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Molecular Characterization of A Glycine and Proline-rich Antibacterial Protein from Larvae of A Beetle, *Protaetia brevitarsis*

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A glycine and proline-rich antibacterial protein was cloned from larvae of a beetle, *Protaetia brevitarsis*. The DNAs encoded a deduced propeptide of 127 amino acid residues with predicted molecular weight of 14.0 kDa and PI of 7.89. Structural analysis of this protein indicated the presence of a recognition sequence for the cleavage site within the constitutive secretory pathway (Arg-Xaa-Lys/Arg-Arg), suggesting that mature portion (72 amino acid residues) is produced by cleavage of signal peptide and propeptide from 127 amino-acid-long precursor protein. Mature portion sequence of this protein showed 72% similarity to that of *Oryctes rhinoceros* Rhinocerosin and 91% to that of *Holotrichia diomphalia* holotricin 2. The mRNA expression was reached the highest level at 4 hrs after *E. coli* injection and then declined gradually.

Key words: *Protaetia brevitarsis,* Glycine and proline-rich protein

Introduction

Insects have a defense mechanism consisting of cellular and humoral systems. Much research has shown that antibacterial peptides are one of the most important humoral systems in insects. Today, more than 200 peptides have been reported from insects.

Insects antibacterial proteins are grouped into five major types (Hultmark, 1993); cecropins, insect defensins, large glycine-rich proteins, small proline-rich proteins and lysozymes. Several proteins have been isolated from different insect species and characterized as glycine-rich antibacterial proteins, *e.g.*, sarcotoxin III from *Sarcophaga peregrina* (Baba *et al.*, 1987), diptericin from *Phormia terranovae* (Dimarcq *et al.*, 1990) and *Drosophila melanogaster* (Wicker *et al.*, 1990), coleoptericin from *Zophobas atratus* (Bulet *et al.*, 1991) and holotricin 2 from *Holotrichia diomphalia* (Lee *et al.*, 1994) and coleoptericin A and B *from Allomyrina dichotoma* (Sagisaka *et al.*, 2001).

In this study, we have cloned and characterized a new gene of glycine and proline-rich antibacterial protein from larvae of a beetle, *Protaetia brevitarsis.*

Materials and Methods

Experimental animals

The larvae of white-spotted flower chafer, *P. brevitarsis*, were maintained at 28°C, 70% relative humidity, and photoperiod of 16L : 8D (Kim *et al.*, 2002) and final instar larvae were used for the experiment.

cDNA library screening, nucleotide sequencing and data analysis

cDNA library constructed using whole bodies of *P. brevitarsis* larvae was used in this study. The clones harboring cDNA inserts were randomly selected and sequenced to generate the expressed sequence tags (ESTs). The plasmid DNA was extracted by Wizard mini-purification kit (Promega Madison, WI). The nucleotide sequence was determined by using a BigDye Terminator cycle sequenc-

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ing kit and automated DNA sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Bio-systems, Forster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI (http:/www.ncbi.nlm.nih.gov/BLAST). GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program. Clustal X (Thomson *et al.*, 1997) was used to align the amino acid sequences of glycine and proline-rich antibacterial protein.

Northern blot analysis

Total RNA was extracted from the whole bodies of larvae after 0, 4, 8, 16 and 24 hrs after injection of *E. coli* using a Trizol reagent (Life technologies, Inc., Gaithersburg, MD, USA) and quantified by ultraviolet spectroscopy. A total of 10 μg aliquots of total RNA were fractionated on 1% agarose/6.7% formaldehyde gels and blotted onto nylon membranes (Schleicher & Schwell BioScience, USA) using 20x sodium chloride sodium citrate buffer. Membranes were hybridized with cDNA probe labeled with [α-^{32}P]dCTP using a random primer labeling kit (Stratagene, USA). Hybridizations were performed using a hybridization oven. Membranes were subsequently exposed to Kodak BIOMAX film (Eastman Kodak Co., Rochester, NY, USA) at 70°C. As an internal marker, 28S rRNA was visualized by ethidium bromide staining.

Results and Discussion

In search of *P. brevitarsis* ESTs, we identified a cDNA showing high homology with previously reported glycine and proline-rich antibacterial protein. The full length sequence of a *P. brevitarsis* glycine and proline-rich antibacterial protein was 527 bp in length, having a 5' untranslated region (UTR) of 36 bp, a 3'UTR of 106 bp, and open reading frame (ORF) of 381 bp encoding a polypeptide of amino acid. The DNAs encoded a deduced propeptide of 127 amino acid residues with predicted molecular weight of 14.0 kDa and PI of 7.89. Structural analysis of this protein indicated that it has a recognition sequence for the cleavage site within the constitutive

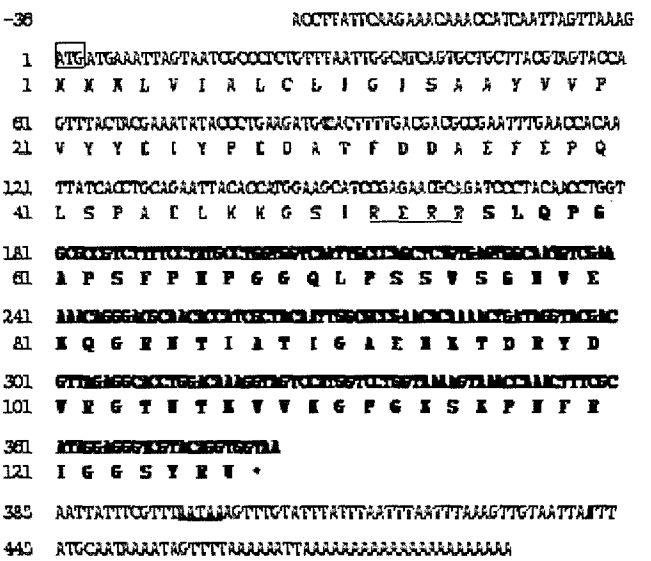

Fig. 1. cDNA sequence and deduced amino acid sequence of *P. brevitarsis* glycine and prolibne rich protein. The ATG start codon is boxed and the termination codon is indicated by asterisk. In the cDNA sequence, the polyadenylation sequence and cleavage site are double-underlined. Mature protein region is shaded.

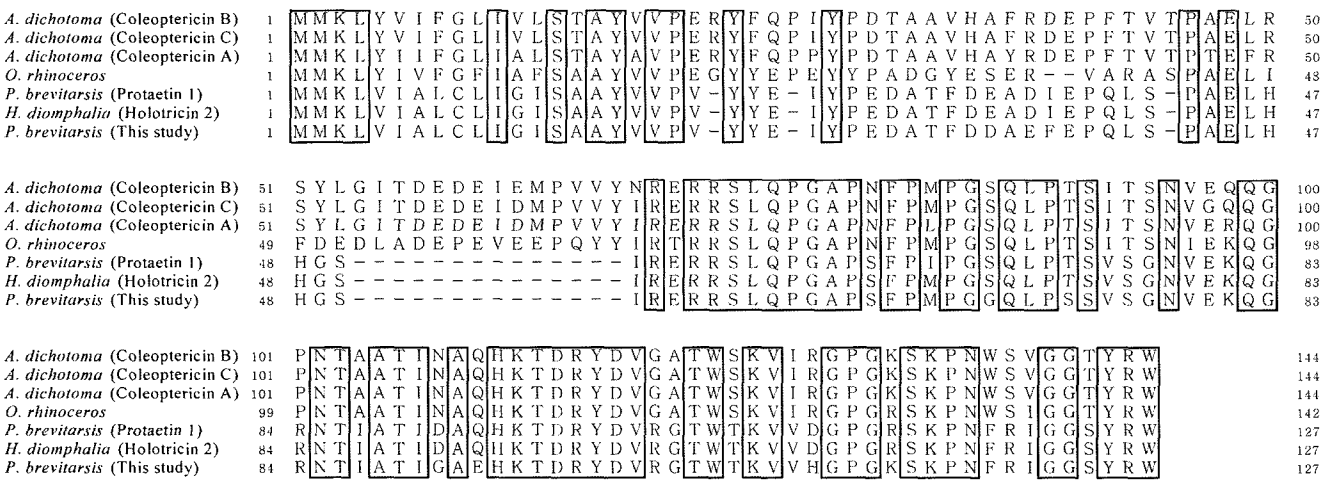

Fig. 2. Multiple alignment of the amino acid sequences of *P. brevitarsis* glycine and proline rich protein with known insect glycine and proline rich protein. The insect glycine and proline rich protein sequences were taken from the following sources: *P. brevitarsis* (this study), *A. dichotoma* [Coleoptericin A] (BAB40436), *A. dichotoma* [Coleoptericin B] (BAB40437), *A. dichotoma* [Coleoptericin C] (BAB40438), *O. rhinoceros* (O76145) and *H. diomphalia* [Holotricin 2] (Q25054).

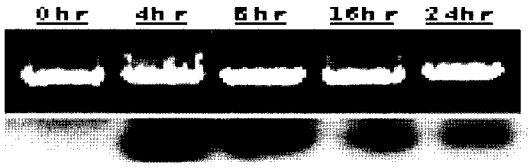

Fig. 3. Time course gene expression analysis of glycine and proline rich protein by Northern analysis. *P. brevitarsis* larvae were injected with 50 μl *E.coli* JM109 (5×10^5 cells) suspended in physiological saline (150 mM NaCl/ 5 mM KCl). Larvae were kept for 0, 4, 8, 16 and 24 hrs. Ten microgram aliquots of total RNA were resolved on formaldehyde containing agarose gels and blotted onto nitrocellulose membranes. The probe was labeled with [α-^{32}P]dCTP. As internal marker, 28S rRNA was visualized by ethidium bromide staining.

secretory pathway (Arg-Xaa-Lys/Arg-Arg; Hosaka *et al.*, 1992), suggesting that the mature portion (72 amino acid residues) is produced by cleavage of signal peptide and propeptide from 127-amino-acid precursor protein. This protein is also rich in glycine (15.2%) and proline (9.7%) as is true for the other reported glycine and proline protein: Rhinocerosin (glycine 11.1% and proline 11.1%) (Yang *et al.*, 1998), Holotricin 2 (glycine 12.5% and proline 9.7%) (Lee *et al.*, 1994), Coleoptericin (glycine 18.0% and proline 8.3%) (Bullet *et al.*, 1991), Coleopterine A and B (glycine 11.1% and proline 11.1%) (Sakisaka *et al.*, 2001). Mature portion sequence of this protein has 72% similarity to that of *Oryctes rhinoceros* Rhinocerosin, 91% similarity to that of *Holotrichia diomphalia* holotricin 2 and 70% similarity to that of *Allomyrina dicotoma* coleoptericin A, suggesting that this gene is an insect glycine and proline rich protein (Fig. 2).

Northern blot analysis was performed to verify differential expression of this protein in *E. coli*-immunized larvae (Fig. 3). The immunized larvae were collected to isolate total RNA 0, 4, 8, 16, and 24 hrs after *E. coli* injection. The mRNA expression was reached the highest level after 4 hrs and then declined gradually. Thus, the increase of this protein expression after *E. coli* injection in insect might be involved in immune response.

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