects Research Laboratory, 1503 S. Providence Road, Columbia, MO 65203, USA*

Correspondence should be addressed to Nor Aliza Abdul Rahim; arnaliza@unimas.my

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Phospholipase A_2 (PLA_2) is a secretory digestive enzyme that hydrolyzes ester bond at *sn-2* position of dietary phospholipids, creating free fatty acid and lysophospholipid. The free fatty acids (arachidonic acid) are absorbed into midgut cells. *Aedes albopictus* and *Culex quinquefasciatus* digestive PLA_2 was characterized using a microplate PLA_2 assay. The enzyme showed substantial activities at 6 and 8 μg/μl of protein concentration with optimal activity at 20 and 25 μg/μl of substrate concentration in *Aedes albopictus* and *Culex quinquefasciatus*, respectively. PLA_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_2 showed higher enzyme activities in pH 8.0 and pH 9.0 from *Aedes albopictus* and *Culex quinquefasciatus*, respectively, at 40°C of incubation. The PLA_2 activity decreased in the presence of 5 mM *(Aedes albopictus)* and 0.5 mM *(Culex quinquefasciatus)* site specific PLA_2inhibitor, oleyloxyethylphosphorylcholine. Based on the migration pattern of the partially purified PLA_2 on SDS-PAGE, the protein mass of PLA_2 is approximately 20–25 kDa for both mosquitoes. The information on PLA_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_2 (PLA_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_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_2s and into PLA_2 receptors and binding proteins reveals entirely new biological actions in which PLA_2 acts as a ligand rather than a catalytic enzyme [2, 3]. Here, we focus attention on PLA_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_2s are responsible for two separate actions in insect physiology. For one, PLA_2s 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_2s are necessary for insects to meet one of their essential nutritional needs. A few insect and invertebrate species express a Δ^{-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