Virology 481 (2015) 166–178

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Expression of the Cydia pomonella granulovirus matrix metalloprotease enhances Autographa californica multiple nucleopolyhedrovirus virulence and can partially substitute for viral cathepsin

Egide Ishimwe, Jeffrey J. Hodgson, A. Lorena Passarelli*

Division of Biology, Kansas State University, Manhattan, KS 66506, United States

ARTICLE INFO

Article history:
Received 16 January 2015
Returned to author for revisions
5 February 2015
Accepted 13 February 2015
Available online 17 March 2015

Keywords:
Baculovirus
Matrix metalloprotease
Chitinase
Cathepsin
Melanization
Liquefaction

ABSTRACT

The Cydia pomonella granulovirus open reading frame 46 (*CpGV-ORF46*) contains predicted domains found in matrix metalloproteases (MMPs), a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins. We showed that CpGV-MMP was active in vitro. Autographa californica multiple nucleopolyhedrovirus (AcMNPV) expressing *CpGV-ORF46* replicated similarly to a control virus lacking *CpGV-ORF46* in cultured cells. The effects of AcMNPV expressing CpGV-MMP on virus infection in cultured cells and *Trichoplusia ni* larvae in the presence or absence of other viral degradative enzymes, cathepsin and chitinase, were evaluated. In the absence of cathepsin and chitinase or cathepsin alone, larval time of death was significantly delayed. This delay was compensated by the expression of CpGV-MMP. CpGV-MMP was also able to promote larvae melanization in the absence of cathepsin and chitinase. In addition, CpGV-MMP partially substituted for cathepsin in larvae liquefaction when chitinase, which is usually retained in the endoplasmic reticulum, was engineered to be secreted.

Introduction

Matrix metalloproteases (MMPs) are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins. They are characterized by the presence of a conserved zinc-binding motif (HEXGHXXGXXHS/T) within the catalytic domain. In addition, most MMPs have an N-terminal propeptide domain with a cysteine residue that maintains MMPs latency as it interacts with the zinc ion in the catalytic domain. Finally, a C-terminal hemopexin-like domain contributes to substrate binding, substrate recognition and binding to tissue inhibitors of MMPs (TIMPs) (Murphy et al., 1992; Van Wart and Birkedal-Hansen, 1990). MMPs are found in a wide range of organisms from invertebrates to vertebrates and plants, and they play a crucial role in extracellular matrix remodeling during processes such as embryonic development, angiogenesis, wound healing and metamorphosis (Nagase and Woessner, 1999; Page-McCaw et al., 2007). However, MMPs have also been implicated in a number of pathological processes, including cancer, neurological diseases, arthritis, bacterial and viral infections, and tumor invasion (Elkington et al., 2005; Frantz et al., 2010).

Baculoviruses are arthropod-specific viruses which primarily infect lepidopteran larvae. They contain relatively large circular double-stranded DNA genomes (80–180 kbp) which are packaged into rod-shaped capsids and replicate in the nucleus of cells (Rohrmann, 2013). Baculoviruses have been classified into four genera based on phylogeny: *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus* (Jehle et al., 2006). Baculoviruses produce two different types of virions during their replication cycle, budded virions (BV) and occlusion-derived virions (ODV). BV allow cell-to-cell virus transmission; they are exported into the cytoplasm and bud from the cell plasma membrane from which they acquire their envelope. ODV are responsible for host-to-host virus transmission. They are produced in the final phases of virus replication, where nucleocapsids accumulate in the nucleus and acquire envelopes from the host cell nuclear membrane. ODV are then packaged in a protein matrix to form occlusion bodies. Occlusion bodies are released by lysis of infected cells and spread into the environment after disintegration and liquefaction of the host (O'Reilly et al., 1994). Larval disintegration and liquefaction require viral encoded enzymes.

Pathogenesis from members within *Alphabaculovirus* and *Betabaculovirus* may differ, although fewer studies have described betabaculovirus pathogenesis. Briefly, alphabaculoviruses and betabaculoviruses infect the midgut epithelium of insect hosts. In alphabaculoviruses, virions reach tissues in the insect hemocoel producing more virions. The product of the very late gene P10 is responsible for nuclear lysis (van Oers et al., 1993), releasing ODV in the environment after the insect cadaver liquefies. In betabaculoviruses, the nucleus of infected

* Corresponding author. Fax: +1 785 532 6653.
E-mail address: lpassar@ksu.edu (A.L. Passarelli).

http://dx.doi.org/10.1016/j.virol.2015.02.022
0042-6822/

cells enlarges, followed by nuclear membrane breakage. The genes and mechanisms affecting this early nuclear membrane rupture are not known. Nucleocapsids are then enveloped and occluded in this nucleocytoplasmic compartment. Betabaculovirus pathogenesis differs in tissue tropism, from viruses being midgut restricted, to infecting midgut epithelium and fat body tissue, to infecting several tissues of the host. In addition, dispersal of ODV may differ from dispersal in diarrheal secretions to dispersal following complete insect liquefaction (reviewed in Federici, 1997).

Degradative enzymes have been reported in baculoviruses, including viral-chitinase (v-chitinase) which digests chitin, the main component of the insect exoskeleton; and viral-cathepsin (v-cathepsin), which is involved in the degradation of internal larval tissues (Ohkawa et al., 1994; Slack et al., 1995). The concerted activity of these two enzymes enables host liquefaction which allows virus release from the infected cadaver and dissemination to other hosts (Hawtin et al., 1997; Kang et al., 1998a). The betabaculovirus Cydia pomonella granulovirus (CpGV) v-cathepsin was shown to be a functional protease and necessary for larval melanization and liquefaction or softening of larval cadavers, depending on the virus background in which it was tested (Hilton et al., 2008; Kang et al., 1998b). Similarly, the CpGV v-chitinase expressed from a Bombyx mori NPV (BmNPV) recombinant virus allowed larval liquefaction and interacted with BmNPV v-cathepsin (Daimon et al., 2007).

The role of another degradative enzyme, the viral MMP, found in betabaculoviruses, has not been studied extensively. To date, there is only one functional study on baculovirus MMPs, characterizing the Xestia c-nigrum granulovirus (XcGV) MMP (XcGV-MMP). XcGV-MMP is a functional MMP involved in larval melanization and thought to have a role in degradation of host basement membranes during the late stages of infection (Ko et al., 2000). Open reading 46 (ORF46) encoded in the CpGV genome predicts a protein, CpGV-MMP, which shows significant similarity to MMPs (Luque et al., 2001). However, its role in viral pathogenesis has not been described.

In this study, we characterized the CpGV-MMP in silico, in vitro, and in vivo to determine its function independently and in conjunction with the viral degradative enzymes, v-cathepsin and v-chitinase. To this end, we analyzed the phylogenetic relationships between CpGV-MMP and other baculovirus MMP homologs and tested CpGV-MMP enzymatic activity in vitro. We also determined the effects of Autographa californica M nucleopolyhedrovirus (AcMNPV) expressing CpGV-MMP on cultured cells and insects in the presence or absence of v-chitinase and v-cathepsin and their interactive roles in insect mortality, liquefaction, and melanization.

Results

MMPs in betabaculoviruses

We screened for putative viral MMPs in the GenBank® protein database. Results revealed the presence of putative *mmps* in the virus families *Baculoviridae*, *Ascoviridae*, *Iridoviridae*, *Hytrosaviridae*, *Poxviridae* (only in the subfamily *Entomopoxvirinae*) and *Nudiviridae*. Viruses within these (sub)families contain diverse large double-stranded DNA viruses that infect invertebrates. In baculoviruses, MMP homologs are present in all species from the genus *Betabaculovirus* (granuloviruses) sequenced to date but absent in species from *Alphabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus*. Baculovirus MMPs cluster more closely with insect MMP-3 than with insect MMP-1 or MMP-2 (Fig. 1A). Insect MMPs are classified into these three major groups (MMP-1, MMP-2 and MMP-3), based on phylogenetic analyses.

All previously identified cellular MMPs, except the human MMP-23 which contains a membrane anchor sequence and is membrane-associated (Ohnishi et al., 2001), contain an N-terminal signal sequence and are secreted. Signal peptide prediction programs uncovered a putative signal peptide in all baculovirus MMPs except in XcGV, Pieris rapae GV and Epinotia aporema GV, which do not contain signal sequences or N-terminal anchor sequences. Thus, the secretion patterns of viral MMPs may differ and may be correlated to their function.

Similar to cellular MMPs characterized to date, baculovirus MMPs have a conserved zinc-binding motif (HEXGHXXGXXHS/T) within the predicted catalytic domain (Fig. 1B). The three underlined histidine residues act as ligands of a zinc ion (Zn^{2+}) cofactor necessary for catalysis. The baculovirus MMP catalytic domain also predicts a conserved methionine-turn (Met-turn) region, where a methionine residue is located seven residues C-terminal to the zinc-binding motif. The Met-turn is thought to enable correct folding of the MMP catalytic domain (Bode et al., 1993).

Although baculovirus MMPs have the conserved catalytic domain, comparison of the predicted baculovirus and cellular MMP sequences revealed that baculovirus MMPs lack other hallmark MMP structural features. First, baculovirus MMPs do not have a cysteine sequence (PRCGV/NPD) necessary for the cysteine switch mechanism. This sequence is conserved within the N-terminal propeptide domain of most cellular MMPs and is involved in maintaining MMP latency. Interaction between the cysteine residue in the conserved cysteine switch motif and the Zn^{2+} in the catalytic domain renders the active-site cleft unavailable for substrate binding; thus, keeping the enzyme inactive. Disruption of the Cys–Zn^{2+} complex (e.g., proteolytic cleavage of prodomain, conformational perturbation, etc.) allows activation of latent MMPs (Van Wart and Birkedal-Hansen, 1990). In addition, baculovirus MMPs lack a hemopexin-like domain at their C terminus. This domain which mediates substrate recognition, specificity, and binding to TIMPs (Murphy et al., 1992) is present at the C-terminal region of most cellular MMPs except human MMP-23, -7 and -26 and some plant MMPs (e.g., *Arabidopsis thaliana*; Maidment et al., 1999). The region downstream of the catalytic domain is short or almost non-existent in some cellular MMPs (e.g., MMP-23, -7, and -26) (Velasco et al., 1999; de Coignac et al., 2000). Baculovirus MMPs, as most cellular MMPs, have longer C-terminal sequences downstream of their catalytic domain; however, no functional domain could be identified in that region using the Simple Modular Architecture Research Tool (SMART) online program (Letunic et al., 2012). Overall, these analyses indicate that even though baculovirus-encoded MMPs lack some conserved cellular MMP domains, they still preserve homology to the MMP family of proteins to be classified as MMPs.

Enzymatic activity of CpGV-MMP

CpGV-MMP is 545 amino acids long with a predicted molecular mass of 65 kDa. The predicted sequence contains an 18-amino acid long signal peptide at its N terminus and a 178-amino acid long (amino acids 125–303) MMP conserved catalytic domain.

To determine if CpGV-MMP is a functional MMP, *CpGV-ORF46* was cloned into the pET-32a bacterial expression vector and fused in frame to a polyhistidine tag at the C terminus. A CpGV-MMP mutant, CpGV-MMP-Mut, in which the conserved glutamic acid in the zinc binding motif was changed to glutamine was also constructed as a control. The glutamic acid residue is thought to be necessary for catalytic activity by enabling nucleophilic attack of a zinc-bound water molecule that cleaves peptide bonds (Dhanaraj et al., 1996). The recombinant proteins were expressed in *Escherichia coli*, purified using metal-affinity columns, and detected following immunoblotting using an anti-His antibody (Fig. 2A and data not shown). Imidazole elution (50 mM) fractions were used for MMP activity assays using a synthetic quenched fluorescently-labeled generic MMP substrate (5-FAM/QXL™520). Upon cleavage, the substrate emits fluorescence that