Abstract

Cytoplasmic polyhedrosis viruses (CPVs) infecting three Indian saturniidae silkworm larvae, *Antheraea mylitta, Antheraea proylei* and *Antheraea assamensis,* were isolated and characterized. Scanning electron microscopic studies revealed the presence of hexahedral (rhombic dodecahedron) shape and various sizes (1-4 μm) of polyhedral bodies from single infected host whereas transmission electron microscopic studies of isolated virions showed all the virions in uniform (icosahedral) shape and size (~55nm). The viral genome was isolated from the purified polyhedra and analyzed through agarose and acrylamide gel electrophoresis. Eleven bands ranging from ~350 bp to 3.9 kb (total 25.35 kb) were detected in the gel without any restriction enzyme treatment of the isolated nucleic acids. These bands were completely degraded after treatment with RNase A at low salt but not at high salt concentration. The banding pattern of the genome remained unaffected by the treatment of DNase I indicating that the viral genome was composed of eleven double-stranded segmented RNA. The RNA banding pattern of CPV isolates from three saturniidae silkworms were similar to each other but different from *Bombyx mori* CPV (BmCPV). These characteristics were also very similar to genome profile of type IV CPVs except the size of different RNA bands and the presence of an extra eleventh RNA segment. The dot blot analysis also showed hybridization of cloned 9th segment cDNAs of *A. mylitta* with RNA of *A. proylei* and *A. assamensis* CPV but not with *B. mori* CPVs. The SDS-PAGE analysis of polyhedral proteins of *A. mylitta* CPV isolates showed the presence of three major and five minor bands with molecular mass from 29.4 kDa to 163 kDa, but the BmCPV polyhedra showed different protein banding pattern. Two heterologous cell lines infected *in vitro* with CPV isolated from *A. mylitta* showed very slow viral growth with the accumulation of polyhedral bodies in the cytoplasm of infected cells indicating a restricted host range of this CPV. The genome segment 9 of the eleven segment double stranded RNA genomes of *A. mylitta* CPV (AmCPV), *A. assamensis* CPV (AaCPV) and *A. proylei* CPV (ApCPV) was converted to double stranded cDNA, cloned and sequenced. This genome segment consists 1473 nucleotides (AmCPV, AaCPV) and 1472 bp (ApCPV) having a long open reading frame (ORF) of 1035 bp, which could encode a protein of 345 amino acids. The deduced molecular weight of the protein was 38 kDa and termed as NSP38. Two potential N-linked glycosylation sites as well as several phosphorylation sites are found within this alanine, leucine and serine rich ORF. Computer assisted prediction of secondary structure showed the presence of nine alpha helices in the central and terminal domain with localized similarity of RNA binding motifs Blue tongue virus (BTV) and Interstitial bursal disease virus (IBDV) RNA polymerases. Nucleotide sequences were 99.6% homologous among these three CPVs but no homology was found with any other protein or nucleotide sequences in the databases. This indicates the infection of Indian saturniidae non-mulberry silkworms by a new type of CPV and segment 9 codes for a novel protein. The ORF from the AmCPV cDNA was expressed as a His-tag fusion protein in *E. coli* and polyclonal antibody was raised against the purified protein. Immunoblot as well as immunofluorescence analysis with the anti-NSP38 showed that the protein was not present in the polyhedra or uninfected cells but in AmCPV infected host midgut cells. NSP38 was expressed in insect cells as soluble protein in a baculovirus expression vector and

detected by immunoblot analysis. The baculovirus expressed NSP38 possessed the ability to bind poly (rI). (rC) agarose that was competitively removed by AmCPV viral RNA. These results indicate that NSP38 is expressed in virus-infected cells as a non-structural protein. By binding to viral RNA it may play a role in the regulation of genomic RNA function and packaging.

Keywords:
Saturniidae silkworms, cytoplasmic polyhedrosis virus, *Antheraea mylitta*, *A. assamensis*, *A. proylei*, double stranded RNA genome, scanning electron microscopy, transmission electron microscopy, cDNA cloning, sequencing, bacterial expression, baculovirus expression, recombinant protein, polyclonal antibody, Western blotting, SDS-PAGE, immunofluorescence, RNA binding assay.