ABSTRACT

The cap is a structure located on 5' terminus of eukaryotic mRNA and it has a substantial role in cellular homeostasis. Hence proteins involved in cap removal, such as Dcp1/2 complex play a vital function in mRNA degradation. Nevertheless their mechanisms of both action and regulation remains obscure. Biochemical studies on Dcp2 protein have shown that Dcp2 interacts specifically with m^7GDP (a product of the cap cleavage). It suggested that m^7GDP structure could be a starting point for preparation of nucleotides displaying higher affinity to Dcp2, which could become valuable tools for biochemical research. A number of cap analogues based on m^7GDP, m^7GTP or m^7Gppppm^{7}G structures was synthesised and their biological properties were examined. These compounds possess a variety of chemical modifications in their phosphate chain, including tio- or boranophosphate groups, which are expected to increase their affinity to cap-interacting proteins. Furthermore, a set of fluorescently labelled cap analogues was also prepared. Since their fluorophores are very compact these nucleotides should be useful for preparation of fluorescent mRNA or as molecular probes. A screening assay revealed that some of prepared nucleotides are inhibitors of Dcp1/2. Further biochemical and structural researches were conducted using the most potent inhibitor ($m^7Gp_Sppp_Sm^7G$, isomer D3). NMR experiments allowed to unravel the nucleotide binding site and its affinity to Dcp2. Kinetic studies showed that the inhibitor is bind by both free enzyme and ES complex. It also have been observed that cap analogues with elongated phosphate chain are hydrolysed by Dcp2. Other biochemical studies showed that some of prepared cap analogues display high affinity to eIF4 translation factor and lower susceptibility to enzymatic degradation. Fluorescent cap analogues obtained in this study are the first such compounds which can be incorporated into RNA *in vitro*. Some of them are also selectively resistant to either Dcp2 or DcpS decapping enzymes. Preliminary biochemical experiments indicated that these fluorescent cap analogues could be applied to studies on cap-protein interactions. Exemplary applications of chemically modified cap analogues to study mRNA degradation was also desribed in this study. These experiments were based on usage of egzogenous mRNA, containing a non-hydrolysable cap analogue, to monitor the degradation of histone mRNA in mammalian cells. Obtained results provided a better understanding of the role of Dcp2 in these processes and the influence of 3' mRNA uridylation on its stability.