Acta virol. **33** : 559—564, 1989

THE PROCESS OF BACULOVIRUS DEPROTEINIZATION

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Received August 1, 1988

Summary. — The localization and function of alkaline protease inside the supervirion capsids (granules, inclusion bodies) of granulosis virus and nuclear polyhedrosis virus have been determined with the help of electron microscopy. It has been shown that deproteinization of baculoviruses proceeds in several steps. The first two stages (the release of virus from supervirion capsids and the release of nucleocapsids from the internal and external coats, were accomplished in the intestine of the insects, while the third stage (the release of nucleic molecules from the nucleocapsids) took place in the nucleoplasm of intestinal epithelial cells.

Key words: *granulosis viruses*; *deproteinization*; *supervirion capsid*; *alkaline protease*

Introduction

Baculoviruses are distinguished by virion inclusions in specific protein supervirion capsids (granules, polyhedra, inclusion bodies) that generally protect the infectious entity from unfavourable environmental factors. The protein matrix of granulosis virus (GV) supervirion capsids (SVC) enters the insect intestine by food. Viruses undergo deproteinization in the alkaline medihm *r*pH 10.5—11.0) of the insect intestinal juice (Bergold, 1947; Kawarabata, 1974; Summers and Volkman, 1976; Granados and Lower, 1981). It was believed up to the present that deproteinization of baculoviruses is caused by the intestinal juice after SVC have been completely dissolved. An alkaline protease has been detected inside GV SVC and nuclear polyhedrosis virus (NPV) superpolyvirion capsids (SPVC) (Yamafiji *et al.*, 1960; Kozlov *et al.*, 1980). The role of the protease has remained unknown. Our aim has been to study the deproteinization process of baculoviruses in the intestine of infected insects using high-voltage electron microscopy which can detect virions while they still remain in SVC.

Materials and Methods

GV was isolated from *Agrotis segetum* and *Hyphantria cunea* larvae. Prior to infection, the larvae were deprived of food of excrements. The larvae were inoculated with the virus suspension in a concentration of 2×10^8 which was administered into the insect intestine with the help of

Fig. 1.
Supervirion capsids (SVC) of granulosis virus (GV) in the high-voltage electron microscope EM-200. The external coat can be seen; magn × 40 000.

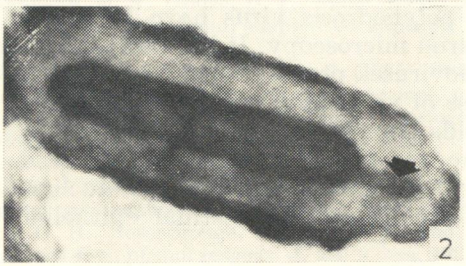

Fig. 2.
GV SVC with a channel connecting the virion with the external space; magn × 110 000.

a specially designed microtube. The viruses in the intestine were tested using a high-voltage electron microscope EM-200 at 150—200 kV after negative contrasting with 2% phosphotungstic acid solution (pH 7.2). The alkaline protease located inside the baculovirus SVC was inactivated by heating of virus suspension to 80 °C for 30—45 min.

Results

Negative contrast staining of GV performed before administration into the larval intestine showed electron dense oval-shaped elongated SVC with a clearly visible coat (Fig. 1). In some cases electron dense virions were observed. One minute after administration of the viral suspension inside the larval intestine about 50% of SVC were detected; the virions could be be well recognized inside them. The latter were encircled by an electron dense substance (Figs. 2 and 3). Occasionally the virions located in the SVC were encircled by round-shaped, electron dense layer about 7—8 nm thick arranged in a specific order (Figs. 3 and 4).

Three min after infection with GV 90% of virions formed SVC. An electron-dense channel 23—30 nm in diameter was seen running from the virions towards the SVC external coat (Fig. 2). In some cases electron dense channels were formed from both ends (or sides) of the virions. The diameter of the electron dense channels widened and the virions left the SPVC in the form of elongated strands (Figs. 4 and 6). In some cases the head-like end of SVC looked broken. After leaving the channel, the external and internal coats of the virion were destroyed (Fig. 5). In the case when the channels

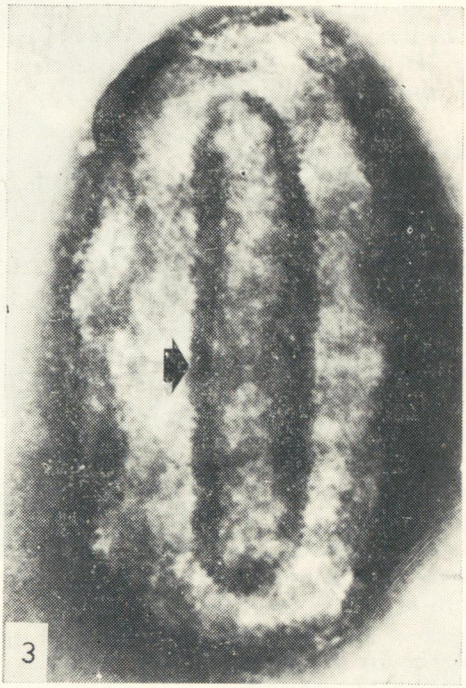

Fig. 3.
GV SVC after the treatment with intestinal juice of *Hyphantria cunea* larva. Inside the SVC protein matrix a virion encircled by electron dense matter is visible; magn × 55 000.

formed at both ends of the virions, SVC split into 2 parts and the virions were released into the external space (Fig. 4).

The viral suspension heated to 80 °C for 30—45 min (which leads to inactivation of alkaline protease) before infection of the insects revealed no electron-dense channels in the protein matrix of either GV SVC or NPV SPVC. In such cases the virions were released only after complete dissolution of the SVC protein matrix. This indicated that electron-dense channels through which the virions left the SVC were only formed in the presence of alkaline protease located in the protein matrix at the ends of the virion and very rarely in their middle.

Six, 12, and 24 min after the infection of larvae no intact GV SVC were detected in the intestine. Only virions with coats or without them were observed. The deproteinization process proceeded in Bergold mixture in the same fashion as in the intestine.

Discussion

Deproteinization of baculoviruses was studied by Bergold (1947). He was the first who showed that GV SVC and NPV SPVC contain virions that can be detected in ultrathin sections or only after their dissolution. He designed a special mixture for dissolution of SVC and SPVC which has been

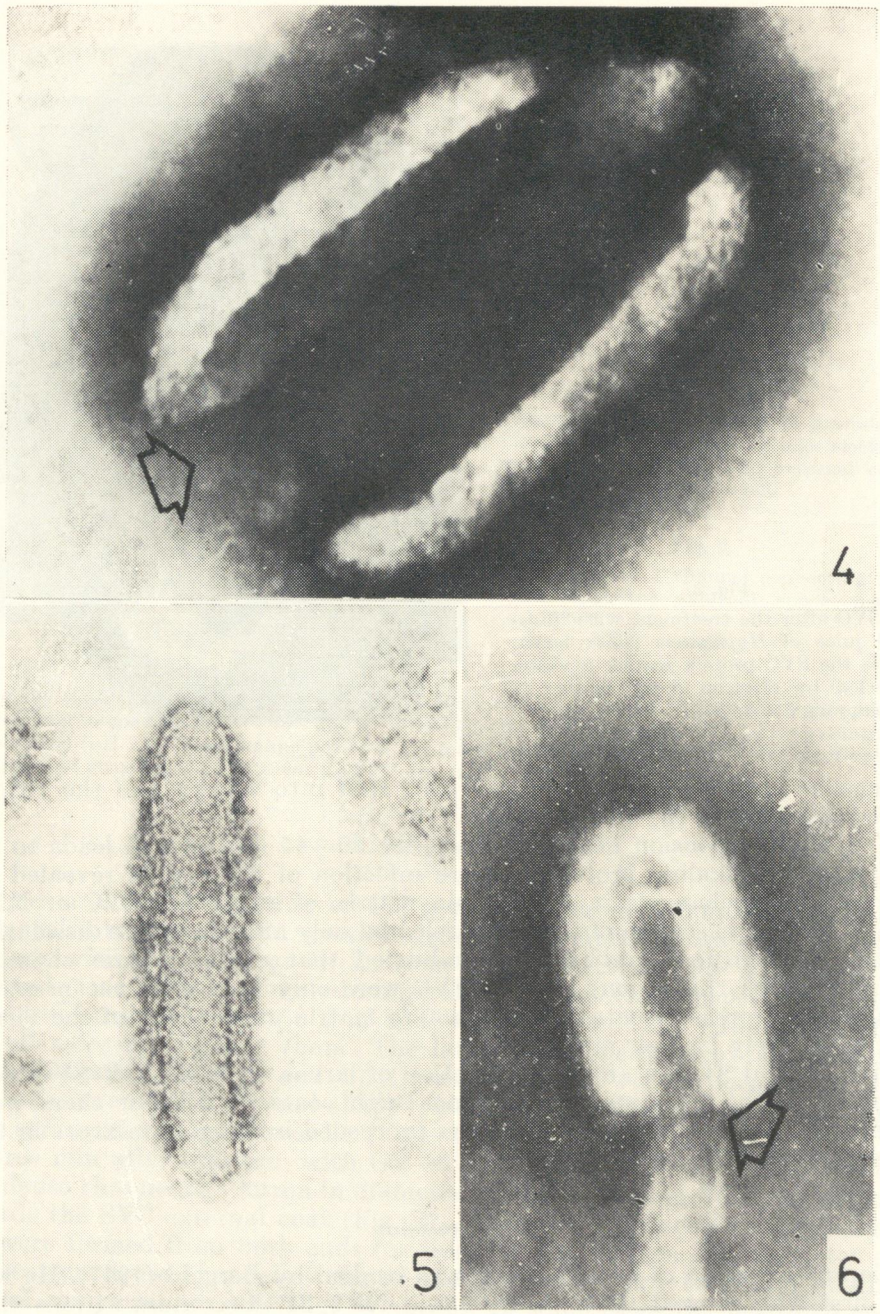

Fig. 4. GV SVC split into two parts thereby releasing the virion; magn × 95 000.
Fig. 5. Virion in the form of a strand leaving the SVC; magn × 65 000.
Fig. 6. A virion with coats released from SVC; magn × 100 000.

used up to present for destruction of baculovirus SVC. Anderson *et al.* (1981), Tweeten *et al.* (1977), Summers and Volkman (1976) described deproteinization of nucleocapsids in the alkaline solution of Na_2CO_3 that seemed similar to deproteinization of nucleocapsids inside intestinal epithelial cells.

Our findings (Chukhry, 1982; Chukhry and Unguryanu, 1986) as well as the results of others (Granados and Lower, 1981) indicate that virus deproteinization depends on the complexity of their structure. The more complex structure of viruses the more stages of deproteinization they undergo. Baculovirus structure resembles a "matreshka": a smaller capsid is enclosed in a larger one and together they provide a reliable protection of the infectious entity (the nucleic acid molecule) from unfavourable environmental factors. Although baculoviruses have a complex structure, when they enter the intestine of susceptible insects, their deproteinization proceeds quickly. The deproteinization rate largely governs the infectivity.

In this paper we found that baculovirus deproteinization is dependent on the activity of alkaline protease located in the SVC protein matrix at the virion ends. In the presence of its enzyme channels are formed along which the virions are released from SVC. The inactivation of alkaline protease by heating at 80 °C for 30—45 min slows down the deproteinization process (its rate becomes several times lower) and no channels are formed. The infection of the insects with baculoviruses is the most intensive during the first 2 min after the inoculation and after 8 min of treatment it decreases. The heating of the digestive fluid to 60 °C or higher leads to inactivation of the protease present in the digestive fluid, thereby changing the deproteinization process (Pritchett *et al.*, 1984). Faust and Adams (1966) have shown that baculovirus deproteinization is primarily dependent on the pH of the intestinal juice and is activated by the enzymes. In some cases the intestinal juice of the insects heated to 100 °C was still capable releasing the virions from SPVC (Estes and Faust, 1966). Gipson and Scott (1975) and also Wood (1980) suggested that GV SVC matrix was susceptible to the action of the protease. GV SVC and NPV SPVC were rapidly destroyed during storage of viral preparations (Chukhry *et al.*, 1984; Chukhry and Unguryanu, 1986).

Our data together with the results of other authors indicate that deproteinization of baculoviruses in the intestine of susceptible insects depends on the pH of the intestinal juice, the activity of the proteases present in the intestine and the activity of protease located inside of baculovirus protein matrix. The nucleocapsid released from SVC comes in contact with the microvili of the intestinal epithelial cells and penetrates into them. Further deproteinization of nucleocapsids proceeds within the intestinal epithelial cell nucleus as demonstrated by Granados and Lower (1981).

Thus, deproteinization of baculoviruses generally proceeds in several stages: release of virions from SVC; release of the nucleocapsid from the external and internal coats; release of the nucleic acid molecule from protein supercapsids. The first two stages are accomplished in the intestinal cavity while the third inside of the epithelial cells. The release of virions

from SVC occurs as follows: 1) the virions leave SVC in the form of a strand through the channel; 2) SVC split into 2 parts and virions are released; 3) SVC are completely dissolved.

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