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Protein tyrosine phosphatases encoded in *Cotesia plutellae* bracovirus: Sequence analysis, expression profile, and a possible biological role in host immunosuppression

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Abstract

A genome project has been launched and aims to sequence total genome of *Cotesia plutellae* bracovirus (CpBV). On this process, several putative open reading frames have been proposed, among which there was a large gene family coding for protein tyrosine phosphatases (PTPs). This study analyzed the deduced amino acid sequences of 14 CpBV-PTPs in terms of conserved domains with other known polydnaviral PTPs and determined their expression patterns in diamondback moth, *Plutella xylostella*, parasitized by *C. plutellae.* The analyzed CpBV-PTPs share the common 10 motifs with classical type of PTPs. However, there are variations among CpBV-PTPs in active site sequence and phosphorylation sites. Quantitative real-time polymerase chain reaction (PCR) indicated that most PTPs in the parasitized *P. xylostella* were expressed from the first day of parasitization and increased the expression levels during parasitization. All 14 PTPs were expressed in both immune-associated tissues of fat body and hemocytes in the parasitized host. During last instar, the PTP enzyme activity of the parasitized *P. xylostella* was significantly lower than that of the nonparasitized. The reduction of the PTP activity was observed in cytosolic fraction, but not in membrane fraction. The hemocytes of parasitized *P. xylostella* markedly lost their spreading ability in response to a cytokine (PSP1: plasmatocyte-spreading peptide 1). The functional link between the reduced PTP activity and the suppressed hemocytic behavior was evidenced by the inhibitory effect of sodium orthovanadate (a specific PTP inhibitor) on hemocyte-spreading behavior of nonparasitized *P. xylostella*. These results suggest that CpBV-PTPs are expressed in the parasitized *P. xylostella* and affect cellular PTP activity, which may be associated with host immunosuppression.
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1. Introduction

For successful parasitization, endoparasitoid wasps are able to attenuate host immune capacity and manipulate host developmental physiology [1]. Some ichneumonoidea species possess polydnavirus

Abbreviations: CpBV, *Cotesia plutellae* bracovirus; PSP, Plasmatocyte-spreading peptide; PTP, Protein tyrosine phosphatase; RT-PCR, Reverse transcriptase-polymerase chain reaction

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(PDV) to play significant roles in such host physiological alterations [2]. PDV, located on the host chromosome, is vertically transmitted with host generation [3]. More than 30,000 species of parasitoid wasps are thought to carry the PDV, although only 50 species have been described systematically [4]. These PDVs are divided into ichnovirus (IV) and bracovirus (BV) depending on host insect family and viral morphology [5].

Cotesia plutellae is a solitary endoparasitoid wasp that parasitizes diamondback moth, *Plutella xylostella* [6]. Parasitized *P. xylostella* larvae exhibit immunosuppression with a prolonged larval period without further metamorphosis [7]. *C. plutellae* bracovirus (CpBV) has been identified and known to be replicated in the ovarian calyx during late pupal stage [8]. CpBV has been suspected as a major factor to reduce host cellular immune capacity in the parasitized *P. xylostella* [9,10].

On-going full genomic sequence study on CpBV has provided several PDV gene families including protein tyrosine phosphatase (PTP), EP1-like [11], and vankyrin [12]. PTP plays critical role in the control of many cellular events, including cell proliferation, differentiation, and metabolism by controlling phosphorylation state of key enzymes with its antagonistic enzyme, protein tyrosine kinase [13]. Various PTPs include classical type, dual-specific type, and low molecular weight type, in which classical PTPs can be subgrouped into cytoplasmic or receptor PTPs and they are characterized by the presence of a domain consisting of 10 conserved motifs [14]. Interestingly, the known BV genomes in *C. congregata* and *Toxoneuron nigriceps* contain 27 and 13 PTP genes, respectively [15]. Based on quite large number of cellular PTPs as in case of *Drosophila* genome that has approximately 38 PTPs [16], the viral PTPs have been speculated that they can interrupt host cellular signals in order to manipulate host physiology for the wasp and PDV development.

In the present study, we have characterized 14 PTPs encoded in the genome of CpBV and analyzed their expression patterns during parasitization period with their possible function as host PTP inhibitor to suppress host immune response.

2. Materials and methods

2.1. Insect rearing

P. xylostella larvae were fed cabbage leaves and reared at $25.0 \pm 1.5\,^{\circ}C$. Late second instar larvae (4 days after oviposition) were parasitized with *C. plutellae* in roughly 1:5 (host: wasp) ratio for 12 h under the rearing condition. The parasitized larvae were reared with the same method until egression of wasp larvae and the resulting cocoons were kept in separate cage until adult emergence, which were used for either extraction of viral DNA or maintaining colony. Adult wasps were fed 40% sucrose solution.

2.2. Plasmatocyte-spreading bioassay

PSP1 (plasmatocyte-spreading peptide 1) was kindly donated by Dr. Kevin Clark from University of Georgia, USA. Ten times high concentration of the synthesized PSP1 was dissolved in 1 × Pringle saline (1.54 M NaCl, 2.6 mM KCl, 1.3 mM $CaCl_2$, and 116 mM dextrose). Hemolymph was prepared in the anticoagulant (98 mM NaOH, 186 mM NaCl, 17 mM Na_2EDTA, and 41 mM citric acid, pH 4.5) and incubated on ice for 40 min. After the hemolymph was centrifuged at 200*g* for 2 min, the resulting hemocyte pellet was resuspended with 70% Ex-Cell 400 medium (JRH Biosciences, Lenexa, KS) in the anticoagulant buffer. Bioassay was performed in 96-well culture plates (Corning, NY, USA), in which 60 μl of reaction mixture was kept in each well. Hemocytes (1.2×10^4 cells in 54 μl) were seeded in each 96-well plate, to which 10^{-6} M of PSP1 (6 μl) was added and incubated for 45 min at 25 °C in dark condition. The spread plasmatocytes were counted under phase contrast microscope (Olympus BX41, Tokyo, Japan). Spread plasmatocytes were identified by the morphological character showing a bilateral cytoplasmic expansion over 20 μm. The percentage of plasmatocyte spreading was scored by counting 100 plasmatocytes from a randomly selected field of view. Each treatment was independently replicated three times.

For PTP inhibitor assay, sodium orthovanadate (Sigma, MO, USA) was used to test its inhibitory potency on hemocyte spreading with seven different doses (10^{-10}–10^{-4} M). PTP enzyme activity of the hemocytes treated with the inhibitor was measured by the method described below.

2.3. Measurement of PTP enzyme activity

PTP activity was measured using tyrosine phosphatase assay system (Promega, WI, USA) according to the manufacturer's instructions. Briefly, enzyme samples were extracted from whole body,

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