Abstract

Antheraea mylitta cytoplasmic polyhedrosis virus (AmCPV) is a segmented double stranded RNA (dsRNA) virus having 11 segments (S1-S11) as its genome. AmCPV is a member of *Cypovirus* genus of the *Reoviridae* family, infecting Indian non-mulberry tasar silkworm *Antheraea mylitta*, and causes substantial economic loss each year. The mechanism of genomic RNA transcription/replication has not been studied in detail for any CPVs. AmCPV S2 encodes 1116 amino acids long (~123 kDa) RNA-dependent RNA polymerase (RdRp) which mediates transcription/replication of all the viral genome segments but its mechanism of RNA synthesis is not elucidated. Therefore, it necessitates the structural and functional characterization of AmCPV RdRp to understand the underlying mechanism for cypoviral RNA-polymerase interaction in the replication/transcription process, which may help to design effective antiviral compound (s) on the basis of structure and biochemical properties of the polymerase. Initially, a three-dimensional model structure of AmCPV RdRp has been predicted by bioinformatics methods and compared to the other structurally known viral RdRps. In addition, docking with biologically relevant molecules and molecular dynamics (MD) simulation have enabled to identify important sequence and/or structural features involved in substrate entry or binding, polymerase reaction and the product release events governed by AmCPV RdRp. These analyses have predicted the presence of three domains in AmCPV RdRp, namely N-terminal, polymerase and C-terminal domains. Cloning, expression, purification and functional analysis of the individual domains of AmCPV RdRp demonstrate that the purified domains interact *in vitro*. The central polymerase domain shows nucleotide binding property as that of the full length polymerase but not the N- and C-terminal domains. The nucleotide binding in the active site is supported by a loop structure of the polymerase, which is further stabilized by a zinc ion (Zn^{2+}). Isolated polymerase domain does not exhibit RdRp activity but the activity can only be reconstituted when all three domains are included in the reaction mixture. It is hypothesized from the MD studies that the motions of the separated polymerase domain may lead to the formation of a less accessible RNA template binding channel which impairs the RdRp activity. Recruitment of RdRp to the specific sequence of viral RNA has been studied using ^{32}P-labeled 3'-UTR of viral RNA and the full length AmCPV RdRp or the domains in a binding assay. It has been demonstrated that the full length viral polymerase specifically binds to the unique secondary structure of the 3'-UTR region of viral RNA. On the other hand, isolated different domains of RdRp exhibit a poor and non-specific interaction with the RNA. Further, the polymerase shows a higher cooperativity to the RNA representing the 5'-UTR (>2.0) than the 3'-UTR (~1.0) of viral RNA, implying a complex mechanism of RNA-polymerase binding and interaction.