PREFACE

The concept that enzymes are proteins got revolutionarily changed with the advent of ribozymes in 1980s and discovery of DNA enzymes in 1990s further strengthened the catalytic potentials of nucleic acids precluding the monopoly of proteins. Although catalytic potential of protein is incomparable, enzymatic role of nucleic acids has got its unique advantage being utilized in gene therapy due to extra-ordinary substrate-specificity and hence less off-target activity. DNAzymes are single-stranded 2-deoxyoligonucleotides with enzymatic activity and absence of 2’-OH group makes it ~1,000 times more stable than protein enzymes and ~100,000 times more than ribozymes. Since its discovery and till date, several DNAzymes with different catalytic potentials are being discovered by in vitro selection process. Due to its highly efficient RNA phosphodiesterase activity, 10-23 DNAzyme made a significant impact for the last decade being utilized as a gene silencing tool.

Although 10-23 DNAzyme is efficient molecular scissor, further exploration is required to determine its therapeutic potential by determining its efficiency in silencing specific genes of therapeutic interest. Also, lack of effective ways to determine the right target site is the major constraint in designing efficient DNAzymes. For getting an initial insight over the probable secondary structure of target mRNA, generally computer predictions are considered. But actual structure of the mRNA differs significantly in the intracellular micro-environment. In several cases, translation initiation codons are targeted by DNAzymes and found to be effective. Thus main purpose of this study is to further determine the therapeutic potentials of DNAzyme and to find out a safe target site without going through laborious experiment based target site selection. Aims and objectives of fulfilling these goals can be summarized as under:
- To find out DNAzyme target site in mRNA. For this, MFOLD software is to be used to determine possible sites of mRNA that can form secondary structure, which should be excluded for designing complementary binding arms of DNAzyme.

- Designing of DNAzymes against different target sites (inducible nitric oxide synthase of murine macrophage, TNF-α and its receptors TNF-R1 and TNF-R2 in THP-1 cells, and core and NS5B of hepatitis C virus) and evaluate their specificities in in vitro reactions using in vitro synthesized or isolated RNAs.

- To perform a comparative study of DNAzyme activity in case of iNOS mRNA targeting translation initiation codon and other computer predicted target sites with primary and secondary structures.

- Transfection of DNAzymes against their respective intracellular target RNAs and quantitate intracellular cleavage activity by RT-PCR.

- Estimation of inhibition of protein expression of DNAzyme targeted mRNA by Western blot analysis.