Genetic knockout and general odorant binding/chemosensory proteins interactions: revealed the function and importance of GOBP2 in the yellow peach moth olfactory system

Dapeng Jing[1], Sivaprasath Prabu[2], Tiantao Zhang[2], Shuxiong Bai[2], Kanglai He[3], and Zhenying Wang[3]

[1]Chinese Academy of Agricultural Sciences Institute of Plant Protection
[2]Affiliation not available
[3]Institute of Plant Protection, Chinese Academy of Agricultural Sciences

April 05, 2024

Abstract

The GOBP2 protein has a unique function in the yellow peach moth, Conogethes punctiferalis. Several general odorant binding proteins (GOBPs) have been identified in various lepidopteran species, but the functional difference between GOBP1 and GOBP2 in recognition of host plant odorants is still unknown. The functions of GOBP1 and GOBP2 in the yellow peach moth were evaluated in this study using the CRISPR-Cas9 system. The results revealed the importance of GOBP2 in the olfaction mechanism in the yellow peach moth. The GOBP1 knocked out larvae's perception towards feeding decreased but did not reach a significant level, while knocking out the GOBP2 and GOBP1/2 genes resulted in huge differences. On the other hand, electroantennograms (EAG) and wind tunnel tests showed that the GOBP2 knocked out adult's sensitivity to odorants decreased more than GOBP1 knocked out individuals. The STRING database text mining results grabbed our attention in the protein-protein interaction studies. In this research, we firstly proved the existence of physical interactions between GOBPs and chemosensory proteins (CSP) through the surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) methods. Interestingly, the GOBP1 and GOBP2 could not interact with each other, but they can interact with CSPs. The interaction result indicates that GOBP2 could physically interact with CSP15, CSP5, and OBP17, whereas GOBP1 could bind only with CSP5 and CSP10, and the association constant (ka) is also more substantial than GOBP1. These results strongly suggest the importance of GOBP2 function in the perception of host plants odorants in the yellow peach moth.

1. Introduction

The general odorant binding proteins (GOBPs), a group of odorant binding proteins (OBPs), play essential roles in host volatiles perception of lepidopteran insects by binding and transporting hydrophobic ligand across the aqueous sensilla lymph to the odorant receptors (ORs) on the dendritic membrane of olfactory neurons (Vogt and Riddiford, 1981; Krieger et al., 2004). GOBP1 and GOBP2 were subsequently defined as conserved non-sex-biased antennal OBPs from lepidopteran GOBPs by sequence comparison (Vogt et al., 1991). While the pheromone binding proteins (PBPs) as another subfamily of OBP has conserved sex-biased antennal OBPs, which bind and transport pheromone molecules to the cognate pheromone receptors (PRs) on the dendritic membrane of olfactory neurons (Liu et al., 2011; Zhang et al., 2016). Phylogenetic analyses show that GOBPs/PBPs from lepidoptera are a unique subfamily, having evolved independently within the order of lepidoptera (Vogt et al., 2015; Zhang et al., 2013; Yasukochi et al., 2018). Besides, the difference between the GOBPs/PBPs form distribution pattern antennae is distinct (Steinbrecht et al., 1995; Nardi et al., 2003). However, evolutionary facts are explained that the GOBPs might be evolved from PBP by gene duplication. This fact was reported in *Manduca sexta* , PBP and GOBP2 have a close relationship and play

a crucial role in coordinated olfactory behaviors (Merritt et al., 1988; Vogt et al., 2002). More interestingly, GOBPs were reported could bind sex pheromones and are possibly responsible for their transportation by some searches, like in *Spodoptera exigua* ,*Chilo suppressalis* , *Bombyx mori* (Liu et al., 2015; Khuhro et al., 2017; Zhou et al., 2009; Nardi et al., 2003). While this function of GOBPs may remain controversial, and *in vivo*functional studies will provide more convincing evidence. Furthermore, OBPs and chemosensory proteins (CSPs) as two different classes of polypeptides have been identified in the lymph of chemosensilla may have some connection. A significant amount of structural information has been accumulated on OBPs and CSPs in the attempt to elucidate their function, but so far, physiological studies have been scant and no convincing models for their action (Pelosi et al., 2006). The gradual application of CRISPR/Cas9 gene editing technique provides a new and more convincing perspective insight for functional verification of the above issues in lepidopteran insects (Wang et al., 2018; Zhang and Reed, 2016).

The yellow peach moth,*Conogethes punctiferalis*(Guenée), is a highly destructive fruit borer can attack more than 100 essential plant species, including peach, durian, chestnut, citrus, papaya, cardamom, ginger (Wan et al., 2016; Lu et al., 2010). Over the past decade, it has become a serious maize pest in China, causing more significant damage than *Ostrinia furnacalis* (Guenée) in some summer corn regions of China (Wang et al., 2006). Previously, our study reported that the GOBP1 of the yellow peach moth plays a crucial role in sex pheromones and plant volatiles recognition based on binding assay (Jing et al., 2019). In the present study, GOBPs (GOBP1 and GOBP2) were further evaluated for testing, using the CRISPR/Cas9 technique along with electrophysiological and behavioral assays. The results provide new insights into the functional role of GOBPs, as well as an essential reference for developing GOBP based behavioral interference control strategies for the yellow peach moth.

2. Materials and methods

2.1 Insect

The yellow peach moth used in this study was reared under laboratory conditions. The larvae were reared using artificial diet maintained at 27 ± 1 °C with a photoperiod of 16 h light: 8 h dark, and 65 ± 5% relative humidity (RH). The pupae were segregated, male and female were kept in separate cages. After emergence, moths were fed on 10% honey water (Braccini et al., 2015).

2.2 Intron detection and preparation of guide-RNAs

Due to the lack of genome database of the yellow peach moth, firstly we have to design primers based on the cDNA sequence of GOBPs, using the DNA as a template for PCR then sequencing to determine its intron sequence. According to some previous research (Zhang and Reed, 2016; Wang et al., 2020a), two Cas9 cut sites were designed for producing long deletions in GOBP1 and GOBP2 target loci. sgRNAs of GOBP1 and GOBP2 were designed by manually searching genomic regions for $GGN_{18}NGG$ or $N_{20}NGG$ protospacer adjacent motif (PAM) sequences on the sense or antisense strands (Figure 1 and Supplementary Table S1). sgRNA template was transcribed *in vitro*with the specific oligonucleotide encoding T7 polymerase-binding site and the sgRNA target sequences following the manufacturer's instruction of GeneArt^{TM} Precision gRNA Synthesis Kit (Thermo Fisher Scientific, USA).

2.3 Embryo microinjection

Fertilized eggs were collected (within 2 hours after laying) and surface sterilized by soaking the eggs in 1% sodium hypochlorite solution for 1 minute and washed with distilled water for three times. The eggs were arranged on double-sided adhesive tape on a microscope slide. Two sgRNAs of target loci of GOBP1 and GOBP2 with the TrueCut^{TM}Cas9 Protein v2 (Thermo Fisher, Shanghai, China) were mixed, using the RNase-free water adjust the final concentration of sgRNA (200 ng/μL) with Cas9 protein (100 ng/μL). Each embryo was injected with approximately 1 nL of the mix solution containing sgRNA and Cas9 protein. The injected eggs were first placed on the ice maintained for 20 min then incubated at 27 ± 1 until hatching.

2.4 Mutation analysis and screening of homozygous mutation line

Microinjected eggs were placed in incubator to hatch, the neonates we maintained in the artificial diet and maintained until pupation. After the yellow peach moth emergence, one hind leg was removed to extract DNA. Then the adults were individually placed in a small plastic box with code until to use, and 10% honey water was provided. Genomic DNA was extracted using the GenoDirect PCR kit following the manufacturer's instruction (Herogen Biotech, Co. Ltd, China). According to the GOBP1 and GOBP2 sgRNA locations within each gene, the forward and reverse primers were designed to detect the deletion of the gene, respectively (Figure 1). The gene deletion was ensured by using pair of primers and expected to amplify a small fragment of GOBP1 and GOBP2 (about 350 bp and 600 bp). A pair of specific primers are developed for each gene to determine if the gene deletion mutation is homozygous or heterozygous (Figure 1). Genotypes of the gene deletion mutation can be discriminated according to the banding pattern of PCR amplified products, and all PCR primers showed in (Supplementary Table S1). The screened moths with the same banding pattern, which small fragments of genomic we expected, was mixed and allowed to produce homozygous mutants.

2.5 Larval growth and development parameters

One hundred newly hatched larvae were selected and monitored the growth and development for 5 generations. This step is performed to examine the gene knockout whether affect the growth or development of larvae. Three biological replicates were used in the experiment.

2.6 Electrophysiological assay

Electroantennograms (EAG) was used to determine the stimulus-dependent potential changes summed over the whole antenna. The ends of an isolated antenna cut from 2- to 3-day-old virgin male and female moth were connected by electroconductive gel (Signal gel, Parker laboratories inc, USA) to the two electrodes, respectively. Odorants for EAG analyses were selected based on a literature review as well as on compounds category. All components were dissolved in hexane to 100 ng/μL. A filter paper strip (2.5 cm × 1.0 cm) containing 10 μL of a test solution was inserted into a Pasteur pipette to deliver the stimuli, and the strip was left for 1 min to let the solvent evaporate before it was used for EAG measurement. The continuous airflow (30 mL/s) and the odor stimulating flow (0.2 s at 10 mL/s) were produced and controlled by a stimulus controller (CS-55, Syntech, Netherlands). At least 45 sec was allowed between two stimuli to provide time for recovery of antennal responsiveness. The antennal signal was amplified tenfold and converted to a digital signal by DC amplifier interface (IDAC, Syntech Inc., Netherlands). The signals were recorded with EAGPro software (version 2.0, Syntech Inc., Netherlands). A standard de canal was tested in the beginning and at the end of each recording to correct for the loss of sensitivity of the antennal preparation. Similarly, a control paraffin oil stimulation was done at the beginning and at the end of each recording to subtract the blank value from the antennal responses (Ren et al., 2017). For each compound, EAG responses of ten antennae from different adult beetles of each sex were recorded.

2.7 Tendency test of larvae to artificial diet

The responsiveness of larvae to the odor of artificial diet was investigated, twenty individuals of the 3^{th} instar larvae which were allowed for starvation (24 h). After starvation, and the larvae were placed in a 20 cm diameter plastic Petri dish, which also contained diametrically opposite two points 11 cm hole, one with some artificial diet, the other set empty for control. After 10 min, larvae crawl to opposite sides of the hole (or in close proximity) were counted, as well as those which had not made any choice. Each experiment was repeated 5 times with unexperienced larvae, results were averaged, and standard deviations were calculated.

2.8 Wind tunnel bioassays

A wind tunnel modified from Hee and Tan (Hee and Tan, 1998) was used to assess the ability of moth to respond to olfactory stimuli. The wind tunnel was constructed of a transparent polyacetate sheet rolled into a cylindrical tube (150 × 30 cm diameter) with ends forming the big plastic box for placing moths (50 × 50 × 50 cm). An electric fan was used to generate a continuous laminar airflow at a speed of 20 cm/s. Apple and sex pheromones mix solution (E10-16:Ald and Z10-16:Ald base on the ratio 9:1 were dissolved in hexane

to 100 ng/μL) (Yasuhiko et al., 1982) were alternately placed at the point (at the downwind end) in the wind tunnel for attracting the moths. When the dark period started 2 h, thirty male/female virgin moths were chosen and placed in the plastic box for testing in dark period. After 1 h, a moth in the tunnel reach or close to the apple or sex pheromones was counted, and each bouquet of female moth or male moth test repeat 5 times.

2.9 Interaction predicted analysis and gene selection

Interaction analysis of GOBP1 and GOBP2 was based on the STRING database (https://string-db.org/), including known and predicted protein-protein interactions. GOBP2 of*Bombyx mori* as a reference template to construct a network interaction map. Based on the results of the interaction, we selected the proteins that might interact with GOBPs and constructed a phylogenetic tree based on the predicted interactions between *Bombyx mori* protein and the identified genes of the yellow peach moth.

2.10 Analysis of protein-protein interaction

Proteins are selected prokaryotic expression *in vitro* and the method as described previously (Jing et al., 2019) (Supplementary Table 2 and Supplementary Figure 1). GOBPs proteins were titrated with Tris-HCl buffer solution by using isothermal titration calorimetry (ITC) (MicroCal ITC_{200}, GE Healthcare, UK). All solutions were thoroughly degassed prior to the titrations to avoid the formation of bubbles in calorimeter cell. The sample cell and reference cell were filled by GOBPs and Tris-HCl buffer of pH=7.4, respectively. Measurements were carried out at 25.0 ± 0.1 °C with a continuous string (600 rpm), a maximum number of injections of 2 μL volume were 20. Control experiments were performed by titrating the Tris-HCl buffer with buffer and subtracted it from the respective GOBPs- Tris-HCl titration before data fitting. The analysis of ITC data was performed using Microcal Origin 7.0 software following the instrument's manufacturer. The data were best fitted for one set of binding sites, and values enthalpy (ΔH), dissociation constant (K_D), entropy (ΔS), and stoichiometry (n) were obtained. The affinity ([?]G) was calculated from the Gibbs equation (Haman et al., 2019).

All the solutions involved in the reaction are desalted and then can be used for surface plasmon resonance (SPR) testing. The mixture of 400 mM EDC with100 mM NHS was injected over series S sensor chip CM5 (BR-1005-30, GE Healthcare, USA) at a flow rate of 10 μL/min. GOBP1 (10 μg/mL) and GOBP2 (10 μg/mL), including CSP1, CSP5, CSP10, and OBP17 were injected over CM5 chip at a flow rate of 10 μL/min, respectively. After each run, the dissociation and the regeneration were performed as described above. Following that, the proteins that could interact were serially diluted based on the initial binding, and the evaluation was carried out as described above.

2.12 Statistical analysis

Student's t-test analyzed larval growth parameters, response to artificial diet and EAG data. Tendency test of larvae and wind tunnel behavior were analyzed using one-way analysis of variance (ANOVA) followed by LSD and Tukey's test. Statistical analysis was performed using SPSS 20.0, and the p value was set at $P = 0.05$ for all comparisons.

3. Results

3.1 Ahomozygous strain of GOBPs deletion

A dual sgRNA-directed CRISPR-Cas9 system was employed to delete the GOBPs genes. According to the genomic arrangement of GOBP1 and GOBP2 (Figure 1), the sgRNA1/2 and sgRNA3/4 were designed to target the respective genes. The two specific sgRNAs and Cas9 protein were co-injected into yellow peach moth early embryos, respectively. In addition, to get both GOBP1 and GOBP2 (GOBP1/2) knockout homozygous population, four specific sgRNAs (sgRNA1/2/3/4) with Cas9 protein were using to co-inject. Among the injected eggs, 266 (96.72%), 180 (94.74%) and 199 (83.61%) neonates developed into adults (G_0) for GOBP1, GOBP2, and GOBP1/2, respectively (Table 1). Based on the PCR amplification results, the primer pair G1/2-F/R revealed about 350 bp band (GOBP1) and about 600 bp band (GOBP2). Sequencing

of those fragments confirmed a deletion event of the genes that were created and inherited in that single pair family. To get homozygous population, 10 homozygous males and females (1:1) were put together to produce the next generation (G_1). Randomly extract DNA and sequenced using primer pair G1/2-F/R from the G1, results showed all the samples have only one expected band. To confirm those adults were homozygous, pair primers T1-F/R and T2-F/R were used for testing. The results showed wild type had one band, but not the same as knocked out GOBP1 and GOBP2 samples (Figure 1). Sequencing the fragments of GOBP1 and GOBP2 confirmed each gene had successfully knockout and the sequences consistent with our expected (Figure 1). All homozygous adults keep generation and can be used for further experiments.

3.2 Development of larvae and food preference

After rearing for five consecutive generations, no difference between the knockout larvae (GOBP1, GOBP2 or GOBP1/2) with WT in pupa and emergence rate, indicating it does not affect the viability of this insect under our rearing conditions (Supplementary Table S3). However, after gene knockout, these larvae diverged in artificial diet choice (Figure 2). When the GOBP1 gene was knocked out, although the larvae had a decline in food selection, it did not reach a significant level, while the larvae that knocked out the GOBP2 or GOBP1/2 genes had apparent differences in food selection. It shows that knocking out of GOBP2 and GOBP1/2 genes affects the larvae sensitivity to food odorants.

3.3 Comparison ofEAG response to odorants of WT andhomozygous strain

We investigated antenna responses to host-plant odorants and some chemicals to determine whether the homozygous strain still detects previous odorants compared with WT strain. EAG was used to test the response to different chemicals. The EAG responses to parts of compounds in both male and female homozygous strain were strongly reduced or absent (Figure 3a). However, compared with males, when the genes were knocked out, female adults respond more strongly to the odorants tested. It shows that females are more sensitive to odorant types and some odorants than males. However, no matter which gene is knocked out, male sensitivity to the odor of sex pheromone has decreased, while females do not respond to it. We further analyzed the correlation between the three homozygous strains with WT strain by using EAG data. The results showed that EAG response of GOBP1/2 strain and WT strain is the highest (Figure 3b), indicating that the GOBP1 population is the least sensitive to odorants. It also indicates that when both genes are knocked out, the types of responses to odorants are significantly reduced. In addition, compared with the GOBP1 strain, the GOBP2 strain has a better correlation with the WT strain, indicating that GOBP2 gene knockout can also greatly reduce the sensitivity to multiple test odorants.

3.4 Comparison susceptibility to host and sex pheromones

Compared with the WT, male and female were not significantly decreased to the apple and sex pheromone, respectively (Figure 4a and d). However, it significantly decreased to apple and sex pheromone for females and males, respectively (Figure 4b and c) in all knockout strain. The results showed that after GOBP1 and GOBP2 genes knockout, adults' sensitivity to apple and sex pheromone are significantly decreased and the different sexes have different choices. Male moths are more sensitive to sex pheromone, while female adults are more sensitive to apple.

3.5 Protein interaction analysis

Based on the analysis results of the predicted protein (Figure 5) and phylogenetic tree (Supplements Figure S2), we finally selected some genes (CSP1, CSP5, CSP10, and OBP17) of the yellow peach moth for protein expression. Subsequently, the expressed proteins were tested for interaction with GOBP1 and GOBP2 proteins using SPR and ITC. The SPR results showed GOBP1 could bind with CSP5 and CSP10; GOBP2 can bind CSP5, CSP10, and OBP17 (Supplements Figure S3). The two proteins cannot bind to each other, and neither can bind to CSP1 (Supplements Figure S3). Multi-concentration SPR determination more accurately showed GOBPs and ligands' binding ability (Table 2 and Figure 6). The K_D of GOBP1 reaction with CSP5 (0.62 μM) and CSP10 (0.26 μM) are more than GOBP2 (0.34 μM and 0.08 μM), respectively, which indicating that GOBP2 has the more vital binding ability with ligands.

The results of ITC are shown in Figure 7. Each peak in the upper part represents the heat generated by a single titration of the same volume of small molecules into the protein solution, and the lower part is the protein binding nonlinear fitting graph. After the first titration, the peak heat value reaches the lowest and then gradually rises. After calculation, the K_D of GOBP1 reaction with CSP5 (1.70 μM) and CSP10 (2.04 μM) are more than GOBP2 (1.39 μM and 1.87 μM), respectively. Those results are consistent with the results of SPR that GOBP1 can directly interact with CSP5 and CSP10, and GOBP2 can directly interact with CSP5, CSP10, and OPB17. However, GOBP1 and GOBP2 cannot directly interact. In addition, all the reaction $\Delta G < 0$ and $|^{H}j{>}Tj^{S}jarespontaneouschemicalreactionsandthisreactionbyenthalpy - driving(Table2)$.

4. Discussion

GOBPs are a member of the OBPs family play important roles as the first barrier for insects to perceive odorants. Therefore, understanding the role of GOBPs in an insect's function requires inseparable from multisensory ecological interaction with the host (Stöckl and Kelber, 2019). In this study, we successfully addressed these behaviors by generating a homozygous strain with CRISPR-Cas9. Applied this genome editing system to produce a large deletion already be useful in *Helicoverpa armigera* and other insects in which the CRISPR-Cas9 system is effective (Wang et al., 2018; Wang et al., 2020b).

The homozygous GOBP1, GOBP2 and GOBP1/2 strain after knockout do not affect the insect's viability under our rearing conditions, but it results in weakening the larva and adults' sensitivity to food odorants. GOBPs are mainly localized in biconical sensilla and postulated to be involved in recognizing general odorants from host plants and the environment (Vogt et al., 1991; Wang et al., 2003; Steinbrecht et al., 1992; Laue et al., 1994). In this study, the larvae after the gene knockout were fed for five consecutive generations, there was no difference compared with the WT strain, and they could successfully emerge without showing a decrease in food sensitivity, mainly because there was more artificial diet around the larvae and this time its touch, taste or other olfactory genes play an important role. When the larvae were placed in a petri dish to keep them away from food and allowed to make choices, we found that they were significantly different from the WT strain. Its sensitivity to food odorants presents difficulties. This result indicates that GOBPs are indeed involved in identifying host odorants. However, not all larvae cannot find the location of the food, and it may be caused by the following reasons: a) some odorants in the diet may be recognized and operated to the receptor by other OBPs or CSPs, which as well as play crucial roles in insect chemoreception, such as recognizing, capturing and transporting hydrophobic chemicals from the environment to olfactory receptors (Pelosi et al., 2006; Pelosi et al., 2005; Liu et al., 2012); b) vision may play a role in identifying food; c) due to limited space, it could happen to find food after crawling aimlessly.

EAG only represents an overall activity of all the sensilla on the antenna and, therefore, even compassionate specialist olfactory receptor neurons may not show up if their total number is low (Deng et al., 2012). When the gene was knocked out, most of the moth's antennae showed a weakened response or even no response to odorants (Figure 3a), indicating that GOBPs are closely related to the recognition of these odorants. Males and females have different responses to the same odorants, and compared to males, females respond more obviously to the tested odorants, which means that females can perceive more odorants than males. The same result can also show in the wind tunnel test. This may be related to their final task. In fact, many moths have shown plasticity of olfactory-guided behavior, dependent not only on the nature of the chemical but also on the physiological status (e.g., age, growth conditions, hormone or mating status) of the individual (Anton et al., 2007; Kubli, 2003; Jing et al., 2020). Our previous studies have also proved this result by stimulating host odorants, ovarian development, and changes in GOBPs expression before and after oviposition (Jing et al., 2019; Jing et al., 2020). For males and looking for hosts to maintain normal physiological activities after emergence, it is more important to find females to complete mating. Therefore, male adults may pay more attention to recognize sex pheromones. For females, looking for food and attracting males to complete mating, it is more important to find suitable oviposition sites, which determines that females need to be able to recognize more odorants. The wind tunnel test experiment in this study also strongly proved sex pheromones are more attractive to males, while host odorants are more attractive to females.

Interestingly, the effect of GOBP2 gene knocked out seems to be greater than GOBP1, and there is no

significant difference between the knockout of both GOBP1 and GOBP2 genes (Figure 3 and 4). Previous research has demonstrated that proteins in the GOBP2 class share high sequence similarity and can bind to a wide range of odorants with a broad specificity (Deng et al., 2012; Zhou et al., 2009; He et al., 2010; Gong et al., 2009) and can bind with the sex pheromones (Feng and Prestwich, 1997; He et al., 2010; Liu et al., 2010). Another experiment also showed that GOBPs from different families of lepidoptera. Lepidoptera seem to have different expression patterns among male and female moths, indicating the different physiological roles of GOBP1 and GOBP2 in the perception of semiochemicals (Yao et al., 2016). This difference may be due to the gender mentioned above, but more importantly, is the function of the protein itself. ITC and SPR are good methods used to thermodynamically and kinetically characterize the protein-ligand or protein-protein interactions' binding mechanism, respectively (Jelesarov and Bosshard, 1999; Krishnamoorthy et al., 2020; Kim et al., 2020). Based on the SPR results, GOBP2 is better than GOBP1 in terms of the number of binding proteins and binding ability. From this result, we can hypothesize that when GOBP2 binds to a variety of other proteins, a complex is formed, which greatly broadens the binding range of the GOBP2 protein, especially with a strong binding ability CSP protein. Because although CSP is a distinct from OBPs, it shares no sequence similarity with OBPs (Lu et al., 2007; Gong et al., 2007). Whereas OBPs are primarily expressed in antennae, CSPs are expressed in various insect tissues, such as antennae, maxillary palps (Maleszka and Stange, 1997), proboscis, pheromone glands (Meillour et al., 2000), and the sub-cuticular epithelium (Wanner et al., 2004), etc. Furthermore, although a vast amount of structural evidence on OBPs and CSPs has been collected in the effort to elucidate their function, physiological studies have been sparse and have failed to offer compelling models for their behavior (Pelosi et al., 2006). The GOBPs can reaction with CSPs by SPR and ITC may provide a new idea for research or contribute to more in-depth research on both.

ITC can detect whether proteins are interacting and measures the heat released or absorbed during the protein-ligand interactions (Krishnamoorthy et al., 2020; Freyer and Lewis, 2008). The results of ITC are consistent tendency with the results of SPR. All reactions with $\Delta G < 0$ are spontaneous reactions, ΔH¡0 are exothermic reactions and ΔS¡0 indicates reactions are process driven by enthalpy, and an unfavorable entropy compensation effect occurs. It also shows that non-covalent bonds such as hydrogen bonds and van der Waals forces may be formed in these reactions. ΔS¡0 indicates that during the binding process, a specific protein is likely to undergo a conformational change, which will reduce the degree of freedom of the molecule, which is a factor that is not conducive to the binding of these proteins. The conformational change of this protein may have a great relationship with the pH value. It was demonstrated previously that the C-terminal dodecapeptide segment of *Bmor* PBP formed a regular helix α7 at pH 4.5, the compact structure allowing the ligand to enter the binding cavity would not be detectable once the complex was formed; and at pH 6.5 did not present the additional α7. The C-terminus of the protein folds into an α-helical domain and enters the bombykol binding site, thus assisting the release of the pheromone molecule from the cavity (Wojtasek and Leal, 1999; Horst et al., 2001). The pH-dependent conformational structural flexibility was also reported in other members of olfactory proteins, such as in *Aedes aegypti*(Leite et al., 2009), *Antheraea polyphemus* (Mohanty et al., 2004). In addition, the structures of PBP1 and PBP2 of *Lymantria dispar* had different changes at pH 5.5 and 7.3 by fluorescence binding assay, showing different affinity to chemicals (Yu et al., 2012). Therefore, we believe that the ΔS will also change under different pH values, especially at low pH, which may be a favorable response. Especially when GOBPs and CSPs are combined, it will be more interesting if their conformation is changed by pH after forming a complex. Therefore, more in-depth experiments need to continue.

In conclusion, we have successfully knockout GOBP1 and GOBP2 genes in *C. punctiferalis* with the CRISPR-Cas9 system. Through the tendency test of larvae to artificial diet, EAG, and the wind tunnel test of the adult, it was found that the knockout effect of the GOBP2 gene is better than GOBP1. The protein interaction test initially explained that this is due to the more substantial binding capacity and broader binding spectrum of GOBP2 than GOBP1. Also, this study provides an additional perspective on insect GOBP, OBP, and CSP genes and their functional contribution to the pest olfactory system.

Funding

This research was funded by the National Key Research and Development Program of China (2018YFD0200602) and China Agriculture Research System (CARS-02).

Competing interests

The authors declare that they have no competing interests.

References

Anton, S., Dufour, M.C., & Gadenne, C. (2007). Plasticity of olfactory-guided behaviour and its neurobiological basis: lessons from moths and locusts. *Entomologia Experimentalis et Applicata,* 123:1-11. doi:10.1111/j.1570-7458.2007.00516.x

Braccini, C.L., Vega, A.S., Aráoz, M.V.C., Teal, P.E., Cerrillo, T., Zavala, J.A., & Fernandez, P.C. (2015). Both volatiles and cuticular plant compounds determine oviposition of the willow sawfly nematus oligospilus on leaves of *Salix spp* . (Salicaceae). *Journal of Chemical Ecology,* 41:985-996. doi:10.1007/s10886-015-0637-z

Deng, S.S., Yin, J., Zhong, T., Cao, Y.Z., Li, K.B. (2012). Function and immunocytochemical localization of two novel odorant-binding proteins in olfactory sensilla of the scarab beetle *Holotrichia oblita Faldermann* (Coleoptera: Scarabaeidae). *Chemical Senses,*37:141-150. doi:10.1093/chemse/bjr084

Feng, L., & Prestwich, G.D. (1997). Expression and characterization of a lepidopteran general odorant binding protein. *Insect Biochemistry and Molecular Biology,* 27:405-412. doi:10.1016/s0965-1748(97)00012-x

Freyer, M.W., Lewis, E.A. (2008). Isothermal titration calorimetry: experimental design, data analysis, and probing macromolecule/ligand binding and kinetic interactions. *Methods Cell Biology* , 84:79-113. doi:10.1016/S0091-679X(07)84004-0

Gong, D.P., Zhang, H.J., Zhao, P., Lin, Y., Xia, Q.Y., & Xiang, Z.H. (2007). Identification and expression pattern of the chemosensory protein gene family in the silkworm, *Bombyx mori* -ScienceDirect. *Insect Biochemistry and Molecular Biology,* 37:266-277. doi.org/10.1016/j.ibmb.2006.11.012

Gong, Z.J., Zhou, W.W., Yu, H.Z., Mao, C.G., Zhang, C.X., Cheng, J.A., & Zhu, Z.R. (2009). Cloning, expression and functional analysis of a general odorant-binding protein 2 gene of the rice striped stem borer,*Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae).*Insect molecular biology,* 18:405-417. doi:10.1111/j.1365-2583.2009.00886.x

Haman, N., Signorelli, M., Duce, C., Franzetti, L. & Fessas, D. (2019). Isothermal calorimetry protocols to monitor the shelf life and aftermarket follow-up of fresh cut vegetables. *Journal of Thermal Analysis and Calorimetry,* 137:1673-1680. doi:10.1007/s10973-019-08064-4

He, X.L., Tzotzos, G., Woodcock, C., Pickett, J.A., Hooper, T., Field, L.M., & Zhou, J.J. (2010). Binding of the general odorant binding protein of *Bombyx mori* BmorGOBP2 to the moth sex pheromone components. *Journal of Chemical Ecology,* 36:1293-1305. doi:10.1007/s10886-010-9870-7

Hee, A.K.W., & Tan, K.H. (1998). Attraction of Female and Male*Bactrocera papayae* to conspecific males fed with methyl eugenol and attraction of females to male sex pheromone components.*Journal of Chemical Ecology* , 24:53-764. doi:10.1023/A:1022302605357

Horst, R., Damberger, F., Luginbühl, P., Güntert, P., Peng, G., Nikonova. L., Leal, W.S., & Wüthrich, K. (2001). NMR structure reveals intramolecular regulation mechanism for pheromone binding and release. *Proceedings of the National Academy of Sciences of the United States of America,* 98:14374-14379. doi:10.1073/pnas.251532998

Jelesarov, I., & Bosshard, H.R. (1999). Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition.J*ournal of Molecular Recognition,* 12:3-18. doi:10.1002/(SICI)1099-1352(199901/02)12:1<3::AID-JMR441>3.0.CO;2-6

Jing, D.P., Zhang. T.T., Bai, S.X., Prabu, S., He, K.L., Dewer, Y., & Wang, Z.Y. (2019). GOBP1 Plays a key role in sex pheromones and plant volatiles recognition in yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Crambidae). *Insects,*10:302-317. doi.org/10.3390/insects10090302

Jing, D.P., Zhang, T.T., Prabu, S., Bai, S.X., He, K.L., Luan, J., & Wang, Z.Y. (2020). PBP genes regulated by the development of the ovaries, sex pheromone release, mating and oviposition behavior in *Conogethes punctiferalis* (Guenée). *Chemoecology,*31:1-9. doi:10.1007/s00049-020-00323-3

Khuhro, S.A., Liao, H., Dong, X.T., Yu, Q., Yan, Q., & Dong, S.L. (2017). Two general odorant binding proteins display high bindings to both host plant volatiles and sex pheromones in a pyralid moth *Chilo suppressalis* (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology,* 20:521-528. doi:10.1016/j.aspen.2017.02.015

Kim, S.Y., Zhang, F., Harris, D.A., & Linhardt, R.J. (2020). Structural features of heparin and its interactions with cellular prion protein measured by surface plasmon resonance. *Frontiers in Molecular Biosciences,* 7:594497. doi:10.3389/fmolb.2020.594497, PMID: 33324681

Krieger, J., Grosse-Wilde, E., Gohl, T., Dewer, Y.M.E., Raming, K., & Breer, H. (2004). Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). *Proceedings of the National Academy of Sciences of the United States of America,* 101:11845-11850. doi.org/10.1073/pnas.0403052101, PMID: 15289611

Krishnamoorthy, G.K., Alluvada, P., Sherieff, S.H.M., Kwa, T., & Krishnamoorthy, J. (2020). Isothermal titration calorimetry and surface plasmon resonance analysis using the dynamic approach.*Biochemistry and Biophysics Reports,* 21:100712. doi:10.1016/j.bbrep.2019.100712

Kubli, E. (2003). Sex-peptides: Seminal peptides of the*Drosophila* male. *Cellular and Molecular Life Sciences,*60:1689-1704. doi.org/10.1007/s00018-003-3052

Laue, M., Steinbrecht, R.A., & Ziegelberger, G. (1994). Immunocytochemical localization of general odorant-binding protein in olfactory sensilla of the silkmoth *Antheraea polyphemus.Naturwissenschaften,* 81:178-180. doi:10.1007/BF01134537

Leite, N.R., Krogh, R., Xu, W., Ishida, Y., Iulek, J., Leal, W.S., & Oliva, G. (2009). Structure of an odorant-binding protein from the mosquito *Aedes aegypti* suggests a binding pocket covered by a pH-sensitive "Lid". *PLoS One,* 4:e8006. doi:10.1371/journal.pone.0008006, PMID: 19956631

Liu, N.Y., He, P., & Dong, S.L. (2011). Binding properties of pheromone-binding protein 1 from the common cutworm *Spodoptera litura* . *Comparative Biochemistry & Physiology Part B Biochemistry & Molecular Biology* 161:295-302. doi:10.1016/j.cbpb.2011.11.007

Liu, R., He, X., Lehane, S., Lehane, M., Hertz-Fowler, C., Berriman, M., Field, L.M., & Zhou, J.J. (2012). Expression of chemosensory proteins in the tsetse fly *Glossina morsitans* is related to female host-seeking behaviour. *Insect Molecular Biology,*21:41-48. doi:10.1111/j.1365-2583.2011.01114.x

Liu, Z., Vidal, D.M., Syed, Z., Ishida, Y., & Leal, W.S. (2010). Pheromone binding to general odorant-binding proteins from the navel orange worm. *Journal of Chemical Ecology,* 36:787-794. doi:10.1007/s10886-010-9811-5

Liu, N.Y., Yang, F., Yang, K., He, P., Niu, X.H., Xu, W., Anderson, A., & Dong, S.L. (2015). Two subclasses of odorant-binding proteins in*Spodoptera exigua* display structural conservation and functional divergence. *Insect Molecular Biology,* 24:167-182. doi:10.1111/imb.12143

Lu, D.G., Li, X.R., Liu, X.X., & Zhang, Q.W. (2007). Identification and molecular cloning of putative odorant-binding proteins and chemosensory protein from the bethylid wasp, *Scleroderma guani* Xiao et Wu. *Journal of Chemical Ecology,* 33:1359-1375. doi:10.1007/s10886-007-9310-5

Lu, J.Q., Wang, Z.Y., He, K.L., & Liu, Y. (2010). Research history, progresses and prospects in the yellow peach moth, *Conogethes punctiferalis. Plant Protection,* 36:37-38. doi:10.4028/www.scientific.net/AMM.37-38.1549

Maleszka, R., & Stange, G. (1997). Molecular cloning, by a novel approach, of a cDNA encoding a putative olfactory protein in the labial palps of the moth *Cactoblastis cactorum* . *Gene,* 202:39-43. doi:10.1016/S0378-1119(97)00448-4

Meillour, P.N., Cain, A.H., Jacquin-Joly, E., François, M.C., Ramachandran, S., Maida, R., & Steinbrecht, R.A. (2000). Chemosensory proteins from the proboscis of *Mamestra brassicae. Chemical Senses,* 25:541-553. doi:10.1093/chemse/25.5.541

Merritt, T.J., LaForest, S., Prestwich, G.D., Quattro, J.M., & Vogt, R.G. (1988). Patterns of gene duplication in lepidopteran pheromone binding proteins. *Journal of Molecular Evolution*146:272-276. doi:10.1007/pl00006303

Mohanty, S., Zubkov, S., Gronenborn, A.M. (2004). The solution NMR structure of *Antheraea polyphemus* PBP provides new insight into pheromone recognition by pheromone-binding proteins.*Journal of Molecular Biology,* 337:443-451. doi:10.1016/j.jmb.2004.01.009, PMID: 15003458

Nardi, J.B., Miller, L.A., Walden, K.K.O., Rovelstad, S., Wang, L., Frye, J.C., Ramsdell, K., Deem, L.S., & Robertson, H.M. (2003). Expression patterns of odorant-binding proteins in antennae of the moth*Manduca sexta. Cell & Tissue Research,* 313:321-333. doi:10.1007/s00441-003-0766-5

Pelosi, P., Calvello, M., & Ban, L.P. (2005). Diversity of odorant-binding proteins and chemosensory proteins in insects.*Chemical Senses,* 30:291-292. doi:10.1093/chemse/bjh229

Pelosi, P., Zhou, J.J., Ban, L.P., & Calvello, M. (2006). Soluble proteins in insect chemical communication. *Cellular and Molecular Life Sciences,* 63:1658-1676. doi:10.1007/s00018-005-5607-0

Ren, L.L., Balakrishnan, K., Luo, Y.Q., & Schütz, S. (2017). EAG response and behavioral orientation of *Dastarcus helophoroides*(Fairmaire) (Coleoptera: Bothrideridae) to synthetic host-associated volatiles. *Plos One,* 12:e0190067. doi:10.1371/journal.pone.0190067

Ribeiro, C., & Dickson, B.J. (2010). Sex peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in *Drosophila* .*Current Biology,* 20:1000-1005. doi:10.1016/j.cub.2010.03.061

Steinbrecht, R.A., Laue, M., & Ziegelberger, G. (1995). Immunolocalization of pheromone-binding protein and general odorant-binding protein in olfactory sensilla of the silk moths*Anthraea* and *Bombyx. Cell & Tissue Research,*282:203-217. doi:10.1007/BF00319112

Steinbrecht, R.A., Ozaki, M., & Ziegelberger, G. (1992). Immunocytochemical localization of pheromone-binding protein in moth antennae. *Cell & Tissue Research* , 270:287-302. doi:10.1007/BF00328015

Stöckl, A.L., & Kelber, A. (2019). Fuelling on the wing: sensory ecology of hawkmoth foraging. *Journal of Comparative Physiology A Neuroethology Sensory Neural & Behavioral Physiology,*205:399-413. doi:10.1007/s00359-019-01328-2

Vogt, R.G., Grosse-Wilde, E., & Zhou, J.J. (2015). The lepidoptera odorant binding protein gene family: gene gain and loss within the GOBP/PBP complex of moths and butterflies. *Insect Biochemistry and Molecular Biology,* 62:142-153. doi:10.1016/j.ibmb.2015.03.003

Vogt, R.G., Prestwich, G.D., & Lerner, M.R. (1991). Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. *Developmental Neurobiology,* 22:74-84. doi:10.1002/neu.480220108

Vogt, R.G., & Riddiford, L.M. (1981). Pheromone binding and inactivation by moth antennae. *Nature,* 293:61-163. doi:10.1038/293161a0

Vogt, R.G., Rogers, M.E., Franco, M., & Sun, M. (2002). A comparative study of odorant binding protein genes: Differential expression of the PBP1-GOBP2 gene cluster in *Manduca sexta* (Lepidoptera) and the organization of OBP genes in *Drosophila melanogaster* (Diptera).*Journal of Experimental Biology,* 205:719-744. doi:10.2174/1389450111009011413

Wan, N.F., Zhang, Y.M., Huang, K.H., Ji, X.Y., Jiang, J.X. (2016). Ecological engineering of trap cropping promotes biocontrol services in peach orchard ecosystems. *Ecological Engineering* . 90:427-430. doi:10.1016/j.ecoleng.2016.01.045

Wang, G.R., Wu, K.M., & Guo, Y.Y. (2003). Cloning, expression and immunocytochemical localization of a general odorant-binding protein gene from *Helicoverpa armigera* (Hubner). *Insect Biochemistry and Molecular Biology,* 33:115-124. doi:10.1016/s0965-1748(02)00182-0

Wang, H.D., Shi, Yu., Wang, L., Liu, S., Wu, S.W., Yang, Y.H., Feyereisen, R., & Wu, Y.D. (2018). CYP6AE gene cluster knockout in*Helicoverpa armigera* reveals role in detoxification of phytochemicals and insecticides. *Nature Communications,*9:4820. doi:10.1038/s41467-018-07226-6

Wang, J., Ma, H.H., Zhao, S., Huang, J.L., Yang, Y.H., Tabashnik, B.E., & Wu, Y.D. (2020a). Functional redundancy of two ABC transporter proteins in mediating toxicity of *Bacillus thuringiensis* to cotton bollworm. *PLOS Pathogens* , 16:e1008427. doi:10.1371/journal.ppat.1008427

Wang, M., Zhang, S.H., Shi, Y., Yang, Y.H., & Wu, Y.D. (2020b). Global gene expression changes induced by knockout of a protease gene cluster in *Helicoverpa armigera* with CRISPR/Cas9. *Journal of Insect Physiology,* 122:104023. doi:10.1016/j.jinsphys.2020.104023

Wang, Z.Y., He, K.L., Shi, J., & Ma, S.Y. (2006). Reasons for aggravation of yellow peach moth on corn and control countermeasures.*Plant Protection,* 32: 67-69.

Wanner, K.W., Willis, L.G., Theilmann, D.A., Isman, M.B., Feng, Q.L., & Plettner, E. (2004). Analysis of the insect OS-D-Like gene family. *Journal of Chemical Ecology* , 30:889-911. doi:10.1023/b:joec.0000028457.51147.d4

Wojtasek, H. & Leal, W.S. (1999). Conformational change in the pheromone-binding protein from *Bombyx mori* induced by pH and by interaction with membranes. *Journal of Biological Chemistry,* 274:30950-30956. doi:10.1074/jbc.274.43.30950

Yao, Q., Xu, S., Dong, Y.Z., Lu, K., & Chen, B.X. (2016). Identification and characterisation of two general odourant-binding proteins from the litchi fruit borer, *Conopomorpha sinensis Bradley* . *Pest Management Science,* 72:877-887. doi:10.1002/ps.4062

Yasuhiko, K., Koshi, A., Katsui, S., & Yoshiharu, M. (1982). (E)-10-Hexadecenal, a sex pheromone component of the yellow peach moth,*Dichocrocis punctiferalis* Guenee (Lepidoptera: Pyralidae).*Applied Entomology & Zoology* , 17:207-217. doi:10.1051/apido:19820405

Yasukochi, Y., Yang, B., Fujimoto, T., Sahara, K., Matsuo, T., & Ishikawa, Y. (2018). Conservation and lineage-specific rearrangements in the GOBP/PBP gene complex of distantly related ditrysian lepidoptera. *Plos One,* 13:e0197528. doi:10.1371/journal.pone.0192762

Yu, Y.X., Ma, F., Cao, Y.X., Zhang, J.H., Zhang, Y.G., Duan, S.N., Wei, Y.D., Zhu, S.F., & Chen, N.Z. (2012). Structural and functional difference of pheromone binding proteins in discriminating chemicals in the gypsy moth, *Lymantria dispar. International Journal Biological Sciences,* 8: 979-991. doi:10.7150/ijbs.4557

Zhang, D.D., Wang, H.L., Schultze, A., Fross, H., Francke, W., Krieger, J., & Lofstedt, C. (2016). Receptor for detection of a type II sex pheromone in the winter moth *Operophtera brumata .Scientific Reports,* 6:18576-18586. doi:10.1038/srep18576

Zhang, L.L., & Reed, R.D. (2016). Genome editing in butterflies reveals that spalt promotes and distal-less represses eyespot colour patterns. *Nature Communications,* 7:11769. doi:10.1038/ncomms11769

Zhang, Y.N., Jin, J.Y., Jin, R., Xia, Y.H., Zhou, J.J., Deng. J.Y., & Dong, S.L. (2013). Differential expression patterns in chemosensory and non-chemosensory tissues of putative chemosensory genes identified by transcriptome analysis of insect pest the purple stem borer*Sesamia inferens* (Walker). *Plos One,* 8:e69715. doi:10.1371/journal.pone.0069715

Zhou, J.J., Robertson, G., He, X.L., Dufour, S., Hooper, A.M., Pickett, J.A., Keep, N.H., & Field, L.M. (2009). Characterization of*Bombyx mori* odorant-binding proteins reveals that a general odorant-binding protein discriminates between sex pheromone components. *Journal of Molecular Biology,* 389:529-545. doi:10.1016/j.jmb.2009.04.015

Tables

Table 1 Statistics of parameters after injection

Gene name	Number of injected eggs (number)	Hatching rate (%)	Pupation rate (%)	Emergence rate (%)	Mutatio
GOBP1	1030	39.12	88.14	96.72	40.62
GOBP2	720	31.94	86.52	94.74	44.45
GOBP1/2	802	47.63	77.22	83.61	52.76

Table 2 Summary of kinetic data of GOBPs protein wit ligands interactions

Interaction	SPR	SPR	SPR	ITC	ITC	ITC	ITC	ITC
	k_a (1/MS)*	k_d (1/S)	K_D (μM)	$^{H}(kJmol^{-1})$	$^{S}(kJmol^{-1})$	- $T^{S}(kJmol^{-1})$	$^{G}(kJmol^{-1})$	$^{G}(kJmol^{-1})$
GOBP1-CSP5	3.55E+03	2.20E-03	0.2	-2817.32	-9.32	2779.18	2779.18	-38.14
GOBP1-CSP10	1.06E+04	2.73E-03	0.26	-3748.62	-12.46	3713.88	3713.88	-34.75
GOBP2-CSP5	6.90E+03	2.33E-03	0.34	-48446.2	-162.18	48355.2	48355.2	-91.04
GOBP2-CSP10	2.50E+04	2.08E-03	0.08	-2786.81	-9.24	2754.25	2754.25	-32.56
GOBP2-OBP17	1.13E+04	8.88E-04	0.08	-5584.48	-18.64	5558.35	5558.35	-26.13

* k_a means association rate constant.

Figure legends

Figure 1 CRIPSPR-Cas9-based knock out of the GOBPs genes in yellow peach moth. **Step1** : positions of each sgRNA and the pair primers for allele-specific PCR detection test. **Step2** : Target sequences of the two sgRNAs (blue), the PAM sequences (red) and the cutting sites by the Cas9 protein are indicated with red triangles; a representative chromatogram from PCR products sequencing of individuals after knocking out population with different pair primers.**Step3** : Genotyping of individual for deletion of each gene according to banding patterns of the PCR products amplified with a set of the different primer pairs. The band pointed with white arrow is represent homozygous.

Figure 2 Sensitivity test of larvae to artificial diet odorants. Asterisk indicates that significant differences in data (*t-test, ***P [?] 0.001, **P [?] 0.01*).

Figure 3 Homozygous strain shows reduced olfactory responses to ecologically relevant single odorants. A heat-map representation of median EAG response values (millivolts) was corrected for the response to the solvent from male and female antennae. Asterisk indicates that significant differences in data when compare with WT (*t-test, ***P [?] 0.001, **P [?] 0.01, *P [?] 0.05*). b redundancy analysis (RDA) on EAG response value in homozygous strain with knocking out GOBP1, GOBP2, and GOBP1/GOBP2. The smaller the angle, the better the correlation. G1, G2, and G1/2 G1 represents the homozygous GOBP1,

GOBP2, and GOBP1/2 strain.

Figure 4 Tendency test of adults to apples and sex pheromones. a and b are the tendency of adults to apple, c and d are the tendencies of adults to sex pheromones (ANOVA, ****P [?] 0.001, 0.01[?] *P [?] 0.05*).

Figure 5 STRING Analysis: Protein-protein interaction networks of GOBP1 and GOBP2. a) GOBP1 interacting network with CSPs and OBPs. b) GOBP2 interacting network with CSPs and OBPs. The GOBP1 and 2 had the average local clustering coefficient of 0.839 and 0.861. The insets at the below show the evidence types in STRING network prediction (p-value - <0.05).

Figure 6 SPR of GOBPs-ligands interaction for kinetic measurements. After diluting the ligand at 2 times the concentration, the measured results are represented by different color curves, and the black curve represents the fitting curve; a is GOBP1 reaction with CSP5 and CSP10, respectively; b is GOBP2 reaction with CSP5, CSP10, and OBP17, respectively. All solutions are measured under the condition of pH=7.4.

Figure 7 ITC profiles for the complexation of GOBPs with other ligands. a is GOBP1 reaction with CSP5 and CSP10, respectively; b is GOBP2 reaction with CSP5, CSP10 and OBP17, respectively. All solutions are measured under the condition of pH=7.4.

Figure S1. SDS-PAGE analysis of purified recombinant CSPs and OBPs.

Figure S2. Phylogenetic tree based on amino acid sequences of OBPs and CSPs from *Bombyx mori* and yellow peach moth. The sequences were aligned using ClustalW. Theevolutionary history was inferred using the neighbor-joining method and MEGA with 1000bootstrap replicates. The OBPs shown in the tree are as follows: *Bombyx mori* (BmorOBP2, NM_001146714; BmorOBP6, NM_001146718; BmorOBP7, NM_001146719; BmorCSP2, NM_001098308; BmorCSP4, NM_001043587; BmorCSP6, NM_001043935; BmorCSP9, NM_001043604; BmorCSP16, NM_001098312), *Conogethes punctiferalis* (CpunOBP1, KF026054; CpunOBP2, KF026055; CpunOBP3, KF026056; CpunOBP4, KP985222; CpunOBP5, KP985223; CpunOBP6, KP985224; CpunOBP7, KP985225; CpunOBP8, KP985226; CpunOBP9, KY130463; CpunOBP10, KY130464; CpunOBP11, KY130465; CpunOBP12, KY130466; CpunOBP13, KY130467; CpunOBP14, KY130469; CpunOBP15, KY130470; CpunOBP16, KY130472; CpunOBP17, KY130473; CpunOBP18, KY130474; CpunOBP19, KY130475; CpunOBP20, KY130476; CpunCSP1, KF026049; CpunCSP2, KF026050; CpunCSP3, KY130477; CpunCSP4, KF026057; CpunCSP5, KF026058; CpunCSP6, KF026051; CpunCSP7, KF026052; CpunCSP8, KF026053; CpunCSP9, KY130478; CpunCSP10, KY130479; CpunCSP11, KY130480; CpunCSP12, KY130481; CpunCSP13, KY130482; CpunCSP14, KY130483; CpunCSP15, KY130484).

Figure S3. SPR of GOBPs-ligands interaction for kinetic measurements.