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Identification and characterization of chitin deacetylase2 from the American White Moth, *Hyphantria cunea* (Drury)

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ABSTRACT Chitin deacetylases (CDAs) are enzymes that catalyze the conversion of chitin into chitosan, thereby influence the mechanical and permeability properties of structures such as the cuticle and peritrophic matrices. The full length cDNAs of chitin deacetylase2 (CDA2) genes from *Hyphantria cunea* were fully cloned by PCR amplification. Two cDNA sequences of *HcCDA2* were searched from transcriptome of *H. cunea* and named as *HcCDA2a* and *HcCDA2b*. The deduced protein sequences showed that HaCDA2a and HaCDA2b are synthesized as preproteins of 524 and 518 amino acid residues with a 18-amino acid signal peptide, respectively. HcCDA2a and HcCDA2b contained a chitin-binding domain (ChBD), a low-density lipoprotein receptor class A domain (LDLa) and a polysaccharide deacetylase-like catalytic domain (CDA). Gene expression analyses results showed that *HcCDA2a* and *HcCDA2b* were both expressed at the head, integument, foregut, midgut, hindgut, Malpighian tubules and fat body, as well as the 1st to 5th days of fifth instar larvae. Western blot analyses revealed that HcCDA2 protein was highly abundant in the head and integument, and the developmental expression result in the fifth instars showed that HcCDA2 was highly present at the first two days. Besides, RT-PCR results showed that *HcCDA2a* and *HcCDA2b* were both expressed in integument and head, whether in molting stage or feeding stage. No visiable phenotypic changes were observed after injection of ds*HcCDA2b*, while lethal phenotypes of cuticle shedding failure and high mortality were resulted from injection of ds*HcCDA2a*. The silence of *HcCDA2a* leads to the ecdysis failure and death of *H. cunea*. These results suggest that HcCDA2 plays an important role during insect development, and provide new candidate targets and basis for developing environment-friendly pesticides.

KEY WORDS chitin deacetylase, *Hyphantria cunea*, RNAi, Molting, phenotype

Introduction

Chitin is one of the most abundant, easily obtained, and renewable natural polymers. It is commonly found in many invertebrates and in the cell walls of most fungi and algae. Insect chitin mainly exists in the procuticle of integument, the peritrophic membrane (PM) and the tracheal tissues, which plays a critical role in maintenance of the shape and size of the exoskeleton and tissues, as well as the molting period (Li et al. 2011). Chitin plays critical role in the shape, size and mechanical properties of epithelial cells structures (Moussian et al. 2005). Enzymes involved in chitin metabolism are attractive targets for the disruption of insect growth by biorational insecticides (Kramer and Muthukrishnan, 1997; Herrera-Estrella and Chet, 1999; Merzendorfer and Zimoch, 2003; Arakane and Muthukrishnan, 2010; Jakubowska et al. 2010). Chitin deacetylase (CDA; EC 3.5.1.41) is a key enzyme involved in