Abstract

The double-stranded segmented RNA genome of cytoplasmic polyhedrosis virus (CPV) infecting the non-mulberry silkworm *Antheraea mylitta* was isolated and separated by gel electrophoresis. Genome segments 6 (S6) and 7 (S7) of the 11 double-stranded RNA genome of *A. mylitta* CPV (AmCPV) were eluted from the gel, converted to cDNA, cloned and sequenced. Northern hybridization of the total AmCPV genome with cloned AmCPV segment 6 and 7 cDNA confirmed the cloning of segment 6 and 7 RNA. Sequencing of AmCPV segment 6 (AmCPV6) cDNA showed that it consisted of 1944 nucleotides with an open reading frame of 607 amino acids and could encode a protein of approximately 68 kDa and we designated as P68. The cDNA of AmCPV segment 7 (AmCPV7) showed that it consisted of 1789 nucleotides with an ORF of 530 amino acids and could encode a protein of approximately 61 kDa and designated as P61. No significant sequence similarity both at nucleotide or amino acid level of S6 or S7 was found by searching public databases except some localized similarities of S6 with S6 of *Operophtera brumata*, *Lymantria dyspar* and *Bombyx mori* CPV, and indicated that AmCPV S6 and S7 encode novel proteins. Motif Scan analysis showed a significant similarity of P61 and P68 with IMPDH enzyme containing one and two Cystathionine beta synthase (CBS) domains, respectively, and it was suggested that by binding to viral RNA through CBS domain, P68 and P61 may help in viral replication or transcription. The ORFs of AmCPV S6 and S7 were expressed in *E. coli* via prokaryotic expression vector and found to produce His-tag fusion protein as insoluble inclusion bodies. Recombinant proteins were purified by Ni-NTA chromatography and high titer polyclonal antibodies were raised in rabbit indicating that both P68 and P61 are highly immunogenic and these antibodies may be used for the detection of AmCPV infection in *A. mylitta* larvae. Immunoblot analysis of AmCPV infected gut cells and purified polyhedra using the raised polyclonal antibodies showed strong immunoreactive bands and indicated that AmCPV S6 and S7 codes for viral structural proteins. S6 was expressed in *Sf*-9 cells via baculovirus system to produce 68 kDa soluble protein and purified to homogeneity but S7 could not be expressed in insect cell to produce correct size protein. Secondary structure prediction by GOR4 and PHD programs showed the presence of 21 α helices and 18 extended β sheets for P68 and 17 α helices and 18 extended β sheets for P61, which was confirmed by CD spectroscopy.

P68 also showed the presence of NTP binding motif and molecular docking analysis by using ATP heteroatom onto a modeled 3D structure showed ATP binding at the predicted domain by hydrogen bonds and hydrophobic interactions. The ability of P68 to bind to ATP was confirmed by fluorescence study using TNP-ATP. P68 also showed nucleotide triphosphatase

activity by ATPase assay suggesting that P68 encoded by AmCPV S6 by binding to viral RNA through its Bateman domain and by its ATP binding and ATPase activity may help in viral replication and transcription.

Key words

Cytoplasmic polyhedrosis virus, *Antheraea mylitta*, P68, P61, CBS domain, NTP binding motif, polyclonal antibody, viral structural protein, CD spectroscopy, molecular docking, TNP-ATP, ATP binding assay, ATPase assay.