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cDNA Sequence of a Novel Immulectin Homologue from the Silkworm, *Bombyx mori*

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A cDNA of novel immulectin homologue (BmIML), a C-type lectin, was cloned from the silkworm, *Bombyx mori*. The immulectin cDNA is an open reading frame of 921 bp encoding 307 amino acid residues. The deduced amino acid sequence from the BmIML cDNA contains two C-type carbohydrate recognition domains (CRDs). The BmIML was most similar (61% protein sequence identity) to the *M. sexta* immulectin-1, whereas BmIML showed relatively lower identity to the *B. mori* lipopolysaccharide-binding protein (25% protein sequence identity). These features of BmIML indicate that BmIML is a novel member of C-type lectin superfamily. Northern blot analysis revealed that the BmIML is specifically expressed in the fat body of *B. mori* larvae.

Key words: Insect, Silkworm, *Bombyx mori*, Immulectin, C-type lectin, cDNA sequences, Fat body, Immunity

Introduction

Insects have a mechanism for defense against microbial infections, which is similar to innate immune system of vertebrates, involving both humoral and cellular responses (Gillespie *et al.*, 1997; Hoffmann, 1995; Hultmark, 1993). In insects, the humoral immune response includes a group of antimicrobial peptides, lectins, and cell adhesion molecules (Cociancich *et al.*, 1994; Karp, 1996).

Lectins have been reported to specifically bind bacterial lipopolysaccharide (LPS) (Jomori and Natori, 1991) and to stimulate hemolymph phenol oxidase activation (Chen *et al.*, 1995). C-type lectins have been isolated from a few insect species. C-type insect lectins contain two tandem carbohydrate recognition domains (CRDs). These C-type lectins have function in insect innate immune system by participating in hemocyte nodule formation (Koizumi *et al.*, 1997, 1999) and activating prophenol oxidase in hemolymph (Yu *et al.*, 1999). C-type lectins of this new type include immulectin-1 (Yu *et al.*, 1999) and immulectin-2 (Yu and Kanost, 2000) from the *Manduca sexta*, and LPS-binding lectins from the silkworm, *Bombyx mori* (Koizumi *et al.*, 1999) and the fall webworm, *Hyphantria cunea* (Shin *et al.*, 1998, 2000).

We report here cloning of a novel cDNA for putative C-type immulectin, named BmIML, from *B. mori*, which contains two CRDs. The deduced amino acid sequence suggested that BmIML is a novel member of the C-type lectin superfamily, with a unique structural feature compared with previous report from *B. mori* (Koizumi *et al.*, 1999).

Materials and Methods

cDNA library screening , nucleotide sequencing and data analysis

A cDNA library was constructed using whole bodies of *B. mori* larvae (Kim *et al.*, 2003). Sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer

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Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program. MacVector (ver. 6.5, Oxford Molecular Ltd.) was used to align the amino acid sequences of immulectins.

RNA isolation and Northern blot analysis

Total RNA was isolated from fat body, midgut, silk gland, ovary, and testis of the *B. mori* larvae using the Total RNA Extraction Kit (Promega, Madison, WI). Total RNA (10 μg/lane) from the *B. mori* was separated on glyoxalation gel (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2 × PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the CRT gene was 1104 bp for *B. mori* immulectin homologue cloned in this study and labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C, and finally exposed to X-ray film.

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-40                                    AATTGTCTGTTAGGTCGTGTCCGGTAGTCGTCACGTCGAA
  1  ATGAAGAGTGCTTTTATTTGTTTCTGCTTTTTGGCGATTGCCAGTGCCCCCCCTCCGAGT
  1  M  K  S  A  F  I  C  F  C  F  L  A  I  A  A  A  P  P  P  S

 61  GTTTCGAAGCAATACCGCTCCGACTACGTATATAATAAAGACACCAACGCGTTTTACAAA
 21  V  S  K  Q  Y  R  S  D  Y  V  Y  N  K  D  T  N  A  F  Y  K

121  CTACACACTGACAGCGCTAAGATATGGGACGCCAAGAGCTCTTGCACCACTGAGGGAGCC
 41  L  H  T  D  S  A  K  I  W  D  A  K  S  S  C  T  T  E  G  A

181  CAGTTGATGGTGCCGGCTTCAGAACAGGATATAATTCAGCTTCATTCGATGTTCAAGAGA
 61  Q  L  M  V  P  A  S  E  Q  D  I  I  Q  L  H  S  M  F  K  R

241  TTTCCAGATCTGGGGAACTATGTTTGGGTGGATGAAGACGGAAAAGACCATGAATCTGCA
 81  F  P  D  L  G  N  Y  V  W  V  D  E  D  G  K  D  H  E  S  A

301  GAGGACAAGCCGATGATTGATTTGTCAGACTCTGTAACGGAAGCGATGAGGTCTAGGTTC
101  E  D  K  P  M  I  D  L  S  D  S  V  T  E  A  M  R  S  R  F

361  GCATTACAAGGGTGCGATGTGGTTACTCGGCAGGGAGAAATCGAAACCTCACCCTGCTAC
121  A  L  Q  G  C  D  V  V  T  R  Q  G  E  I  E  T  S  P  C  Y

421  AACCGATTACCTTTCATTTGCAAAGTGGAAGCCATTGATGCGCCTTACGATACGCACTGT
141  N  R  L  P  F  I  C  K  V  E  A  I  D  A  P  Y  D  T  H  C

481  GGGGTTTTTGCTATAGGGTACCAATACGTGGAGAGCACTGGAAGCTGCTATAAGATCTCC
161  G  V  F  A  I  G  Y  Q  Y  V  E  S  T  G  S  C  Y  K  I  S

541  AAAGTAGCCTATTCCTGGACCCAAGCTTATGACGAATGTCAAGCCGAGAATGCCCACCTG
181  K  V  A  Y  S  W  T  Q  A  Y  D  E  C  Q  A  E  N  A  H  L

601  GTGGTCCTTAATTCGGAAGCGGAGATGTTGGTCGTGAAGAATTTGACGAACGCAGCGGCC
201  V  V  L  N  S  E  A  E  M  L  V  V  K  N  L  T  N  A  A  A

661  CCAGTTGACGAAGCCCAGACCACATATTTCTTTTATGCCGGTTTCAGAGCACAGGAACCC
221  P  V  D  E  A  Q  T  T  Y  F  F  Y  A  G  F  R  A  Q  E  P

721  ACTAAAAACGAAACTCCCGTGTTCAAGACGATTTTCAATCAAACATTACAAGAAGCCGGT
241  T  K  N  E  T  P  V  F  K  T  I  F  N  Q  T  L  Q  E  A  G

781  TTCAGTGGCTGGTCCGAAAACGAACCTAACAACGCGAACAACATCGAATTTTGCGGCACG
261  F  S  G  W  S  E  N  E  P  N  N  A  N  N  I  E  F  C  G  T

841  CTCTTCAAAAACGACGGCAAACTGAATGACGTCGACTGCTCCGATAAATACGCCTTCATA
281  L  F  K  N  D  G  K  L  N  D  V  D  C  S  D  K  Y  A  F  I

901  TGCGAAAAGGAAGTGCATATGTAAACCGTTCTGTGCTAAAAACGTACTTATATAGCAGTT
301  C  E  K  E  V  H  M  *

961  ATTACGAAACTTAAGTCTGAATTTTTATTGTCATAAATTAAAATAATATTTAAAACCGCA
1021 TCTTTTGTAATTACACGTTATCGCTGAAAAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of BmIML cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. The poly(A) addition signal is underlined.

Results and Discussion

During EST (expressed sequence tag) screening of a *B. mori* larval whole body cDNA library, a clone was isolated, which contained a 1,104 bp insert. Database searching revealed that this clone contained an open reading frame (ORF) encoding an amino acid sequence similar to carbohydrate recognition domains (CRDs) of C-type lectins. The C-type immulectin homologue cDNA was sequenced and its sequences exhibited similarity to previously reported immulectins. The nucleotide and deduced amino acid sequences of *B. mori* immulectin (now named BmIML) are presented in Fig. 1. The cDNA is 1,104 bp long, with an ORF of 921 bp encoding 307 amino acid residues.

A multiple sequence alignment of the deduced protein sequence of BmIML cDNA with four C-type insect immulectin sequences is shown in Fig. 2. Alignment of the deduced amino acid sequence from the BmIML cDNA revealed that BmIML contains two C-type CRDs, indicating that BmIML is a putative member of the C-type lectin superfamily. This feature of BmIML is similar to the *M. sexta* immulectin-1 (Yu *et al.*, 1999) and 2 (Yu and Kanost, 2000), and to lectins from two insect species: LPS-binding protein (LBP) from *B. mori* (Koizumi *et al.*, 1999) and *H. cunea* (Shin *et al.*, 1998, 2000). In addition, five C-type insect lectins contained ten-conserved cysteine residues to allow the formation of the five disulfide bonds. The hydropathy plot (data not shown) of BmIML by the Kyte and Doolittle method (1982) showed a 16-residue putative signal peptide.

Alignment of the BmIML sequences with those of C-type insect immulectins from several other species indicates the extent of the identity that exists (Fig. 3). The BmIML was most similar (61% protein sequence identity) to the *M. sexta* immulectin-1 (Yu *et al.*, 1999), whereas BmIML showed relatively lower identity to the *M. sexta* immulectin-2 (23% protein sequence identity) (Yu and Kanost, 2000), *B. mori* LBP (25% protein sequence identity) (Koizumi *et al.*, 1999) and *H. cunea* hdd15 (21% protein sequence identity) (Shin *et al.*, 1998, 2000).

To confirm the expression of the BmIML gene at transcriptional level, Northern blot analysis was performed using total RNA prepared from the fat body, midgut, silk gland, ovary and testis of *B. mori* larvae (Fig. 4). A

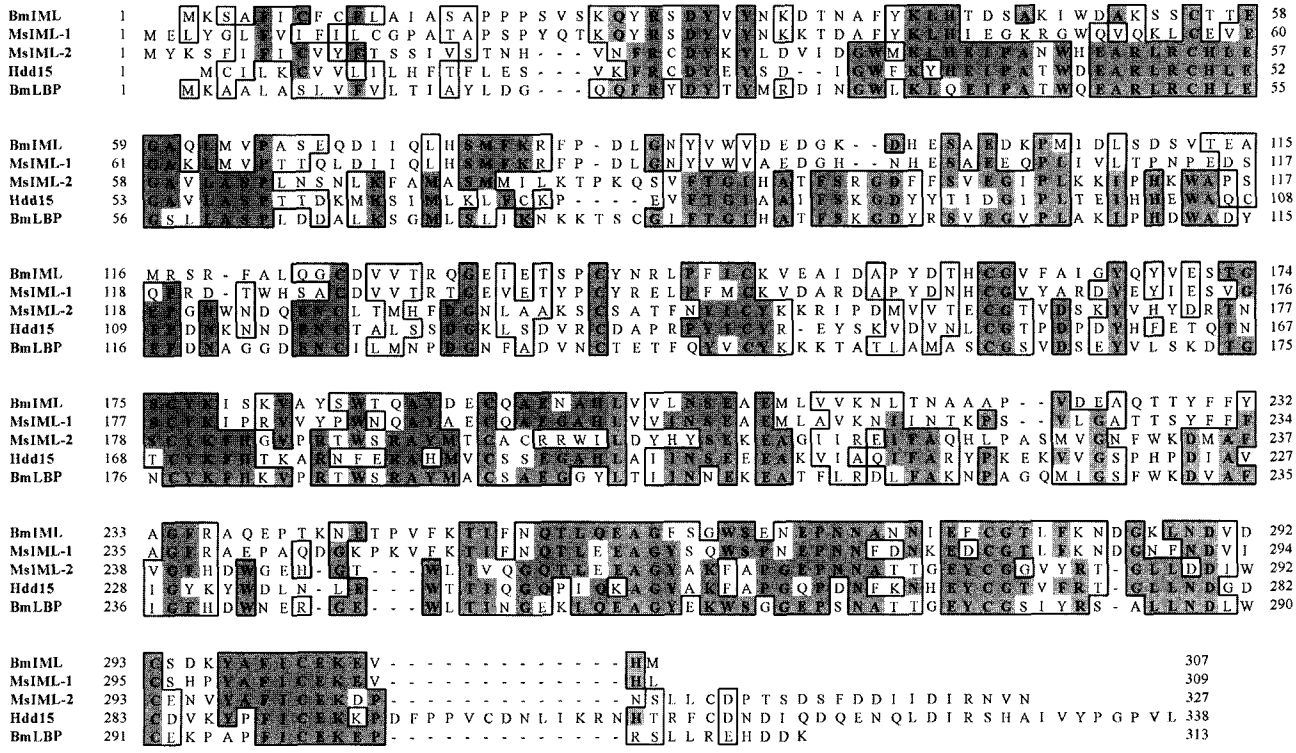

Fig. 2. Comparison of BmIML and other C-type lectins. Alignment of CRD in BmIML and four C-type lectins; BmIML, *B. mori* immulectin; MsIML-1, *M. sexta* immulectin-1 (Yu *et al.*, 1999); MsIML-2, *M. sexta* immulectin-2 (Yu and Kanost, 2000); Hdd15, *H. cunea* Hdd15 (Shin *et al.*, 1998), BmLBP, *B. mori* lipopolysaccharide-binding protein (Koizumi *et al.*, 1999). Identical amino acid residues are indicated by boxes and the numbering is based on the sequence of each protein. The two amino acid residues for the ligand binding specificity are marked with diamond. Invariant cysteine residues are marked with inverted triangle, whereas the extra two cysteines in the long-form CRD2 are marked with circle.

Percent similarity

	1	2	3	4	5
1. BmIML		73	38	39	42
2. MsIML-1	61		38	37	39
3. MsIML-2	23	23		54	65
4. Hdd15	21	23	42		57
5. BmLBP	25	24	50	40	

Percent identity

Fig. 3. Identity of BmIML and other C-type lectins. Pairwise comparison among amino acid sequences of BmIML and four C-type lectins; BmIML, *B. mori* immulectin; MsIML-1, *M. sexta* immulectin-1 (Yu *et al.*, 1999); MsIML-2, *M. sexta* immulectin-2 (Yu and Kanost, 2000); Hdd15, *H. cunea* Hdd15 (Shin *et al.*, 1998), BmLBP, *B. mori* lipopolysaccharide-binding protein (Koizumi *et al.*, 1999).

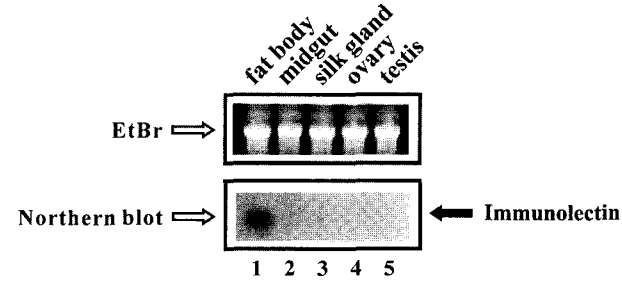

Fig. 4. Northern blot analysis of the BmIML. Total RNA was isolated from fat body (lane 1), midgut (lane 2), silk gland (lane 3), ovary (lane 4) and testis (lane 5) of *B. mori* larvae. The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred onto a nylon membrane, and hybridized with the appropriate radiolabelled probe (lower panel). BmIML messages are indicated by arrow on the right side of the panel.

hybridization signal was detected as a single band in mRNA from the fat body. The Northern hybridization exhibited an expression of the BmIML gene in the fat body, evidencing the fat body as a major synthesis site for defense system against microbial infections (Yu *et al.*, 1999).

In conclusion, we report the cDNA sequence of a novel C-type immulectin in *B. mori*. We hope that the molecular characterization of BmIML in this study will expand the understanding of C-type insect immulectins.

Acknowledgements

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