Identification and RNAi-Based Functional Analysis of Four Chitin Deacetylase Genes in Sogatella furcifera (Hemiptera: Delphacidae)

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Abstract

Chitin deacetylases (CDAs) are chitin-degrading enzymes that play a key role in insect molting. In this study, we identified and characterized four full-length cDNAs of CDAs from Sogatella furcifera (Horváth). Developmental expression showed that SICDA1 and SICDA2 were expressed at all nymph developmental stages, SICDA3 and SICDA4 were mainly expressed in the third-instar to fifth-instar nymph stages, whereas tissue-specific analyses indicated that four CDA genes were mainly high expressed in the integument and head during the fifth-instar nymph. RNA interference (RNAi) results revealed that SICDA1, SICDA2, and SICDA4 are associated with molting defect and high mortality with nymph–adult molting. Furthermore, transcripts of chitin synthase 1 variants (SICHS1a, SICHS1a, and SICHS1b) were significantly downregulated and causing significant changes in the expression levels of trehalases (TRE1 and TRE2) in the SICDA1, SICDA2, and SICDA4 dsRNA treatment groups. By contrast, no significant phenotypic characteristics were observed after dsSICDA3 injection. Taken together, our results suggest that SICDA1, SICDA2, and SICDA4 play a vital role in nymph–adult transition, and these genes could regulate chitin biosynthesis expression levels.

Key words: Sogatella furcifera, chitin deacetylase, molting, wing expanse, chitin biosynthesis

Chitin, a linear polymer of N-acetylglucosamine linked with β-1,4 glycosidic bonds, is widely distributed in fungi, nematodes, insects, brachiopods, and mollusks (Merzendorfer 2006). In insect, chitin has been a major component of the integument, peritrophic membrane (PM), and tracheal tissues, and it plays an important role in maintaining the shape and protecting against external forces and is critical for molting (Moussian 2010, Merzendorfer 2011). The periodic degradation and synthesis of chitin is essential for insects’ proper growth and development (Arakane and Muthukrishnan 2010, Zhu et al. 2016).

Chitin deacetylases (EC3.5.1.41, CDAs) are key enzymes involved in chitin metabolism, belonging to carbohydrate esterase family 4 (CE4). They catalyze the N-deacetylation of chitin to form chitosan (Tsigos et al. 2000). The first insect CDA sequence was identified from the midgut of Trichoplusia ni (Lepidoptera: Noctuidae) (Guo et al. 2005). Since then, several CDAs have been identified in many insect orders, including Lepidoptera (Zhong et al. 2014, Tetreau et al. 2015), Coleoptera, Hymenoptera, Dipteran (Dixit et al. 2008), Hemiptera (Xi et al. 2014), and Orthoptera (Yu et al. 2016, Ding et al. 2014). Insect CDAs were divided into five groups (I–V) based on sequence homology and domain structures. Groups I and II include a chitin-binding domain (CBD), a low-density lipoprotein receptor domain (LDLa), and a deacetylase-like catalytic domain (CDA). Groups III and IV contain a CBD and a CDA, but group V only has a CDA (Tetreau et al. 2015). Group I CDAs (CDA1 and CDA2) play a critical role in molting, as demonstrated by RNA interference (RNAi) knockdown in Tribolium castaneum (Coleoptera: Tenebrionidae) (Arakane et al. 2009), Stegobium paniceum (Coleoptera: Anobiidae) (Yang et al. 2018), Choristoneura fumiferana (Lepidoptera: Tortricidae) (Quan et al. 2013), Nilaparvata lugens (Hemiptera: Delphacidae) (Xi et al. 2014), and Locusta migratoria (Orthoptera: Acrididae) (Yu et al. 2016, Yu et al. 2018). In Drosophila melanogaster (Diptera: Drosophilidae), a serpenpine (serp) mutation in CDA1 or a vermi-form (verm) mutation in CDA2 leads to elongated and tortuous tracheal tubes (Luschnig et al. 2006, Wang et al. 2006). Group V CDAs, a midgut PM protein, and group V CDAs are involved in changes to the physicochemical properties of chitin in the midgut.
PM of *Mamestra configurata* (Lepidoptera: Noctuidae) and *Bombyx mori* (Lepidoptera: Bombycidae), which affects the binding degree and integrity of proteins (Toprak et al. 2008, Liu et al. 2019). Despite these numerous reports, the number and function of CDA genes in *Sogatella furcifera* (Horváth) remain unknown.

The white-backed planthopper (WBPH), *S. furcifera*, is an important pest of rice and causes severe losses in the rice production by sucking plant juices, oviposition, and transmitting viruses (Zhou et al. 2008). Currently, control of *S. furcifera* has mainly relied on insecticides, but due to its irrational usage, *S. furcifera* has developed resistance against many insecticides and caused environmental pollution (Zhang et al. 2017, Nakata et al. 2019). Therefore, it is urgent to explore new pest-control strategies. For example, RNAi technique is able to effectively knock down expression of vital genes insects and it holds considerable potential for controlling pest insects (Niu et al. 2018). Because chitin is absent in vertebrates, CDAs genes could be considered as targets for RNAi-based pest control.

In this study, we identified and characterized the full-length cDNA sequences of four CDA genes from *S. furcifera* and investigated their expression profiles at different developmental stages and tissues. Furthermore, RNAi of CDAs genes was performed to elucidate roles during nymph-adults molting.

**Materials and Methods**

**Insects Rearing**

*Sogatella furcifera* were collected in 2013 from a rice field in the Hua Xi district, Guiyang, Guizhou Province, China. All insects were reared on TN1 rice seedlings in a climate chamber at a temperature 25 ± 1°C and 70 ± 10% relative humidity (RH) with a photoperiod of 16 h (light) and 8 h (dark).

**Cloning and Sequencing**

First, the *S. furcifera* genomic and transcriptomic annotation database (Wang et al. 2017, Zhou et al. 2018) was identified. Second, reported CDA nucleotide and protein sequences for CDA genes from *T. castaneum*, *D. melanogaster*, *N. lugens*, *L. migratoria*, *Anopheles gambiae* (Diptera: Culicidae) were used as a reference (Supp Table S2 [online only]) to assemble transcriptome and genome databases using Geneious R9 software (Kearse et al. 2012). Subsequently, each of the putative CDA-like sequences was identified used BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi/).

Reverse-transcription PCR (RT–PCR) was used to validate CDA gene sequences screened from the genome and transcriptome database of *S. furcifera*. Primers were designed using Primer Premier 6.0 (Premier Biosoft, CA, USA; Supp Table S1 [online only]). RT–PCR was performed using Taq DNA polymerase (Catalog numbers: B600001, Sangon Biotech, Shanghai, China), with reaction program as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55–60°C for 30 s, and elongation at 72°C for 1–2 min, with extension at 72°C for 10 min. PCR product purified were using a DiaSpin Column DNA Gel Extraction Kit (Catalog numbers: B110092, Sangon Biotech, Shanghai, China), and each purified DNA product was sequenced in both directions by Sangon Biotech (Shanghai, China).

Full-length cDNA sequences of *S. furcifera* CDA genes were cloned by rapid amplification of cDNA ends (RACE) using a SMARTer RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) following the manufacturer’s instructions. Gene-specific primers (GSPs) for amplification of 5′RACE and 3′RACE were designed based on the known fragments of CDA genes, the primers listed in Supp Table S1 (online only). The first PCR was performed using the universal primer mix provided (UPM) and the GSP. PCR conditions were as follows: followed by 25 cycles at 94°C for 30 s, annealing at 60–70°C for 30 s, and elongation at 72°C for 3 min. Then, the first PCR products were diluted 50–100 times for use as a cDNA template for the second PCR, which was performed using the common short primer and the GSP. Conditions for the second PCR were the same as those for the first PCR. PCR products were purified using a DiaSpin Column DNA Gel Extraction Kit, and each purified product was ligated into the pMDTM18-T Vector (Takara, Japan) and transformed into DH5α competent cells (Sangon Biotech, Shanghai, China). Positive clones were selected for Sangon Biotech sequencing.

**Sequence Analysis and Phylogenetic Tree Construction**

ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) was used to accurately identify open reading frames and protein-coding amino acids for the CDA genes. The cDNA sequence and deduced amino acid sequences of *SfCDAs* were analyzed using DNAMAN 6.0 (LynnonBiosoft, Quebec, Canada). The molecular weight (MW) and isoelectric point (PI) of the protein were predicted using ExPaSy (https://web.expasy.org). Domain structures were predicted using SMART (http://smart.embl-heidelberg.de/). Alignments of deduced amino acid sequences for the CDA were assessed using the BoxShade service (https://www.expasy.org/resources/boxshade). A phylogenetic tree of eight insects protein CDA sequence was constructed using MEGA 6.0 software (Tamura et al., 2013), adopting the maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP) algorithm with 1000-fold bootstrap resampling.

**Developmental and Tissue-Specific Expression Analysis of Four *SfCDA***

To perform developmental and tissue-specific expression analysis of *SfCDAs*, samples at 21 different developmental stages (including eggs, first- to fifth-instar nymphs, 1- to 4-d old male and female adults) were collected. Six different tissues including integument, gut, fat body, head, leg dissected from the fifth-instar nymphs, and integument, gut, fat body, head, leg, wing, ovary, and testis were collected from third to fourth day male or female adult. Total RNA was isolated using an HP Total RNA kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer’s instructions. The concentration and quantity of total RNA were assayed using a spectrophotometer (NanoDrop 2000, Thermo, USA). One microgram of total RNA was used to synthesize first-strand complementary DNA (cDNA) by using PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Japan) according to the manufacturer’s protocols. The expression profiles of the genes for the four *SfCDAs* were determined using quantitative real-time PCR (RT–qPCR), The following conditions were applied: initial denaturation for 10 min at 95°C followed by 40 cycles, at 95°C for 30 s, and 60°C for 30 s with CXFX96 RT–qPCR system (Bio-Rad, Hercules, CA, USA) using FastStart Essential DNA Green Master Mix (Roche, Indianapolis, IN, USA). Melting curve stage was tested for 0.5 s at 65°C to 95°C. The ribosomal protein L9 (RPL9, Accession number: KM885285) was used as an internal reference gene. All RT–qPCR experiments analyses were repeated in triplicate, and 2^−ΔΔCt method was used to determine the relative quantification (Livak and Schmittgen., 2001). The primers for RT–qPCR are shown in Supp Table S3 (online only). At the same time, we used semi-quantitative RT–PCR to detect the four *SfCDAs* expressions in different tissues. The RNA extraction and first-stand cDNA synthesis method were followed as described above.

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RNA Interference

Specific primers for each CDA gene were designed using Primer Premier 6.0, with each primer containing a T7 polymerase promoter sequence. The dsRNA primers are shown in Supp Table S3 (online only). The dsRNAs were synthesized using a Transcript Aid T7 High Yield Transcription Kit (Thermo, Waltham, MA, USA), following the manufacturer’s protocol. The integrity of the dsRNA was verified on a 1.2% agarose gel, and dsRNA concentrations were determined using a NanoDrop 2000 spectrophotometer. The dsRNA for GFP (Accession number: CAA58789) was used as a nonspecific negative control gene.

To study the biological role of CDA genes in the molting process of *S. furcifera*, we used first day fifth-instar nymphs for micro-injection experiments. Each insect was injected with 1000 ng/μl gene-specific dsRNA using a microinjector (IM-31 Injector; Narishige, Tokyo, Japan). There were 120 insects for three biological replicates. Each biological replicate included forty insects. Post-injection insect transferred onto at glass tubes in fresh rice seedlings and mortality rates were recorded and phenotypes were observed on a daily basis. To determine silencing efficiency, 10 nymphs were randomly chosen for RNA extraction at 48 and 72 h after dsRNA injection. mRNA-level detection of target genes was performed using RT–qPCR of extracts.

### Table 1. Characteristics of chitin deacetylase genes in *S. furcifera*

| Group | Gene   | cDNA length | Coding region | 5'UTR | 3'UTR | Aa   | pl   | Mw (KDa) | GenBank ID |
|-------|--------|-------------|---------------|-------|-------|------|------|----------|------------|
| Group I | SfCDA1 | 2745        | 165–1811      | 164   | 934   | 548  | 5.4  | 56.297   | MN508364   |
|       | SfCDA2 | 2506        | 160–1803      | 159   | 703   | 547  | 5.1  | 62.326   | MN482711   |
| Group III | SfCDA3 | 2172        | 117–1601      | 116   | 571   | 494  | 5.5  | 56.454   | MN482712   |
| Group IV | SfCDA4 | 3888        | 367–2799      | 366   | 1089  | 810  | 6.2  | 91.806   | MN482713   |

**Fig. 1.** Phylogenetic analyses of genes for chitin deacetylases (CDAs) from *Bombyx mori* (Bm), *Drosophila melanogaster* (Dm), *Tribolium castaneum* (Tc), *Anopheles gambiae* (Ag), *Locusta migratoria* (Lm), *Nilaparvata lugens* (Nl), *Daphnia pulex* (Dp), and *Sogatella furcifera* (Sf). The genes for CDAs were classified into five groups (I–V). The GenBank accession numbers for all of the insect protein sequences for CDAs used are listed in Supp Table S2 (online only).
Expression of Chitin Biosynthesis Genes After RNAi of SfCDA1, SfCDA2, SfCDA3, and SfCDA4

To determine the effects of RNAi on the transcript expression of genes involved in chitin biosynthesis-chitin synthase 1 (SfCHS1) (Wang et al., 2019) and trehalase (SfTRE), after 72 h injected dsSfCDA1, dsSfCDA2, dsSfCDA3, and dsSfCDA4, nymphs were collected to extract RNA and synthesize cDNA. The expressions levels of these genes were analyzed by RT-qPCR as described above. Primer are listed in Supp Table S4 (online only).

Statistical Analysis

Statistical analyses were performed using SPSS 21.0 (Chicago, IL, USA), and data are presented as standard errors of the mean. One-way ANOVA followed by a Tukey test was used to analyze relative levels of these genes.

Fig. 2. Deduced domain architectures and catalytic domain of four CDAs from S. furcifera. (A) The deduced amino acid sequences were used to predict the domain architectures of four CDAs in S. furcifera using SMART software. (B) Multiple sequence alignments of catalytic domains of the deduced genes for CDAs in T. castaneum and S. furcifera. The red boxes marked 1–5 indicate that the catalytic domain motif is TFDD, H[S/T]xxH, RxP[Y/F], FxYD[S/A], or Lxxxx[P/F]H, respectively.
expression levels at different development stages and different tissues. Student’s t-test was used to compare the dsRNA treatment and control groups. Kaplan–Meier (log-rank: Mantel–Cox) tests used GraphPad Prism 8.0 to analyze survival rates.

Results
Identification and Analysis of SfCDA Sequence
In total, we identified four CDA genes (SfCDA1, SfCDA2, SfCDA3, and SfCDA4) from the S. furcifera genome and transcriptome database. Each CDA gene fragment was confirmed by RT–PCR and sequencing, and used the RACE method to amplify four CDA gene full-length sequences. The gene names, cDNA length, encoding protein, theoretical isoelectric point, molecular weight, and accession number for the four S. furcifera CDA genes are shown in Table 1.

Three phylogenetic tree analysis was performed to investigate the evolutionary relationship of CDAs from S. furcifera and other organisms (Fig. 1). The insect CDA genes were categorized into five groups (I–V). SfCDA1 and SfCDA2 were clustered into group I, SfCDA3 belonged to group III, and SfCDA4 was placed in group IV. No S. furcifera CDAs were classified into groups II and V.

Developmental and Tissue Expression Patterns of SfCDAs
The developmental expression patterns of four SfCDAs were determined using RT–qPCR (Fig. 3). SfCDA1 and SfCDA2 had similar expression patterns, with transcript expressed at all nymph stages of development at a stable level. SfCDA3 and SfCDA4 were expressed...
at a low level from egg to the second-instar nymphs, but \textit{SfCDA3} and \textit{SfCDA4} mRNA expressions were higher from third-instar to fifth-instar nymphs. Subsequently, \textit{SfCDA1}, \textit{SfCDA2}, \textit{SfCDA3}, and \textit{SfCDA4} were expressed at gradually decreasing levels in the male and female adult stages.

The expression patterns of the four CDA genes were determined by RT–qPCR in different tissues (Fig. 4). \textit{SfCDA1} were expressed at highly varied levels among nymph and adult tissues. In fifth-instar nymph tissues, the \textit{SfCDA1}, \textit{SfCDA2}, \textit{SfCDA3}, and \textit{SfCDA4} were all highly expressed in the integument, head, fat body, leg, and gut (Fig. 4). In adults tissues, all four CDA genes were mainly expressed in the wing, leg, and head, while they were expressed at extremely low levels in the other adult tissues (Fig. 4).

**Functional Analyses of CDA Genes Through RNAi**

To explore the biological function of the four CDA genes on \textit{S. furcifera} molting, we performed functional analyses of CDAs by RNAi. The dsRNAs for \textit{SfCDA1}, \textit{SfCDA2}, \textit{SfCDA3}, and \textit{SfCDA4} were injected into first day fifth-instar nymphs, and dsGFP as a control. RT–qPCR results showed that transcriptional was significantly inhibited at 48 and 72 h after dsRNA injection (Fig. 5).

At 96 h following inhibition of \textit{SfCDA1} and \textit{SfCDA2}, the survival rate of nymphs was 18% and 16%, respectively (Fig. 6A and B). Two lethal phenotypes were observed after the injection of dsRNAs for \textit{SfCDA1} and \textit{SfCDA2}. In Phenotype 1, nymph old cuticles of the head and thorax are only slightly splitted open and eventually lead to death. In Phenotype 2, old nymph cuticles did not split from the head and thorax and died before molting (Fig. 6E). Injection of ds\textit{SfCDA1} resulted in 82% of nymphs lethal phenotypes. Of 82% of nymph lethal phenotypes, the proportion of P1 was 58% and in P2, 24%, respectively (Fig. 6F). Injection of ds\textit{SfCDA2} resulted in 84% of nymphs lethal phenotypes. Of 84% of nymph lethal phenotypes, the proportion of P1 and P2 was 55% and 29%, respectively (Fig. 6F).

At 96 h after injection of dsRNAs for \textit{SfCDA4}, the normal emerged rate of nymphs was 23.4% (Fig. 6D). Three phenotypes were observed after injection of ds\textit{SfCDA4}. P1 and P2 were similar to Phenotype 1 and Phenotype 2, respectively. However, Injection of ds\textit{SfCDA4} resulted in 52% of nymph lethal phenotypes. Of 52% of nymph lethal phenotypes, the proportion of P1 was 30% and in P2, 22%, respectively (Fig. 6F).

\begin{figure}[h]
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\caption{Tissue-specific expression analyses of four chitin deacetylases genes in \textit{S. furcifera}. Data are presented as standard error of the mean of three biological replications, analyzed with one-way analysis of variance (ANOVA), and followed by Tukey’s multiple comparison test. The lowercase letters above the bars indicate significant differences (\(P < 0.05\)).}
\end{figure}
to those caused by dsSfCDA1 and dsSfCDA2 (Fig. 6E). In addition, another abnormal phenotype was observed in adults. In P3, although nymphs could morph into adults, but had curled wings and could not spread them normally. (Fig. 6E). Injection of dsSfCDA4 resulted in 63% of nymphs lethal phenotypes and 13% with adult curled wings. Of 63% of nymphs lethal phenotypes, the proportion of P1 and P2 were 37% and 26% (Fig. 6F).

Nymphs injected with dsRNAs for SfCDA3 could successfully molt and develop into adults, and no significant phenotypic changes were observed. Their survival rate was 84% at 96 h following injection of dsSfCDA3 (Fig. 6C).

Knockdown of SfCDA1, SfCDA2, SfCDA3, and SfCDA4 Affects the Expression of Chitin Biosynthesis Genes

We used RT–qPCR to detect the expression of chitin synthesis pathway genes (SfCHS1, SfCHS1a, SfCHS1b, SfTRE1, and SfTRE2) after RNAi of SfCDAs genes for 72 h in first day fifth-instar nymphs. The transcript levels of SfCHS1, SfCHS1a, and SfCHS1b were down-regulated and significantly treated with dsSfCDA1, dsSfCDA2, and dsSfCDA4 compared with the control (Fig. 7A, B, and D). In addition, expression of SfTRE1 and SfTRE2 was significantly decreased compared with controls when SfCDA1 and SfCDA4 were silenced, while expression of SfTRE1 and SfTRE2 was increased significantly compared with controls after SfCDA2 was silenced (Fig. 7A, B, and D). This suggests that SfCDA1, SfCDA2, and SfCDA4 are involved in the regulation of the chitin biosynthesis genes during the nymph–adult transition. However, there was no significant change in the expression of the five chitin biosynthesis path genes after injection of SfCDA3 RNAi, relative to levels in controls (Fig. 7C).

Discussion

CDA is a key enzyme involved in chitin metabolism. Numerous CDA family genes have been identified in many insect species. There are four CDA genes in N. lugens (Xi et al. 2014), six in D. melanogaster (Dixit et al. 2008), nine in T. castaneum (Arakane et al. 2009), nine in Manduca sexta (Lepidoptera: Sphingidae)
Fig. 6. Effects of SfCDA1, SfCDA2, SfCDA3, and SfCDA4 RNAi on the nymph–adult transition in S. furcifera. (A–D) Survival rate after dsSfCDA1, dsSfCDA2, dsSfCDA3, and dsSfCDA4 or dsGFP injection. Survival rates were estimated using the Kaplan–Meier method. (E) For injection of dsRNAs for SfCDA1, SfCDA2, and SfCDA4, two lethal phenotypes were observed. P1: The old cuticle split open, but the nymph failed to shed the old cuticle and died before molting (red arrows). P2: The old cuticles did not split and the nymph failed to reach the molting stage, dying before eclosion. P3: Although the nymph could emerge as an adult, the individual adult had wing deformity (red arrows).
Insect Group I CDAs

Group I contains genes encoding the proteins CDA1 and CDA2 in insects. In this study, SfCDA1 and SfCDA2 were expressed at all nymph developmental stages and in the integument, head, fat body, leg, and gut. Silencing of SfCDA1 and SfCDA2 caused high mortality or phenotypic defects (Fig. 6E). Similar results have been documented in hemimetabolous insects, such as with CDA1 and CDA2 in N. lugens (Xi et al. 2014), Oxya chinensis (Orthoptera: Acridoidea) (Ding et al. 2014; Yu et al. 2014), and L. migratoria (Yu et al. 2016; Yu et al. 2018). These findings further support the conclusion that CDA1 and CDA2 are indispensable during molting. In previous research, it was shown that group I CDAs in some insects have redundant functions. For example, in D. melanogaster, both serp (CDA1) and verm (CDA2) lead to elongated and tortuous tracheal tubes (Luschnig et al. 2006, Wang et al. 2006). Interestingly, Gal4/UAS expression system was used to tissue-specific RNA interference with serp and verm expression, demonstrating that serp and verm play distinct roles in wing development of D. melanogaster (Zhang et al. 2019). In the current study, SfCDA1 and SfCDA2 were both expressed adult wing, the silencing of SfCDA1 and SfCDA2 in the fifth-instars nymphs of S. furcifera resulted in 82% and 84% nymph mortality, but no adult wing deformity has been observed, respectively. We did not know whether application of Gal4/UAS expression system treatment with SfCDA1 and SfCDA2 has a similar effect on S. furcifera, which remains unclear and is worth further researching.

Insect Group III CDAs

Different insects show different expressions levels of group III CDAs at different developmental stages and in different tissues. In M. sexta, MsCDA4 is expressed in all developmental stages and in the integument (Tetreau et al. 2015). The expression of CmCDA4 in the head of Cnaphalocrocis medinalis (Lepidoptera: Pyralidae) and in the adult stage is significantly higher than that in the larval stage (Yu et al. 2016). However, while TcCDA4 is highly expressed in the epidermis in both the larval and adult stages of T. castaneum, no obvious phenotypic changes are observed from silencing TcCDA4 (Arakane et al., 2009). Similarly, L. migratoria CDA4 is highly expressed in the integument, but no visible abnormal phenotypic changes were observed after the injection of dsLmCDA4, and no effect is seen on content or organization of the chitin (Yu et al. 2017). In our study, SfCDA3 was highly expressed in five nymph tissues (integument, head, fat body, leg, and gut) and were periodically expressed during the nymph molting stage, but knockdown of SfCDA3 had no obvious effect on the development of S. furcifera. These data demonstrate that Group III CDA is not involved in molting development. Moreover, one DcCDA3 from Diaphorina citri (Hemiptera: Kerimidae) is highly expressed in the integument and third-instar nymph stage, but silencing of DcCDA3 does not lead to any observable phenotypic change. Interestingly, injection...
of Escherichia coli and Staphylococcus aureus induces a significantly changed level of expression of DcCDA3 in the midgut, suggesting that CDA3 may be involved in the immune response of D. citri (Yu et al. 2020). Whether the CDA3 of S. furcifera also has similar roles remains unknown and is worth further investigation.

Insect Group IV CDAs

In N. lugens, RNAi silencing of NICDA4 causes arrested development in nymph–nymph transition and more than 95% mortality (Xi et al. 2014). In this study, we found that silencing of SfCDA4 resulted in a lethal phenotype in 63% of nymphs and in 13% of abnormal wing adults. Similarity, among the chitin-degrading enzymes, Cht7 in S. furcifera and Plutella xylostella (Coleoptera: Plutellidae), β-N-acetylglucosaminidase 2 (NAG2) in Lasioderma serricorne (Coleoptera: Anobiidae) lead to wing development deformity (Chen et al. 2017, Zhu et al. 2019, Yang et al. 2019), indicating a chitin deposition critical role in adult wing development. By contrast, in T. castaneum, no phenotypic abnormality is observed after injection of dsTcCDA5 (Arakane et al., 2009). Likewise, in L. migratoria, silencing of LmCDA5 shows no effect on locust development or cuticle structure (Yu et al. 2017), suggesting that the same CDA group gene may have different functions in different insects.

Effects of SfCDA1, SfCDA2, SfCDA3, and SfCDA4 RNAs on Transcripts of Chitin Biosynthesis

Our data indicate that SfCDA1, SfCDA2, and SfCDA4 play a vital role in nymph–adult transition (Fig. 6E), but SfCDA3 has no effect. The following question remained: Is there an influence on the transcript level of chitin synthesis after knockdown of the four SfCDAs? We found that injection of dsSfCDA1, dsSfCDA2, and dsSfCDA4 reduced the level of transcript of SfCHS1, SfCHS1a, and SfCHS1b, and it increased or decreased the expression of SfTRE1 and SfTRE2 compared with controls, but RNAi suppression of SfCDA3 expression did not affect expression of chitin biosynthesis genes (Fig. 7A–D). Similarly, in Leptinotarsa decemlineata (Coleoptera: Chrysomelidae), the mRNA levels of three transcripts (LdCHS2a, LdCHS3a, and LdCHS4a) are significantly lower, and those of two trehalose transcripts (TRE1 and TRE2) are upregulated compared with controls, in LdCDA1- and LdCDA2b-depleted larvae (Wu et al. 2019a, Wu et al. 2019b). In S. furcifera, RNAi inhibited the expressions of SfCht5, SfCht7, SfCht10, and SfHDGF2 and caused a change in the expression level of chitin synthesis SfCHS1, SfTRE (Yang et al. 2020). In L. serricorne, two chitin synthesis genes (CHS1 and TRE1) were significantly downstream after knockdown of LsNAG2 (Yang et al. 2019). Thus, these results showed that CHS and TRE expression levels were regulated by the Cht, NAG, and CDA genes.

In summary, we successfully cloned and characterized four full-length CDA genes from S. furcifera. These genes belong to the three of five distinct groups in the CDA family, but S. furcifera lacks orthologues in groups II and V found in hemimetabolous insects. SfCDA1 and SfCDA2 were expressed at all nymph developmental stages, and SfCDA3 and SfCDA4 were mainly expressed in the third-instar to fifth-instar nymph stages. Furthermore, SfCDA1, SfCDA2, SfCDA3, and SfCDA4 were predominantly expressed in the integument and head, followed by the adult wing, whereas RNAi results showed that SfCDA1, SfCDA2, and SfCDA4 are involved in the nymph–adult molting transition and these genes could regulate chitin biosynthesis expression levels.

Supplementary Data

Supplementary data are available at Journal of Insect Science online.

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Author Contributions

X.-B.Y. and H.Y. conceived and designed the experiments. C.Z. and G.-Y.L. measured the gene expression levels; X.-B.Y. examined the genes function and writing-original draft, editing. X.-B.Y., C.Z., H.Y., G.-Y.L., and D.-C.J. finalized the manuscript. All authors read and approved the final manuscript.

References Cited

Arakane, Y., R. Dixit, K. Begum, Y. Park, and C. A. Specht, 2009. Analysis of Functions of the chitin deacetylase gene family in Tribolium castaneum. Insect Biochem. Mol. Biol. 39: 355–365.

Arakane, Y., and S. Muthukrishnan, 2010. Insect chitinase and chitinase-like proteins. Cell. Mol. Life Sci. 67: 201–216.

Chen, C., H. Yang, B. Tang, W. J. Yang, and D. C. Jin, 2017. Identification and functional analysis of chitinase 7 gene in white-backed planthopper, Sogatella furcifera. Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 208: 19–28.

Dixit, R., Y. Arakane, C. A. Specht, C. Richard, K. J. Kramer, and R. W. Beeman, 2008. Domain organization and phylogenetic analysis of proteins from the chitin deacetylase gene family of Tribolium castaneum and three other species of insects. Insect Biochem. Mol. Biol. 38: 440–451.

Ding, G. W., R. R. Yu, M. I. Yang, E. B. Ma, J. Yang, and J. Z. Zhang, 2014. Molecular characterization and functional analysis of chitin deacetylase 1 gene in Oxya chinensis (Orthoptera: Acrididae). Acta Entomologica Sinica. 57: 1265–1271.

Guo, W., G. Li, Y. Pang, and P. Wang, 2005. A novel chitin binding protein identified from the peritrophic membrane of the cabbage looper, Trichoplusia ni. Insect Biochem. Mol. Biol. 35: 1224–1234.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, and S. Sturrock, 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28: 1647–1649.

Luschnig, S., T. Bätz, K. Armbruster, and M. A. Krasnow, 2006. serpentine and vermillon encode matrix proteins with chitin binding and deacetylation domains that limit tracheal tube length in Drosophila. Curr. Biol. 16: 186–194.

Lüvak, K. J., and T. D. Schmitten, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^−ΔΔCt Method. Methods. 25: 402–408.

Liu, L., Q. Mingbo, L. Tian, Q. Chen, X. M. Guo, and J. Yang, 2019. Biochemical characterization of three midgut chitin deacetylases of the Lepidopteran insect Bombyx mori. Insect Physiol. 113: 42–48.

Merzendorfer, H. 2006. Insect chitin synthases: a review. J. Comp. Physiol. B. 176: 1–15.

Merzendorfer, H. 2011. The cellular basis of chitin synthesis in fungi and insects: common principles and differences. Eur. J. Cell. Biol. 90: 759–769.

Moussian, B. 2010. Recent advances in understanding mechanisms of insect cuticle differentiation. Insect Biochem. Mol. Biol. 40: 363e375.

Nakata, M. M., N. H. Chung, and Y. Kobori, 2019. Insecticide Application and Its Effects on the Density of Rice Planthoppers, Nilaparvata lugens and Sogatella furcifera, in Paddy Fields in the Red River Delta, Vietnam. J. Pestic. Sci. 44: 129–135.

Niu, J., G. Shen, O. Christiaens, G. Smagghe, L. He, and J. Wang, 2018. Beyond insects: current status, achievements and future perspectives of RNAi in mite pests. Pest. Manag. Sci. 74: 2680–2687.
Quan, G., T. Ladd, J. Duan, F. Wen, D. Doucet, and M. Cusson. 2013. Characterization of a spruce budworm chitin deacetylase gene: stage- and tissue specific expression, and inhibition using RNA interference. Insect Biochem. Mol. Biol. 43: 683–691.

Tsigos, I., A. Martinou, D. Kafetzopoulos, and V. Bouriotis. 2000. Chitin deacetylases: new, versatile tools in biotechnology. Trends Biotechnol. 18: 305–312.

Tetterau, G., X. Cao, Y. R. Chen, S. Muthukrishnan, H. Jiang, and G. W. Blissard. 2015. Overview of chitin metabolism enzymes in Manduca sexta: identification, domain organization, phylogenetic analysis and gene expression. Insect Biochem. Mol. Biol. 62: 114–126.

Toprak, U., D. Baldwin, M. Erlandson, C. Gillott, X. Hou, C. Coutu, and D. D. Hegedus. 2008. A chitin deacetylase and putative insect intestinal lipases are components of the Mamestra configurata (Lepidoptera: Noctuidae) peritrophic matrix. Insect Biochem. Mol. Biol. 17: 573–585.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA 6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30: 2725–2729.

Wang, S., S. A. Jayaram, J. Hemphäil, K. A. Senti, V. Tsarouhas, H. Jin, and C. Samakovlis. 2006. Septate-junction-dependent luminal deposition of chitin deacetylases restricts tube elongation in the Drosophila trachea. Curr. Biol. 16: 180–185.

Wang, L., N. Tang, X. L. Gao, Y. X. Ye, B. Yu, and C. X. Zhang. 2015. Genome sequence of a rice pest, the white-backed planthopper (Sogatella furcifera). Giga Science. 6: 1–9.

Wu, J. J., L. L. Mu, Z. C. Chen, K. Y. Fu, W. C. Guo, C. Li, and G. Q. Li. 2019b. Silencing chitin deacetylase 2 impairs larval–pupal and Pupal-adult molts of a rice pest, Sogatella furcifera (Hemiptera: Delphacidae). Insect Mol. Biol. 23: 695–705.

Wu, J. J., Z. C. Chen, Y. W. Wang, K. Y. Fu, W. C. Guo, and G. Q. Li. 2019a. Disruption of ecdysis in Leptinotarsa decemlineata by knockdown of chitin deacetylase 1. J Asia-Pac Entomol. 22: 443–452.

Wang, Z., H. Yang, C. Zhou, W. J. Yang, D. C. Jin, and G. Y. Long. 2019. Molecular cloning, expression, and functional analysis of the chitin synthase 1 gene and its two alternative splicing variants in the white-backed planthopper, Sogatella furcifera (Hemiptera: Delphacidae). Sci. Rep. 9: 1087.

Xu, Y., P. L. Pan, Y. X. Ye, B. Yu, and C. X. Zhang. 2014. Chitin deacetylase family genes in the brown planthopper, Nilaparvata lugens (Hemiptera: Delphacidae). Insect Mol. Biol. 23: 695–705.

Yu, R. R., W. G. Ding, Y. P. Guo, E. B. Ma, and J. Z. Zhang. 2014. Molecular characterization and functional analysis of chitin deacetylase 2 gene in Oxya chinensis. Sci. Agric. Sin. 47: 1321–1329.

Yu, R. R., G. W. Ding, W. M. Liu, M. Zhang, X. M. Zhao, and P. F. Zhao. 2017. Molecular Characterization and Biological Function of Chitin Deacetylase Genes in Locusta migratoria. Sci. Agric. Sin. 50: 2498–2507.

Yu, H. Z., M. H. Liu, X. Y. Wang, and X. Yang. 2016. Identification and expression profiles of chitin deacetylase genes in the rice leaf folder Cnaphalocrocis medinalis. J. Asia-Pac. Entomol. 19: 691–696.

Yu, R. R., W. M. Liu, D. Q. Li, X. M. Zhao, and G. W. Ding. 2016. Helicoidal organization of chitin in the cuticle of the migratory locust requires the function of the chitin deacetylase 2 enzyme (LmCDA2). J Biol. Chem. 291: 24353–24363.

Yu, R. R., W. M. Liu, X. M. Zhao, M. Zhang, D. Q. Li, and R. Zubir. 2018. LmCDAI organizes the cuticle by chitin deacetylation in Locusta migratoria. Insect Mol. Biol. 28: 301–312.

Yu, H. Z., N. Y. Li, B. Li, S. Toufeeq, Y. X. Xie, Y. L. Huang, Y. M. Du, X. D. Zeng, B. Zhu, and Z. J. Lu. 2020. Immune Functional Analysis of Chitin Deacetylase 3 from the Asian Citrus Psyllid Diaphorina citri. Int. J. Mol. Sci. 21: 64.

Yang, W. J., K. K. Xu, X. Yuan, and C. Li. 2019. Knockdown of β-N-acetylglucosaminidase 2 Impairs Molting and Wing Development in Lasioderma serricorne (Fabricius). Insects. 10: 396.

Yang, W. J., K. K. Xu, X. Yan, C. X. Chen, Y. Cao, and Y. L. Meng. 2018. Functional characterization of chitin deacetylase 1 gene disrupting larval-pupal transition in the drugstore beetle using RNA interference. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 219: 10–16.

Yang, X. B., C. Zhou, G. Y. Long, H. Yang, and D. C. Jin. 2020. Characterization and functional analysis of chitinase family genes involved in nymph–adult transition of Sogatella furcifera. Insect Sci. 1–16.

Zhou, G. H., J. J. Wen, D. J. Cai, P. Lin, and D. L. Xu. 2008. Southern rice black-streaked dwarf virus: a new proposed Fijivirus species in the family Reoviridae. Chin. Sci. Bull. 53: 3677–3683.

Zhu, K. Y., H. Merzendorfer, W. Zhang, J. Zhang, and S. Muthukrishnan. 2016. Biosynthesis, turnover, and functions of chitin in insects. Annu. Rev. Entomol. 61: 177–196.

Zhu, B., J. P. Shan, R. Li, P. Liang, and X. W. Guo. 2019. Identification and RNAi-based function analysis of chitinase family genes in diamondback moth, Plutella xylostella. Pest. Manag. Sci. 75: 1951–1961.

Zhou, C., H. Yang, Z. Wang, G. Y. Long, and D. C. Jin. 2018. Comparative transcriptome analysis of Sogatella furcifera (Horvath) exposed to different insecticides. Sci. Rep. 8: 8773.

Zhuo, Z. X., W. H. Wang, X. Tan, Q. Y. Xia, Z. H. Xiang, and P. Zhao. 2014. Identification and molecular characterization of a chitin deacetylase from Bombyx mori peritrophic membrane. Int. J. Mol. Sci. 15: 1946–1961.

Zhang, M., Y. Ji, X. Zhang, P. Ma, Y. Wang, B. Moussian, and J. Zhang. 2019. The putative chitin deacetylases Serpentine and Vermiform have non-redundant functions during Drosophila wing development. Insect Biochem. Mol. Biol. 110: 128–135.

Zhang X. L., X. Liao, K. K. Mao, P. Yang, D. Y. Li, E. Ali, H. Wan, and J. H. Li. 2017. Neonicotinoid insecticide resistance in the field populations of Sogatella furcifera (Horvath) in Central China from 2011 to 2013. J Asia-PAC Entomol. 20: 955–958.

Zhang, Z. Y., J. M. Yan, Q. Liu, Y. H. Zhang, J. Gong, and Y. Hou. 2019. Genome-wide analysis and hormone regulation of chitin deacetylases in silkworm. Int. J. Mol. Sci. 20: 1679.