Acta Tropica 167 (2017) 121–127

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Expression of heat shock proteins (HSPs) in *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) larvae in response to thermal stress

Arun Sivan, Ananganallur Nagarajan Shriram*, Nagarajan Muruganandam, Ramanathan Thamizhmani

Regional Medical Research Centre (Indian Council of Medical Research), Post Bag No. 13, Port Blair, Andaman & Nicobar Islands 744 101, India

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form
18 December 2016
Accepted 19 December 2016
Available online 24 December 2016

Keywords:
Heat shock proteins
Aedes aegypti
Aedes albopictus
Relative quantification
Larvae

ABSTRACT

Climatic changes are responsible, to a certain extent for the occurrence and spread of arboviral pathogens world over. Temperature is one of the important abiotic factors influencing the physiological processes of mosquitoes. Several genes of heat shock protein (HSP) families are known to be expressed in mosquitoes, which aid in overcoming stress induced by elevated temperature. In order to understand expression of HSP family genes in the Andaman population of *Aedes aegypti* and *Aedes albopictus*, we used quantitative real-time polymerase chain reaction (qPCR) to examine expression levels of HSPs in response to thermal stress under laboratory and in actual field conditions. HSP genes *AeaHsp26*, *AeaHsp83* and *AeaHsc70* were examined by comparing relative transcript expression levels at 31 °C, 33 °C, 34 °C, 37 °C and 39 °C respectively. Enhanced up-regulation of HSPs was evident in third instar larvae of *Ae. aegypti* with rise in water temperatures (31 °C, 33 °C, 34 °C) in the containers in the nature and thermally stressed (37 °C and 39 °C) in laboratory conditions. In *Ae. albopictus* up-regulation of HSPs was observed in field conditions at 34 °C only and when thermally treated at 37 °C, while down regulation was evident in larvae subjected to thermal stress in laboratory at 39 °C. Data on expression levels revealed that larvae of *Ae. aegypti* was tolerant to thermal stress, while *Ae. albopictus* larvae was sensitive to heat shock treatment. Statistical analysis indicated that *AeaHsp83* genes were significantly up-regulated in *Ae. aegypti* larvae after 360 min exposure to high temperature (39 °C). The difference in expression levels of *AeaHsp26*, *AeaHsc70* and *AeaHsp83* genes in *Ae. albopictus* larvae was statistically significant between different exposure temperatures. All of these genes were significantly up-regulated at 37 °C. These results indicate that *AeaHsp26*, *AeaHsc70* and *AeaHsp83* are important markers of stress and perhaps function as proteins conferring protection and enhance survival of the Andaman population of both the *Aedine* species. Biological implications of these findings could impact the vector competencies.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Heat shock proteins (HSPs) are a group of proteins which aid the organisms acclimate to stress and protects them from environmentally induced cellular damages. HSPs function as molecular chaperones stimulating precise refolding and prevention of aggregation of denatured proteins (Johnson et al., 1998; Feder and Hofmann, 1999). It has been documented that several families of HSPs are expressed in insects, including mosquitoes, and probably play a collective role in mounting response to thermal stress (Mahroof et al., 2005; Yadav et al., 2005; Rinehart et al., 2006a, 2006b; Robich et al., 2007; Zhao et al., 2010). Besides, HSPs are also expressed in response to other environmental stress factors among insects including mosquitoes (Parsell and Lindquist, 1993; Feder and Hofmann, 1999; Rinehart et al., 2007; Sim et al., 2007). Consequently, the expression of HSPs in the immatures assumes significance to the overall understanding of the mosquito response to heat shock and other types of environmental stress.

World Health Organization (WHO) has recognized dengue as one of the fastest-growing viral threats globally. According to the WHO (WHO 2016), 50–100 million dengue infections and about 500,000 cases requiring hospitalization occur each year world-

* Corresponding author at: Regional Medical Research Centre (Indian Council of Medical Research), Post Bag No.13, Port Blair, 744 101, Andaman & Nicobar Islands, India.
E-mail addresses: shriraman@icmr.org.in, anshriram@gmail.com, anshriram@gmail.com (A.N. Shriram).

http://dx.doi.org/10.1016/j.actatropica.2016.12.017
0001-706X/© 2016 Elsevier B.V. All rights reserved.

wide. *Aedes aegypti* is an important vector of dengue virus, and the more severe dengue hemorrhagic fever (Tatem et al., 2006). Alongside, *Aedes albopictus* has also been considered a potential vector of dengue and several virus isolations from this mosquito species have been made in Southeast Asia (Hawley, 1988; Kow et al., 2001; Ahmad et al., 1997). Dengue and chikungunya predominantly occur in tropical and subtropical regions (Kumar and Gopal, 2010; WHO, 2016). Southeast Asia region (SEAR) which reports largest number of dengue cases, hospitalizations and infant mortality (Gubler, 1997; Arankalle et al., 2007; Sam and AbuBakar, 2006; WHO, 2016) lies in the tropics and sub tropics (Encyclopedia Britannica, 2015), where temperature rise and climate change have been observed (Cruz et al., 2007). Competent vectors viz. *Ae. aegypti* and *Ae. albopictus* are predominantly distributed in this region (Scholte and Schaffner, 2007; Womack, 1993). The temperature in this region normally range from 21 °C to 35 °C, which could raise up to 40 °C in summer.

The Andaman and Nicobar Islands (A & N) is a Union Territory of India, an archipelago of more than 500 islands, stretching over 700 km from north to south, in Bay of Bengal. There are 38 inhabited islands with an approximate human population of 380,000. During the study period (in 2013), the average temperature ranged from 23 °C to 30 °C, and during summer it peaked to 35 °C (National Informatics Centre, Andaman & Nicobar Islands, MEIT, Government of India). Natural accidental exposure of the breeding containers to high temperatures may perhaps increase the risk of thermotolerant mosquitoes becoming more susceptible to pathogens due to lower intrinsic incubation period, which could aid in rapid transmission of arboviral pathogens.

Dengue fever is an emerging public health problem in the Union Territory of A & N islands (Vijayachari et al., 2011; Chaaithanya et al., 2011; Muruganandam et al., 2014) and high levels of vector infestation indices were observed. *Ae.aegypti* has a wide distribution within urban Port Blair, the head quarter of the islands (Shriram and Sehgal, 1999), and during the past few years, this species has been infiltrating into the *peri*-urban and rural areas (Shriram et al., 2008). *Ae. albopictus* infestation has been observed in the *peri*-urban areas adjoining Port Blair (Shriram et al., 2009).

The *Aedes* larval stages during the course of development come across several environmental stressors that impact the survival which in turn results in morphological, physiological and behavioural changes among those which overcome different types of environmental stress. Temperature can be considered as one such parameter, which is common world over. The larvae may be exposed to temperature variations in the tropical regions during hotter months, when there are chances that larvae are exposed for longer durations to temperature rise. This has implications on the larval development, survival ability in larvae as well as the resulting adult, size of the adult, gonotrophic cycle and competencies in transmission of pathogens (Reeves et al., 1994; Westbrook et al., 2010; Muturi and Alto, 2011; Muturi et al., 2012). Larval stages are also important for the maintenance of virus in nature *via* transovarial transmission (Thenmozhi et al., 2000). Heat shock studies indicate that, temperature perhaps stimulates expression of proteins that aid in tiding over the stress and crucial for the survival, mediated through heat shock proteins (HSPs) (Zhao et al., 2010). Rise in water temperature in the containers supporting *Aedes* breeding during the summer months, may influence the expression of HSPs.

In view of these factors, studies were undertaken to assess the impact of thermal stress on survival and development on late third instar larvae of *Ae. aegypti* and *Ae. albopictus*. Briefly, larvae exposed at 37 °C and 39 °C, on re-exposure at 43 °C and 45 °C, the larvae exposed at 39 °C were more thermotolerant than larvae exposed at 37 °C, implying that higher temperature in summer could be a significant determinant for the survival, development and distribution of both the species (Arun Sivan et al., unpublished data).

In the present study, to further our understanding on the expression of HSP family genes expressed in the larvae of *Ae. aegypti* and *Ae. albopictus*, we used quantitative real-time polymerase chain reaction (qPCR) to examine expression levels of HSPs in response to thermal stress under laboratory and field conditions. We also compare the expression levels of HSPs that are critical to survival of both the mosquito species and the analyses are reported in this manuscript.

2. Materials and methods

2.1. Mosquito strains

Ae. aegypti and *Ae. albopictus* (South Andaman population) reared in the Entomology laboratory of the Regional Medical Research Centre (ICMR), Port Blair were used in the study. The F_1 generation of late 3rd instar larvae, originally collected from the wild in South Andaman district were used for heat shock experiments.

2.2. Field samples

Late 3rd and 4th instar larvae of *Ae. aegypti* and *Ae. albopictus* were predominantly collected from the breeding receptacles viz. plastic drum, metal drum, cement tanks, discarded plastic containers, discarded tin cans etc. that had temperature > 30 °C during the period between March to May 2013.

2.3. Heat shock experiments

To assess how thermal stress affects gene expression and quantify various HSPs, late third instar larvae of *Ae. aegypti* and *Ae. albopictus* were subjected to thermal stress under different temperature regimes to optimize the response and facilitate detection of the differentially expressed genes. Briefly, larvae of both species were exposed to temperatures i.e. 37 °C and 39 °C for 360 min. Concurrently, *Ae. aegypti* larvae collected from field, naturally exposed to high temperatures (31 °C, 33 °C and 34 °C) during summer months. While, the field collected *Ae. albopictus* larvae had natural exposure to 34 °C, i.e. water temperature in the container was 34 °C. In order to assess the differences in gene expression and HSPs third instar larvae were kept at 27 °C (controls at room temperature). Three replicates were accomplished for each experiment and the experiments were repeated thrice for reproducibility. Twenty larvae from each pool were processed for extraction of RNA.

2.4. RNA extraction and cDNA synthesis

Total RNA was extracted using TRIZOL (Invitrogen) and Qiagen mini preparation kit following manufacture's instruction (Qiagen, USA). Reaction mix of 20 μl containing 10 μl of RNA templates was converted into cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). These transcribed products were stored at −20 °C for future use. The primers used for the detection of heat shock proteins, viz. *AeaHsp*26, *AeaHsc*70, *AeaHsp*83 and internal control *AeaActin*, have been described previously (Zhao et al., 2010).

2.5. Quantitative qPCR amplification

Real-time PCR was performed with Applied Biosystems 7500 System. The 25 μl reaction volume contained 12.5 μl Syber green Universal master mix (CAT no:4367659, Applied Biosystems, USA), 0.5 μl of each primer and 2 μl of template cDNA of test and control

Download English Version:

https://daneshyari.com/en/article/5670954

Download Persian Version:

https://daneshyari.com/article/5670954

Daneshyari.com