Abstract

The trimethylguanosine (TMG) cap is a motif present at the 5' end of small nuclear and nucleolar RNAs, which are involved in RNA splicing. The TMG cap plays a crucial role in RNA processing and stability as it protects the RNA molecule from degradation by exonucleases and facilitates its export from the nucleus. In the cytoplasm in each cell the TMG cap plays a role in the recognition of snRNA by snurportin, a protein that initiates nuclear import formatting also complex with importin β. TMG cap analogs have been used as affinity resins in protein separation and purification from extracts or as nuclear localization signal for improving nuclear import of some polar and large biomolecules (DNA, streptavidin), however they can be used also in biochemical experiments and biological assays as molecular tools to substitute the natural TMG capped RNAs.

The main aim of my work was synthesis, physicochemical (free compounds) and biophysical characterization (complexed) of a series of dinucleotide TMG cap analogs and their conjugates with Fluorescent Molecular Rotors (FMR) in experiments with snurportin both *in vitro* and in cells. This functionalization was intended to open the possibility of detecting snurportin–ligand interactions *in vitro* and potentially *in vivo*. I have obtained large set (over 50) of final compounds with FMRs in different positions and different nucleotides part and evaluated as molecular probes for snurportin. Some compounds have been additionally modified either in bridging region (methylenebisphosphonate, CH_2), by elongating to tetraphosphate or at 5'-O positions by changing O to S. These modifications were expected to ensure resistance to enzymatic degradation by human Nudt16 and/or maintain or enhance affinity towards snurportin.

Among FMRs five GFP-like chromophores (derived from green fluorescent protein) and two julolidine derivatives have been tested. The evaluation of binding affinities for snurportin showed unexpectedly a strongly stabilizing effect for TMGpppG-derived dinucleotides containning the GFP-like FMR at the 2'-O-position of guanosine. These newly discovered compounds are potent snurportin ligands with nanomolar K_D (dissociation constant) values, which are two orders of magnitude lower than that of natural TMGpppG. The effect is diminished by ~50-fold for the corresponding 3'-regioisomers. To deepen the understanding of the structure–activity relationship, I have synthesized and tested FMR conjugates lacking the TMG cap moiety, which revealed, that this newly observed effect persist also in m^7G caps and in less extend in GMP analogs, yet with unspecific interactions (ie. m^7G cap-FMR conjugates were proved to bind competitively also with eIF4E). These experimental studies, have been supported by molecular docking, and both suggest, that the enhanced affinity arises from additional hydrophobic contacts provided by the GFP-like FMR moiety regardless of functional groups in phenyl ring. The strongest snurportin ligand, which also gave the greatest fluorescence enhancement (F_m/F_0) when saturated with the snurportin, were tested in living cells to detect interactions and visualize complexes by monitoring fluorescence lifetimes. This approach has potential applications in the study of RNA processing and RNA– protein interactions which is also discussed. Finally, 9 TMG cap-FMR conjugates have been identified in my studies as potent and specific snurportin ligands and among them 4 conjugates with DMHBI as FMR has shown beneficial fluorescence properties in complexes *in vitro*, meanwhile one exemplary conjugate from these have been tested in living cells and has been found to associate in complexes comparable in sizes with snurportin.