Abstract

Antheraea mylitta cytoplasmic polyhedrosis virus (AmCPV), which infects and destroys Indian tasar silk worm, belongs to the genus *Cypovirus* of the family *Reoviridae* and contains eleven segmented double stranded RNA in its genome. Genome segment 2 (S2) of AmCPV was converted to cDNA, cloned and sequenced. S2 consisted of 3798 nucleotides with one long ORF of 1116 amino acids and could encode a protein ~123 kDa. BLAST analysis showed 29% and 30% identity of S2 sequence encoding RNA dependent RNA polymerase (RdRp) of *Lymantria dispar* CPV (LdCPV) and *Choristoneura occidentalis* CPV (CoCPV) respectively. AmCPV S2 contained all eight conserved motifs (motif A-G), which are normally present in all other RdRps including the catalytic GDD motif (Motif C). Phylogenetic analysis of amino acid sequences of AmCPV S2 with that of other members of the family *Reoviridae* showed that AmCPV, DpCPV-1, BmCPV-1, LdCPV-1 and CoCPV are closely related to each other and form a cluster, suggesting a common origin of all these insect *Cypovirus*. The ORF of S2 was expressed as 123 kDa insoluble His-tagged fusion protein in *E. coli*, purified through Ni-NTA chromatography and used to raise polyclonal antibody. Soluble protein was obtained by expressing the S2 ORF in insect cells via baculovirus expression system and confirmed by immunoblot analysis. Purified soluble protein exhibited RdRp activity in an *in vitro* RNA polymerase assay using homopolymeric RNA and S2 mini genomic RNA as templates. The RdRp activity of AmCPV S2 encoded protein was found to be strictly primer dependent when homopolymeric RNA template was used as a template. Maximum activity was observed at 37 ^{0}C, at pH 6.5 in presence of 3 mM $MgCl_2$, 20 mM KCl and 80 mM NaCl. *De novo* synthesis of RNA (primer independent) was also observed when AmCPV mini genomic RNAs were used as a template without any intrinsic terminal transferase activity at the rate of 120 nt/min. The importance of the conserved GDD motif in the enzyme activity was demonstrated by changing these residues by site directed mutagenesis because the mutated AmCPV S2 encoded proteins bind with the AmCPV RNA less efficiently (Kd value 0.34 µM) than the wild type (Kd value 2.02 µM) and show reduced activity of RdRp. The AmCPV RdRp activity was found to be inhibited by known inhibitors, phosphonoacetic acid (PAA). The fluorescence spectroscopic analysis showed that this protein is stable upto 40 ^{o}C and 4 M urea concentration and undergoes denaturation at high temperature and 6 M urea. Molecular characterization of AmCPV S2 encoded RdRp will help to screen and develop new anti-viral compounds for the improvement of tasar sericulture in India.

Key words: *Antheraea mylitta* cypovirus, RNA dependent RNA polymerase, cloning, sequencing, expression, GDD motif, In vitro activity of expressed RdRp.