J.Biol.Control, 5 (1), 23-27, 1991

Characterization of Nuclear Polyhedrosis Virus of *Mythimna (Pseudaletia) separata*

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ABSTRACT

Electron microscopic study of the purified nuclear polyhedrosis virus of *Mythimna (Pseudaletia) separata* revealed that the polyhedral inclusion bodies (PIB) contained the multiply-embedded nucleocapsid particles (*M*NPV) which were found enveloped. Biochemically, the proteins and nucleic acid of the virus were analyzed, respectively, for their composition or properties. The envelope and matrix proteins were studied by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The properties of the DNA of Ms*M*NPV and Ha*M*NPV were studied after *EcoR*-I treatment by agarose slab gel electrophoresis. The electrophoretic separation profile of the DNA fragments revealed a unique array of bands with respect to the molecular size.

Key Words : *Mythimna separata* NPV, electron microscopy, biochemical characterization

The rice armyworm, *Mythimna (Pseudaletia) separata* (Walker) is infected by a nuclear polyhedrosis virus and the routes of infection, sites of virus multiplication, signs and symptoms, behavioural changes in the host, and the vertical transmission of the pathogen, have all been shown to fully match with those for a typical baculovirus (Neelgund, 1975; Shreesam *et al.*, 1983). The virus has shown no biological activity against some insects (Dhaduti, 1981) and a vertebrate (Kumar and Mathad, 1979).

Although Hatfield and Entwistle (1988) have brought about a comparison of the biological and biochemical properties of the NPV isolates from China, Japan and India, an exhaustive identification with respect to the morphology, viral proteins and biological properties of the virus was considered necessary for the comprehensive characterization. One such study of *M. separata* NPV (Ms*M*NPV) is presented in this article.

MATERIALS AND METHODS

Stock culture of the host *M. (P.) separata* was maintained on the semisynthetic diet (Neelgund, 1975). The sterilized eggs were allowed to hatch under optimum conditions (39°C, 75%RH) to obtain disease-free larvae which were subjected to the virus infection. The NPV stocks were prepared from the larvae died of polyhedrosis in their fifth or sixth instars. Large scale harvesting, purification, storing and counting of the polyhedral inclusion bodies (PIB) were all performed as described by Neelgund (1975).

The purity of polyhedra preparation and the morphology of the virus were observed under an electron microscope employing negative staining procedure as described by Summers and Paschke (1970). One per cent Uranyl acetate and/or neutral 1% phosphotungstic acid was used to stain the samples and, the stained samples were studied under Siemens Elmiskop I electron microscope.

The PIB were purified by banding in sucrose gradients (Summers and Smith, 1978) and the virus particles embedded in polyhedra were isolated as suggested by Summers and Smith (1975). The virus particles were purified by layering the preparations on sucrose

gradients in 0.1 *M* Tris (pH 7.8) buffer containing 0.01 *M* EDTA (density 1.17 - 1.2 g/ml) and ultracentrifuging at 100,000 g for 2 h at 4°C. Complete purification of the virus was achieved by repeating the density gradient centrifugation.

The virus stock corresponding to about 500 µg of protein/ml was treated with 2% NP-40 in 0.01 *M* Tris buffer (pH 7.8) containing 0.01 *M* EDTA and incubated the suspension at 37° C for 18 h. The treated solutions were further processed as per the method of Summers and Smith (1978) to separate the envelope proteins from nucleocapsids and capsids. To enrich the yield of the nucleocapsid proteins, the purified Ac*M*NPV and Ms*M*NPV were treated with NP-40 in the presence of NaCl and EDTA (Summers and Smith, 1978). The purified preparations were subjected to electrophoretic analysis. Polyhedrin was purified from the highly purified polyhedra as reported by Summers and Smith (1976). DNA was purified from the purified virus preparations according to the method of Summers and Anderson (1973) and Smith and Summers (1978).

Fig 1. Electronmicrograph of Ms*M*NPV showing rod shaped virions embedded in polyhedral coat (49, 759 X)

The purified DNAs (1.0 µg) from Lambda phage, multiple embedded nuclear polyhedrosis virus of *Autographa californica* (Ac*M*NPV), *Heliothis armigera* (Ha*M*NPV), and *M. separata* (Ms*M*NPV) were treated with *EcoR*-I as explained in the report of Smith and Summers (1978).

Polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE of proteins were performed as described by Laemmli (1970) with details as explained in Summers and Smith (1978). The protein bands were stained with Coomassie brilliant blue R-250 and destained in acetic acid and photographed. Molecular weights of polyhedrin and structural proteins were determined by the Weber and Osborn (1969) method, by comparing with standard proteins. The restriction endonuclease digest was electrophoresed in 1% agarose slab gels applying 50 Volts at 15°C for about 20 h. The discrete fragmented DNA bands were visualized under long wave UV illumination employing a transilluminator (UV Products San Gabriel, Calif.). Kodak Pan contrast process film was used to photograph the electrophoretogram.

RESULTS AND DISCUSSION

Virus morphology

As it appears in the electron microscopic picture (Fig. 1), the MsNPV particles were found to be enveloped to form multicapsid nuclear polyhedrosis virus (*M*NPV) particles that are, in turn, embedded in the protein matrix, polyhedrin. The polyhedra recorded a mean diameter of 1.71 ± 0.16 µm and enveloped nucleocapsids measured 274.75 ± 16.12 nm (length) and 71.00 ± 14.00 nm (width).

Biochemical properties

a. Structural polypeptides of the baculovirus capsids

The protein isolates were treated with SDS for electrophoretic analysis of the capsid

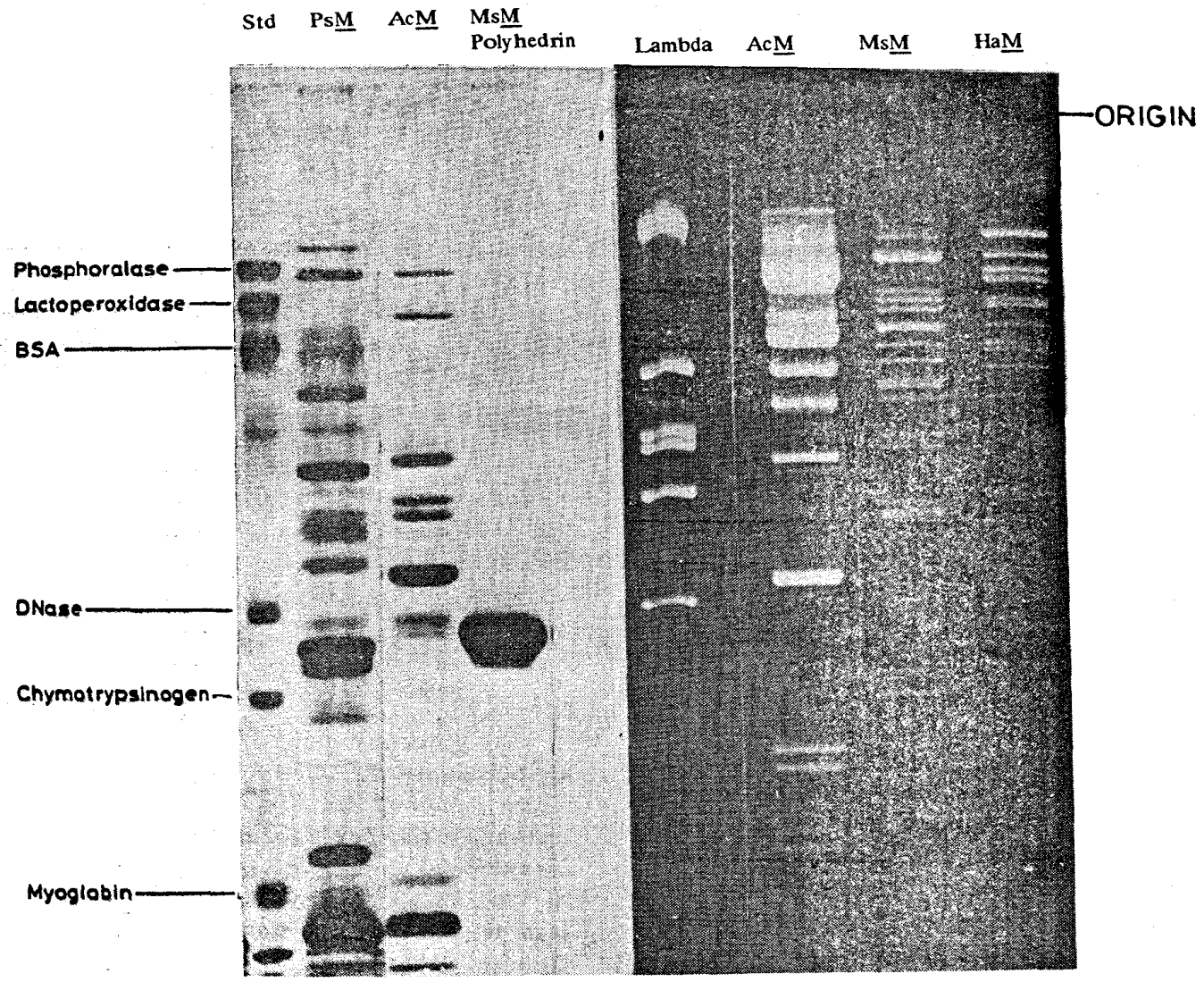

Fig 2. Electrophoretic profile of structural polypeptides of Ms*M*NPV, Ac*M*NPV, and the Ms*M*NPV polyhedrin protein [Left lane : Standard proteins-phosphorylase (90 kd), lactoperoxidase (77 kd), bovine serum albumin (68 kd) DNase (31 kd), chymotrypsinogen (25 kd) and myoglobin (17.2 kd)]

Fig 3. Electrophoretic profile of the Lambda Ac*M*NPV, Ms*M*NPV and Ha*M*NPV DNAs after *Eco*R-I digestion

proteins. Figure 2 is the profile of the electrophoretic mobilities of nucleocapsids of Ac*M*NPV and Ms*M*NPV. It is clear from the profiles of protein bands that the protein make-up of both the viral particles is different. The protein band in the far right lane in the electrophoretogram is due to the purified polyhedrin protein, that is found to have a molecular weight of 30,000 daltons with reference to the standard protein lane located at the far left hand side of the picture.

b. Nucleic acids

The purified DNA preparations (1.0 µg) of Lambda, Ac*M*NPV, Ms*M*NPV and Ha*M*NPV were treated with *Eco*R-I enzyme and electrophoresed in 1% agarose. The restriction fragmentation pattern of each DNA was further analyzed by scanning densitometry (Fig. 3). Except for a few *Eco*R-I restriction fragments that showed an almost similar electrophoretic mobilities, other fragments, more than 12 of

them, were observed to exhibit distinctly different electrophoretic migration rates. Therefore, it is obvious from the electrophoretogram that the restriction fragments of the DNAs belong to the three different viral isolates.

The shape of the polyhedra and configuration of the virions in the polyhedra were found to resemble those of other *M*NPVs. The electronmicrographs indicated that the dimensions of the *separata* virus were different from those of the other virus particles. Though Fig.1 provides a tentative means of identifying the virus as a member of *Baculoviridae* family, a conclusive identification called for biochemical and biological characterization (Entwistle and Evans, 1985).

One of the routine ways of identifying a virus, is to study the genetic complexity that gets manifested in terms of large number of specific and nonspecific structural polypeptides. Electrophoretic mobility (Fig. 2) of the structural polypeptides of Ms*M*NPV was found to differ from that of other baculoviruses, such as Ac*M*NPV. The molecular size and composition of the structural polypeptides of the *separata* virus did not compare with those of Ac*M*NPV or, with those of any other baculoviruses, such as, Ro*M*NPV, Ag*M*NPV, Ha*M*NPV, Tn*S*NPV, Hz*S*NPV, TnGV and SfGV determined by Summers and Smith (1978).

Certain preliminary observations (Summers *et al.,* 1986) on the *EcoR*-I fragmentation of the Ms*M*NPV-DNA were corroborated by the detailed investigations of Hatfield and Entwistle (1988) who compared the restriction endonuclease fragmentation (*EcoR*-I and *Bam* HI) pattern of the DNAs of PuNPV, MsNPV (China), MsNPV (Japan)and MsNPV (India). In addition, the restriction endonuclease (*EcoR*-I) fragmentation pattern (Fig. 3) of the DNAs of Ms*M*NPV, Ac*M*NPV and Ha*M*NPV in the present study clearly demonstrated that the genetic make-up of the *separata* virus is different from those of others. A comparison of the electrophoretic mobilities of the restriction fragments of DNA of Ms*M*NPV with those of other baculoviruses like Ac*M*NPV, Ro*M*NPV, Tn*M*NPV, SfGV etc. (Smith and Summers, 1978) provides an additional proof in support of the identification of the *separata* virus as a distinctly different virus.

Furthermore, the Ms*M*NPV appeared rather very specific to *M. separata* as it failed to utilize the cell culture of cabbage looper which allowed the propagation of Ac*M*NPV, and it has been proved innocuous to the other insects like *Bombyx mori, Antheraea mylitta, Philosamia ricini* and *Apis cerana indica* (Dhaduti, 1981).

On the basis of the present study on the morphology and biochemistry, and the biological properties of the virus described earlier, it can be concluded that the Ms*M*NPV might be a distinct baculovirus infecting specifically, *M. separata.*

ACKNOWLEDGEMENTS

We profusely thank Dr.M.D.Summers and Dr.G.E.Smith, The Cell Research Institute and Department of Botany, The University of Texas, Austin, Texas for their assistance in the biochemical work involving the cell lines of *T. ni* TN-368-10, Ac*M*NPV, Ha*M*NPV etc. We also wish to extend our sincere thanks to Dr.Jean R.Adams, Insect Pathology Laboratory, United States Department of Agriculture, Northeastern region, Beltsville, Maryland for help in preparing the electromicrographs.

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