ABSTRACT: *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV), a cypovirus of *Reoviridae* family, infects non-mulberry Indian silk worm, *Antheraea mylitta*, and contains eleven segmented double stranded RNA (S1-S11) in its genome. All of its genome segments except segments 4 and 5 have been previously characterized. In the present work genome segments 4 and 5 (S4 and S5) have been cloned, sequenced and characterized. S4 consists of an ORF of 1110 amino acids and could encode a protein of ~ 127 kDa (p127), and S5 consists of an ORF of 606 amino acids and could encode a protein of ~65 kDa (p65). Bioinformatics analysis of S4 shows presence of 5' RNA triphosphatase (RTPase) domain (LRDR), S-adenosyl-L-methionine (SAM) binding (GxGxG) motif and K-D-K-E tetrad of 2'-O-methyltransferase (MTase), and suggests that S4 may encode RTPase and MTase. Similar analysis of S5 shows presence of KLRS and HxnH motifs of guanylyltransferase (GTase) and suggests that S5 may encode viral GTase. The ORF of both S4 and S5 have been expressed in *E. coli* to produce soluble 127kDa and 65kDa his tagged fusion protein, respectively and purified through Ni-NTA chromatography. Biochemical analysis reveals that p65 possesses GTase activity and transfer GMP moiety to the di phosphate (A/G) ended viral RNA after the formation of p65-GMP complex. Site directed mutagenesis at K21A in KLRS motif, and H93A or H105A in HxnH motif completely abolishes the autoguanylylation activity and indicates importance of these residues at these sites. Thermodynamic analysis shows p65-GTP interaction is primarily driven by enthalpy whereas the p65-RNA interaction by favorable entropy. Biochemical analysis of recombinant p127 shows its 5' RTPase as well as SAM dependent guanine N-7 and ribose 2'-O-MTase activity. MTase assay using *in vitro* transcribed AmCPV S2 RNA having 5' G*pppG end shows that guanine N-7- methylation occurs prior to the ribose 2'-O-methylation to yield m7GpppG/ m7GpppGm RNA cap. Mutagenesis of the SAM binding (GxGxG) motif (G832A) completely abolished N-7 and 2'-O-MTase activity indicating importance of these residues for capping. From the kinetic analysis the K_m of N-7-MTase for SAM and RNA was calculated as 4.41 and 0.39 uM, respectively. These results suggest that AmCPV S4 encoded p127 catalyses RTPase and two cap methylation reactions, and along with GTase activity provided by p65 helps in the capping the 5' end of newly synthesized viral RNA. Thus molecular and functional characterization of these two proteins encoded by S4 and S5 will help to understand AmCPV replication and may be used as targets for the development of anti-viral compounds to improve tasar sericulture.

Key Words: *Antheraea mylitta* cypovirus, Methyltransferase, Guanylyltransferase, Cloning and sequencing, Expression, Functional characterization, Kinetic analysis.