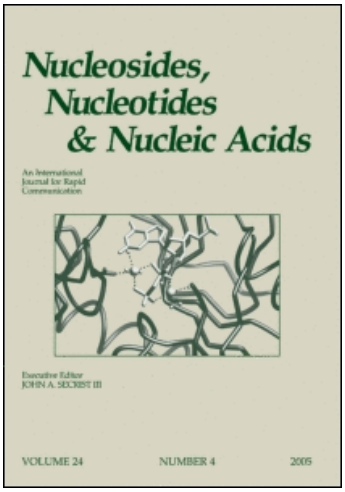

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LNA and α-L-LNA: Towards Therapeutic Applications

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ABSTRACT

LNA and α-L-LNA are promising candidates for the development of efficient oligonucleotide-based therapeutic agents. Here, we report dose-dependent inhibition of HIV-1 Tat-dependent *trans* activation by a 12-mer chimeric α-L-LNA/DNA oligomer. This oligomer exhibits a dose-dependency similar to that of the corresponding 12-mer chimeric LNA/2′-*O*-Me-RNA oligomer. In addition, we show that incorporation of α-L-LNA or LNA monomers into each of the two binding arms of a "10–23" DNAzyme markedly increases cleavage of the target RNA.

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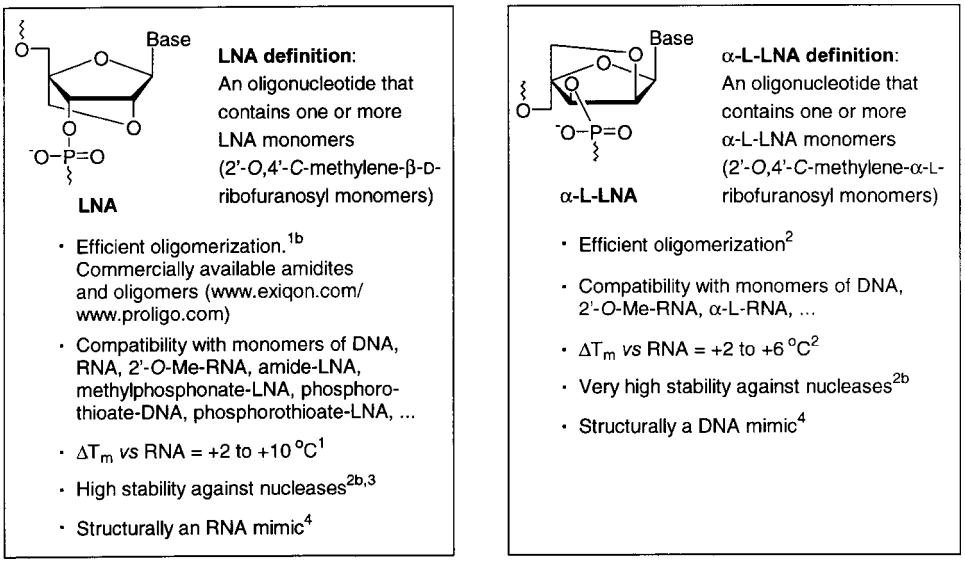
INTRODUCTION

LNA[1] (locked nucleic acid) and α-L-LNA[2] (α-L-*ribo* configured locked nucleic acid) are diastereoisomeric oligonucleotide analogues, both of which display high-affinity recognition of RNA. More precise definitions and key characteristics of these oligomers are given on the following page, together with the structures of LNA and α-L-LNA monomers.

An increase in the A-like character of the LNA:RNA hybrids is observed by high-resolution NMR when the LNA content of the chimeric LNA/DNA strands is augmented. The shift to A-like character indicates that LNA monomers influence the sugar conformations of neighbouring DNA monomers, and this correlates well with the observation that the increase in helical thermostability *per* LNA *monomer* relative to native reference duplexes reaches a maximum for LNAs containing less than 50% LNA monomers.[1,3,4] The A-type conformation adopted by a partly modified nonamer LNA:RNA hybrid[4] corroborates such a "structural saturation" effect. Further indirect support comes from the gradual increase in helical thermostability *per* α-L-LNA *monomer* observed for chimeric α-L-LNA/DNA strands[2] in which no conformational steering of the sugar rings of the DNA monomers are induced by the presence of the α-L-LNA monomers.[4c]

Encouraging reports on the therapeutic potential of LNA have appeared recently.[5] For recruitment of RNase H, LNA/DNA/LNA gapmers should be used, and, if required, phosphorothioate linkages can be incorporated.[3,5b] The high-affinity binding of cognate RNA makes it possible to achieve a steric block with very short LNAs, and this has been demonstrated for an LNA/2′-*O*-Me-RNA chimera,[5c] LNA/DNA chimera[5d] and fully modified LNA.[5d]



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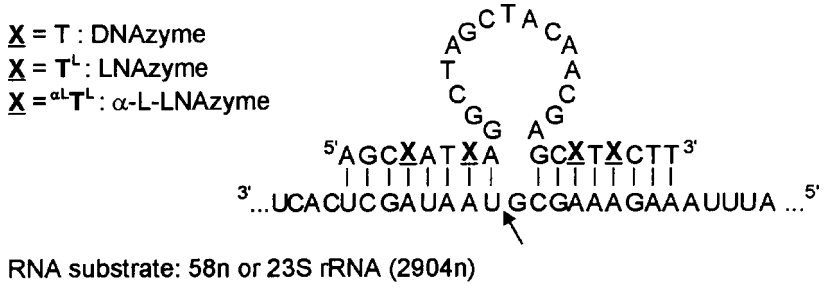
RESULTS AND DISCUSSION

α-L-LNA as a steric blocking agent. The HIV-1 *trans*-activation responsive element (TAR) is a 59-nucleotide stem–loop RNA that interacts with the HIV *trans*-activator protein Tat as well as with other cellular factors to stimulate transcriptional elongation from the viral long terminal repeat (LTR).[6] Inhibition of these interactions therefore blocks full-length HIV transcription and hence viral replication.[7] We have earlier applied 12-mer LNA oligomers as steric blockers of the HIV-1 TAR domain.[5c] A chimeric LNA consisting of five LNA monomers and seven 2′-*O*-Me-RNA monomers inhibited both Tat-dependent transcription in vitro, as well as Tat-dependent HIV-1 LTR *trans* activation in HeLa cells, in a dose-dependent, sequence-specific manner.[5c] Recently, we studied the corresponding 12-mer chimeric α-L-LNA/DNA (5′-$\underline{\mathbf{C}}$T$\underline{\mathbf{C}}$C$\underline{\mathbf{C}}$AGG$\underline{\mathbf{C}}$T$\underline{\mathbf{C}}$A-FAM; $\underline{\mathbf{C}}$ = 5-methyl-cytosin-1-yl α-L-LNA monomer). Both in vitro and in HeLa cells, this α-L-LNA/DNA oligomer displayed dose-dependent activities very similar to those reported for the chimeric LNA/2′-*O*-Me-RNA oligomer.[5c]

LNAzymes. Deoxyribozymes (DNAzymes) are catalytically active DNA molecules that can function as specific RNA endonucleases. The ''10–23'' DNAzyme is a 31-nucleotide long oligomer consisting of a 15-nucleotide catalytic core between two binding arms.[8] Incorporation of LNA or α-L-LNA monomers into the binding arms of the DNAzyme yielded an LNAzyme and an α-L-LNAzyme (see figure below; $\mathbf{T^L}$ = thymin-1-yl LNA monomer; $^{\alpha L}\mathbf{T^L}$ = thymin-1-yl α-L-LNA monomer; the arrow points at the cleavage site in the RNA substrates). In comparison with the corresponding DNAzyme, these LNAzymes showed strongly enhanced efficiency of RNA cleavage (single- and multiple-turnover conditions), when the target was presented both in a synthetic 58-nucleotide RNA, and in the much larger, naturally occurring 23S rRNA (in the latter case, the cleavage site was situated in a highly structured RNA region).[9]

CONCLUSION

The efficient inhibition of Tat-dependent transcription in vitro and in HeLa cells by LNA and α-L-LNA oligomers, and the enhanced cleavage obtained for the LNAzymes, strongly indicate improved access of LNA-type oligomers to RNA targets.



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This has important implications for the general application of LNAs as tools for gene regulation.

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