Gloverin-like Protein Gene in *Bombyx mori* (*BmGLP*) and Inducible Expression in the Fat Bodies

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ABSTRACT

Gloverin is an antibacterial peptide which has been previously isolated in *Hyalophora gloveri*, and is known to exert an inhibition effect on the growth of Gram-negative bacteria (GNB). When the expressed genes in the immunized *Bombyx mori* were screened, gloverin like gene (*BmGLP*) was found and it was thought to respond against the bacterial invasion. This study presents some data about the gene organization and expression of this candidate for a novel antibacterial peptide in *Bombyx mori*. The complete sequence, which was determined via RACE, revealed that this *BmGLP* was identical to the recently reported clone *BmGloverin2* mRNA. The transcription of this gene was increased transiently, but to a high degree, around 7~11 hours after infection, as evidenced by the results of Northern blotting hybridization analysis. Based on the tentative coding sequence, we amplified the genomic sequence and determined that the gene harbored 3 introns. Interestingly, we found retrotransposon relative sequences in introns 1 and 3. Both Southern blotting analysis and sequencing data indicated that the *BmGLP2* gene had another relative- or pseudo- sequences in its genome. We also attempted the generation of recombinant peptides in a prokaryotic cell system, but we obtained only truncated peptides and were unable to determine the existence of any antibacterial activity with the cell extract under physiological conditions. In further studies, the expression system and activity test conditions should be made more representative of the actual insect organism. Finally, we have proposed the possibility that some type of evolutionary link connects retrotransposons activity in insect immunity gene variation.

Key words: antibacterial peptide, insect immunity, retrotransposon, gloverin, *Bombyx mori*.

INTRODUCTION

The insect immune system has been a subject of intense attention not only due to the many features it shares in common with the immune systems of mammals, but also due to the unique weapons that are restricted to certain species such as *B. mori* (moricin), *Drosophila* (drosocin), and others (Bulet et al., 1993; Hara and Yamakawa, 1995). In order to gain some understanding into the general immune mechanisms of these species, and to track down or develop novel...
antimicrobial agents, researchers have examined a variety of insects. Although insects tend to lack adapted immune systems such as the antibody production mechanisms observed in mammals, insect exhibit both cellular and humoral immune mechanisms against pathogenic invasion and integument damage (Ling et al., 2005; Brey et al., 1993). The processes inherent to these reactions tend to be quite complicated including pathogenic elicitor, pattern recognition, signaling cascades, and induction of gene expression (Iwanaga et al., 2005; Kanost et al., 2004; Yamakawa and Tanaka, 1999).

Consisting of insect humoral immunity, in a fashion similar to that of the complement system of mammals, antimicrobial peptides are extremely species-specific and diverse in structure, and more than 170 such peptides have already been identified (Ueda et al., 2005). Although many extensive studies have already been performed, the identification of novel antimicrobial peptides would expand the amount of proteins currently associated with the processes of insect immunity. Analyzing differences in the expression of genes between naïve and bacterially challenged insects has been a pivotal and helpful process in the screening of novel immune proteins (Zhu et al., 2003). Induction under the infection conditions is one of the most important features in immune response.

The domestic silkworm, *Bombyx mori*, is a well-studied insect, easily reared and experiences a relatively large larval stage. These features are able to provide enough hemolymph and fat bodies, the primary immune tissue for study. This lepidopteran insect has mechanical protection system mediated by proPO activation, which results in melanization, and humoral defense system associated with other peptides (Kanost et al., 2004). Currently, at least 4 antibacterial peptides have been isolated in *B. mori*; cecropins, moricins, lebocins, and an attacin.

In the previous study, we cloned several unknown sequences from the cDNA library of immunized silkworm fat bodies. Among these, we determined that the gloverin like gene (CK240582) could be induced by exposure to Gram-negative bacteria (Hwang et al., 2004). This study focuses on the gloverin like peptide (GLP) gene of *B. mori* (*BmGLP*), and its inducible expression subsequent to bacterial infection. Gloverin is one of the antibacterial peptides that was reported in *Hyalophora* pupae at 1997 as an antibacterial peptide (Axen et al., 1997), and then subsequently obtained from other insects, including *Helicoverpa armigera*, *Trichoplusia ni*, and *Galleria mellonella* (Seiz et al., 2003; Lundstorm et al., 2002; Machintosh et al., 1998). Here, we report the isolation of a homologue of this peptide in *Bombyx mori*; focusing on its gene structure, transient expression subsequent to infection, and expression in an experimental prokaryotic system. Our data constitute a viable basis for future study into the antimicrobial activity of *BmGLP*, as well as the defense mechanisms utilized by the silkworm.

**MATERIALS AND METHODS**

**Insects, cells, and bacterial immunization**

Korean domestic silkworms (*Bombyx mori*), provided for bacterial immunization, were reared at a temperature 25°C. Fifth instar larvae were employed in this experiment. The *Escherichia coli* strain, DH5α (10⁵ cells/mL in insect physiological saline containing 150 mM NaCl and 5 mM KCl), was allowed to grow in LB medium at 37°C with agitation (200 rpm), and was injected into the silkworms.

**DNA and RNA preparation from larval fat body**

Immunized larvae were dissected with a needle and a sharp forceps, and we collected the fat bodies and washed them in physiological saline. Subsequent to homogenization with liquid nitrogen, we extracted the total RNA using EasyBlue™ (INTRON BioTech, Korea), and purified the genomic DNA using Wizard Plus SV (Promega, USA) according to the manufacturer’s protocols.

**Rapid Amplification of cDNA Ends (RACE) and sequencing**

We then designed several primers for genomic PCR, on the basis of the coding sequences of the mRNA. The