International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Assessment of Lysozyme Activity in Bacteria Challenged Haemolymph of *Achaea janata* Larvae

Ragamalika G, Ravindar G[1], Nagaraja Rao P[2]

Molecular Entomology Lab, Department of Zoology, Osmania University, Hyderabad, Telangana, India

Abstract: ***Lysozyme was the first humoral antibacterial factor to be studied in insects and it was considered the main immune factor in the haemolymph. We have been investigating lysozyme activity in bacterial challenged haemolymph of Achaea janata larvae. This is a major crop pest and polyphagus lepidoptera insect. Results showed that the initially no activity after 5 min lysozyme activity was gradually increase and it reached maximum at 15 min post bacterial challenge haemolymph. Later lysozyme activity was constantly decreased. The present study we can conclude that Lysozyme shows its efficiency if not immediately but within 5-15 or 20 min time interval thus protecting the host cell and it may triggers the insect immune humoral response by lysis the lysozyme by the action of M.luteus bacteria challenge.***

Keywords: Lysozyme, insect, Achaea janata, humoral immunity and heamolymph

1. Introduction

Lysozyme is an enzyme that attacks bacterial cell walls. In that one of the cell organelle is having the enzyme to cleave or lysis the cell wall by the hydrolyzing capacity is called Lysozyme (Hultmark, 1996). It was the first humoral antibacterial factor to be studied in insects and was considered the main immune factor in the haemolymph (Jiang, 2008). It degrades the cell wall by cleaving the sugar backbone of the peptidoglycan component. Specifically, lysozyme adds water to (hydrolyze) the glycosidic bond between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). In general, lysozymes use three possible killing mechanisms against microorganisms (*Qian et al.,* 2009). In which, binding of cationic lysozyme with polyanionic peptidoglycon layer of bacteria is common and fundamental one or first method of action. Stimulation of autolysin activity and bacterial membrane perturbation is second mode of action and hydrolysis of peptidoglycan is the third one cleavage of peptidoglycan in the cell.

Lysozyme is a common constituent of biological tissues and secretions, it has been found in hemolymph of lepidoptera. In lepidopteron insects such as *Hylophora cecropia*, *Bombyx mori*, *Manduca sexta*, *Spodaptera exigua* over expression of c-type lysozyme after immune challenge has been evidence in various tissues-fat body, hemocytes, malphigian tubes, midgut etc (*Bae and Kim* 2003; *Lavine et al*., 2005). *Achaea janata* is a polyphagus insect (Lepidoptera), commonly called as Caster Semilooper, due to their mode of locomotion and feed on caster (*Ricinus communis*).

The aim of this study is to understand how a microbial activity in insect haemolymph and it triggers the immune humoral response by lysis the lysozyme by the action of *M.luteus*, the immune system of insect *Achaea janata* was by using *Micrococcus luteus* (Gram positive) bacteria.

2. Material and Methods

2.1 Test Insect Rearing

The test insect for the present study was *Achaea janata* (Linnaeus), Order: Lepdoptera, Family: Noctuidae, Genus: *Achaea*, Species: *A.janata*, is a Noctuid moth, the caterpillars of which are termed semi looper due to their mode of locomotion and feed on caster *Ricinus communis* as they feed off the fresh castor oil plant, it is a polyphagous insect. The entire life cycle from egg to adult takes in 48-50 days (Singh, 2007).

2.2 Preparation of Bacterial Cultures

Fresh bacteria cultures (freeze-dried cultures) were obtained from the institute of microbial type culture collection (MTCC) Chandigarh, India. These bacterial cultures were maintained on 1.5%LB medium at 37°C in a bacteriological incubator (REMI, Modal) used. The bacteria were used in mid logarithmic phase at a titer of 5×10^5 cfu ml^{-1}.an aliquot amount of 10µl of *Micrococcus luteus (M.luteus)* was used to inoculate each larva.

Collection of haemolymph from test insect:
Haemolymph was collected by cutting abdominal thoracic pro-leg control and bacterial challenged *Achaea janata* larvae (Dorrah 2009).The free flowing haemolymph has been collected into ice cold sterile polyprophylene containing anticoagulant, haemolymph was stored at -20 until further use.
Lysozyme activity was estimated using one of the Turbido metric method by Sepctroscopy (Azambuja et al 1991). In briefly, the spectroscopy method has been adopted to determine the concentration of lysozyme in the insect hemolymph. Initially standardization of total protein concentration was done by Bradford's method absorbance recorded @450nm. The concentration of lysozyme activity was determined in the test sample of insect haemolymph against the standard. 0.5µl of haemolymph was taken and 50µl of Phosphate buffer saline was added to, followed by the addition of 0.9µl of *Micrococcus luteus* equally to all the test tubes. Then the tubes were incubated at room

Licensed Under Creative Commons Attribution CC BY

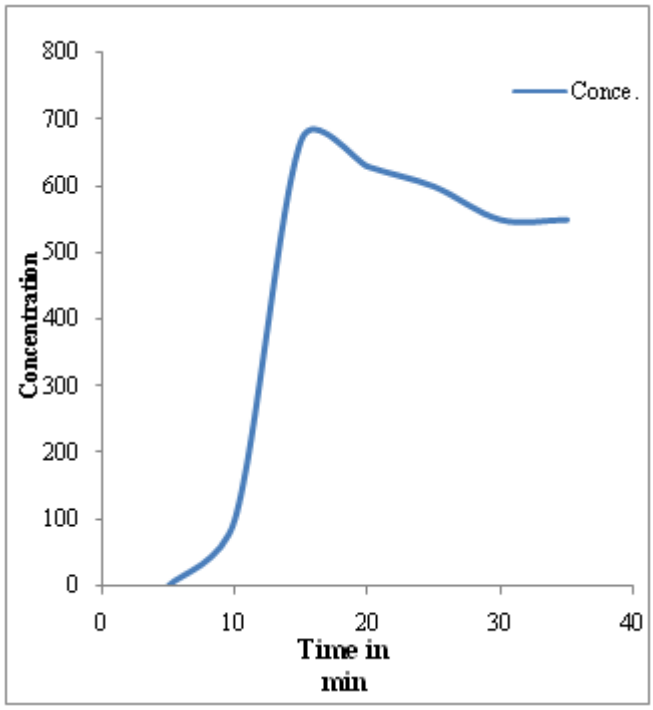
temperature and lysozyme activity was noted at different time intervals with a difference of 5 min each till a time period of 35 min. The lysozyme activity was noted by recording the absorbance@450nm.

3. Results and Discussions

The standardization graph shows a straight line where all the points co-inciding the straight line and from graph, the concentration was calculated which is 300ug/0.5ul. By using the standard graph,the effect of time on enzyme concentration was correlated and observations were made at different time intervals of 5-35 min with a difference of 5 min respectively.

The lysozyme concentrations at different time intervals were noted by taking absorbance at 450nm by spectroscopy (Azambuja et al 1991) at 5 min interval in BCH (Bacterial challenged haemolymph)the lysozyme concentration was zero, and rest of concentrations at different time intervals are 100ug concentration at 10 min, 670ug at 15 min,630ug at 20 min,600ug at 25 min, and 30,35 min the concentration was 550ug.

Graph1: Lysozyme activity in bacteria challenged haemolymph of *Achea janata* larvae at different intervals. Each point is a mean of three replicates ±SE



According to the graph, the first interval that was recorded at 5 min there was no concentration observed after BCH was zero concentration .Then the concentration of lysozyme is reached 100ug at 10 min time interval, after that gradually increased 670ug at 15 min. At the time of 20-25 min the concentrations come to 630ug, 600ug that was deceased, and 30, 35 min the concentrations come to a constant level.

Generally enzyme activity is affected by many factors like temperature, pH, substrate, enzyme concentration and time (Prager and Jolles 1996). The present study is based on the affect of time and its effect on activity with variation in the time intervals. The activity increases with time, the enzyme activity depending upon the concentration significantly varies (Barbara Masschalck). According to the Azambuja et al 1991 observation of the graph plotted with time on X-axis and O.D values on Y-axis evident that the activity differs. Chadwick, (1970) and Hoffman et al., (1996) stated that the lysozyme activity level was rapidly increased in various insects upon bacteria challenge.

Summarizing the activity of Lysozyme according to the concentration at different time intervals, it can be understood that if not certainly but there is a significant effect of time on enzyme activity which will be highly effective not initially but after a time period that is of 5-15 or 20 min and variably stable thereafter, as per the last time interval of 35 min taken for the present study.

As discussed earlier the enzyme activity is affected by many other factors which show their effect on its catalytic activity. In the present study, Time factor played an important role in ultimately responding or showing its (Lysozyme) antibiotic effect, through which ever media the bacteria is invading the host. Finally, we may conclude that Lysozyme shows its efficiency if not immediately but within 5-15 or 20 min time interval thus protecting the host cell.

It has been assumed that lysozyme either in the naive insects or expressed during microbial infection hydrolyzes peptidoglycon of bacteria and generates a chemical signal which triggers antimicrobial proteins and peptides synthesis in fat body proved that lytic activity of lysozyme relatively produces soluble peptidoglycan fragments, which will provoke antibacterial peptide production.

4. Acknowledgements

We acknowledge Prof. P. Nagaraja Rao for their suggestion and guidance. This research work was supported by University Grant Commission (UGC), New Delhi, India

References

[1] Azambuja,P., Garcia, E.S., Ratcliffe, N.A., and Warthen, J.D., Jr. (1991). Immune-depression in Rhodnius prolixus induced by growth inhibitor, azadirachtin. J. Insect Physiol., 37:771-777
[2] Bae,S., and Kim, Y., (2003). Lysozyme of the beet armyworm, Spodoptera exigua activity induction and cDNA structure. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 135:511-519
[3] Bradford, M.M (1976). A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein binding. Analyt.Biochem., 72:248-254
[4] Chadwick, J.S. (1970): Relation of lysozyme concentration to acquired immunity against pseudomonas aeruginosa in *Galleria mellonella*. J. Invertebr. Pathol. 15,455-456
[5] Dorrah, A. Moataza (2009). Phenoloxidase and Lysozyme activity in naive and immune responsive larval Parasarcophaga Hertipes (Diptera:Sarcophagidae. Efflatounia, 9:10-17

Licensed Under Creative Commons Attribution CC BY

[6] Hoffman, J. A., Reichert J.M., Hetru C (1996). Innate immunity in higher insects. Curr.Opin. Immunol. 8, 8-13
[7] Hultmark, D.(1996). Insect lysozymes. In: Lysozymes: model Enzymes in Biochemistry and Biology. (Jolles, P., and Birkhauser, B., Eds), 87-102
[8] Jiang, H. (2008). The biochemical basis of antimicrobial responses in Manduca sexta. Insect Sci., 15:134-145
[9] Lavine, M.D., Chen, G., and Strand, M.R., (2005). Immune challenge differentially affects transcript abundance of three antimicrobial peptides in hemocytes from the moth *Pseudoplusia includes*. Insect Biochem.Mol.Biol. 35: 1335-1346
[10] Prager, E.M., Jolles, P.(1996). Animal lysozymes C and g an overview. In: Jolles P(ed) Lysozyme: model enzymes in biochemistry and biology. Birkhauser Verlag, Basel, 9-31
[11] Singh, R., (2007). Lysozyme in pulmonary host defense. Am. J. Respir. Crit. Care Med. 169:435-436
[12] Qian, R., Xiaofan, Z., Jinxing, W., (2009). Molecular characterization and expression analysis of chicken-type lysozyme gene from housefly (*Musca domestica*). J. Genet. Genomics 36:7-16

Licensed Under Creative Commons Attribution CC BY