Gene Reports 5 (2016) 70–74

Contents lists available at ScienceDirect

Gene Reports

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

Identification and evaluation expression level of arrestin 1 gene during the development stage of *Anopheles stephensi*

S. Shabani [a,b,*], A. Karimi [c], A. Rashki [f], N.M. Meshkinkhood [a,b], F. Niknam [d], A. Poursharif [e], F. Mahboudi [b], N. Dinparast Djadid [b]

[a] Department of Biology, Faculty of Science, University of Zabol, Iran
[b] Biotechnology Research Center (BRC), Pasteur Institute of Iran, Iran
[c] Pasteur Institute of Iran and Department of Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Biology, Faculty of Science, Islamic Azad University of Damghan, Iran
[e] Department of Biology, Faculty of Science, University of Guilan, Iran
[f] Department of Physiopathology, Faculty of Vet-Medicine, University of Zabol, Iran

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 29 August 2016
Accepted 6 September 2016
Available online 12 September 2016

Keywords:
Anopheles stephensi
Arrestin 1
Visual
RACE

ABSTRACT

Olfaction and visual play are an important role in many insect behaviors, including host-seeking behavior, and blood-feeding. Identification of molecular mechanism of these kinds of behaviors is a crucial issue that should be considered to find new methods in controlling vector-born diseases. Previous studies on arrestin 1 from *Anopheles gambiae* (main malaria vector in Iran, Indian subcontinent and China) have shown that this gene plays a prominent role in the visual process of this mosquito. Despite its importance, this gene and its protein have not yet been identified and characterized in *Anopheles stephensi* (main malaria vector in Iran, Indian subcontinent and China). In this study, we used Rapid Amplification of cDNA Ends (RACE) technique to identify total cDNA of this gene, applied real time PCR to evaluate expression level of this gene in different stages of mosquito development, and characterized it using bioinformatics tools. The result showed that arrestin 1 from *Anopheles stephensi* (arr1*As*) gene and its protein are remarkably similar to that of the arrestin 1 gene from *Anopheles gambiae* (arr1*Ag*) and is expressed during all stages of mosquito development in *Anopheles stephensi* except for the embryo stage. Although it needs to further test the determining function of arrestin 1, based on the similarity of sequences (cDNA and amino acid) and expression pattern of this gene between *Anopheles gambiae* and *Anopheles gambiae*, it seems that it has the same function in *Anopheles stephensi*.

1. Introduction

Malaria is a main public health problem in third world countries and is the top ranked priority tropical disease of the World Health Organization (Hay and Snow 2006). Despite of the global effort to eradicate malaria disease by different methods from chemical to DNA vaccine, it remains a significant epidemic that is responsible for hundreds of thousands of deaths every year. Therefore, finding new targets and novel methods in controlling this disease should be considered at priority of new research.

Olfaction and visual play is a crucial role in most insect behaviors among mosquito vectors and other insects (Zwiebel and Takken 2004). Several studies have shown that arrestins are key elements in both of visual and olfactory processes (Pippig et al. 1993; Mombaerts 1999; Pilpel and Lancet 1999; Tian et al. 2012).

In the olfaction process, joining transmembrane-domain G-protein-coupled receptors (GPCRs) and heterotrimeric G proteins leads to the activation of downstream effector enzymes, producing several messengers to induce the depolarization or hyperpolarization of olfactory neurons, leading to start an olfaction process (Pilpel et al. 1998; Mombaerts 1999). For interruption olfactory process, coupling between GPCRs and heterotrimeric G proteins should be separated. Desensitization of GPCRs by arrestins separates coupling between GPCRs and heterotrimeric G proteins, leading to an interrupted visual process (Pippig et al. 1993).

In the visual process, the coupling between rhodopsin, light-sensitive receptor protein, and a heterotrimeric G-protein leads to activation of reactions, leading to visual process. To terminate visual process, visual arrestins are coupled to phosphorylated rhodopsin and separate it from reactions.

Several arrestins, including arr1, arr2 and Krz, have been characterized in insects, especially in *Drosophila melanogaster* (Krishnan and Ganguly 1990; Yamada et al. 1990; Roman et al. 2000; Merrill et al.

Abbreviations: An., anopheles; Arr1As, arrestin 1 gene from *Anopheles stephensi*; Arr1Ag, arrestin 1 gene from *Anopheles gambiae*; 5R-1, 5′ RACE primer-1; 3R-1, 5′ RACE primer-1; Arr1, arrestin 1; Krz, Kurtz arrestin; C-arr1-F1, conserve primer arrestin 1-Forward 1; C-arr1-F2, conserve primer arrestin 1-Forward 1; C-arr1-R1, conserve primer arrestin 1-Reverse 1; C-arr1-R2, conserve primer arrestin 1-Forward 2.

* Corresponding author at: Department of Biology, Faculty of Science, University of Zabol, Iran.
E-mail address: sadeq.shabani280@gmail.com (S. Shabani).

http://dx.doi.org/10.1016/j.genrep.2016.09.002
2452-0144/

2003; Manzini and Schild 2010). Insects have two visual arrestins (arrestin 1 and arrestin 2) and one non-visual arrestin (Krz) (Gurevich and Gurevich 2006). In *Drosophila melanogaster arr1* is considered as a minor arrestin due to low expression level compared with *arr2* gene, involving in visual signaling process. Also, arrestin 1 from *Drosophila melanogaster* recognizes phosphorylated rhodopsin and several regulating rhodopsin level of the membrane (Dolph et al. 1993; Satoh and Ready 2005; Shieh et al. 2014). One of the arrestins in the visual process of *Anopheles gambiae* and *Drosophila melanogaster* is *arr1* (Merrill et al. 2002). Arrestin 1 recognizes phosphorylated rhodopsin and several regulating rhodopsin level of the membrane in *Drosophila melanogaster* and *Anopheles gambiae* (Dolph et al. 1993; Satoh and Ready 2005; Shieh et al. 2014). Despite the importance of arr1 gene in visual and olfactory processes of mosquito vectors, this gene and its protein have not yet been characterized in *Anopheles stephensi* (main malaria vector in Iran, Indian subcontinent and China). In this study, we identified the full cDNA of *arr1As*, characterized it at bioinformatics level, and evaluated expression levels of this gene both in male and female mosquitoes at different stages of mosquito development. Accordingly, although it needs further biological tests, with regard to high similarity in sequences (nucleotide and protein) and expression pattern of arr1As and other insects, especially *Anopheles gambiae*, it may be concluded that this gene has the same function in visual and olfactory processes as *Anopheles gambiae*.

2. Material and method

2.1. Sample collection

The Anopheles adult were collected using an aspirator from the malaria endemic areas of Iran, including Iranshahr, Chabahar, Khash, Zabol, Zahedan, Sarbaz, Saravan, and Nikshahr districts that are located in Sistan and Baluchistan province, south-east of Iran (Fig. 1).

2.2. Morphological and molecular identification

First, morphological studies were carried out based on identification key for the female Anopheles of Southwestern Asia and Egypt as previously described (Glick 1992). In the next step, molecular identification of Anopheles was performed according to rDNA-ITS2 method for identification of *Anopheles stephensi* (Dinparast Djadid et al. 2003).

2.3. Insect rearing, RNA extraction and cDNA synthesis

After finishing the identification process, adult mosquitoes were put in a cage and were reared according to the mosquito rearing (*A. gambiae*) protocol as previously described (Das et al. 2007) in national Insectarium of Pasteur Institute of Iran. Total RNA was extracted from every stage (egg, first, second, third, and fourth instar larvae, pupae, male and female) of *A. stephensi* using RNeasy the Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instruction. The cDNAs were synthesized from 1 μg of total RNA by using cDNA synthesis kit (EURx, Poland) according to the manufacturer's instruction.

2.4. Conserve primer designing and PCR

Four conserve primers(C-arr1-F1, C-arr1-F2, C-arr1-R1, and C-arr1-R2) were designed based on conserved regions of *arr1As* by Gene Runner software (version 3.05, 1994; Hastings Software Inc.) (Supplementary data). Desired regions of cDNA of arr1*As* were amplified by the conserve primers.

PCR amplification was performed by total volume 50 μL reaction mix which includes 0.2 M forward and reverse primer, 0.2 mM nucleotides, 1.5 mM $MgCl_2$ and 1 μL Taq DNA polymerase in GeneAmp® PCR System 2400 Thermal Cycler (Perkin, Elmer). PCR condition was started in 94 °C for 5 min then followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min. Amplifications were completed with a final extension at 72 °C for 10 min.

2.5. Specific primer designing and RACE

Two specific primers (5R-1, 3R-1) were designed from the specific region of arr1*As*, achieving in the previous step to support RACE technique according to guidelines of RACE-TaKaRa kit's instruction (Supplementary data). Then, 5′-RACE and 3′-RACE were performed using the Clontech SMART™ RACE cDNA Amplification Kit (TaKaRa) according to manufacturer's instructions obtaining full cDNA of arr1*As*.

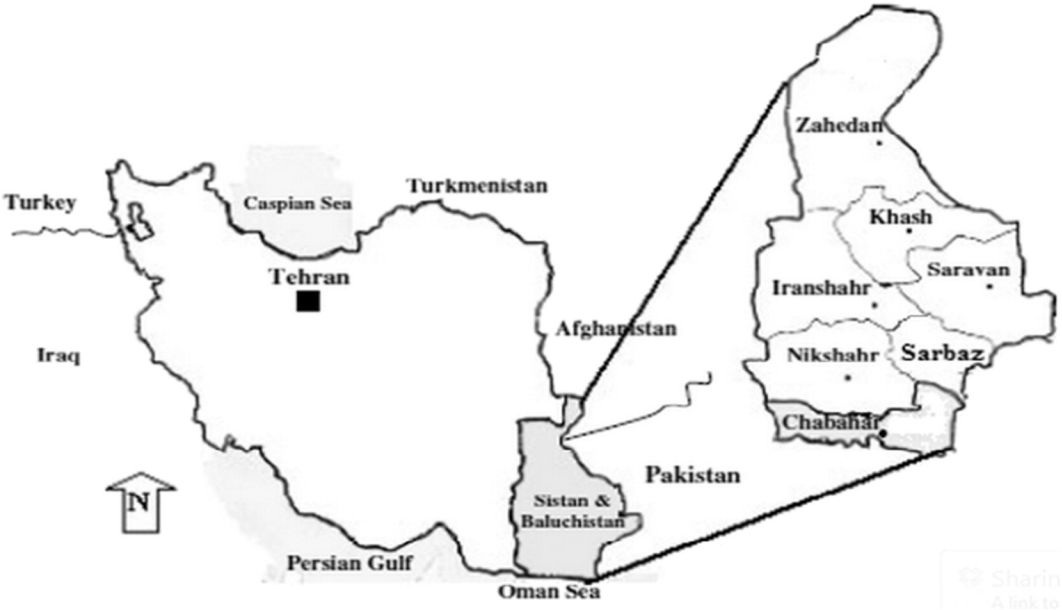

Fig. 1. Map of the study area. *A. stephensi* was collected from eight districts in Sistan and Baluchistan Province (southeast of Iran), including Iranshahr, Chabahar, Khash, Zabol, Zahedan, Sarbaz, Saravan, and Nikshahr districts.