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THE PROCESS OF VIRUS ASSEMBLY IN INSECT VIRUS MIXED INFECTIONS

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Summary. — Interference occurred upon infection of the cabbage moth caterpillars (*Mamestra brassicae* L.) with a mixture of nuclear polyhedrosis virus (NPV) and cytoplasmic polyhedrosis virus (CPV), resulting in the impairment of virus assembly, and formation of abnormal nucleocapsids. At the same time protein supercapsids were produced normally, but contained no infectious virions. When insects were infected with related viruses, the virions developed as usual, but the protein supercapsids revealed abnormal forms.

Key words: *nuclear polyhedrosis virus*; *cytoplasmic polyhedrosis virus*; *granulosis virus*; *mixed infection*

Introduction

Both in the nature and laboratory, mixed infections with two or more viruses may be found in the populations of pest insects. At interaction of infecting viruses, phenomena of synergism, of interference, or an independent development of both viruses may be observed.

In order to obtain high-quality virus preparations and to use them effectively against pest entomofauna, it is necessary to know when which kind of interaction occurs. Mixed infections in insects are most frequent with nuclear polyhedrosis virus (NPV) and granulosis virus (GV) and with NPV and cytoplasmic polyhedrosis virus (CPV). NPV and GV contain DNA, their nucleocapsids are rod-shaped; they affect the same tissues: fatty tissue, hypoderma, gonads, etc., but not the gut epithelial cells. CPV virions are spherical, contain RNA and affect exclusively the epithelial cells of the gut. NPV, GV, and CPV have a very complicated structure. They differ from other virus families in that their morphogenesis is not completed at the stage of virion formation but continues until their insert into protein superviriocapsids.

When insects are infected with NPV and GV, their independent replication (Injak, 1973) or synergism (Tarasevich *et al.*, 1979) are most frequently observed. In the latter instance recombinants may be generated (Burdenko *et al.*, 1979). In cases of interference the process of virus assembly is disturbed (Tchukhriy, 1978*a*); the resulting protein supercapsids (polyhedra,

inclusion bodies) contain no virions. Similar results were obtained in tissue culture cells (Quiot *et al.*, 1980). This work was aimed at the study of the process of virus assembly in mixed infections of insects with related (NPV and GV) and unrelated (NPV and CPV) viruses which is very important for manufacture of effective virus preparations.

The terminology used in this paper has been proposed and discussed at the Conferences on Insect Pathology (Tchukhriy, 1977, 1978*b*, 1982). "Superviriocapsid" is used insead of "granule", "polyviriocapsid" (PVC) instead of "inclusion bodies", "polyhedra" when many virions are enclosed in protein formations; "polyvirion" (polygenome virion) instead of "virion bundles" or "nucleocapsid bundles". I think that the proposed terms define more clearly the virus structure at different stages of morphogenesis and they cannot be confused with other structures of cellular or non-cellular origin.

Materials and Methods

Caterpillars of cabbage moth (*Mamestra brassicae* L.) in 2—3 instars were used for the study of mixed infections with NPV and CPV. The infection was carried out as follows: 1) monoinfection with NPV, 2) monoinfection with CPV, 3) simultaneous infection with NPV and CPV, 4) initial infection with NPV in 24 hr followed by CPV, 5) initial infection with CPV in 24 hr followed by NPV, 6) uninfected caterpillars of the same instars served as controls.

Caterpillars of the turnip moth of 2—3 instars were used for mixed infection with NPV and GV.

Virus administration. For each experimental variant, 40 caterpillars were used which were kept individually in glass jars at the temperature of 26 °C. Infection was performed by feeding with an artificial diet containing the virus(es) in given doses (in monoinfections: with NPV — 3×10^4, with CPV — 2×10^5, with GV — 5×10^3; half of these doses was used in mixed infections). The disease in caterpillars was diagnosed preliminarily according to external symptoms (in CPV — change in the colour of the gut, in NPV — changes in the consistence of the fatty body). In doubtful cases electron microscopic examination was performed. Protein supercapsids were dissolved in 0.25 mol/l sodium thioglycolate, pH 7.2, contrasted with 2% phosphotungstate acid solution, pH 7.2, for 2 min directly on formwar-coated electron microscopic grids. Simultaneously with the observation of mixed NPV and CPV infections in caterpillars, studies were performed in embryonal cell cultures of *Bombyx mori* of strain species CMBm 36.

For electron microscopic examinations, pieces of the fatty body and gut of the infected caterpillars as well as the sediments of infected cell cultures were fixed with 2% glutaraldehyde solution in cacodylate buffer, pH 7.2 and postfixed in 2% OsO_4 solution. Dehydration and further embedding into Epon were done by conventional method. Ultrathin sections cut in LKB-III ultramicrotome were contrasted with 2% uranyl acetate and lead citrate and examined in electron microscope EMV-100 LM.

Results

Biological assays showed that after infection of the insects with NPV and CPV mixtures in different combinations, the mortality rate was lower than in monoinfection experiments (Fig. 1). In NPV monoinfection, caterpillars began to die at 5 days post infection (p.i.). The total mortality was 95%. The virus was found in fatty tissue cells and hypoderma. No disorders in the virus development cycle were observed. Protein supercapsids contained randomly arrayed virions or polyvirions (Fig. 2-I). Caterpillar mortality due to CPV was 70%.

Experiments with mixed infection of caterpillars with NPV and CPV showed disturbances in the process of virus assembly. Thus, after inicial

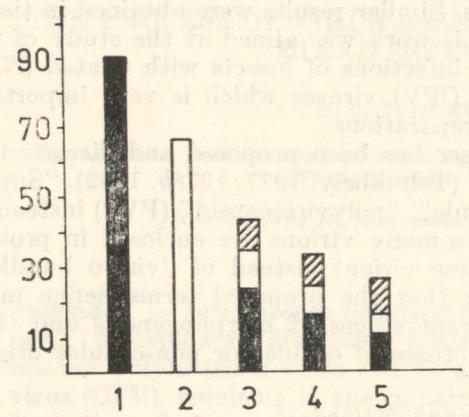

Fig. 1.

Lethality dependence of NPV and CPV mixed infection of cabbage moth caterpillars onthe sequence of virus inoculation

Abscissa: 1 — NPV monoinfection; 2 — CPV monoinfection; 3 — mixed infection with NPV followed at 24 hr by CPV; 4 — simmultaneous infection with NPV and CPV; 5 — infection with CPV after 24 hr followed by NPV. Black column — NPV, empty column — CPV, dashed column — mixed infection. Ordinate: % lethality.

infection with NPV followed at 24 hr by CPV, mortality of caterpillars was 45%; from these 64% died of NPV, 20% of CPV, and 16% of mixed infection. In fatty tissue and hypodermic cells the process of assembly of NPV was found to be impaired. PVC were found, which contained no virions or a few ones only. Sometimes, in PVC electron-transparent areas were observed corresponding in their shape and size to virions (Fig. 2-III). In this experimental variant, the assembly of CPV was impaired.

In the trial with simultaneous NPV and CPV infection of caterpillars, the assembly process of both viruses was disturbed. Caterpillar mortality occurred in 35%, of these 43% died due to NPV, 30% due to CPV, and 27% due to mixed infection. In neither case of mixed virus infection no assembly of viruses took place. In the periphery of PVC, invaginations could be seen, which accompany virions in monoinfections. No virions were found in the vicinity of the invaginations in mixed infections (Fig. 2-IV).

When the initial infection with CPV was followed after 24 hr with NPV, the total mortality was 26%, of which 29% was due to NPV, 26% to CPV, and 45% to mixed infection. In the gut epithelial cells NPV and CPV were found in the same cell (Fig. 3-I). Rod-shaped NPV nucleocapsids were present in vacuoles containing membrane-like formations. In the cytoplasm of the gut epithelial cells CPV polyviriocapsids were seen, containing a small number of virions. Alike to the experiments with NPV, they contained electron transparent areas of the same shape and size as virions. Free virions were found in the cell cytoplasm. In this case, fatty tissue and hypoderma cell nuclei contained PVC without virions (Fig. 3-II). No electron-transparent areas were found.

Electron microscopic examinations of embryonal cell cultures infected with NPV and CPV in all variants confirmed the data obtained in caterpillars. In mixed infection one cell contained components of NPV and CPV without virus assembly, however. NPV nucleocapsids of filamentous shape were found in the cell nuclei, and spherical nucleocapsids of CPV in the

cytoplasm (Fig. 4-I). When turnip moth caterpillars were infected with NPV and GV in different combination, the percentage of caterpillar mortality was higher than in monoinfections (Tarasevich *et al.*, 1979). Electron microscopic examinations of NPV- and GV-infected tissues showed virions to develop normally whereas supercapsids produced aberrant forms. Instead of one virion, GV supercapsids contained 2 or more virions (Fig. 4-II). Thus, in infections of caterpillars with NPV and GV the process of virus assembly was not disturbed, however, protein supercapsids of abnormal forms were found.

Discussion

The absence of virions in supercapsids of baculoviruses have been already reported (Cunningham, 1970; Pioseca-Serafin and Payne, 1975; Yamamoto and Tanada, 1978). However, these authors demonstrated only that supercapsids contained no virions. We succeeded in elucidating the pattern and degree of the impairment of the virus assembly process.

Until recently it was known that NPV, before developing in fatty tissue and hypoderma cells, multiplied in the gut epithelial cells where nucleocapsids were mainly formed which were then "transported" to other insect tissues (Granados, 1970; Summers, 1971). CPV affects the gut epithelial cells exclusively. Thus, NPV and CPV may be found in the gut epithelial cells in the initial stages of infection only. In order to study the morphogenesis of these viruses in mixed infection, caterpillars were infected at different time intervals. After virus invasion to the insects gut cavity, polyvirio-capsids dissolve releasing virions which enter epithelial cells of the gut where their development begins. In successive infection, the first virus develops normally until a second virus is introduced. Electron microscopic examinations showed partial disturbance of the assembly process of those viruses which get into the insects first and complete disturbance of the assembly of viruses inoculated later.

In simultaneous infection with NPV and CPV, the process of assembly of both viruses is impaired. Separate components of viruses and only in rare cases normal viruses were found in cells of the fatty tissue and hypoderma as well as in epithelial cells of the gut. Caterpillar mortality was higher when the initial infection was with NPV, because the cycle of development of this virus was shorter. The lowest mortality rate occurred in caterpillars first infected with CPV, because its development cycle is much longer than that of NPV, and apparently it has a delay effect. The phenomenon of interference has well been studied in simultaneous infection of bacteria with unrelated phages resulting in blocking of one or both viruses. The success of infection depended upon the phage which was first to infect the cells. The mechanism of this phenomenon has been discussed in the literature (Miller, 1980). A more complicated type of interference occurs in infection of insects with unrelated viruses (NPV and CPV) one of which, as a rule, develops only in cell nuclei and the other only in the cytoplasm. These viruses are capable of developing in the same cell, however, only separate

components of the viruses are formed while the process of virus assembly is disturbed. Even in those cases where NPV develops in the fatty tissue and hypoderma and CPV in the gut epithelial cells, no complete assembly of the viruses occurs. Nucleocapsids of abnormal shape are formed, whereas supercapsids develop normally but contain no virions.

Our experiments have not confirmed the hypothesis that NPV virions are the centers of supercapsid crystallization (Bergold, 1963; Harrap, 1972). Supercapsids and virions may form independently, the latter after inserting into supercapsids form an intricate complex. The phenomenon of disturbed virus assembly in mixed infection with NPV and CPV is very important in production of virus preparations because simultaneous presence of interfering viruses may reduce the effectiveness of viral preparations.

In mixed infection of insects with related viruses (NPV and GV) abnormal supercapsids are found (Fig. 4-II), within which one or several virions are present. Formation of multiploid virions is of great interest for specialists because it indicates the capacity of these viruses to form heterozygotes which are the first step in formation of recombinants. Mixed infection with related viruses (NPV and GV) in production of virus preparations is more preferable, because it results in a more effective virus preparation. Thus, in mixed infection of insects with unrelated viruses interference occurs resulting in an impaired virus assembly and formation of abnormal nucleocapsids; supercapsids develop normally, in contrast to mixed infections with related viruses (NPV and GV). In the latter, viriocapsids develop as usual and protein supercapsids produce abnormal forms.

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Explanation of Electron Micrographs (Plates XLI—XLIII):

Fig. 2. Ultrathin section of fatty tissue cells of cabbage moth caterpillars infected with NPV and CPV.
I — NPV monoinfection, ×30,000; II — CPV in monoinfection, ×32,000; III — area of polyviriocapsid after NPV infection followed by CPV: electron transparent spots at the site of virion location, ×32,000; IV — a portion of a cell after simultaneous infection with NPV and CPV; no virions found inside the polyvirion capsid; ×16,000.

Fig. 3. Ultrathin section through caterpillars infected with CPV and NPV.
I — A part of the cytoplasm of a gut epithelial cell after infection with CPV followed at 24 hr by NPV; nucleocapsids of NPV and CPV superviriocapsids are found in the same cell, ×18,000; II — NPV protein supercapsids in a fatty tissue cell after initial infection of caterpillars with CPV followed by NPV. No virions observed inside polyviriocapsids, ×23,000.

Fig. 4. Part of a tissue culture cell infected with NPV and CPV (I).
I — Cell nucleus with filamentous nucleocapsids and protein supercapsid with no virions inside; in the cytoplasm — abundant CPV nucleocapsids, ×15,000; II — part of a fatty tissue cell of turnip moth caterpillars infected with NPV and GV. Two virions inserted in one supercapsid are seen, ×43,000.