Dissertation presented at Uppsala University to be publicly examined in C10:301, BMC, Husargatan 3, Uppsala, Thursday, June 5, 2008 at 13:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract

Honcharenko, D. 2008. Conformationally Constrained Nucleosides, Nucleotides and Oligonucleotides. Design, Synthesis and Properties. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 440. 71 pp. Uppsala. ISBN 978-91-554-7219-1.

This thesis is based on six original research publications describing synthesis, structure and physicochemical and biochemical analysis of chemically modified oligonucleotides (ONs) in terms of their potential diagnostic and therapeutic applications. Synthesis of two types of bicyclic conformationally constrained nucleosides, *North-East* locked 1',2'-azetidine and *North* locked 2',4'-aza-ENA, is described. Study of the molecular structures and dynamics of bicyclic nucleosides showed that depending upon the type of fused system they fall into two distinct categories with their respective internal dynamics and type of sugar conformation. The physicochemical properties of the nucleobases in the conformationally constrained nucleosides found to be depended on the site and ring-size of the fused system.

The incorporation of azetidine modified nucleotide units into 15mer ONs lowered the affinity toward the complementary RNA. However, they performed better than previously reported isosequential 1',2'-oxetane modified analogues. Whereas aza-ENA-T modification incorporated into ONs significantly enhanced affinity to the complementary RNA. To evaluate the antisense potential of azetidine-T and aza-ENA-T modified ONs, they were subjected to RNase H promoted cleavage as well as tested towards nucleolytic degradation. Kinetic experiments showed that modified ONs recruit RNase H, however with lower enzyme efficiency due to decreased enzyme-substrate binding affinity, but with enhanced turnover number. Both, azetidine-T and aza-ENA-T modified ONs demonstrated improved 3'-exonuclease stability in the presence of snake venom phosphodiesterase and human serum compared to the unmodified sequence.

Oligodeoxynucleotides (ODNs) containing pyrene-functionalized azetidine-T (Aze-pyr **X**) and aza-ENA-T (Aza-ENA-pyr **Y**) modifications showed different fluorescence properties. The **X** modified ODNs hybridized to the complementary DNA and RNA showed variable increase in the fluorescence intensity depending upon the nearest-neighbor at the 3'-end to **X** modification (dA > dG > dT > dC) with high fluorescence quantum yield. However, the **Y** modified ODNs showed a sensible enhancement of the fluorescence intensity only with complementary DNA. Also, the **X** modified ODN showed decrease (~37-fold) in the fluorescence intensity upon duplex formation with RNA containing a G nucleobase mismatch opposite to the modification site, whereas a ~3-fold increase was observed for the **Y** modified probe.

Keywords: antisense oligonucleotides, conformationally constrained nucleosides, azetidine, aza-ENA, target affinity, RNase H, exonuclease stability, pyrene-functionalized nucleotides, fluorescence, mismatch discrimination

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