Abstract

The double stranded segmented RNA genome of cytoplasmic polyhedrosis viruses (CPVs) infecting the three Indian non-mulberry silkworms, *Antheraea mylitta, Antheraea assamensis* and *Antheraea proylei* were isolated and separated by gel electrophoresis. Genome segment 10 of the 11 double stranded segmented RNA genomes encoding viral polyhedrin polypeptide of these three CPV isolates were cloned and sequenced. One cDNA clone (1502 bp) was found common to *A. mylitta* CPV (AmCPV), *A. assamensis* CPV (AaCPV) and *A. proylei* CPV (ApCPV) isolates. But in addition two variant forms (1120 bp and 1415 bp) of cDNA were obtained from AmCPV isolate only. The 1502 bp cDNA clone of AmCPV consisted of an open reading frame of 254 amino acids and encodes a protein of approximately 29 kDa. On the other hand 1120 bp cDNA and 1415 bp cDNA clones consisted of an ORF of 339 and 319 amino acids and encode polyhedrin proteins of approximately 39 and 37 kDa, respectively. Nucleotide sequence of AmCPV10-1 cDNA clone was ~99% identical to AaCPV and ApCPV segment 10 cDNAs, and 71% and 94% similar to AmCPV10-2 and AmCPV10-3, respectively. But no sequence homology (either at nucleotide or aminoacid level) was detected with polyhedrins from other CPVs or NPVs (nuclear polyhedrosis viruses) or any other sequences available in the databases. Northern hybridization of the total AmCPV genome with cloned AmCPV segment 10 cDNA confirmed the existence of variant forms of segment 10 RNA in AmCPV. These results indicate that a novel type of CPV infects Indian non-mulberry silkworms and its segment 10 encodes a novel protein polyhedrin. All the cDNA clones of segment 10 had a duplication of almost 382 nucleotides in tandem at their 3' ends with mutation and deletion found in the variant forms. The ORFs from all three cDNA clones of AmCPV were expressed in *E. coli* as his-tagged fusion proteins via prokaryotic expression vector. These proteins showed immuno-reactivity with polyclonal antibody raised against naturally occurring AmCPV polyhedrin, suggesting that three variant forms of AmCPV segment 10 RNAs generated by genome rearrangements are functionally active and may produce three novel polyhedrins. AmCPV10-1 cDNA was expressed in insect cells via baculovirus expression system and shown to produce cubical polyhedra by scanning electron microscopy but AmCPV10-2 and AmCPV10-3 did not express in insect cells. Similar result was obtained from naturally occurring AmCPV infected gut cells of *A. mylitta* larva where only expression of ~ 29 kDa polyhedrin was detected by immunoblot assay. These results suggested that although variants of AmCPV segment 10 RNA are present in AmCPV genome but only AmCPV10-1 can express polyhedrin in insect cells with the capacity of supramolecular assembly.

On the basis of AmCPV10 cDNA sequences specific primers were designed to detect AmCPV infection by RT-PCR with the sensitivity of 32 picograms of total AmCPV dsRNA. An anti-AmCPV polyhedrin monoclonal antibody secreting hybridoma cell line was also generated by fusing spleen cells of recombinant polyhedrin immunized Balb/C mice and P3-X63Ag8.653 mouse myeloma cells. This anti-polyhedrin monoclonal antibody, USF7, was characterized as IgG2B heavy chain and lambda light chain isotype, and could detect as low as 125 ng of naturally occurring AmCPV polyhedral bodies by Enzyme linked immunosorbent assay (ELISA). Thus it appears that these immunological and molecular techniques may be used for the detection of AmCPV in the infected larvae at a very early stage to prevent viral spread and improve the sericulture.

Key words

Saturniidae silkworms, cytoplasmic polyhedrosis virus, *Antheraea mylitta*, *A. assamensis*, *A. proylei*. Polyhedrin cDNA, Anti-polyhrdin monoclonal and polyclonal antibody.