Abstract

Unusual DNA secondary structures especially G-quadruplex and i-motif have attracted widespread interest in the field of biology and biological chemistry. Their pivotal role in various biological processes (replication, transcription and recombination etc.) is now slowly being proved and they are also being identified as novel drug targets. Only very recently (first quarter of 2013) evidences started to accumulate for the presence and function of G-quadruplex structure in vivo. There are many facets of these conformational motifs still to explore, which might help in understanding their existence and biological significance. In the present study, we focussed on the structural aspects of G-quadruplexes. Studies were conceived to explore the G-quadruplex formation ability of 23-mer G-rich sequence having G5-tract present at promoter region of Human Myosin HC β Gene (MYH7). Mutation associated with this gene causes Hypertrophic cardiomyopathy, Myosin storage myopathy and dilated cardiomyopathy. This study demonstrated that guanine rich (G-rich) and cytosine rich (C-rich) strands in the proximal promoter MYH 7 are able to form G-quadruplex and i-motif structures independently. We demonstrated a unique three stranded G-quadruplex structure formed by 23-mer G-rich sequence of Human (HM23) as well as the Rabbit counterpart (RM23) of MYH7 gene promoter. The involvement of G5 stretch in the novel three stranded structure was confirmed by introducing mutation in HM23 G-rich sequence and it was observed that absence of G5 stretch does not form this unique trimeric G-quadruplex.

The C-counterparts of G-rich sequence of MYH7 gene promoter adopt i-motif (C-tetraplexes) structures of varied molecularity. Surprisingly, RM34C (extended version of RM23C) was shown to adopt an i-motif structure as well as homoduplex at neutral pH. The sequence forms i-motif in the temperature range of 4-15 °C while at 25 °C it transforms to a homoduplex.

To study further the polymorphism associated with G-quadruplex structures, we investigated the effect of G-tract length and intervening bases Thymine (T) on the G-quadruplex topology. Similarly, their complementary C-rich sequences were also studied to explore the effect of C-tract length and intervening bases Adenine (A) on i-
motif formation. It was found that G-rich sequences are highly polymorphic and adopt structures of varied molecularity. The G-quadruplexes formed by these sequences showed a higher thermal stability in presence of K\(^{+}\). The C-rich counterparts of these sequences adopt bimolecular, tetramolecular and intramolecular i-motif structures and demonstrated stability with A1→A3 in loop while presence of A4 causes destabilization of the structure.

In the Ligand-G-quadruplex interaction studies, G-quadruplex interactive-compound TMPYP4 was shown to stabilize G5-tract containing parallel G-quadruplexes specifically. Sanguinarine specifically recognized, stabilized and facilitated G-quadruplex of antiparallel topology. Interestingly, Sanguinarine stabilizes intermolecular G-quadruplexes at a greater extent than the intramolecular species. As an approach to see the binding affinity of peptides towards various G-quadruplexes, we also studied interaction of G-quadruplexes by a 11-mer designed peptide (RGKGWGWGKGR) and found that the peptide (WG) specifically unfolds G-quadruplexes of parallel topology.

Overall this study presents an in-depth understanding of structural polymorphism associated with G-quadruplexes and i-motif (C-tetraplex) unusual DNA structures. Study of such structures, formed at G-C rich locations of genomic origin, may suggest a nucleic based mechanism for recognition of specific genes. Recognition of these multistranded structures by small molecules creates interest in designing and development of molecular agents which can display high degree of affinity and selectivity towards a special structural topology.