α-L-LNA (α-L-*ribo* Configured Locked Nucleic Acid) Recognition of RNA. A Study by NMR Spectroscopy and Molecular Dynamics Simulations

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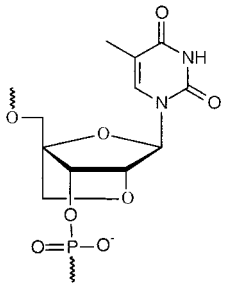
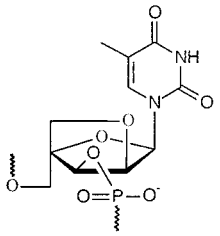
Efficient antisense oligonucleotides should bind to their cognate mRNA to suppress translation of the given mRNA.[1] The antisense oligonucleotide (AO) must be resistant toward exonucleolytic degradation and possess enhanced affinity toward the cognate mRNA. Furthermore, the AO:RNA hybrid structure should be unperturbed relative to the unmodified DNA:RNA hybrid to invoke RNase H recognition with subsequent degradation of the RNA strand.[2] Recently, LNA, a conformationally locked nucleic acid analogue, has been introduced.[3] LNA possesses a number of pleasing features, e.g. substantially increased helical thermostability for both LNA:DNA duplexes and LNA:RNA hybrids, and resistance toward exonucleolytic degradation. However, LNA, as an RNA mimic, promotes the A-like character of a deoxyribose strand.[4] This feature is detrimental to RNase H recognition of LNA:RNA hybrids as RNase H is known to recognize DNA:RNA hybrids adopting an overall duplex geometry intermediate of A- and B-forms. As RNase H binds to the minor groove of nucleic acids it has been proposed that indeed the intermediate minor groove width of DNA:RNA hybrids is the key element in the recognition process.[2a]



LNA, $\mathbf{T^L}$
(β-D-*ribo* configuration)



α-L-LNA, $^{\alpha L}\mathbf{T^L}$
(α-L-*ribo* configuration)

Recently, a number of conformationally locked isomers of LNA have been synthesized and evaluated.[5] Of these, α-L-LNA caught most attention with helical thermostability toward complementary DNA and RNAs only second to that of LNA.[6] In the present work, we have investigated a partly modified α-L-LNA:RNA hybrid by NMR spectroscopy and molecular dynamics (MD) simulations, and subsequently, we have studied the fully modified hybrid of identical base composition by an MD simulation.

An NMR sample the of d($C_1{}^{\alpha L}\mathbf{T^L}_2G_3A_4{}^{\alpha L}\mathbf{T^L}_5A_6{}^{\alpha L}\mathbf{T^L}_7G_8C_9$):r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) hybrid was prepared by standard procedures.[4a] The NOESY- (with mixing times of 80 ms and 250 ms), DQF-COSY-, TOCSY-, watergate NOESY-, and $^1H-^{13}C$ HSQC-spectra at either 500 or 800 MHz were assigned by conventional methods.[7] The 1D spectrum exhibits sharp lines (line widths 2−3 Hz), and in the 2D spectra there are no traces of single strands at 25 °C. The NOESY spectra in H_2O and D_2O display the characteristics of a right-handed nucleic acid duplex with all the nucleobases in the anti conformation and partaking in Watson−Crick base pairing. Furthermore, the spectra are indicative of a regular helical conformation of predominantly A-form as shown by interstrand adenine H2 cross-peaks. Selective DQF-COSY and E-COSY spectra were acquired and the values of the deoxyribose coupling constants were determined. Ensuing, the sugar conformations of the deoxyriboses were identified using a method previously reported.[4a,d]

The α-L-LNA nucleotides are locked in an *N*-type ($_3$E) conformation.[8] According to the Karplus equation of Donders et al.,[9] this sugar conformation yields $J_{H1'H2'}\simeq 3$ Hz. This is in qualitative agreement with measurements of antiphase splittings in a DQF-COSY spectrum. All but terminal ribose sugars in the RNA strand have $J_{H1'H2'} < 2$ Hz and thus are in pure *N*-type conformations. For terminal riboses we observe slightly larger $J_{H1'H2'}$ coupling constants showing *N/S* sugar equilibria are pertaining for these sugars.

The six remaining deoxyriboses were thoroughly analyzed by iterative simulations of the selective DQF-COSY and E-COSY spectra. The fractions of *N*- and *S*-type sugar conformation found by analysis with the PSEUROT program are listed in Table 1.[10] It is observed that the introduction of α-L-LNA nucleotides perturbs the sugar equilibria only slightly. Except for the terminal residues, only G8 is significantly altered compared with the unmodified hybrid. In contrast, the introduction of LNA nucleotides completely alters the sugar conformations of all deoxyriboses in the strand. Thus, this analysis of sugar conformations points to an α-L-LNA:RNA duplex structure much like that of the unmodified DNA:RNA hybrid. This observation is supported by the similarity of the CD spectra of the DNA:RNA and the α-L-LNA:RNA hybrids.

Molecular Dynamics (MD) simulations of 1 ns duration were performed of the partly modified d($C_1{}^{\alpha}\mathbf{LT^L}_2G_3A_4{}^{\alpha}\mathbf{LT^L}_5A_6{}^{\alpha}\mathbf{LT^L}_7G_8C_9$):r($G_{10}C_{11}A_{12}U_{13}A_{14}U_{15}C_{16}A_{17}G_{18}$) duplex and the fully modified hybrid of the identical base sequence using AMBER6[11] with standard A-form starting geometry. For reference, an MD simulation was also performed of the unmodified hybrid. Each trajectory in the MD simulations was stable within 1 ns, with RMSDs along the trajectories between 1.6 and 1.8 Å for nonterminal residues. For each trajectory the final 500 ps were used for analysis.

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(6) We measured the melting temperature of the partly modified d($C^{\alpha L}\mathbf{T^L}$-$GA^{\alpha L}\mathbf{T^L}A^{\alpha L}\mathbf{T^L}$GC):r(GCAUAUCAG) hybrid to be 44 °C under standard conditions;[4a] this is an increase of $\Delta T_m = 5.6$ °C per modification compared with the unmodified hybrid ($T_m = 27$ °C).
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Table 1. The Sugar Conformations of d(C$^{\alpha L}$T^{L}GA$^{\alpha L}$T^{L}A$^{\alpha L}$T^{L}GC):r(GCAUAUCAG)[a]

	α-L-LNA:RNA			DNA:RNA[4a]			LNA:RNA[4a]		
	% N	P_N [b]	P_S	% N	P_N [b]	P_S	% N	P_N [b]	P_S [b]
C1	25(13)	32	159	64(10)	−10	169	47(8)	−16	152
T2				16(12)		179			
G3	37(10)	10	156	23(10)		162	>90		
A4	24(9)		153	38(11)	−15	182	>90		
T5				31(13)	4	170			
A6	30(8)	−13	144	27(12)		179	>90		
T7				20(14)		164			
G8	46(8)	−7	172	15(11)		184	>90		
C9	43(12)	20	182	36(19)	39	156	70(10)	−7	148

[a] Standard deviations are shown in brackets. No analysis of locked sugars was performed. [b] If the mole fraction of the conformer was below 30%, the pseudorotation angle was not considered to be determined with sufficient accuracy.

Table 2. Backbone Angles in the Deoxyribose Strands of the Three Hybrids in the MD Simulations

	DNA:RNA	partly modified α-L-LNA:RNA[a]	fully modified α-L-LNA:RNA
α	g$^-$	g$^-$, t (G3,A6,G8)	t
β	t	t	t
γ	g$^+$	g$^+$, t (T2, T5, T7)	t
δ	g$^+$ [b]	g$^+$,[b] g$^-$ (T2, T5, T7)	g-
ϵ	t	t	t
ζ	g$^-$	g$^-$, g$^+$ (T2, T5, T7)	g+

[a] For the partly modified hybrid, nucleotides with angles deviating from the uniform values are shown in brackets. [b] Broad distributions due to sugar repuckering.

Examination of the sugar puckers in the trajectory of the unmodified hybrid reveals deoxyriboses with a broad distribution of the pseudorotation angle, the most populated conformations being around $P \simeq 125-150°$ with the distributions stretching toward the *N*-type range. In the partly modified α-L-LNA:RNA hybrid, the three modified nucleotides have a very narrow distribution near $P \simeq 14°$, confirming the locked nature of these sugar rings. The remaining deoxyriboses have broader distributions of their sugar puckers, with maxima of $P \simeq 125-150°$, however, with G3, A6, and G8, the 3′-flankers of the modifications, displaying slightly sharper distributions than C1, A4, and C9. Thus it seems that the α-L-LNA nucleotides slightly organize the 3′-flanking sugar rings in a more *S*-like conformation. In the fully modified hybrid, uniformly tight distributions, centered at $P = 14°$, are observed for the α-L-LNA strand. In this, as well as in the other hybrids, the riboses adopt *N*-type conformations showing little flexibility except at the termini of the strands.

From inspection of the backbone angles of the hybrids, it is evident that the malleable deoxyribose backbone adjusts itself so as to present the nucleobases of the α-L-LNA nucleotides for efficient stacking and Watson−Crick base pairing. The deoxyribose backbone angles from the MD simulations are presented in Table 2. The manner in which the backbone accommodates the modifications is evident. This rearrangement of the sugar−phosphate backbone is mirrored in the intrastrand phosphorus distances. The distances across α-L-LNA nucleotides have values of 7.0−7.1 Å, while the remaining DNA distances in the modified hybrids are in the range 6.6−6.8 Å, reminiscent of the 6.5−6.8 Å found for deoxyriboses in the unmodified hybrid. Intrastrand phosphorus distances of ∼7 Å are normally found in B-form dsDNA duplexes, as the C2′-*endo* sugar pucker favors this distance, while C3′-*endo* sugar puckering favors a shorter distance of ∼6 Å, as is indeed found in the ribose strands of the three hybrids.

Thus the conformational alteration of the deoxyribose strand imposed by the α-L-LNA modifications is toward a more B-like hybrid. This observation is supported by a comparison of minor groove widths, with an average value of 14.9 Å for the unmodified hybrid and 14.3 and 14.0 Å for the partly and the fully modified α-L-LNA:RNA hybrids, respectively. Though slightly narrower than in the unmodified hybrid, 14.0 Å still represents a value intermediate of A-form (16.8 Å) and B-form (11.0 Å) minor groove widths.

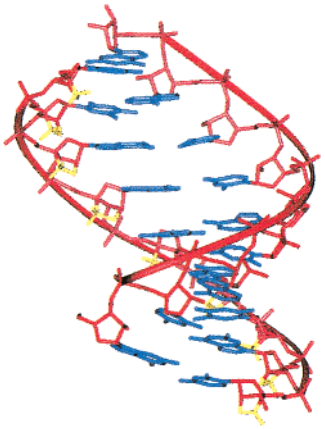

Figure 1. View into the major groove of the average MD structure of the fully modified α-L-LNA:RNA hybrid. The nucleobases are shown in blue, the sugar−phosphate backbone in red, and the modified 2′-*O*,4′-*C* methylene bridge in yellow.

A thorough examination of the helical parameters, calculated by the CURVES program,[12] of the last 500 ps of each of the trajectories reveals no changes attributable to the introduction of α-L-LNA modifications. On the contrary, some probably sequence specific variations can be identified along the duplexes for each of the hybrids.

The overall appearance of the unmodified hybrid is similar to the NMR high-resolution structure determined by us,[13] though the minor groove width is somewhat larger in the MD simulation, 14.9 Å compared with 14.5 Å in the NMR structure. Furthermore, we observe a distinct narrowing of the minor groove in the center of the duplex in the NMR structure of the unmodified hybrid. This is not seen in the MD simulation of the DNA:RNA hybrid where the minor groove is quite uniform in its appearance.

The MD simulation of the partly modified α-L-LNA:RNA hybrid yields results in accordance with the NMR experiments, i.e. repuckering of the deoxyriboses, locked α-L-LNAs and riboses, and an overall regular A-like duplex structure. In both the partly and the fully modified hybrids, the 2′-*O*,4′-*C* methylene bridge is positioned on the brim of the major groove with ample space for its protrusion (Figure 1).

In comparison, LNA can be dubbed a paragon A-type mimic[4] while α-L-LNA appears to be a perfect B-type mimic. Given the results detailed above and the knowledge of RNase H, it is conceivable that either partly or fully modified α-L-LNA:RNA hybrids can act as RNase H substrates. With the substantial helical stability gained by the α-L-LNA modifications, we therefore propose α-L-LNA to be a most interesting antisense molecule.

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Supporting Information Available: Experimental details, part of the NOESY spectrum of the duplex, experimental and theoretical DQF- and E-COSY spectra, and figures of sugar pucker distributions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Results to be published.