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Molecular cloning and characterization of a C-type lectin from the cotton bollworm, *Helicoverpa armigera*

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Abstract
C-type lectins participate in pathogen recognition and other defense responses in innate immunity as well as in cell–cell interactions. A new cDNA encoding a 335-residue polypeptide containing two tandem C-type lectin domains was cloned from the haemocytes of *Helicoverpa armigera* (Ha-lectin). Northern hybridizations revealed that the mRNA of Ha-lectin was expressed constitutively in haemocytes, and was up-regulated following injections of bacteria, yeast, or virus. Ha-lectin expression was also induced in the fat body when larvae were injected with bacteria, yeast or 20-hydroxyecdysone and a non-steroidal ecdysone agonist, RH-2485. The Ha-lectin was detected in granular haemocytes. The recombinant protein (rHa-lectin) expressed in *Escherichia coli* had hemagglutinating and sugar-binding activities. The native Ha-lectin protein was identified in haemocytes and plasma using a polyclonal antiserum raised against rHa-lectin by immunoblotting techniques. All together, our results suggest that the Ha-lectin gene is involved in innate immunity, and may also participate in the molting process.

1. Introduction

C-type lectins (CLECTs) comprise a superfamily of Ca^{2+}-dependent carbohydrate binding proteins. CLECTs function as pattern recognition molecules or receptors for pathogens in innate immunity, and are involved in cellular interactions [1,2]. In most vertebrates, 14 groups (I–XIV) of CLECTs have been distinguished based on their C-type lectin domain (CTLD) architecture [3,4]. Recent studies on *Fugu* have revealed 3 new C-type lectin classes [5].

Analyses of whole-genome model organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, show that the CLECT superfamily is as abundant and diverse in invertebrates as it is in vertebrates [6]. Because of the absence of an adaptive immunity system in invertebrates,

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the innate immune system must assume the entire burden of defending the host against pathogens. The most universal and effective method of distinguishing self from non-self is being able to recognize characteristic carbohydrate structures. To live in an environment with a variety of pathogens, invertebrates have evolved an effective repertoire of defense mechanisms, in which the CLECTs are major systemic players. This may be one reason for the high abundance of CTLD-containing proteins in invertebrates.

To date, CLECTs have been reported in only a few insect species. For example, immulectins have been purified from larval hemolymphs of the tobacco hornworm *Manduca sexta* [7–10], lipopolysaccharide (LPS)-binding protein (LBP) has been extracted from *Bombyx mori* [11], and a putative lectin clone, Hdd15, has been isolated from *Hyphantria cunea* [12]. These lectins all share common dual-CTLD architectures. However, LBP from *Periplaneta americana* [13], and a lectin from *Sarcophaga peregrina* [14,15] contain only a single CTLD.

Lectins in the plasma of lepidopterans and cockroaches have been shown to participate in innate immune responses such as phagocytosis [16], haemocyte nodule formation [11,17], encapsulation and melanization [9,10,18], and the activation of prophenoloxidase [7,8]. *Sarcophaga* lectin may also be involved in the development of the imaginal discs of *Sarcophaga peregrine* [19].

Attempts to classify insect haemocytes have been based generally on morphological characteristics, and five circulating types of haemocytes are recognized in some lepidopteran insects, including *B. mori* [20] and *Anticarsia gemmatalis* [21]. The five types of haemocytes are prophaemocytes or stem cells, plasmatocytes or predifferentiated cells, and three specialized cells, granulocytes, spherule cells and oenocytoids. Haemocyte composition differs among and within insect species, as the haemocyte types and numbers change over the course of development.

In this study, we cloned and characterized a C-type lectin from the larval haemocytes of the cotton bollworm, *Helicoverpa armigera*. This *H. armigera* C-type lectin, designated as Ha-lectin, contains dual CTLD domain architecture. The expression, distribution and characterization of Ha-lectin were studied by Northern blot analyses, in situ hybridization, recombinant expression and immunoblotting analysis.

2. Materials and methods

2.1. Chemicals

RH-2485 powder (95% pure) was donated by the Rohm and Haas Company (Spring House, Pennsylvania, USA). Other materials were purchased from the following companies: Unizol reagent (Biostar Company, Shanghai, China); Quickprep™ Micro mRNA Purifiation Kit and Hybond N^+ nylon membrane (Amersham, Buckinghamshire, England); PCR-Select cDNA Subtraction Kit and SMART PCR cDNA synthesis kit (BD Bioscience Clontech, Mountain View, USA); pGEM-T Easy (Promega Biosciences, Madison WI, USA); N-Glycosidase F (recombinant), DIG-RNA labeling Kit (Roche, Boehringer Mannheim, Mannheim, Germany); Ex Taq Polymerase (TaKaRa Biotech, Dalian, China); RNA Marker (ToYoBo Company, Osaka, Japan); Mannose, galactose, glucose, maltose, xylose, sucrose and lactose (Amresco, USA); *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, laminarin, trehalose, 20-hydroxyecdysone (20E), LPS from *Escherichia coli* Serotype 055:135, curdlan and peptidoglycan from *Staphylococcus staphylolyticus* (Sigma, St. Louis, MO).

2.2. Insects

H. armigera specimens were obtained originally from the Wuhan Institute of Virology, Chinese Academy of Science, Wuhan, China, and were maintained in our laboratory at $26 \pm 1\,^{\circ}C$ under light and dark conditions for 14:10 h. The larvae were reared on the artificial diet described by Zhao et al. [22].

2.3. Preparation of RNA

The haemolymphs were collected into ice-cold phosphate buffered saline (PBS; 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) treated with diethyl pyrocarbonate (DEPC). The haemocytes were collected by centrifugation at 700*g* for 3 min (4 °C). After the haemolymph was completely discharged from the cutting prolegs, we collected heads, fat bodies, epidermis and midguts for RNA extraction. Total RNA was extracted from the various tissues of the fifth instar molting larvae (with head cap slippage), sixth instar feeding larvae (48 h post-ecdysis) and other developmental stages. In the challenge experiment, each sixth instar feeding larva was injected with 1×10^5 *E. coli*, *Pichia pastoris*, *Bacillus thuringiensis*, *Staphylococcus aureus* cells and 1×10^6 *H. armigera* nuclear polyhedrosis virus (HaNPV) in 5 μl PBS into the hemocoel, and the same volume of PBS as a negative control. The total RNA population was then extracted from the haemocytes at 0, 6, 12, 18, 24, 32 and 48 h intervals. At the same time, the total RNA from the fat bodies was extracted at 12 and 24 h. In the 20E and RH-2485 induction experiments, 20E was dissolved in dimethyl sulfoxide (DMSO) at 10 mg/ml, the stock solution was diluted with water to a concentration of 0.1 μg/μl, and 5 μl of this solution was injected into the larva. The same amount of DMSO, without 20E, represented the controls. RH-2485 was dissolved in isopropanol at 5 mg/ml, the stock solution was diluted with water to a concentration of 0.02 μg/μl and 5 μl of the solution was injected into the larva. The same amount of isopropanol (without RH-2485) was used in the controls. Total RNA was extracted from the haemocytes and fat bodies at 0, 6, 12, 18, 24, 32 and 48 h intervals.

2.4. Suppression subtractive hybridization (SSH)

To study larval molting- and metamorphosis-related genes from *H. armigera*, the suppression subtractive hybridization (SSH) technique was applied to identify the differentially expressed genes during larval molting and metamorphosis. The mRNA samples from haemocytes obtained from the molting fifth and day 1 feeding sixth instar larvae were isolated using the Quickprep™ Micro mRNA Purifiation kit. The tester and driver cDNAs were produced using a SMART