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cDNA cloning and characterization of the antibacterial peptide cecropin 1 from the diamondback moth, *Plutella xylostella* L

Fengliang Jin [a,*,1], Qiang Sun [a,1], Xiaoxia Xu [a], Linmiao Li [a], Gang Gao [a], Yingjie Xu [a], Xiaoqiang Yu [b], Shunxiang Ren [a,*]

[a] College of Natural Resources and Environments, South China Agricultural University, Engineering Research Center of Biological Control, Ministry of Education, Guangzhou 510642, China
[b] School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110, USA

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ABSTRACT

Cecropins are linear cationic antibacterial peptides that have potent activities against microorganisms. In the present study, a 480 bp full-length cDNA encoding diamondback moth (*Plutella xylostella*) cecropin 1 (designated as Px-cec1) was obtained using RT-PCR. A Northern blot analysis showed that the Px-cec1 transcript was predominantly expressed in fat bodies, hemocytes, midgut and epidermis with the highest expression level in fat bodies. The expression of Px-cec1 mRNA in fat bodies was significantly increased 24 h after microbial challenge, with the highest induced expression by *Staphylococcus aureus*. A circular dichroism (CD) analysis revealed that the recombinant Px-cec1 mainly contained α-helixes. Antimicrobial assays demonstrated that recombinant Px-cec1 exhibited a broad spectrum of anti-microbial properties against fungi, Gram-positive and Gram-negative bacteria, but it did not exhibit hemolytic activity against human erythrocytes. Furthermore, Px-cec1 caused significant morphological alterations of *S. aureus*, as shown by scanning electron microscopy and transmission electron microscopy. These results demonstrated that Px-cec1 exerts its antibacterial activity by acting on the cell membrane to disrupt bacterial cell structures.

Introduction

Insects are constantly exposed to potentially harmful pathogens via contact, ingestion and inhalation [1,2]. The survival of insects in a microbe-thriving environment depends on humoral and cellular responses to effectively kill the invading microbes because insects lack the adaptive immunity capable of producing antibodies [3–5]. An analysis of the humoral immune response within the framework of a septic injury in organisms has led to the current paradigm in which two distinct intracellular signal transduction pathways, the immune deficiency (IMD)[2] and Toll pathways, regulate the transcription of hundreds of genes by controlling the nuclear uptake of the NF-κB transcription factors Relish, Dorsal and Dorsal-related immunity factor (DIF) [1,5–9].

The classical effector molecules in the humoral responses are antimicrobial peptides (AMPs), which are constitutively expressed or induced in fat bodies and hemocytes and released into the hemolymph to kill invading microorganisms effectively [2,4,9–11]. Most AMPs are synthesized shortly after microbial infection and act rapidly to neutralize a broad range of microbes. The AMPs discovered to date have been divided into several groups based on their length, their secondary and tertiary structure, and the presence or absence of disulfide bridges [12–14]. These AMPs exhibit bactericidal, fungicidal, virucidal and tumoricidal properties, and the fact that they have the potential to overcome bacterial resistance makes them promising candidates for therapeutic drugs [12–15]. In *Drosophila melanogaster*, drosomycin exhibits fungicidal activity at micromolar concentrations and is active mainly on filamentous fungi. Other AMPs, such as cecropins, attacins, drosocins, and diptericins, are active primarily against Gram-negative bacteria, whereas defensin is effective against Gram-positive bacteria [4–6,10,11].

Cecropins are a family of basic antibacterial peptides produced by insects [16,17]. They have low molecular weights, are water-soluble, and possess a broad spectrum of antibacterial activities, but they are unable to lyse eukaryotic cells [17–19]. Cecropin was first isolated from *Hyalophora cecropia* [17,20], and since then, many cecropin peptides and genes have been reported in lepidopteran and dipteran species [2,18,21–23]. Cecropins exhibit activity against Gram-positive and Gram-negative bacteria by forming ionic pores to destroy the ionic balance of the bacterial membrane

* Corresponding authors. Fax: +86 20 85280293.
E-mail addresses: jflbang@yahoo.com.cn (F. Jin), rensxcn@yahoo.com.cn (S. Ren).
[1] These authors contributed equally to this work.
[2] *Abbreviations used:* IMD, immune deficiency; DIF, Dorsal-related immunity factor; AMPs, antimicrobial peptides; Bt, *Bacillus thuringiensis*; RACE, rapid amplification of cDNA end; NJ, Neighbor Joining; PBS, phosphate-buffered saline; TOF, time of flight; CD, circular dichroism; MIC, minimal inhibitory concentrations; TRX, thioredoxin.

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[16]. Moreover, studies related to AMPs such as cecropins may provide insight into the innate immunity of invertebrates, and these AMPs may be used to design novel broad-spectrum antibiotics for use in humans.

The diamondback moth, *Plutella xylostella* (L.), is a worldwide pest of cruciferous crops. With the abuse of pesticides, insects have developed high resistance to most conventional pesticides, including *Bacillus thuringiensis* (Bt) toxin in the field [24,25]. Therefore, a study of the structure and potential function of effector molecules, such as cecropins, of *P. xylostella* in the antimicrobial process may improve the understanding of the pest immune mechanism and will provide a regulatory inhibitor of immunology as potential environmentally safe pest control agents. In the present study, a cecropin1 gene from *P. xylostella* (Px-cec1) was cloned and characterized. Px-cec1 cDNA was obtained using RT-PCR. The tissue-distribution and transcriptional induction of Px-cec1 in response to a microbial challenge were described. In addition, the recombinant Px-cec1 protein expressed in *Escherichia coli* was purified and assayed for antibacterial activity. Finally, the mechanism of recombinant Px-cec1 against *Staphylococcus aureus* was examined.

Materials and methods

Sample preparation

The larvae of the diamondback moth, *P. xylostella*, were collected from the common cabbage fields and reared successively on common cabbage at 23 ± 2 °C, 70% RH, and a photoperiod of 16:8 h (light:dark) in a greenhouse. Each last instar larva was injected with log phase *E. coli* $K_{12}D_{31}$ (2×10^6 cells) into the thoracic region. The hemolymph was collected from the prolegs of the normal and immune-challenged larvae 24 h post-injection into 1/10 volume of anticoagulant buffer (10% sodium citrate, pH 7.0, and 200 mM phenylthiourea as a melanization inhibitor). The hemocytes were

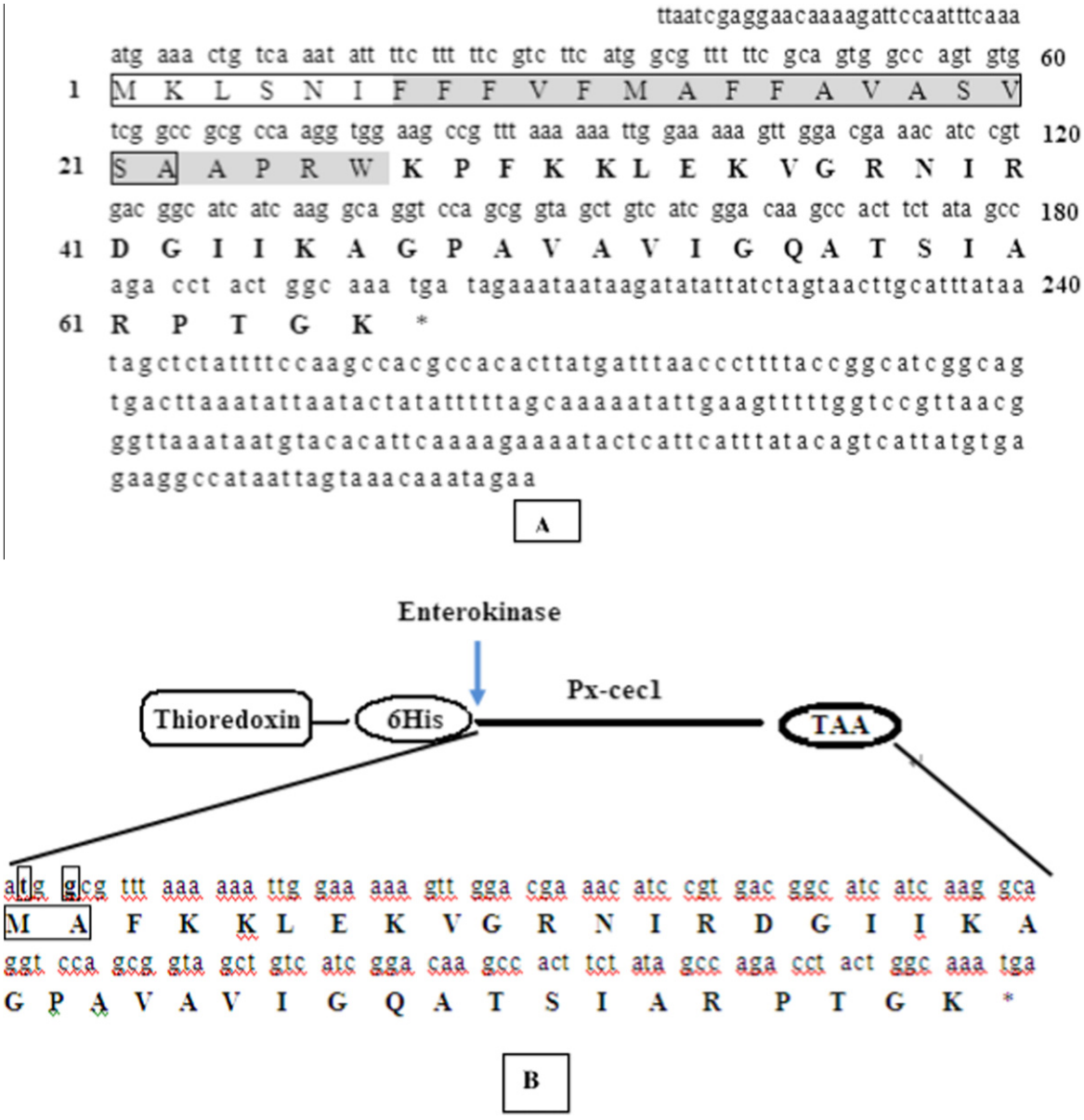

Fig. 1. Nucleotide and deduced amino acid sequences encoding cecropin1 from the diamondback moth (*P. xylostella*) (A)and Schematic representation of the vector used for expression of the mutated Px-cec1(B). (A)The mature peptide is indicated in bold type, the signal sequence is boxed and the predicted transmembrane domain was shaded. The translation stop codon is marked with an asterisk. (B)When we expressed the mature protein of Pxcec1 in BL21(DE3), we mutated the nucleotide sequence ——— **aagcc**g(amino acid KP) into nucleotide sequence —**atggcg** (amino acid MA). As a result, the mature protein of the Pxcec781 was 39 aa(**MA**FKKLEKVGRNIRDGIIKAGPAVAVIGQATS IARPTGK).