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Research Article

Toxicity of Dithiothreitol (DTT) Onlipolytic Activity of *Maruca vitrata* (Fabricius)

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Abstract

Losses of agriculture product because of pest have been evaluated worldwide. The partial characterization of larval lipase of *Maruca vitrata* (Fabricius) shows higher activity at pH 7.9 and K_m 9.56×10^{-2} mM. The gradual increase in lipase activity was observed from 4 to 8-day larvae, decrease from 8 to 13th day and maximum activity in 8-day in control and dithiothreitol treated larvae of *M. vitrata*. The lipase activity of 9-day control larvae is 0.280 and dithiothreitol treated larvae is 0.274 µmol FFA/mg protein/25 min. The lipase activity of 8-day control larvae was 1.02 fold more than dithiothreitol treated larvae. The inhibitory role of dithiothreitol on larval lipase *Maruca vitrata* has been attempted in present work.

Keywords: Lipase, larva, Dithiothreitol (DTT), *M. vitrata* (F).

Introduction

Spotted pod borer, *Maruca vitrata* (Fabricius) cause significant economic loss, especially cause damage to economical parts such as flowers, buds and pods [1]. The yield loss due to *M. vitrata* was estimated to be 9-84% [2]. In India cowpea is one of important leguminous plant. It is one of the ancient crops known to man. Cowpea is one of the most important crops cultivated by small scale farmers as a subsistence crop [3]. Lipase (EC 3.1.1.3), which catalyses the hydrolysis of fatty acid ester bonds and widely distributed among animals and plants [4]. Most characteristic property of lipase is that it acts on substrate at the interface between aqueous and the lipid phase [5]. The efficacy of a toxic substance Dithiothreitol (DTT) has been shown to be toxic to cultured cells by inducing the generation of reactive oxygen species that ultimately cause cell death. DTT is routinely used as a thiol molecule in molecular-biological laboratories. It is applied for protein denaturation [6]. However, the information about the toxicity of lipase activity during larval development of *M. vitrata* is rather scanty hence present work is undertaken for the study.

Material and Methods

Insect rearing

Rearing of pod borer, *M. vitrata* (Fabricius) was attempted according [7] in laboratory condition at temperature 28°C and 78% and humidity on their food cowpea pods. Larval stages from 4 to 13-day larvae were taken for study.

Toxicity of dithiothreitol on lipase activity

Toxicity of dithiothreitol (DTT) on lipase activity were attempted according to [8]. Partially purified 0.25 mL enzyme was pre-incubated for 30 minutes in 0.25 mL solution of 2 mM dithiothreitol (DTT) and 1 mL of phosphate buffer (7.9 pH) in separate glass stoppered conical flask and after preincubation 0.25 mL of substrate was added to above reaction mixture [9] in total

volume of 1.75 mL. At the end liberated fatty acids was measured calorimetrically [10]. The absorbance was read at 540 nm.

Partial purification of lipase

By using ammonium sulphate precipitation method partial purification of lipase was attempted [11]. Enzyme assay included incubation period 25 minutes, 37^0C temperature, 2 mL Cu-TEA reagent. The flasks were in rotary shaker. Contents shake after addition of 10 mL chloroform and kept for separation of aqueous and organic phase. After 5 min chloroform phase was transferred to centrifuge tube. After 2 mL of water was added without mixing and the tubes were centrifuge for few minutes. Then 2 mL of chloroform phase was taken in stoppered test tube. After that 1 mL of colour reagent was added. At the end liberated fatty acids were measured calorimetrically. The absorbance was read at 540.

Result and Discussion

Result

The partial characterization of larval lipase of *Maruca vitrata* (Fabricius) shows higher activity at pH 7.9 and K_m 9.56×10^{-2} mM. The gradual increase in lipase activity was observed from 4 to 8-day larvae, decrease from 8 to 13th day and maximum activity in 8-day in control and dithiothreitol treated larvae of *M. vitrata*. The lipase activity of 8-day control larvae is 0.280 and dithiothreitol treated larvae is 0.274 µmol FFA/mg protein/25 min. The lipase activity of 8-day control larvae was1.02-fold more than dithiothreitol treated larvae.

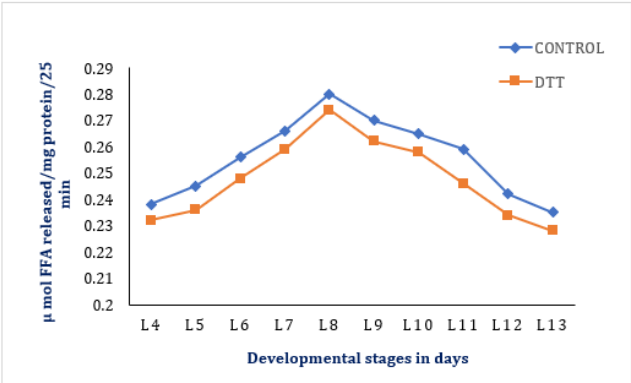

Figure 1: Toxicity of dithiothreitol (DTT) on larval lipase activity of *M. vitrata*.

Discussion

Metal ions like Fe^{2+}, Fe^{3+} and Cu^{2+} inhibits the lipase enzyme activity [12]. The lipase enzyme was inhibited by Fe^{2+} ions [13]. In *Rhodnius prolixus* lipase activity increased with Ca^{2+} ions [5]. Lipase activity was maximum at pH 8.2, Km value 0.310 mM, V_{max} value 1.479U/mg protein and 40% activity inhibited by Fe^{3+} in gypsy moth, *Lymantria dispar* midgut lipase [15].

In the present study, lipase showed maximum activity at pH 7.9 and K_m 9.56×10^{-2} mM in the larvae of *M. vitrata* suggests lipase maximally active at an alkaline pH. Low km value indicates more affinity of enzyme with substrate. In the present study, lipase activity decreased with dithiothreitol larvae of *M. vitrata* indicates potent inhibitors react with some functional group in the active site to block substrate site or to leave it catalytically inactive also high concentration of divalent ions form salt bridges between carboxylic groups of amino acids and reduces enzyme activity. This work may be useful to control *M. vitrata* in the future.

Conclusion

The lipase activity of 8-day dithiothreitol treated larvae was 1.02 fold less than control larvae. The changes in lipase activity may be due to different rate of lipolysis to cope up with the high energy demand or less energy requirement in larval developmental stages of *M. vitrata.* This study will help to design new and more specific strategies for insect control. The present study can be further extended for understanding the molecular analysis and sequencing of proteins.

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