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Parallel nucleic acid recognition by the LNA (locked nucleic acid) stereoisomers β-L-LNA and α-D-LNA; studies in the mirror image world

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Two LNA (locked nucleic acid) stereoisomers (β-L-LNA and α-D-LNA) are evaluated in the mirror-image world, that is by the study of two mixed sequences of LNA and α-L-LNA and their L-DNA and L-RNA complements. Both are found to display high-affinity RNA-recognition by the formation of duplexes with parallel strand orientation.

Conformationally restricted oligonucleotides have enabled high affinity recognition of DNA and RNA.[1,2] In the LNA-family of stereoisomeric *locked nucleic acid* analogues the nucleoside monomers are locked in *N*-type conformations (Fig. 1),[3–10] and both LNA[3–5] and α-L-LNA sequences[6–8] (*i.e.* LNA with β-D- and α-L-configurations, respectively)‡ have demonstrated unprecedented antiparallel hybridisation with both DNA and RNA complements. This duplex stabilisation is also evident for mixmers of LNA or α-L-LNA nucleotides and natural 2′-deoxyribonucleotides. In order to investigate the scope of parallel nucleic acid recognition we recently introduced α-LNA (or α-D-LNA; LNA with α-D-configuration).‡[9,10] The formation of parallel duplexes has been reported for α-DNA (*i.e.* the α-anomer of DNA) with complementary DNA and RNA,[11–13] and subsequently, mixed fully modified pyrimidine α-LNA sequences were found to recognise complementary RNA, but not DNA, forming strong parallel stranded duplexes.[10]

With the furanose rings locked in *N*-type (C-3′-*endo*) conformations, LNA is essentially a perfect RNA-mimic,[3–5] whereas the situation for α-LNA is more complicated. Thus, in LNA–DNA mixmers, the LNA-monomers have been found to tune the neighbouring DNA-monomers towards *N*-type conformations thereby inducing the formation of overall A-type duplexes.[14] On the other hand, it is unlikely that α-configured nucleosides exist in a perfect *N*-type conformation due to the reverse influence of the anomeric effect, and α-LNA monomers are unable to tune neighbouring α-DNA monomes towards *N*-type conformations. Furthermore, α-LNA is not an obvious conformational mimic of either α-DNA or α-RNA.

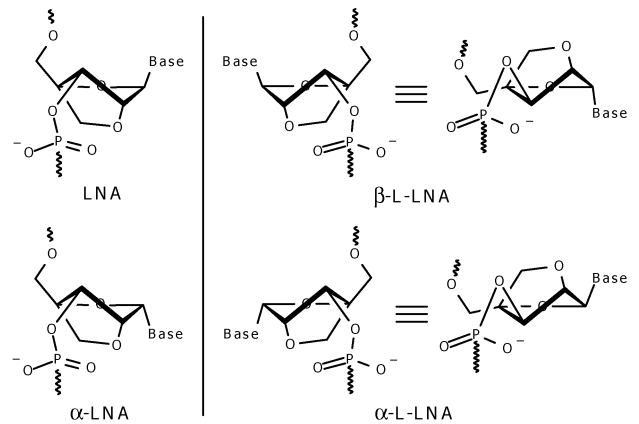

Fig. 1 Structures of LNA-stereoisomers.

NMR studies of duplexes containing α-L-LNA sequences and complementary DNA or RNA have led to the conclusion that this LNA stereoisomer can be regarded as a DNA-mimic.[15,16] With LNA being an RNA mimic and α-L-LNA being a DNA mimic, we deduce the "α-anomer" of α-L-LNA, *i.e.* β-L-LNA, to be an α-DNA mimic and subsequently an even stronger candidate for parallel nucleic acid recognition than α-LNA. In this communication we explore this hypothesis by comparing the hybridisation properties of β-L-LNA and α-LNA sequences of mixed base composition. However, the synthesis of β-L-LNA monomers has not been realised, and the studies were performed with LNA and α-L-LNA in the mirror-image world. We have previously studied oligothymidylate sequences by this strategy[17] but here we introduce mixed sequences allowing conclusions about general hybridisation behaviour including strand orientation.

A decamer α-L-LNA sequence (Table 1) was prepared from the appropriate thymine, adenine and 5-methylcytosine phosphoramidite building blocks[8] on a universal support in order to obtain a completely modified α-L-LNA sequence. The LNA-sequence of the same base composition was obtained in a similar way by a standard LNA-synthesis protocol.[4] Four complementary L-DNA and L-RNA sequences were designed as both parallel and antiparallel complements as well as with single A/T or A/U mismatches.§ Standard DNA and RNA sequences were used as reference strands (Table 1). The applied standard sequence was designed as a non-self-complementary sequence.

As expected, both the α-L-LNA sequence and the LNA sequence were found to recognise their antiparallel DNA and RNA complements with very high affinity (T_m = 66–87 °C, Table 1) and with the expected selectivity for match over mismatch sequences (ΔT_m = −16 °C). With parallel complements, the situation was more complicated. Thus, complexes with mismatch sequences were more stable than with match sequences with T_m′s up to 51 °C for the LNA:RNA complex. However, we deduce these complexes to be antiparallel wobble structures rather than regular parallel duplexes. When the α-L-LNA sequence was mixed with complementary L-configured DNA and RNA sequences, the observations earlier made for an α-LNA pyrimidine sequence[10] were supported. Thus, no complex could be detected with either the antiparallel complements or with parallel complementary DNA. With the parallel RNA complement, a melting temperature of 44 °C was observed. When the LNA sequence was mixed with the L-configured complements, the general properties of the new β-L-LNA analogue were examined. No complexes were detected with antiparallel DNA and RNA complements, whereas stable duplexes with both parallel DNA and RNA (with almost identical thermal stabilities, 42 °C and 44 °C, respectively) were formed. The base-pairing selectivity, which was questioned in our first study on an oligothymidylate sequence,[17] was here confirmed to be satisfactory (−15 °C and −12 °C, respectively) for a mixed sequence.

Thus, β-L-LNA and α-LNA demonstrate equal strength in parallel RNA-recognition, but only the former forms a duplex with

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Table 1 Hybridisation data for LNA, α-LNA, α-L-LNA and β-L-LNA sequences and reference strands with parallel and antiparallel DNA and RNA complements

	α-L-LNA[b] T_m/°C[a]	LNA[b] T_m/°C[a]	DNA[b] T_m/°C[a]	RNA[c] T_m/°C[a]
DNA (p)[d]	— [g]	—	—	—
mm[f]	—	27	—	—
DNA (ap)[e]	**66**	**70**	37	25
mm[f]	50	54	20	14
RNA (p)[d]	40	45	—	—
mm[f]	47	51	—	12
RNA (ap)[e]	**81**	> **87**	38	44
mm[f]	65	71	22	31
L-DNA (p)[d]	**—**	**42**	—	— [h]
mm[f]	—	27	—	—
L-DNA (ap)[e]	—	—	—	— [h]
mm[f]	—	—	—	—
L-RNA (p)[d]	**44**	**44**	—	— [h]
mm[f]	36	32	—	—
L-RNA (ap)[e]	—	—	—	— [h]
mm[f]	—	—	—	—

[a] Melting temperatures (T_m values) obtained from the maxima of the first derivatives of the melting curves (A_{260} *vs.* temperature) recorded in a medium salt buffer (Na_2HPO_4 (10 mM), NaCl (100 mM), EDTA (0.1 mM), pH 7.0) using 1.5 μM concentrations of each strand (assuming identical extinction coefficients for all modified oligonucleotides). All T_m values are given as averages of double determinations. [b] DNA, LNA and α-L-LNA sequences correspond to 5′-^{m}CA^{m}CTATT^{m}C^{m}CA-3′; ^{m}C = 5-methylcytosine monomers. [c] RNA: 5′-CACUAUUCCA-3′. [d] Parallel DNA and L-DNA: 5′-GTGATAAGGT-3′; Parallel RNA and L-RNA: 5′-GUGAUAAGGU-3′. [e] Antiparallel DNA and L-DNA: 5′-TGGAATAGTG-3′; Antiparallel RNA and L-RNA: 5′-UGGAAUAGUG-3′. [f] Mismatch sequences: 5′-GTGATTAGGT-3′, 5′-GUGAUUAGGU-3′, 5′-TGGATTAGTG-3′ and 5′-UGGAUUAGUG-3′. [g] "—" corresponds to the absence of any detectable melting temperature above 10 °C. [h] Confirmed also in a high salt buffer (Na_2HPO_4 (10 mM), NaCl (700 mM), EDTA (0.1 mM), pH 7.0).

parallel DNA. Thereby, our hypothesis, that β-L-LNA is a better mimic of α-DNA than is α-LNA, has been confirmed. The present results also demonstrate that the family of the four *ribo*-configured LNA-stereoisomers is an extraordinary group of RNA-recognising nucleic acid analogues. When compared to the series of the four RNA-stereoisomers, the introduction of a locked *N*-type furanose conformation favours duplex formation in all cases. Thus, a mixed α-RNA sequence has been found to form only a low affinity parallel stranded duplex with complementary RNA,[13,18] whereas an oligothymidylate sequence of α-L-RNA was found to give no complex with complementary RNA.[19] On the other hand, strong duplexes with RNA have now been demonstrated with the locked analogues α-LNA and α-L-LNA, parallel and antiparallel respectively, with the latter demonstrating the most remarkable improvement. As the enantiomer of natural RNA, β-L-RNA has been more intensively studied than the other unnatural RNA-stereoisomers.[20–22] Thus, β-L-RNA as well as β-L-DNA sequences have recently been successfully applied as aptamers in the so-called spiegelmer approach.[23] However, only a weak Watson–Crick recognition between β-L-RNA and complementary RNA has been found and the preference for a parallel strand orientation suggested.[20,21] In our investigation, however, no duplex formation between L- and D-configured DNA or RNA sequences was observed (Table 1). Nevertheless, the results with β-L-LNA confirm that (longer) duplexes formed between β-L-RNA and RNA should have parallel strand orientation.

By the introduction of α-LNA and β-L-LNA, we have explored the upper level of high affinity parallel nucleic acid recognition by oligonucleotides with locked *N*-type conformations. Obviously, the thermal stabilities of the parallel duplexes are lower than those of the corresponding antiparallel duplexes formed with α-L-LNA or LNA. However, progress might be obtained with α-D- (or even β-L) configured nucleoside analogues with locked *S*-type furanose conformations. An example of an α-nucleoside conformationally restricted towards an *S*-type conformation has recently been presented.[24] Nevertheless, we have demonstrated that LNA can potentially recognise spiegelmers,[23] *i.e.* L-DNA and L-RNA oligomers.

The hybridisation data now available for the four *ribo*-configured LNA stereoisomers reveal that hybridisation in the world of pentofuranosyl nucleic acids is a matter of the conformational equilibria of the pentofuranoses rather than their configuration. Further research considering the RNA-selective recognition properties of α-LNA, and parallel nucleic acid recognition in general, is in progress.

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Notes and references

‡ Throughout this paper, we exclude the terms β and/or D when nucleic acid configurations are stated. Thus, LNA is defined as oligonucleotides containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomers and α-LNA is similarly defined by the α-D configured stereoisomer.

§ The L-DNA phosphoramidite building blocks were purchased from ChemGenes. The four L-RNA sequences were purchased from Noxxon Pharma. LNA sequences can be purchased from Proligo.

1 P. Herdewijn, *Biochim. Biophys. Acta*, 1999, **1489**, 167.
2 M. Meldgaard and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3539.
3 S. K. Singh, P. Nielsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455.
4 A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen and J. Wengel, *Tetrahedron*, 1998, **54**, 3607.
5 S. Obika, D. Nanbu, Y. Hari, J. Andoh, K. Morio, T. Doi and T. Imanishi, *Tetrahedron Lett.*, 1998, **39**, 5401.
6 V. K. Rajwanshi, A. E. Håkansson, B. M. Dahl and J. Wengel, *Chem. Commun.*, 1999, 1395.
7 V. K. Rajwanshi, A. E. Håkansson, R. Kumar and J. Wengel, *Chem. Commun.*, 1999, 2073.
8 M. D. Sørensen, L. Kværnø, T. Bryld, A. E. Håkansson, B. Verbeure, G. Gaubert, P. Herdewijn and J. Wengel, *J. Am. Chem. Soc.*, 2002, **124**, 2164.
9 P. Nielsen and J. K. Dalskov, *Chem. Commun.*, 2000, 1179.
10 P. Nielsen, N. K. Christensen and J. K. Dalskov, *Chem. Eur. J.*, 2002, **8**, 712.
11 F. Morvan, B. Rayner, J.-L. Imbach, D.-K. Chang and J. Lown, *Nucleic Acids Res.*, 1986, **14**, 5019.
12 G. Lancelot, J.-L. Guesnet and F. Vovelle, *Biochemistry*, 1989, **28**, 7871.
13 F. Morvan, H. Porump, G. Degols, I. Lefebvre, A. Pompon, B. S. Sproat, B. Rayner, C. Malvy, B. Lebleu and J.-L. Imbach, *J. Med. Chem.*, 1993, **36**, 280.
14 K. Bondensgaard, M. Petersen, S. K. Singh, V. K. Rajwanshi, R. Kumar, J. Wengel and J. P. Jacobsen, *Chem. Eur. J.*, 2000, **6**, 2687.
15 M. Petersen, A. E. Håkansson, J. Wengel and J. P. Jacobsen, *J. Am. Chem. Soc.*, 2001, **123**, 7431.
16 K. M. E. Nielsen, M. Petersen, A. E. Håkansson, J. Wengel and J. P. Jacobsen, *Chem. Eur. J.*, 2002, **8**, 3001.
17 V. K. Rajwanshi, A. E. Håkansson, M. D. Sørensen, S. Pitch, S. K. Singh, R. Kumar, P. Nielsen and J. Wengel, *Angew. Chem., Int. Ed.*, 2000, **39**, 1656.
18 F. Debart, B. Rayner, G. Degols and J.-L. Imbach, *Nucleic Acids Res.*, 1992, **20**, 1193.
19 L. Keinicke, M. D. Sørensen and J. Wengel, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 593.
20 C. A. A. van Boeckel, G. M. Visser, R. A. Hegstrom and J. H. van Boom, *J. Mol. Evol.*, 1987, **25**, 100.
21 G. W. Ashley, *J. Am. Chem. Soc.*, 1992, **114**, 9731.
22 S. Pitch, *Helv. Chim. Acta*, 1997, **80**, 2286.
23 A. Vater and S. Klussmann, *Curr. Opin. Drug Disc. Dev.*, 2003, **6**, 253.
24 B. M. Keller and C. J. Leumann, *Synthesis*, 2002, 789.