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Special Focus Review

Locked nucleic acid as a novel class of therapeutic agents

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Abbreviations: LNA, locked nucleic acid; mRNA, messenger RNA; AONs, antisense oligonucleotides; HIV, human immunodeficiency virus; RNAi, RNA interference; siRNA, small interfering RNA; sisiRNA, small internally segmented interfering RNA; miRNA, microRNA; TAR, trans-activation responsive element; SELEX, systematic evolution of ligands by exponential enrichment

Key words: LNA, oligonucleotides, antisense, siRNA, miRNA, aptamers, RNA targeting

Locked Nucleic Acid (LNA) is a nucleic acid analogue with unprecedented binding affinity and excellent specificity toward complementary RNA and DNA oligonucleotides. The remarkable properties of LNA have led to applications within various gene silencing strategies both in vitro and in vivo. In the present review, we highlight the uses of LNA for regulation of gene expression with emphasis on RNA targeting.

Introduction

Nucleic acid derivatives have gained a lot of interest for the treatment of many diseases, and the first oligonucleotide to enter the clinic was Vitravene®,[1] an antisense oligonucleotide for the treatment of cytomegalovirus infection. The use of chemically modified nucleic acids is needed as naturally occurring DNA or RNA have some limitations like poor RNA binding affinity, inefficient cellular uptake and very limited nuclease resistance. Among the numerous modifications known, LNA has shown broad usefulness within chemical biology.[2-9]

LNA: Structural Features and Key Properties

LNA (Fig. 1) contains a ribose ring which is locked by a O2'-C4'-methylene linkage, imposing conformational restriction to adopt an *N*-type sugar puckering.[2-5,10] Structural investigation of LNA oligonucleotides by NMR spectroscopy revealed their similarities with natural nucleic acid duplexes, and confirmed the RNA mimicking structures adopted by LNA.[11,12]

LNA offers key properties needed for successful therapeutic exploitation of oligonucleotides, including (1) unprecedented binding affinity towards RNA (and DNA), (2) excellent base pairing specificity, (3) high bio-stability (resistance towards nucleolytic degradation, (4) low toxicity (at least for many LNA oligonucleotides) in animals and (5) convenient chemistry for manufacturing and modification.

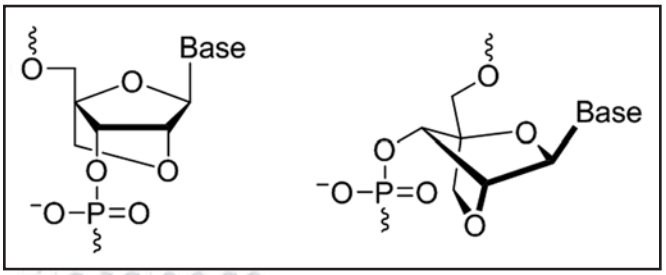

Figure 1. Two representations of an LNA monomer. The structure is shown schematically to the left, whereas the structure and its locked sugar ring conformation is shown in three dimensions to the right.

LNA in Antisense Technologies

'Antisense' is a term normally used for single stranded nucleic acid based approaches that interfere with the processing of RNA in a sequence selective manner. For effective modulation of gene expression, the advantages of synthetic oligonucleotides are exploited through binding of antisense oligonucleotides (AONs) to a specific mRNA or pre-mRNA by Watson-Crick base pairing. Upon binding, the oligonucleotide can modulate RNA processing, inhibit translation, or promote degradation. Antisense-based gene silencing strategies using LNA-containing oligonucleotides have already been the topic of detailed reviews.[9,13,14]

LNA-antisense experiments have largely been focused on mRNA inhibition by RNase H recruitment, though non RNase H mechanisms have also been reported. These studies highlight a broad potential of LNA oligonucleotides for effective gene silencing both in vitro and in vivo. Recently, Jacobsen et al. for example showed that LNA AONs are effective inhibitors of HIV-1 expression.[15]

DNAzymes are catalytically active DNA molecules that are able to cleave RNA in a sequence-specific manner after binding to complementary sequences. Studies conducted using LNA-modified DNAzymes, termed LNAzymes showed an enhanced efficiency of

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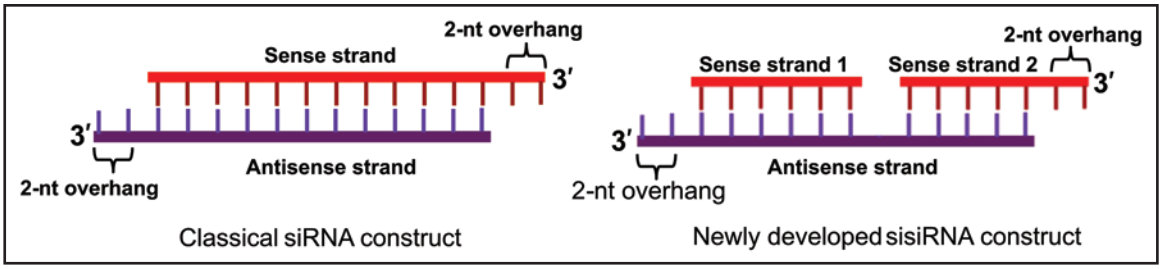

Figure 2. Schematic representations of siRNA and sisiRNA constructs. In a classical siRNA construct, the sense strand complementary to the antisense strand is single stranded. In a sisiRNA construct, a nick has been introduced in the sense strand which is therefore constituted by two separate short strands complementary to the antisense strand. Both constructs are depicted with two nucleotide 3'-overhangs and with a shortened duplex region; this in typical siRNA and sisiRNA constructs contains 19 base pairs.

RNA cleavage.[16] In line with previous findings, Jacobsen et al. reported efficient inhibition of HIV-1 expression by targeting LNAzymes to functionally selected binding sites,[15] whereas targeting of miRNAs by using LNAzymes has recently been reported by Maiti and co-workers.[17]

RNA interference (RNAi) has been developed as a highly potent approach to knock down gene expression.[18] RNAi mediated by small interfering RNAs (siRNAs) can target complementary mRNA and induce its degradation upon incorporation of the siRNA into the RNA-induced silencing complex (RISC). siRNAs themselves are candidates for incorporation of modified nucleotides for improved bio-stability and effective RNA targeting. In this direction, the use of LNA-modified siRNA, termed siLNA, has been investigated. Recently, Mook et al. evaluated the effect of LNA-modified siRNA both in vitro and in vivo.[19] They showed that minimal LNA-modifications at the 3'-end of siRNAs are effective to stabilize the siRNA, and that multiple LNA-modifications may lead to decreased efficacy in vitro and in vivo. The study also highlighted reduced off-target gene regulation when using LNA-modified siRNA (compared to the unmodified siRNA). Very recently, Bramsen et al. introduced a three stranded siRNA construct termed 'small internally segmented interfering RNA' (sisiRNA, Fig. 2) in which the antisense strand is complexed with two short sense strands of approximately 10–12 nt in length.[20] In the sisiRNA approach only the antisense strand is functional as the nick completely eliminates unintended mRNA targeting by the sense strand. LNA nucleotides were incorporated to stabilize the sisiRNA constructs which proved efficient for gene silencing upon transfection into a H1299 lung carcinoma cell line.

MicroRNAs (miRNAs) constitute a class of short regulatory RNAs (~22 n) that control gene expression post-transcriptionally during development, differentiation and metabolism.[21,22] Similar to classical AONs developed for the inhibition of coding RNAs, synthetic oligonucleotides are the only rational approach for specific inhibition of individual miRNAs and therefore have the potential to be developed into an important new class of miRNA targeting drugs. Elmén et al. have recently described effective miRNA silencing in non-human primates by short LNA-modified oligonucleotides (LNA antimiRs; LNA/DNA mixmers) designed to target liver-expressed miR-122.[23]

LNA Aptamers

Aptamers[24-26] are short DNA or RNA oligonucleotide sequences which can bind tightly to a specific molecular target because of their ability to form three dimensional structures. The remarkable properties and applications of LNA oligonucleotides highlighted above substantiate the desire to explore aptamers composed of LNA-modified nucleotides to rival aptamers composed of unmodified RNA or DNA. Darfeuille et al. introduced LNA modification of an already selected RNA aptamer targeted to the TAR RNA element of HIV-1.[27,28] Surface plasmon resonance (SPR) experiments identified LNA/DNA mixmers binding to TAR RNA with a dissociation constant in the low nanomolar range. Schmidt et al. later described the capability of LNA nucleotides to improve the in vivo stability of aptamers.[29] Further work showed that an aptamer modified with LNA nucleotides targeting the TAR RNA element of HIV-1 displayed good binding properties and competed with the viral protein Tat for binding to TAR.[30] Furthermore, the same group later reported a TAR RNA aptamer as an LNA/2'-*O*-methyl RNA mixmer which displayed improved HIV-1 TAR element binding.[31] All of these results underscore the desire to develop procedures to allow evolution of LNA aptamers by SELEX-based[32,33] processes.

Future Prospects

The remarkable properties of LNA with respect to high affinity and specificity make this analogue unique for applications in molecular biology research, biotechnology and RNA targeting. We believe that LNA constructs will be important molecules as the prospects of nucleic acid based drugs will be realized. This is underlined by the recent reports on efficient miRNA targeting using LNA probes and the fact that LNA/DNA mixmers have been forwarded into human clinical trails.[34] We are attempting to develop ways of evolving LNA-based aptamers and have recently published reports on enzymatic methods for synthesis of LNA containing oligonucleotides.[35,36]

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