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Cloning and characterization of *NYD-OP7*, a novel deltamethrin resistance associated gene from *Culex pipiens pallens* ☆

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Abstract

One mosquito *opsin* gene, *NYD-OP7*, has been cloned from *Culex pipiens pallens*. An open reading frame (ORF) of 1116 bp was found to encode a putative 371 amino acids protein which exhibits high identity with opsins from *Aedes* and *Anopheles* mosquitoes. Transcript expression of *NYD-OP7* was determined by real-time PCR in all life stages of deltamethrin-susceptible and -resistant strains of the *Culex* mosquito. The results demonstrated that this gene is expressed at all developmental stages, and it is expressed predominantly at the pupae and adult stages. Meanwhile, in pupae and adults, *NYD-OP7* is overexpressed in deltamethrin-resistant strain than in -susceptible strain. Importantly, stable expression of *NYD-OP7* in the mosquito C6/36 cells can confer moderate deltamethrin resistantce. Our study provides the first direct evidence that increased expression of an *opsin* gene may play some role in the development of deltamethrin resistantce in *Cx. pipiens pallens*.
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Keywords: *Culex pipiens pallens*; Opsin; Reverse transcription PCR; Transfection; Deltamethrin resistantce

1. Introduction

Opsins, the visual pigments of animal photoreceptors, belong to the G-protein coupled receptor family which is characterized by a seven-helix transmembrane topology and by the ability to activate heterotrimeric G-proteins [1–3].

Since the first sequence of an opsin, bovine rhodopsin, was determined by conventional protein sequencing and cDNA sequencing, more than 1000 opsins have been identified [4,5]. In invertebrates, some photoreceptor opsins have been well-characterized, both in terms of their sequences and cellular functions [1]. When sequence alignments are made of the invertebrate and vertebrate opsins, it is evident that individual sequence may be quite different, but the tertiary structure appears conserved [5,6].

Insecticide resistance is a major obstacle to the control of vector-borne diseases [7–9]. Many studies have suggested that the insecticide resistance phenotype evolves rapidly based on the selection of major effect genes [10,11]. However, recent genome-wide transcription profiling indicated that a broader range of genes may be involved and that insecticide resistance may be more complex than previously considered [12–14].

As part of an ongoing study of insecticide resistance in *Cx. pipiens pallens*, we have employed suppression subtractive hybridization (SSH) and cDNA microarray to identify differentially expressed genes between deltamethrin-susceptible and -resistant strains of *Cx. pipiens pallens*. *Opsin* is one of only ten genes that showed over-expression in deltamethrin-resistant strain [15]. In the present study, we isolated and sequenced the complete sequence of this gene and

☆ The nucleotide and deduced amino acid sequences presented in the manuscript have been submitted to the GenBank database under Accession No. AY749413.

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termed it *NYD-OP7*[2]. We then characterized the expression pattern of *NYD-OP7* gene in different developmental stages of *Cx. pipiens pallens*. In addition, we investigated the role of *NYD-OP7* in deltamethrin resistance by transfecting this gene into the mosquito C6/36 cells and tried to determine if over-expression of *NYD-OP7* can change the sensitivity of the cells to deltamethrin.

2. Materials and methods

2.1. Mosquito strains

The deltamethrin-susceptible strain of *Cx. pipiens pallens* was obtained from the Shanghai Insect Institute of the Chinese Academy of Sciences and was maintained in our laboratory. The mosquitoes had never been exposed to any insecticides and the median lethal concentrations (LC_{50}) of deltamethrin (Roussel Uclaf, France) was 0.0008 mg/L. The strain was reared and bred at 25–27 °C in a 16 h light/8 h dark photoperiod. The deltamethrin-resistant strain was derived from the susceptible early fourth instar larvae by selection with deltamethrin for 10 generations until the resistant ratio (LC_{50} of filial generation/LC_{50} of parental generation) reached 400 [16].

2.2. Construction of cDNA library

Total RNA were extracted from 300 adult females of resistant strain using the RNeasy maxi kit (Qiagen, Germany) according to the manufacture's instructions. The integrity of total RNA was determined by denaturing agarose gel electrophoresis and the yield and purity of total RNA was estimated by spectrophotometry. The poly (A+) mRNA was purified from total RNA with the PolyA-tract mRNA isolation system (Promega, USA), and 2 μg polyA mRNA was used for cDNA library construction with λExcell/*Not*I/*Eco*RI/CIP vector system (Pharmacia, USA) following the manufacturer's protocols. The cDNA library contained approximately 5×10^5 clones and was amplified.

2.3. Cloning and sequencing of NYD-OP7 cDNA

To isolate the full-length of culex *opsin* gene, we used the standard SP6 and T7 library vector primers and the primers designed based on the sequence of the SSH fragment we reported previously (GenbBank Accession No. BE247812). The sequence of the oligonucleotide primers was: forward 5′-GTCGCTCTGGTGACCATTTC-3′; reverse 5′-CACG ATCGGGTTGTAGACAG-3′. PCR conditions were: initial denaturation at 94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 90 s with final 10 min extension at 72 °C. The PCR products were separated by 1% agarose gel electrophoresis and purified using a QIA quick Gel extraction kit (Qiagen, Germany). Products were then cloned into the pGEM-T easy vector (Promega, USA) and sequenced at Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). Then the sequences of above three fragments were assembled to generate a putative full-length cDNA of opsin. A pair of PCR primers: forward 5′-TTAAGTCTGAAGTAGTTC-3′ and reverse 5′-CTAGCAAAGATCAGCAATC-3′ were designed for the amplification of full-length cDNA according to the assembled sequence of opsin. PCR was carried out using LA *Taq* polymerase (TaKaRa, Japan) using the following protocol: 94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 90 s with final 10 min extension at 72 °C. Then the PCR product was purified, cloned into the pGEM-T easy vector and sequenced.

2.4. Sequence alignment and phylogenetic tree

The standard protein-/protein BLAST sequence comparison (blastp; www.ncbi.nlm.nih.gov / BLAST/) and PSI-BLAST programs were used to search for sequences in the GenBank and SWISS-PROT databases with similarities to the translated sequences of *NYD-OP7* [17]. Sequence alignment and phylogenetic tree analysis using the neighbour-joining method was carried out by the CLUSTAL W program [18]. The opsins included in our analysis were: twelve sequences from *An. gambiae* genome [2], six sequences from *Drosophila melanogaster*, three sequences from *Manduca sexta*, six sequences from *Papilio glaucus* and two sequences from *Aedes aegypti* [6].

2.5. RNA extraction and cDNA synthesis

Total RNA were extracted from embryos, first, second, third and fourth instar larvae, pupae, and adults of both susceptible and resistant strains using the RNeasy mini kit (Qiagen, Germany) according to the manufacturer's protocol, and contaminant genomic DNA was removed by DNase I treatment. cDNA was synthesized from 2 μg of total RNA with M-MLV reverse transcriptase (Promega, USA) and random oligonucleotide primers according to the manufacturer's protocol.

2.6. Real-time PCR of NYD-OP7 in mosquito developmental stages

Aliquots of cDNA were amplified on an ABI PRISM 7300 (Applied Biosystems) using the SYBR green PCR kit (PE Applied Biosystems) with 25 μl reaction mixtures. The PCR mixture contained 1× SYBR PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM (each) dNTP, 0.2 μM primers, and 0.3 μl of *Taq* polymerase (2.5 U/μl). The primers used were: *NYD-OP7*: forward 5′-GTGGGCTTTCGCACTCTT-3′, reverse 5′-GTTCACGCATCTGTTCCTC-3′; β-actin: forward 5′-C GCTTCCTCGTCTACACTGG-3′, reverse 5′-GTGTTGG CGAACAGATCCTT-3′. For amplification, the following program was employed: 40 cycles of 95 °C for 15 s, 60 °C for

[2] *Abbreviation used:* NYD, the abbreviation of the Chinese name of Nanjing Medical University.

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