Insect Biochemistry and Molecular Biology 81 (2017) 41–50

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

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

Identification of the binding domains and key amino acids for the interaction of the transcription factors BmPOUM2 and BmAbd-A in *Bombyx mori*

Yong He [a], Huimin Deng [a, *], Qihao Hu [a], Zidan Zhu [a], Lin Liu [a], Sichun Zheng [a], Qisheng Song [b], Qili Feng [a, **]

[a] Guangzhou Key Laboratory of Insect Development Regulation and Application Research, School of Life Sciences, South China Normal University, Guangzhou, 510631, China
[b] Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA

ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form
10 December 2016
Accepted 12 December 2016
Available online 14 December 2016

Keywords:
BmPOUM2
BmAbd-A
Transcription factor
Binding domain
Protein interaction
Bombyx mori

ABSTRACT

The transcription factor BmPOUM2 interacted with another transcription factor BmAbd-A to regulate the expression of the wing cuticle protein gene *BmWCP4* in *Bombyx mori*. In this study, the binding domains and amino acids for the interaction between BmPOUM2 and BmAbd-A were reported. Two isoforms of BmPOUM2 were identified. The short isoform (BmPOUM2-S) lacks a 114-amino acid sequence containing a POU-homeodomain and a nuclear localization signal peptide (NLS), as compared to the full-length isoform (BmPOUM2). Both BmPOUM2 and BmPOUM2-S proteins bound to the BmAbd-A through the POU-specific domain. When the six amino acids (Lys166, Gly173, Gln176, Ser192, Glu200 and Asn208) that are highly conserved in POU family genes were mutated, BmPOUM2 did not bind to BmAbd-A. BmAbd-A interacted with BmPOUM2 by the homeobox domain or the LCR2 (low complexity region) domain. When seven amino acids (Phe156/248, His158/250, Ala175/263, Cys180/265, Glu190/268, Trp196/274 and Val214/289) that are shared in the homeobox and LCR2 domains were mutated, BmAbd-A did not bind to BmPOUM2. Overexpression of either BmPOUM2 or BmAbd-A or both increased the activity of *BmWCP4* promoter in CHO cells. ChIP assay and EMSA showed that BmAbd-A protein bound to the Hox cis-regulatory element in the *BmWCP4* promoter, while the BmPOUM2 bound to the nearby POU CRE. A model for the interaction and action of BmPOUM2 and BmAbd-A in regulation of the *BmWCP4* expression is proposed.

1. Introduction

Insect molting and metamorphosis are initiated and regulated by molting hormone 20-hydroxydysone (20E). 20E binds to a heterodimer of two nuclear receptors, ecdysone receptor (EcR) and ultraspiracle (USP), to initiate the expression of a cascade of genes, eventually leading to molting and metamorphosis (Riddiford et al., 2000; Thummel, 2001).

POU (Pit-Oct-Unc) transcription factor has been found to be involved in the molting and metamorphosis in insects (Cheng et al., 2015; Deng et al., 2012; Danielsen et al., 2014; Bauke et al., 2015). POU transcription factors are characterized by a POU-specific domain and POU-homeodomain (Herr et al., 1988). Both of these two POU domains are required for high-affinity and site-specific binding to the octamer motif (ATGCAAAT) and closely related sequences in the promoters and enhancers of many genes. In addition to DNA binding, the POU domain also mediates specific protein-protein interactions between Oct factors or between Oct factors and other transcription factors or cofactors (Botfield et al., 1992; Verrijzer et al., 1992; Schonemann et al., 1998; Zhao, 2013).

Transcription factor Abdominal-A (Abd-A), belonging to the homeobox gene family (Hox proteins), plays important roles in the development of embryo, differentiation of body segment and formation of abdomen in insects (McGinnis et al., 1984; Casares et al., 1997; Pan et al., 2009; Tomita and Kikuchi, 2009). A homeodomain conserved in Hox proteins usually binds to the promoter of genes,

* Corresponding author.
** Corresponding author.
E-mail addresses: denghuiminmin@163.com (H. Deng), qlfeng@scnu.edu.cn (Q. Feng).

http://dx.doi.org/10.1016/j.ibmb.2016.12.007
0965-1748/

activating the expression of the genes. When Hox proteins interact with other transcription factors to form a complex, the specificity of the regulation of the target genes would be enhanced (Ponzielli et al., 2002).

In previous study (Deng et al., 2012), we found that BmPOUM2 interacted with the Hox protein BmAbd-A in regulation of the expression of *BmWCP4*. However, how these two transcription factors interact and the effect of the interaction on the expression of *BmWCP4* is unknown. In present study, the regions and key amino acids that are critical for the interaction of BmPOUM2 and BmAbd-A were identified and a regulatory mechanism of the expression of *BmWCP4* through the interaction was proposed.

2. Materials and methods

2.1. Experimental insects and treatments

The silkworm *B. mori* strain Dazao was provided by the Research and Development Center of the Sericultural Research Institute of the Academy of Agricultural Sciences of Guangdong Province, China. Larvae were reared on fresh mulberry leaves at 25–27 °C under a photoperiod of 12 h light and 12 h dark. Under these conditions, larvae wandered on day 6/7 of the 5th instar and pupated 3 days thereafter. In this study, W0 was the time when larvae stopped feeding. P0 was the moment at which larvae just shed the remnants of the larval integument, marking the beginning of the pupal stage.

2.2. Bioinformatic analysis of DNA and protein sequences

The domains of BmAbd-A and BmPOUM2 were analyzed with the SMART (http://www.smart.embl-heidelberg.de/). The secondary structures of BmAbd-A and BmPOUM2 were predicted by PredictProtein (https://www.predictprotein.org/). The putative Hox cis-regulatory element was analyzed using the MatInspector program (http://www.genomatrix.de/).

2.3. Genomic DNA isolation and southern blot analysis

Genomic DNA was prepared from *B. mori* larvae using the phenol extraction method as described by Sambrook et al. (1989). For Southern blots, aliquots (10 μg each) of genomic DNA were digested with *Hind* III, *Xba* I or *Eco*R I for 12 h at 37 °C, fractionated on a 1% agarose gel, transferred to a Hybond N^+ nylon membrane (GE Healthcare Life Sciences, USA) and hybridized using a ^{32}P-dCTP labeled DNA probe, which was generated from a full-length *BmPOUM2* cDNA using the Random Primer DNA Labeling Kit (TaKaRa Co. Dalian, China). The hybridization conditions and signal detection were conducted as described by Deng et al. (2011).

2.4. Reverse transcription PCR (RT-PCR)

Total RNA was extracted from the epidermis containing wing discs tissues or the wing discs of larvae according to the instructions (TaKaRa Co. Dalian, China). RNA quantification and reverse transcription were the same as described previously (Deng et al., 2011). The primers used for amplifying *BmPOUM2* cDNA were: 5′-GAAGCATTCGCCAAACA-3' (forward) and 5′-TTAGTGCGCTGCCAGTG-3' (reverse). Template DNA was denatured at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s for each cycle. PCR products were separated on 1.0% agarose gels and stained with ethidium bromide.

2.5. Truncation of protein sequences, site-directed mutagenesis and recombinant protein expression

To identify the protein-protein binding regions, the open reading frame (ORF) of *BmPOUM2-S* was truncated into the two fragments: *BmPOUM2-S-S1* (1–139 aa) and *BmPOUM2-S-S2* (140–238 aa), named S1 and S2, respectively. The region (216–329 aa) of BmPOUM2 that is not present in BmPOUM2-S was named BmPOUM2-H (P-H). The POU domain was located on the *BmPOUM2-S-S2* region. Six amino acids (Lys166, Gly173, Gln176, Ser192, Glu200 and Asn208) in the POU domain of BmPOUM2 were mutated into the amino acids (N166, R173, H176, T192, D200 and K208), respectively, and the mutated DNA fragment was synthesized by Beijing Liuhe Huada Gene Technology (Shenzhen, China) and named POU-M6.

The ORF of BmAbd-A was truncated into two sets of fragments: a set of two fragments: BmAbd-A-S1 (1–216 aa) and BmAbd-A-S2 (217–352 aa), named AS1 and AS2, respectively and another sect of four fragments, named SA (1–110 aa), SB (111–216 aa), SC (217–291 aa) and SD (292–352 aa). The Homeobox domain was located on BmAbd-A-S2 and BmAbd-A-SC regions. Seven amino acids (Phe, His, Ala, Cys, Glu, Trp and Val) in the BmAbd-A-SB and BmAbd-A-SC were mutated (Phe to Ile, His to Asp, Ala to Pro, Cys to Ser, Glu to Gln, Trp to Arg, Val to Leu) and the mutated DNA fragments were synthesized by Beijing Liuhe Huada Gene Technology (Shenzhen, China) and named SB-M7 and SC-M7, respectively.

The ORFs of BmPOUM2, BmPOUM2-S, BmPOUM2-H (P-H, 216–329 aa), BmPOUM2-S1 (S1, 1–139 aa), BmPOUM2-S2 (S2, 140–238 aa), BmPOUM2-POU (P-POU, 158–214 aa) and BmPOUM2-POU-M6 (POU-M6) were cloned into pPROEXHTa expression vector (Life Technologies, Burlington, Canada) in fusion with a 6×His tag at the C-terminal end, generating the recombinant expression vectors, respectively. The ORFs of BmAbd-A, BmAbd-A-S1 (AS1), BmAbd-A-S2 (AS2), BmAbd-A-SA (SA), BmAbd-A-SB (SB), BmAbd-A-SC (SC), BmAbd-A-SD (SD), SB-M7 and SC-M7 were cloned into pET28a expression vector (Novagen, Darmstadt, Germany) with a 6×His tag on the N-terminal ends to express His-tagged proteins, respectively. Expression of His-tagged proteins were induced by adding IPTG at the final concentration of 1.0 mM and purified by affinity chromatography using Ni-NTA resin (Novagen Co., Darmstadt, Germany).

2.6. Far-western blot analysis

To confirm that BmAbd-A protein interacted with BmPOUM2 protein, far-Western blots were performed according to the method of Wu et al. (2007). The purified recombinant BmPOUM2, BmPOUM2-S, BmPOUM2-H, BmPOUM2-S1, BmPOUM2-S2, BmPOUM2-POU, BmPOUM2-POU-M6, BmAbd-A, BmAbd-A-S1, BmAbd-A-S2, BmAbd-A-SA, BmAbd-A-SB, BmAbd-A-SC, BmAbd-A-SD, ASB-M7 or ASC-M7 protein (1 μg) were separated in 12% (wt/vol) SDS/PAGE and proteins were transferred to nitrocellulose membranes. One set of membranes was used for direct immunoblotting using rabbit polyclonal antibody against BmAbd-A or BmPOUM2, and the other set of membranes was used for far-western blot analysis. The membranes were washed in TBST for 1 h with the buffer changed every 20 min to remove SDS, blocked with 3% (wt/vol) BSA in TBST for 1 h, and then probed with purified BmPOUM2 or BmAbd-A (1 μg/mL) in TBST containing 0.1% BSA and incubated at pH 9.0 overnight at 4 °C with a gentle rocking. The membranes were then washed in TBST three times, with each wash lasting 5 min, and then probed with rabbit polyclonal antibody against BmPOUM2 or BmAbd-A, which was used as the primary antibody to detect protein interactions by using the procedures as described for direct immunoblotting.