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The α-L-*ribo*-isomers of RNA and LNA (locked nucleic acid)

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ABSTRACT

The phosphoramidite approach has been used for the automated synthesis of α-L-LNA, α-L-RNA, and oligomers composed of mixtures of α-L-LNA, α-L-RNA and DNA monomers. Binding studies revealed very efficient recognition of single-stranded DNA and RNA target oligonucleotide strands. α-L-LNAs were shown to be significantly stabilized towards 3'-exonucleolytic degradation. Duplexes formed between RNA and α-L-LNA induced *E. coli* RNase H-mediated RNA cleavage, albeit very slow, at high enzyme concentration.

INTRODUCTION

Following the discovery of LNA (locked nucleic acid, β-D-*ribo* isomer, Figure),[1,2] we have recently introduced the stereoisomeric analogue termed α-L-LNA (α-L-*ribo* configured locked nucleic acid, α-L-*ribo* isomer, Figure).[3] By virtue of their dioxabicyclo[2.2.1]heptane skeletons, the conformations of the furanose rings of an LNA monomer and an α-L-LNA monomer are efficiently locked in an *N*-type and an *S*-type (or *N*-type, C3'-*endo*)[4] conformation, respectively (Figure). In preliminary experiments in homothymine sequence contexts, the helical thermostabilities of α-L-LNA:DNA and α-L-LNA:RNA duplexes have been shown to approach those of the corresponding LNA:DNA and LNA:RNA duplexes.[3] We have subsequently studied the hybridization properties of partly and fully modified 9-mer and 11-mer α-L-LNA (and α-L-RNA) sequences towards complementary single-stranded DNA, RNA, LNA and α-L-LNA complements. In addition, *in vitro* studies on the 3'-exonucleolytic stability and ability to activate RNase H have been performed.[5]

RESULTS AND DISCUSSION

Hybridization studies. In hybridization studies towards complementary DNA and RNA, high-affinity recognition was obtained for α-L-LNA. For example, stereoirregular α-L-LNA composed of a mixture of α-L-LNA monomers [α-L-*ribo* configuration] and DNA monomers [β-D-*ribo* configuration] were shown to display ΔT_m values of +1 to +3 °C towards DNA and +4 to +5 °C towards RNA when compared to the corresponding unmodified DNA:DNA and DNA:RNA reference duplexes. Furthermore, α-L-LNA:α-L-LNA and α-L-LNA:LNA, like LNA:LNA, have been shown to constitute exceptionally stable duplex structures. Reduced binding affinities were observed for the corresponding α-L-RNA oligomers, but the ability of stereoirregular pentofuranosyl nucleic acids to hybridize with complementary RNA was demonstrated.

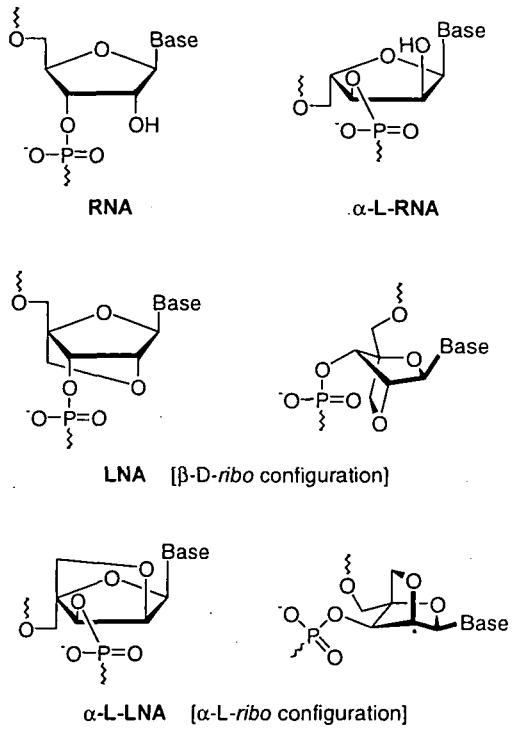

Figure. The structures of the nucleotide monomers of RNA, α-L-RNA, LNA and α-L-LNA. Also shown are the locked *N*-type (C3'-*endo*/^3E) and "*S*-type" (C3'-*exo*/$_3E$; *N-type*, C3'-*endo*, ^3E)[4] furanose conformations of an LNA and an α-L-LNA monomer, respectively.

3'-Exonucleolytic stability *in vitro*. α-L-LNAs, composed either of alternating α-L-LNA and DNA monomers (mixmeric α-L-LNA) or entirely of α-L-LNA monomers, were shown to be significantly stabilized towards 3'-exonucleolytic degradation. Experimentally, a solution of the oligonucleotides (~ 0.2 OD) in 2 mL of a buffer (0.1 M Tris-HCl; pH = 8.6; 0.1 M NaCl; 14 mM $MgCl_2$) was treated with 1.2 U snake venom phosphodiesterase (30 μL of a solution in the following buffer: 5 mM Tris-HCl; pH = 7.5; 50 % glycerol (v/v)) at 25 °C. The increase in absorbance at 260 nm during digestion was used for qualitative determinion of 3'-exonucleolytic stability.

Cleavage of α-L-LNA/RNA hybrids by *Escherichia coli* RNase H. Duplexes formed between RNA and fully modified α-L-LNA or mix-meric α-L-LNA induced *E. coli* RNase H-mediated cleavage, albeit very slow, of the RNA targets at high enzyme concentration. Thus, significant cleavage of the RNA strand was only seen after prolonged exposures, *i.e.* > 4 h. By contrast, no cleavage was detected with the corresponding LNA/RNA duplexes. Experimentally, the radiolabeled ribonucleotide target strand (2.5 pmol) and the complementary oligonucleotide (5 pmol) were incubated at 37 °C for 15 min in a total volume of 25 μL (pH 7.5) containing 10 mM Tris-HCl, 25 mM KCl and 0.5 mM $MgCl_2$. Cleavage reactions were initiated by addition of 10 U RNase H (*E. coli* ribonuclease H, Life Technologies) to the mixture while control tubes received no enzyme. Aliquots were taken at appropriate time intervals, mixed with an equal volume of stop mix (EDTA 50 mM, xylene cyanol FF 0.1%, and bromophenol blue 0.1% in formamide 90%) and chilled in a dry ice/acetone bath. Samples were analyzed by denaturing 20% polyacrylamide gel electrophoresis (PAGE) containing urea (50%) with TBE buffer at 1000 V for 1.25 h followed by visualization by phosphorimaging (Packard Cyclone, OptiQuant software).

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2. We have defined LNA as an oligonucleotide containing one or more 2'-*O*,4'-*C*-methylene-β-D-ribofuranosyl nucleotide monomer(s).
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4. The furanose conformation of an α-L-LNA monomer could alternatively be assigned as *N*-type (C3'-*endo*, ^3E) because of its L-configuration. However, for a direct comparison with the conformations of the natural DNA/RNA monomers and the parent LNA monomers, the furanose conformation of an α-L-LNA monomer is considered herein as equivalent to an *S*-type conformation.
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