Insect Biochemistry and Molecular Biology 73 (2016) 12–19

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

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

Functional validation of the carbon dioxide receptor in labial palps of *Helicoverpa armigera* moths

Chao Ning, Ke Yang, Meng Xu, Ling-Qiao Huang, Chen-Zhu Wang*

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form
5 April 2016
Accepted 5 April 2016
Available online 7 April 2016

Keywords:
Helicoverpa armigera
CO_2 receptor
Xenopus expression system
Two-electrode voltage-clamp recording
In situ hybridization
Single sensillum recording

ABSTRACT

Adult moths possess an organ in their labial palps, the labial-palp pit organ, which is specialized for sensing carbon dioxide (CO_2). They use CO_2 as a cue to detect healthy plants and find food or lay eggs on them. The molecular bases of the CO_2 receptor in *Drosophila melanogaster* and *Aedes aegypti* have been reported, but the molecular mechanisms of the CO_2 receptor in Lepidoptera remains elusive. In this study, we first re-examined three putative *Helicoverpa armigera* CO_2 gustatory receptor genes (*HarmGr1*, *HarmGr2*, and *HarmGr3*), and then analyzed expression patterns of them. RT-PCR results verified they were predominantly expressed in the labial palps of *H. armigera.* Thus, we used *in situ* hybridization to localize the expression of three genes in the labial palps. We found that all three genes were co-expressed in the same cells of the labial palps. Next, we employed the *Xenopus laevis* oocyte expression system and the two-electrode voltage-clamp recording to study the function of the three genes. Results showed that only oocytes co-expressing HarmGr1 and HarmGr3 or co-expressing HarmGr1, HarmGr2 and HarmGr3 gave robust responses to $NaHCO_3$. Finally, we confirmed that the sensory cells in labial palps of both females and males show dose dependent responses to CO_2 stimuli by using single sensillum recording. Our work uncovers that HarmGr1 and HarmGr3 are indispensable and sufficient for CO_2 sensing in labial palps of *H. armigera*.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Almost all organisms produce carbon dioxide (CO_2) when they generate the energy required for their life processes by oxidizing the biomass. As a ubiquitous sensory cue, CO_2 not only helps hematophagous insects detect and orientate to host vertebrates, but also plays important roles in foraging and oviposition of phytophagous insects (Guerenstein and Hildebrand, 2008).

Insects possess specialized receptor cells that can detect low concentrations of CO_2 (Guerenstein and Hildebrand, 2008). These cells are located in the maxillary palps in mosquitos and labial palps in Lepidoptera adults (Bogner et al., 1986; Grant et al., 1995; Kellogg, 1970; Kent et al., 1986). Basiconic sensilla on the maxillary palps of female *Aedes aegypti* respond to concentrations of CO_2 as low as 150–300 ppm and can detect increments as small as 50 ppm (Grant et al., 1995). In the labial palps of adult Lepidoptera, there is an organ called labial-palp pit organ (LPO), which is specialized for sensing CO_2 (Bogner et al., 1986; Kent et al., 1986). Before foraging and ovipositing, moths probe the surface of a plant with their LPOs to measure the gradient of CO_2 levels generated by host plants (Stange et al., 1995; Thom et al., 2004).

The molecular mechanisms underlying chemoreception of CO_2 stimuli at the level of receptor-cell dendrites were first unraveled from *Drosophila melanogaster* (Jones et al., 2007b; Kwon et al., 2007). Two gustatory receptor (Gr) genes, *DmelGr21a* and *DmelGr63a* are co-expressed in all CO_2 receptor cells of *D. melanogaster*. Such configuration is necessary and sufficient for the CO_2 receptor cells to respond to CO_2, and it is thought that the two Grs form a heterodimeric receptor (Jones et al., 2007b; Kwon et al., 2007). Three putative CO_2 receptor genes have been identified in the maxillary palps of three mosquito species, *Anopheles gambiae*, *Aedes aegypti*, and *Culex pipiens quinquefasciatus* (Kent et al., 2008; Robertson and Kent, 2009), and their function has been studied in *Aedes aegypti* (Erdelyan et al., 2012). *AaGr1*, *AaGr2* and *AaGr3* are co-expressed in the same receptor cells of the maxillary palps in *A. aegypti*. RNA interference (RNAi)-mediated gene knockdown of either *AaGr1* or *AaGr3* resulted in a loss of CO_2 sensitivity in both

* Corresponding author. State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing, China.
E-mail address: czwang@ioz.ac.cn (C.-Z. Wang).

http://dx.doi.org/10.1016/j.ibmb.2016.04.002
0965-1748/© 2016 Elsevier Ltd. All rights reserved.

male and female mosquitoes, but RNAi-mediated knockdown of *AaGr2* expression had no impact on CO_2 reception, suggesting that *AaGr1* and *AaGr3*, like the *Drosophila* orthologues, function as a heterodimer (Erdelyan et al., 2012; Jones et al., 2007a; Kwon et al., 2007; McMeniman et al., 2014). Three putative CO_2 receptor genes have also been identified in five Lepidoptera species, *Bombyx mori, Helicoverpa armigera, Danaus plexippus, Heliconius melpomene,* and *Popilio xuthus* (Anderson et al., 2009; Briscoe et al., 2013; Robertson and Kent, 2009; Xu and Anderson, 2015), but their functional identity has not been completely validated. Xu and Anderson (2015) reported that three putative CO_2 receptor genes, *HarmGR1*, *HarmGR2* and *HarmGR3*, were specifically expressed in the labial palps of *Helicoverpa armigera*. When the three Grs were expressed individually in insect Sf9 cells, only HarmGR3 was significantly activated by $NaHCO_3$ (Xu and Anderson, 2015). Therefore, whether the co-expression of multiple Grs is required for CO_2 detection in *H. armigera* and other lepidopteran species is still unknown.

In the present study, we first re-examine the coding sequences of three putative CO_2 Grs in *H. armigera*, and then analyze their expression patterns in tissues of adults and larvae and used *in situ* hybridization to localize the cells expressing the three genes in labial palps. We further used *Xenopus* oocyte expression system and two-electrode voltage-clamp recording to study the functions of three Grs in different combinations. Finally, we verified electrophysiological responses of the CO_2-sensitive cells in labial palps of *H. armigera*.

2. Materials and methods

2.1. Animals rearing

H. armigera were reared at 27 ± 1 °C with a photoperiod of 16 h: 8 h, D: L in the laboratory of Institute of Zoology, Chinese Academy of Sciences, Beijing. Eggs were hatched on a pad of cotton gauze, and larvae were individually transferred into glass tubes (i.d. 2.5 cm, length 8 cm) with an artificial diet mainly containing wheat germ, yeast and tomato paste as previously reported (Jiang et al., 2015). Pupae were sexed and males and females were put into separate cages for eclosion. 10% honey was used as the diet for adults. One-to-three-day-old virgin adults and fifth instar larvae were used in the experiments.

Xenopus laevis frogs were kindly provided by Prof. Qinghua Tao's laboratory in School of Life Sciences, Tsinghua University in Beijing, and reared with pork liver as food in our laboratory at 20 ± 1 °C. *Xenopus laevis* were anesthetized by submersion in 1 g/L 3-aminobenzoic acid ethyl ester, and the oocytes were surgically collected before experiments.

2.2. Chemicals

$NaHCO_3$ (>99.5%) (Sigma, USA), ethephon (>98%) (Guangtaiweiye Company, Beijing, China), 2,3-butanedione (>97%) (Sigma, USA), D-(−)-fructose (>97%) (Sigma, USA), D-(+)-glucose (>99.5%) (Sigma, USA), (Z)-11-hexadecenal (Z11-16:Ald, >92%) (Shin-Etsu Chemical, Tokyo Japan), (Z)-9-hexadecenal (Z9-16:Ald, >90%) (Shin-Etsu Chemical, Tokyo Japan), citral (>96%) (Sigma, USA), geraniol (>96%) (Sigma, USA) were used in experiments. Before experiments, stock solutions (0.5 M) of $NaHCO_3$, ethephon, 2,3-butanedione, D-(−)-fructose, and D-(+)-glucose were diluted in ND96 Buffer (96 mM NaCl/2 mM KCl/1 mM $MgCl_2$/1.8 mM $CaCl_2$/5mMHEPES pH 7.5). The stock solutions (200 mM) of citral, geraniol, Z11-16:Ald, and Z9-16:Ald were first prepared in dimethyl sulfoxide (DMSO) and stored at −20 °C, and then dissolved in ND96 Buffer before use. ND96 Buffer and ND96 Buffer containing 0.1% DMSO were used as negative controls respectively.

2.3. Re-examination of putative carbon dioxide receptor genes

To understand the molecular mechanism of CO_2 detection in *H. armigera*, we first used the transcriptome data of *H. armigera* from the NCBI sequence read archive (SRA) (http://www.ncbi.nlm.nih.gov/sra/?term=SRP041166) provided by Liu et al. (2014) and from our laboratory to recheck the coding sequences of three putative CO_2 receptor genes, *HarmGR1* (KF768713.1), *HarmGR2* (KF768714.1) and *HarmGR3* (KF768715.1), which were previously identified by Xu and Anderson (2015) from the transcriptome of *H. armigera* in Australia. The SRA Toolkit was used to convert the data to fastq format. The data of twelve tissues including ours were trimmed using Trimmomatic-0.30, merged, assembled using Trinityrnaseu-r2013-02-25, removed redundancy using Tgicl, and finally capped using Cap3 to get the unigene. The annotation of unigenes was performed using Blastx, and the database is the non-redundant protein sequences (nr) and UniProtKB/Swiss-Prot (swissprot). The hit sequences were compared with the coding sequences of the CO_2 Grs of *D. malanogaster* and *A. aegypti* using Genwise-2-4-1. The coding sequences and amino acid sequences of the three Grs of *H. armigera* were aligned using DNAMAN version 8.

2.4. RT-PCR and qRT-PCR of putative carbon dioxide receptor genes

We used RT-PCR to study the expression pattern of three putative CO_2 receptor genes in different tissues or organs of *H. armigera* adults and larvae. Labial palps, female antennae, male antennae, female proboscis, male proboscis, female foreleg tarsi, and female wings of the adults, and maxillae, foreguts, midguts, hindguts, and fat body of the fifth instar larvae were separately collected from up to 150 individuals, and then stored at −80 °C. Frozen tissues were transferred to a liquid nitrogen cooled mortar and ground with a pestle. The homogenate was covered with 1 mL of TriZol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA extractions were performed following the manufacturer's instructions. Total RNA of each tissue or organ was dissolved in H_2O, and RNA integrity was verified by gel electrophoresis. RNA quantity was determined by absorbance at 260 nm, 280 nm, and 230 nm on a Nanodrop ND-2000 spectrophotometer (Nano-Drop products, Wilmington, DE, USA). cDNA was synthesized with M-MLV reverse transcriptase (Promega, Madison, WI, USA) from the total RNA.

RT-PCR was performed using gene-specific primers designed with Primer Premier 5.0 based on the full length mRNA sequences of three putative CO_2 receptor genes from our *H. armigera* transcriptome. The primer sequences are listed in Table 1. *Actin* gene of *H. armigera* was used as the control to monitor the quality of each cDNA samples. The PCR program was as follows: 98 °C for 2 min; 94 °C for 3 min; 26 cycles at 94 °C for 30s, 53 °C for 30s, 72 °C for 90s, and 72 °C for 10 min for Actin, and 98 °C for 2 min; 94 °C for 3 min; 35 cycles at 94 °C for 30s, 53 °C for 30 s, 72 °C for 90s, and 72 °C for 8 min for *HarmGr1*, *HarmGr2* and *HarmGr3*. The RT-PCR reactions were repeated three times, and the products were analyzed by using 1.2% agarose gels (Jiang et al., 2015).

qRT-PCR was further performed to quantify the relative expression levels of *HarmGr1*, *HarmGr2* and *HarmGr3* in labial palps of *H. armigera* adults. Total RNA was isolated from labial palps of 50 pairs of female and male adults using RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany), in which genomic DNA was removed by gDNA Eliminator. cDNA was synthesized with M-MLV reverse transcriptase (Promega, Madison, WI, USA) from the total RNA. Real-time PCR were carried out using Mx3005P qPCR System (Agilent Technologies, CA, USA). The primer sequences are listed in Table 1. All reactions were performed in triplicate in a total volume of 20 μL containing 10 μL SYBR Premix Ex TaqII(TaKaRa, Otsu, Japan) and 0.4 mM of each primer under the following conditions: 95 °C

Download English Version:

https://daneshyari.com/en/article/8321304

Download Persian Version:

https://daneshyari.com/article/8321304

Daneshyari.com