Korean J. Genetics 29 (2) : 255-261 (June 2007)

Molecular Cloning and Expression Patterns of a Serpin-2 from the Beet Armyworm, *Spodoptera exigua*

Mi Young Noh[1#], Hong Ja Kim[3], Yong Hun Jo[1#], Cheol Hong Park[1], Hee Jung Park[1], Seongon Kim[2], Jong Dae Park[2], Iksoo Kim[1], Sook Jae Seo[3], Yong Seok Lee[4] and Yeon Soo Han[1*]

[1]Department of Agricultural Biology, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea
[2]Jeonnam Agricultural Research and Extension Services, Naju 520-715, Korea
[3]Division of Applied Life Science, Gyeongsang National University, Jinju, 660-701, Korea
[4]Department of Parasitology and Malariology, PICR, College of Medicine and Frontier Inje Research for Science and Technology, Inje University, Busan, 614-735, Korea

Received May 8, 2007; accepted May 21, 2007

ABSTRACT

Serpins are known to play a critical role in insect immunity and physiology. Here, we present a 1,296 nucleotide sequence of a serpin gene from the beet armyworm, *Spodoptera exigua*. The putative gene product is a 376 amino acid protein containing the conserved reactive center loop (RCL) residues found in serpin, and has the highest identity to *Bombyx mori* serpin-2 (67%). In addition, it has a homology to serpins of other insects such as *Lonomia obliqua* (66%), *Manduca sexta* (52%), and *Plutella xylostella* (41%). RT-PCR analysis shows that it is not induced in response to laminarin and lipoteichoic acid (LTA), whereas *SeCecropinB* is induced under the same condition. In addition, it is not induced by nucleopolyhedrosis virus of *S. exigua* (SeNPV), whereas *SeCecropinB* and inhibitor of apoptosis (SeNPV-IAP) are induced by SeNPV infection. The lack of dramatic induction of *S. exigua* serpin-2 under these immune challenged conditions suggests that it may have an unknown function during the developmental stages albeit it remains to be further elucidated.

Key words: *Spodoptera exigua*, serpin.

INTRODUCTION

Serpins are serine protease inhibitors of the serpin superfamily present vertebrate and invertebrate including insects. They are about 400 amino acid residues long with a reactive center loop (RCL) near their carboxyl terminus. Serpins are suicide-substrate inhibitors by forming stable covalent complexes with proteases after the cleavage of a scissile bond in the reactive center loop (Zou and Jiang, 2005). They play pivotal and diverse roles during various innate immune responses in arthropod. It includes protection against microbial proteinases, regulation of insect endogenous proteinases, hemolymph coagulation, phenoloxidase activation, and

*To whom correspondence should be addressed.
#Mi Young Noh and Hong Ja Kim are egually contributed to this work.
E-mail: hanys@chonnam.ac.kr. Source of grant funded: This work was supported by Korea Research Foundation Grant (KRF-2004-F00003).

proteolytic activation of insect cytokines (Kanost, 1999).

In Dipteran species, a number of serpin have been cloned and studied. In *Drosophila melanogaster*, attention has been paid to elucidate the roles of serpin (Spn27A, SPN42DaA, and Spn43Ac) in Toll pathway (Goto et al., 2003; Levashina et al., 1999; Ligoxygakis et al., 2002; Robertson et al., 2003), and melanization (De Gregorio et al., 2002; Nappi et al., 2005). In *Anopheles gambiae*, many vector biologists have cloned and examined whether mosquito serpins act as either negative regulator or positive regulator of malaria parasite (*Plasmodium berghei*) using gene silencing technique (Abraham et al., 2005; Michel et al., 2005; Noh et al., 2006; Osta et al., 2004; Vlachou and Kafatos, 2005). Finally, Raikehl and his colleagues have reported that the Toll immune pathway and its activation of *Aedes* Serpin-27A are closely associated with Toll antifungal immune pathway in *Aedes aegypti* (Bian et al., 2005; Shin et al., 2005).

In Lepidopteran species, many serpin genes (Serpin 1~6) from the tobacco hornworm, *Manduca sexta* have been extensively studied. They are divided into two groups based on their functions in the context of insect innate immunity. One group of *M. serpin* genes are involved in regulating the preoteolytic activation of prophenoloxidase (proPO) to phenoloxidase (PO) during the larval stage. For instance, *M. sexta* serpin gene-1 is expressed in larval fat body and hemocytes (Kanost et al., 1995). It has been well known that *M. sexta* serpin-1 has 12 alternative splicing forms which give 12 different serpin proteins in hemolymph. One of the serpins, serpin-1j, is involved in inhibiting proPO activation (Jiang et al., 2003). In addition, cloning and sequence analysis shows that *M. sexta* serpin-3 has homology to *Drosophila melanogaster* serpin 27A, a regulator of melanization. ProPO activation is inhibited by recombinat serpin-3 (Zhu et al., 2003). Both serpin-4 and serpin-5 mRNAs in *M. sexta* are constitutively expressed but induced by bacterial challenge. Recombinant serpin-4 and serpin-5 decreased pro-PO activation (Tong and Kanost, 2005). Finally, biochemical analysis of *M. sexta* serpin-6 recombinant protein shows that it inhibited prophenoloxidase-activating proteinase-3 (PAP-3) and blocked proPO activation. (Wang and Jiang, 2004). The other is *M. sexta* serpin-2 (43 kDa). Unlike the serpins mentioned above, it is not present in hemolymph. *In situ* hybridization showed that the serpin-2 mRNA is present in granular hemocytes of immune-stimulated larvae (Gan et al., 2001). However, the precise role of *M. sexta* serpin-2 has not so far been elucidated (Kanost et al., 2004).

Compared to the famous model (*M. sexta*) for insect serpin investigation, not much attention has been made to the serious agricultural pest, *Spodoptera exigua*. In addition, we have recently been involved in host-parasite interactions between *S. exigua* and nucleopolyhedrosis virus. This context led us to begin a study of *S. exigua* serpin-2 that may have important functions in insect immunity and physiology during the life cycle of *S. exigua*. Using PCR-based cloning method, we identified *S. exigua* serpin-2 as the ortholog of *Bombyx mori* serpin-2 and *M. sexta* serpin-2. Expressions patterns of *S. exigua* serpin-2 were analyzed in response to three different immune elicitors such as laminarin, lipoteichoic acid (LTA) and SeNPV.

MATERIALS AND METHODS

Rearing of *Spodoptera exigua*

The beet armyworm, *Spodoptera exigua* was reared on the leaf of cabbage at 26 ± 1°C and 70% relative humidity with a photoperiod of 16L:8D.

PCR-based cloning of *S. exigua* serpin-2

Degenerate primers (Serpin- FW: 5′-GCCGCTCGAG-AAYGCIGTSTAYTTYAARG-3′; Serpin-Rev: 5′ -GCCGCTCGAGGCYCCYTCYTCRTTIACYTC-3′) were used to amplify a partial fragment of serpin. To obtain the complete cDNA of the serpin, rapid amplification of cDNA ends (RACE) PCR was performed using the 5′-RACE system and 3′-RACE system (Invitrogen) according to the manufacturer's instructions. Single-stranded cDNA for the 5′-RACE PCR was reverse transcribed from total RNA isolated from *S. exigua* larva using 5′-RACE system (Invitrogen). For 3′-RACE, cDNA was synthesized from 5μg of total RNA with adaptor primer (invitrogen) (Lee et al., 2006). PCR amplification conditions were as follows: 94°C for 3 min; 33 cycles at 94°C for 1 min, 55°C for 1 min 72°C for 2 min and 72°C for 10 min. PCR products cloned into the vector pCR2.1-TOPO (Invitrogen), and sequenced. The CAP3, a DNA sequence assembly program was used to generate a complete contig of *S. exigua* serpin-2.