Journal of Insect Physiology 77 (2015) 1–8

Contents lists available at ScienceDirect

Journal of Insect Physiology

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

Two homologous carboxylesterase genes from *Locusta migratoria* with different tissue expression patterns and roles in insecticide detoxification

Jianqin Zhang [a,1], Pingting Ge [a,1], Daqi Li [a], Yaping Guo [a], Kun Yan Zhu [b], Enbo Ma [a,*], Jianzhen Zhang [a,*]

[a] Research Institute of Applied Biology, Shanxi University, Taiyuan, Shanxi 030006, China
[b] Department of Entomology, 123 Waters Hall, Kansas State University, Manhattan, KS 66506, USA

ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 1 February 2015
Accepted 25 March 2015
Available online 1 April 2015

Keywords:
Locusta migratoria
Carboxylesterase
RNA interference
Insecticide detoxification

ABSTRACT

Carboxylesterases (CarEs) play a crucial role in detoxification of xenobiotics and resistance to insecticides in insects. In this study, two cDNAs of CarE genes (*LmCesA4* and *LmCesA5*) were sequenced from the migratory locust, *Locusta migratoria*. The cDNAs of *LmCesA4* and *LmCesA5* putatively encoded 538 and 470 amino acid residues, respectively. The deduced amino acid sequences of the two CarE genes showed 45.0% identities, possessed highly conserved catalytic triads (Ser-Glu-His), and clustered in phylogenetic analysis. These results suggest that they are homologous genes. Both CarE genes were expressed throughout the developmental stages. However, *LmCesA4* was predominately expressed in the midgut (including the gastric caeca) and fat bodies, whereas *LmCesA5* was mainly expressed in the gastric caeca. The *in situ* hybridization results showed that the transcripts of the two genes were localized in apical and basal regions of the columnar cells in the gastric caeca. Gene silencing followed by insecticide bioassay increased the mortalities of deltamethrin-, malathion-, and carbaryl-treated locusts by 29.5%, 31.0% and 20.4%, respectively, after the locusts were injected with *LmCesA4* double-stranded RNA (dsRNA). In contrast, the injection of *LmCesA5* dsRNA did not significantly increase the susceptibility of the locusts to any of these insecticides. These results suggest that these genes not only show different tissue expression patterns but also play different roles in insecticide detoxification.

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1. Introduction

Carboxylesterases (CarEs) belong to a superfamily of multifunctional and ubiquitous enzymes in all living organisms, including animals, plants and microbes (Oakeshott et al., 2005b; Marshall et al., 2003; Bornscheuer, 2002). These enzymes play important roles in many metabolic reactions (e.g., xenobiotic detoxification and pheromone degradation), neurogenesis and developmental regulations (Yu et al., 2009). Although many CarE genes exist in insects, their phylogenies show three distinct groups and 14 major clades (A–N) on the basis of amino acid sequence similarity and catalytic competence (Oakeshott et al., 2005b). However, CarEs are not only highly different in their functions, but also in the gene numbers among different insect species. For example, based on the genome sequences, the numbers of CarE genes that have been determined in *Aedes aegypti*, *Drosophila melanogaster*, *Anopheles gambiae*, *Apis mellifera* and *Bombyx mori* are 49, 35, 51, 24 and 69, respectively (Yu et al., 2009; Strode et al., 2008; Tsubota and Shiotsuki, 2010a). Apparently, the silkworm *B. mori* has the largest number of CarE genes among these species.

As one of the most important metabolic detoxification systems in insects, CarEs have been extensively studied in various insect species (Pan et al., 2009; Wu et al., 2010). In recent years, it has been found that insect CarEs could be involved in resistance to many insecticides including organophosphates (OPs), carbamates and pyrethroids, through gene amplification, improvement of mRNA stability and point mutation (Hemingway and Karunaratne, 1998; Ranson et al., 2002; Li et al., 2007; Alon et al., 2008; Zhu et al., 1999). Lin et al. (2001) found that the activities of CarEs in the OP and pyrethroid resistant strains of *Blattella germanica* were higher than those of the susceptible strains, suggesting that the elevated activities of CarEs in the resistant strains may be responsible for resistance to these insecticides. A high level of malathion carboxylesterase (MCE) activities has also been observed in malathion-resistant housefly, *Musca domestica* L. (Taskin and Kence, 2004). In *Nilaparvata lugens*, OP and carbamate resistance is based on the amplification of a CarE gene, *Nl-EST1* (Vontas et al., 2000). The ability of *LcαE7* from *Lucilia cuprina* to

* Corresponding authors.
E-mail addresses: maenbo2003@sxu.edu.cn (E. Ma), zjz@sxu.edu.cn (J. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jinsphys.2015.03.013
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confer OP resistance derives from its immediate active site similar to acetylcholinesterase and higher binding affinity for the OP paraoxon (Jackson et al., 2013).

Locusta migratoria is an important agricultural pest worldwide, and mainly damages gramineous plants (Chen, 2000). Frequent applications of insecticides have inevitably resulted in the development of resistance in some natural populations of the locust (Ma et al., 2004). Further research demonstrated that CarEs are related to insecticide resistance in the locust (Yang et al., 2008, 2009). Our recent research has demonstrated that two CarE genes play important roles in malathion resistance as revealed by RNA interference (RNAi) followed by bioassay (Zhang et al., 2011).

In this study, we (1) identified and sequenced two full-length cDNAs of CarE genes from *L. migratoria*; (2) determined the tissue and developmental expression patterns and localized the transcripts of two CarE genes in the gastric caeca; and (3) explored the detoxification function of the two CarE genes using RNAi followed by insecticide bioassay. These results are expected to help us better understand the detoxification function of insect CarEs and reveal the mechanism of insecticide resistance in this important agricultural insect pest.

2. Materials and methods

2.1. Insects

A locust colony, originally collected from the field in 1997, had been maintained on an artificial diet without exposure to any insecticides for 11 years in Insect Protein Co., Ltd., Cangzhou, Hebei Province. The colony was then introduced to Beishao Locust Breeding Facility, Jinyuan District, Taiyuan, Shanxi Province in 2008. Eggs were purchased and hatched in a growth chamber at 30 °C at Shanxi University, Taiyuan, Shanxi Province. Locusts were reared on wheat sprouts in plastic cages (22 × 22 × 22 cm) at 28 ± 1 °C under a 14:10 h (light:dark) photoperiod and 50% humidity.

2.2. Sequencing of full-length cDNAs of LmCesA4 and LmCesA5 genes

Total RNA was extracted from the whole body of fifth instar nymphs using Trizol reagent (TakaRa, Otsu, Shiga, Japan) and 4 μg RNA was used to synthesize the first-strand cDNA using RevertAid™ H Minus M-MuLV reverse transcriptase (Fermentas, Glen Burnie, MD, USA) and an oligo-$(dT)_{18}$ primer (TaKaRa). *LmCesA4* and *LmCesA5* cDNA fragments were first obtained from the locust transcriptome database that was developed from many samples collected from different developmental stages (eggs, five nymphal instars and adults). The complete cDNA sequences of *LmCesA4* and *LmCesA5* were amplified with pairs of primers which are shown in Table 1. A 25 μL PCR mixture was comprised of 1 μL of diluted cDNA template, 12.5 μL of Taq Master Mix (TianGen, Beijing, China), 5 μM of each primer, and 10.5 μL of sterilized water. PCR was performed by initially denaturing the cDNA template for 1 min at 94 °C followed by 35 cycles, each consisting of denaturing at 94 °C for 1 min, annealing at 60 °C for 30 s and extension at 72 °C for 90 s, and a final extension step of 5 min at 72 °C. Amplified PCR fragments from each reaction were purified using E. Z. N. A.™ Gel Extraction Kit (Omega Bio-Tek, Norcross, GA, USA), and the purified fragment was subcloned into *pEASY*-T3 cloning vector (TransGen, Beijing, China) for sequencing from both directions.

2.3. Tissue-dependent expression analysis of LmCesA4 and LmCesA5

For tissue distribution studies, nine selected tissues including the brain (BR), foregut (FG), gastric caeca (GC), midgut (MG),

Table 1
Primer sequences used in this study.

Amplification of primers	Gene name	[a]Primer sequences (5′–3′)	PCR product size (bp)
qPCR analysis	*LmCesA4*	**F:** TGAAAGGTGACCTCTGGTTTGTCT **R:** AAGTACATCAGTTCATCTCCATGGC	184
	LmCesA5	**F:** AAGGAAAGAGTGACAGAGTCCACAC **R:** AGTTTCATCAGAGTGAACTGCTCCT	216
	β-actin	**F:** CGAAGCACAGTCAAAGAGAGGTA **R:** GCTTCAGTCAAGAGAACAGGATG	156
	RP49	**F:** CGCTACAAGAAGCTTAAGAGGTCAT **R:** CCTACGGCGCACTCTGTTG	168
	EF1α	**F:** AACATCGTCGTCATTGGTCA **R:** GTTCAGCCTTCAGCTTGTCC	175
Full-length verification	*LmCesA4*	**F:** GCACAACGCCTCCATCCAG **R:** TTACCTTTCCTGTAGTTCCAATGTG	2176
	LmCesA5	**F:** TCAGGTTCCAGCCACCAGAG **R:** ACAAGTCACAGACACAAATCATCAC	1511
dsRNA	*LmCesA4*	**F:** *TAATACGACTCACTATAGGG* GCCACACGAGGAAAAACTACG **R:** *TAATACGACTCACTATAGGG* CGACAGTTTTCTCGCTGCTCT	412
	LmCesA5	**F:** *TAATACGACTCACTATAGGG* GTCTTTCCACGAACAAGTCAACTG **R:** *TAATACGACTCACTATAGGG* TGTGACGAATGTAGAGTTGCTGC	505
	GFP	**F:** *TAATACGACTCACTATAGGG* GTGGAGAGGGTGAAGG **R:** *TAATACGACTCACTATAGGG* GGGCAGATTGTGTGGAC	571
Probe synthesis for *in situ* hybridization	*LmCesA4*	**F:** GCCACACGAGGAAAAACTACG **R:** CGCTGCTCTGATGGTAGGGT	400
	LmCesA5	**F:** CCTACTGTGGAACATTCAATACCTG **R:** TGTGACGAATGTAGAGTTGCTGC	374

[a] F: forward primer; R: reverse primer. The T7 polymerase promoter sequences are highlighted in italic.

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