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Structural features of the salivary gland hypertrophy virus of the tsetse fly revealed by cryo-electron microscopy and tomography

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ABSTRACT

Glossina palipides salivary gland hypertrophy virus (GpSGHV) infects tsetse flies, which are vectors for African trypanosomosis. This virus represents a major challenge in insect mass rearing and has hampered the implementation of the sterile insect technique programs in the Member States of the International Atomic Energy Agency. GpSGHV virions consist of long rod-shaped particles over 9000 Å in length, but little is known about their detailed structural organization. We show by cryo electron microscopy and cryo electron tomography that the GpSGHV virion has a unique, non-icosahedral helical structure. Its envelope exhibits regularly spaced spikes that protrude from the lipid bilayer and are arranged on a four-start helix. This study provides a detailed insight into the 3D architecture of GpSGHV, which will help to understand the viral life cycle and possibly allow the design of antiviral strategies in the context of tsetse fly infections.

1. Introduction

The *Glossina pallipides* salivary gland hypertrophy virus (GpSGHV) of the tsetse fly is a long, rod-shaped virus containing a circular dsDNA. It belongs to the recently described Hytrosaviridae family (Abd-Alla et al., 2009). Infection of the tsetse fly results in accumulation of the virus in the salivary gland causing the characteristic salivary gland hypertrophy (SGH) syndrome. The virus also infects the ovarioles and gonadal tissues. Infected flies may be symptom-less but in many instances infection shortens lifespan, reduces fertility or entails fly sterility (Abd-Alla et al., 2010). The sustainable control of tsetse flies, the sole cyclic vectors of the African trypanosomosis (also known as sleeping sickness in human or nagana in animals), relies on the use of the area–wide integrated pest management approach including the sterile insect technique (SIT). SIT involving the large scale production and release of sterile males in target areas has already demonstrated some value (Vreysen et al., 2000). However, contamination of tsetse colonies with GpSGHV is a common cause hampering the mass production even though recent progress using a clean feeding technique and an antiviral drug have proved promising to control this virus infection (Abd-Alla et al., 2014).

GpSGHV particles have been visualized in the past by negative stain electron microscopy (Jaenson, 1978) and more recently using cryo electron microscopy (cryo-EM) (Kariithi et al., 2013). Virions appear as long rigid rod-shaped particles (about 1000 Å in width and 10000 Å in length) consisting of a nuclecapsid surrounded by a tegument layer and an outer lipid envelope. They have been shown to encapsidate a circular double-stranded 190 kbp DNA genome that comprises 160 non-overlapping open reading frames (Abd-Alla et al., 2008, 2016; Odindo et al., 1986). The DNA sequence clearly distinguishes GpSGHV from other insect virus families and groups it together with another salivary gland hypertrophy virus of the domestic housefly *Musca domestica* (MdSGHV) into the Hytrosaviridae family (Abd-Alla et al., 2009; Garcia-Maruniak et al., 2009). Proteomic analysis of viral particles has identified 61

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structural proteins suggesting a complex organization with two major immuno-dominant proteins of 50 kDa and 130 kDa respectively (Kariithi et al., 2010). Unfortunately, despite numerous attempts, so far no suitable cell culture system allowing the in vitro multiplication of GpSGHV is available. Therefore, studies of the virus and its infectious cycle rely on examination of hypertrophied salivary glands. GpSGHV capsids assemble in the nucleus and acquire a lipid envelope probably after moving through nuclear pores and budding through endoplasmic / Golgi membranes (Kariithi et al., 2013). To gain a more detailed understanding of the ultrastructural features of the GpSGHV, we have examined virus particles isolated from hypertrophied salivary glands by cryo-EM and cryo-electron tomography (cryo-ET) under conditions where the particles are preserved in a frozen-hydrated state. This has allowed us to more precisely define the physical parameters of the virus structure and detect some previously unrecognized features such as the asymmetry in the virus particle, the four-start helical organization of the external spike layer and the helical organization of the inner capsid. This study should provide a first basis for the ultimate goal of establishing a detailed structural model of this unique virus.

2. Results

Examination by negative staining electron microscopy of GpSGHV particles extracted from freshly homogenized hypertrophied salivary glands revealed rod-like structures that were often twisted or crooked, probably due to drying after adsorption on the carbon film of the grid and negative stain processing of the samples (Fig. 1**A & B**). Although the fine details of the virions were poorly visible, in a number of instances, the helical organization of the virus surface could be visualized. The negative staining technique of intact particles usually does not provide access to the virus internal structure, however, when broken, the nucleocapsid outer and inner layers were apparent (Fig. 1**C**). In addition, it is worth noting that the particles display a marked asymmetry, one end being rounded and the other appearing conical (Fig. 1 and Supplementary Fig. 1). Examination of the same preparations using cryo-EM which preserves the hydrated state (Dubochet et al., 1988) enabled the observation of intact, native GpSGHV particles (Fig. 1**D**). The rod-shaped particles were consistently rigid and measured about 980 Å in width and 9230 Å in length. The polarity of the particles was also clearly apparent and additional fine sub-structure on either end could be observed.

To address the 3D structural organization of the virus in more detail we performed cryo-ET (Fig. 2). The virus shows a coating layer, a membrane region, a tegument and an inner nucleocapsid (see annotations in Fig. 2**G**). The outer section displays uneven density along its long axis due to the presence of regularly spaced spikes extending from the viral envelope (Fig. 2**A**). The spikes appeared to exhibit a two-fold symmetry suggesting that they may be assembled as dimers (Fig. 2**G & I**). The spikes are arranged in a left-handed helical manner, following the helix orientation from the R to the C tips of the viral rod (left and right end, respectively, in Fig. 1 **&** Fig. 2**G/H**). Taking into account the compaction of the spikes in rows and the rising angle ($\approx 20^{\circ}$) that the rows adopt with respect to the long axis of the particles, the helix necessarily derives from an assembly of more than one strand, as estimated from tomograms to be 4 strands for the outer envelope (Fig. 2). This is consistent with the fact that the same row appears 4 rows later after a full helical turn (Fig. 2**C & D**). The outer section as well as the middle section (Fig. 2) allowed the precise determination of the number of helix turns (48 if considered as a one start helix and 12 for a four start helix) as well as the number of spikes per turn (23). Thus, the entire

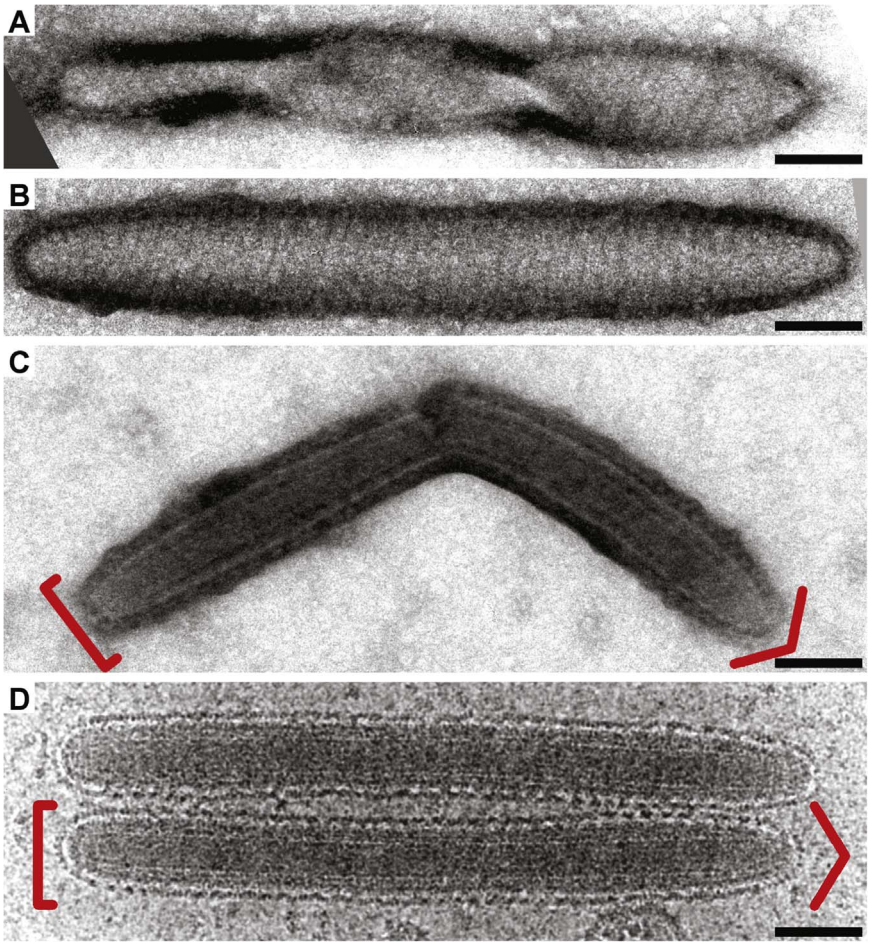

Fig. 1. Negative stain and cryo imaging of SGHV particles. **A** Negative stain image of the SGHV particle showing extensive deformation. **B** Negative stain image of the SGHV particle showing the turn of the coating protein. **C** Negative stain image of a broken SGHV particle showing the internal structure; labels in red show the R (round, left) and C (conical, right) ends of the virus. **D** Cryo-EM image showing intact SGHV particle. The polarity of the SGHV particles with two distinctively different ends is visible including a fine sub-structure of the coating layer. All scale bars are 1000 Å.