Journal of Invertebrate Pathology 134 (2016) 15–22

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Budded baculovirus particle structure revisited

Qiushi Wang [a,b], Berend-Jan Bosch [b], Just M. Vlak [a], Monique M. van Oers [a], Peter J. Rottier [b], Jan W.M. van Lent [a,*]

[a] Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands
[b] Virology Division, Department of Infectious Disease and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 16 July 2015
Revised 24 November 2015
Accepted 1 December 2015
Available online 29 December 2015

Keywords:
Baculovirus
Budded virus
Ultrastructure
Cryo-EM
Spike structure

ABSTRACT

Baculoviruses are a group of enveloped, double-stranded DNA insect viruses with budded (BV) and occlusion-derived (ODV) virions produced during their infection cycle. BVs are commonly described as rod shaped particles with a high apical density of protein extensions (spikes) on the lipid envelope surface. However, due to the fragility of BVs the conventional purification and electron microscopy (EM) staining methods considerably distort the native viral structure. Here, we use cryo-EM analysis to reveal the near-native morphology of two intensively studied baculoviruses, *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) and *Spodoptera exigua* MNPV (SeMNPV), as models for BVs carrying GP64 and F as envelope fusion protein on the surface. The now well-preserved AcMNPV and SeMNPV BV particles have a remarkable elongated, ovoid shape leaving a large, lateral space between nucleocapsid (NC) and envelope. Consistent with previous findings the NC has a distinctive cap and base structure interacting tightly with the envelope. This tight interaction may explain the partial retaining of the envelope on both ends of the NC and the disappearance of the remainder of the BV envelope in the negative-staining EM images. Cryo-EM also reveals that the viral envelope contains two layers with a total thickness of ≈6–7 nm, which is significantly thicker than a usual biological membrane (<4 nm) as measured by X-ray scanning. Most spikes are densely clustered at the two apical ends of the virion although some envelope proteins are also found more sparsely on the lateral regions. The spikes on the surface of AcMNPV BVs appear distinctly different from those of SeMNPV. Based on our observations we propose a new near-native structural model of baculovirus BVs.

1. Introduction

Baculoviruses are a family of enveloped, large double-stranded DNA viruses that predominantly infect insects. The genome of baculoviruses ranges from 80 to 180 kb in size and contains between 90 and 180 genes (Herniou et al., 2012). The viral genome is packed into rod-shaped nucleocapsids (NC) of 30–70 nm in diameter and 200–400 nm in length (Boucias and Pendland, 1998; Jehle et al., 2006). Two phenotypes of infectious enveloped virions are produced during the infection cycle: the occlusion-derived viruses (ODV) that initiate infection of the midgut of the host upon oral ingestion of occlusion bodies (OBs) and budded viruses (BV) that are responsible for cell-to-cell spread and further systemic infection. ODVs obtain their *de novo* assembled envelopes in the nucleus, while BVs acquire their envelope from the plasma membrane upon budding into the extracellular space or neighboring cells (Okano et al., 2006). Although the two phenotypes contain identical viral genomes and nucleocapsids, they differ in the lipid and protein composition and structure of their envelopes (Slack and Arif, 2007). The ODV envelope is more rigid than the BV envelope due to the presence of more saturated fatty acids (Braunagel and Summers, 1994; Slack and Arif, 2007). Several envelope-associated proteins of BVs (GP64, F), but no envelope-associated proteins of ODVs were found to be *N*-glycosylated (Hou et al., 2013). Unlike BVs, ODVs have tegument proteins, which package nucleocapsids and create a matrix layer between the nucleocapsid and the envelope (Slack and Arif, 2007). One or multiple ODV virions are further embedded into a proteinaceous OB. Due to the highly compact structure and OB protection the intact ODVs are more easily isolated in their native form than BVs.

The *Baculoviridae* family is divided into four genera: *Alphabaculovirus* (encompassing viruses with lepidopteran hosts), *Betabaculovirus* (lepidopteran hosts), *Gammabaculovirus* (hymenopteran hosts) and *Deltabaculovirus* (dipteran hosts) on the basis of the genome phylogeny (Herniou et al., 2012). The alphabaculoviruses are

* Corresponding author.
E-mail address: jan.vanlent@wur.nl (J.W.M. van Lent).

http://dx.doi.org/10.1016/j.jip.2015.12.001
0022-2011/

divided into two subgroups, group I and group II according to the sequence and phylogenetic analysis of conserved genes (Zanotto et al., 1993; Jehle et al., 2006). The subdivision between these two groups is correlated with employment of two different BV envelope glycoproteins, i.e. GP64 (group I) and F (group II), for virus-cell fusion and receptor binding (Lung et al., 2002). These two envelope glycoprotein types are highly distinct in sequence and structural features (Kadlec et al., 2008; Westenberg, 2004). In group I alphabaculoviruses both GP64 and F-like proteins are present; only GP64 mediates membrane fusion and the F-like protein appears to have lost its fusion function (Jehle et al., 2006; Lung et al., 2003; Wang et al., 2008). In members of group II alphabaculoviruses, the betabaculoviruses and the deltabaculoviruses the major envelope protein is the F protein, which mediates membrane fusion (Jehle et al., 2006; Lung et al., 2002).

The baculovirus F proteins differ from GP64 proteins not only in amino acid sequence, but also in their biochemical and structural properties, except that both proteins are activated at acidic pH (Blissard and Wenz, 1992; IJkel et al., 2000). F proteins require proteolytic cleavage to prime the membrane fusion (IJkel et al., 2000). Based on the structural elements and organization, F protein and GP64 proteins were characterized as class I and class III viral fusion proteins, respectively (Bosch et al., 2003; Kadlec et al., 2008). Both classes of fusion proteins are composed of trimers with their ectodomains oriented perpendicular to the viral membrane. Two trimers assemble into spikes on the surface of BV envelope. The fusogenic forms of class I and class III viral fusion proteins are of mainly α-helical structure or a mixture of α-helices and β-sheets, respectively [reviewed in (White et al., 2008)].

Previously, the baculovirus BV ultrastructure was studied using negative-staining electron microscopy EM (Adams et al., 1977; Beaton and Filshie, 1976; Fraser, 1986; Harrap, 1972). The BV envelopes of several group I and II alphabaculoviruses in baculovirus-infected cells and tissue of hosts were reported bulbous at one end. The surface of the envelope is serrated with notches, which are concentrated at the bulbous end (Adams et al., 1977). These BV notches, called spikes, on the surface were demonstrated to be composed of envelope proteins including GP64 and F protein (Volkman et al., 1984; Pearson et al., 2001).

BV and ODV structures have been studied by negative-staining EM (Adams et al., 1977; Beaton and Filshie, 1976; Fraser, 1986; Harrap, 1972). However, due to the fragility and flexibility of BV particles, the procedure of negative-staining and subsequent drying severely impairs the structural integrity of the viral envelope and causes virions to collapse. Intact BV particles are therefore rare and usually have lost part of their envelope. In the current study we have used cryo-EM of vitrified BV suspensions to re-visit the morphology of virions of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV), as representatives of group I (GP64) and group II (F), alphabaculoviruses to provide new insights on the ultrastructural organization and assembly of BV virions.

2. Materials and methods

2.1. Virus production and purification of budded baculovirus

Hemolymph-derived SeMNPV BVs and cell culture-derived AcMNPV BVs were obtained from Els Roode (Wageningen University) and subsequently used for virus production in cell culture. The titers of the SeMNPV and AcMNPV BV suspensions were first determined on Se301 and Sf9 cells, respectively, using end point dilution assays. Se301 cells were then infected with SeMNPV BVs at an MOI of 5 tissue culture infection dose 50% ($TCID_{50}$) units per cell in HyClone CCM3 insect culture medium (Thermo Scientific). Sf9 cells were infected with AcMNPV BVs at an MOI of 5 $TCID_{50}$ units per cell in Insect-XPRESS insect culture medium with L-glutamine (Lonza). At 48 h post-infection (p.i.) cell culture supernatants, where BVs were expected to be present, were clarified at 3000*g* for 15 min at 4 °C to remove cells and cellular debris. This supernatant was directly used for cryo-EM.

2.2. Fractionation of NCs from budded baculoviruses

The clarified cell culture supernatant from the procedure above was incubated with 1% Nonidet P-40 (NP-40) in TE buffer (10 mM Tris–HCl at pH 7.4, 137 mM NaCl, 2 mM EDTA) for 30 min at room temperature to remove the viral envelope. Six ml of NP-40 treated BV suspension were layered onto 3 ml of 25% and 50% iodixanol solution in TE buffer and centrifuged at 16,000*g* for 3 h at 4 °C in SW41Ti rotor (Beckman Coulter). The fractions were collected every 3 ml from the top to bottom. The NCs were most abundant in the third fraction from the top of the centrifuged tube upon examination with negative-staining EM as described below. The NC fraction was diluted in 30 ml phosphate-buffered saline (PBS) at pH7.4 (Lonza) and centrifuged at 100,000*g* for 1 h at 4 °C in an SW32Ti rotor (Beckman Coulter). The pellet was suspended in PBS.

2.3. Electron microscopy of budded baculovirus

For negative-staining EM, formvar/carbon coated 400-mesh copper grids were exposed to a glow discharge in air for 20 s. Ten μl of a BV or NC suspension was placed on the grids and incubated for 2 min. Negative-staining was performed with 1% phosphotungstic acid (PTA, pH 7.2) for 1 min. The specimens were examined in a JEOL 1011 transmission EM equipped with an Olympus Keenview (1K × 1K) and Veleta (2K × 2K) digital camera.

For cryo-EM, specimens were vitrified using a Vitrobot (FEI Company). Four μl of a suspension containing virions or NCs was placed on a quantifoil carbon or lacey carbon grid and allowed to absorb for 30 s. After blotting the grid was plunged into liquid ethane. The frozen specimen was examined at −180 °C in a JEOL 2100 TEM equipped with a Gatan CT3500 cryo-holder and a Gatan US4000 (4K × 4K) digital camera. Images were recorded at low dose with DigitalMicrograph software (Gatan) and analyzed with iTEM platform (Olympus).

3. Results

3.1. General architecture of baculovirus BVs

To study the near-native morphology of AcMNPV and SeMNPV BVs, virions were produced in Sf9 and Se301 cells respectively for 48 h, isolated from the supernatant and subjected to cryo-fixed by plunge freezing. Fig. 1 shows images of these BV particles, which exhibit remarkably different features as compared to the current knowledge on BV morphology. The majority (≈95%) of virions of both AcMNPV and SeMNPV with intact envelope show an extended 'ovoid' shape (Fig. 1A–C) instead of a 'rod' shape as described previously (Adams et al., 1977). The 'pocket' between NC and lateral envelope appears to be electron-lucent, yet not empty because the electron density is darker than the ambient surrounding the BV particles. Small vesicles are apparently included in the lateral space of some virions (Fig. 1E), indicating that the pockets might be filled with soluble content. The interactions between NC and envelope appeared to be limited to the two ends of NC and therefore the virions tend to have heterogeneous shapes around the lateral area even with cryo-fixation (Fig. 1D). The virions marked with an arrowhead in Fig. 1D appear to get compressed at the inner lateral space, which generated high tension at one end of NC. As a