Universality of LNA-mediated high-affinity nucleic acid recognition

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LNA (locked nucleic acid) is a novel class of nucleic acid mimic structurally closely resembling RNA; incorporation of three LNA monomers together with six ribonucleotide monomers afforded the first ribo-LNA sequence; unprecedented thermal stabilities of duplexes towards complementary DNA and RNA without compromising base-pairing selectivity were obtained for ribo-LNA, thus establishing the universality of LNA-mediated efficient targeting of natural nucleic acids.

LNA (Fig. 1) has been recently introduced as a novel nucleic acid mimic able to induce unprecedented increases in the thermal stability of duplexes towards both DNA and RNA in different 2′-deoxynucleotide sequence contexts.[1,2]‡ Other appealing characteristics of LNA include efficient automated oligomerization, satisfactory aqueous solubility and stability towards 3′-exonucleolytic degradation.[1,2] Further definitions and details are given in Fig. 1 and Table 1.

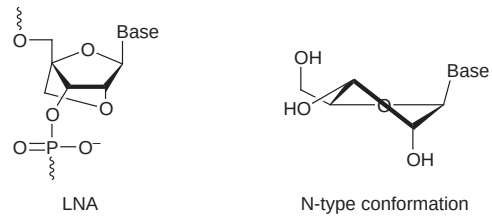

Fig. 1 Structure of the thymine LNA monomer $\mathbf{T^L}$ used in this study. LNA is defined as an oligonucleotide containing one or more LNA monomers. Deoxy-LNA is defined as an LNA consisting of LNA monomers and 2′-deoxynucleotide monomers. Ribo-LNA is defined as an LNA consisting of LNA monomers and ribonucleotide monomers. Also shown is the preferred monomer conformation in A-type duplexes (generally RNA/RNA and DNA/RNA, N-type conformation).

In entries 5–8 (Table 1) the hitherto reported LNA hybridization data (melting temperatures, T_m) for nonamer mixed sequences are summarized[1,2] and compared with the results for the corresponding unmodified reference duplexes. It should be noted that ΔT_m in Table 1 and in the text denotes increases in T_m per LNA monomer incorporated. In this comparative study, the thymine LNA monomer $\mathbf{T^L}$ is used as a representative example, but it is noteworthy that analogous results have been obtained for other pyrimidine and purine LNA monomers.[2] Incorporation of three $\mathbf{T^L}$ monomers together with six deoxynucleotides (to give a nonamer deoxy-LNA) induces remarkable increases in the thermal affinity towards both DNA and RNA (ΔT_m = +5.3 and +7.3 °C, respectively, entries 5 and 6). The fully modified LNA displayed yet higher thermal stabilities (entries 7 and 8). It has been clearly shown that LNA obeys the Watson–Crick base-pairing rules discriminating with excellent selectivity between fully matched and singly mis-matched complementary nucleic acids.[1,2]

Consequently, fully modified LNA as well as deoxy-LNA are able to sequence-selectively recognize complementary DNA/RNA with unprecedented thermal affinities. It is at this point important to note that comparable DNA and RNA recognition has so far only been demonstrated for 2′-fluoro N3′-P5′-phosphoramidates (ΔT_m = +3 to +5 °C).[3] We believe that the explanation offered by these authors, namely a strong pre-organization of the pentofuranose ring of the modified monomers into an N-type conformation[3] (Fig. 1), is viable also for LNA,[1,2] leading eventually to entropically favourable duplex formation.[4]

To explore the universality of LNA-mediated nucleic acid recognition, we decided to examine the properties of the ribo-LNA 5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC) (**LNA-1**) consisting of three thymine LNA monomers $\mathbf{T^L}$ and six ribonucleotide monomers. Synthesis of **LNA-1** was efficiently performed by the phosphor-

Table 1 Hybridization data of LNA and reference strands[a]

Entry	Duplex	T_m/°C	ΔT_m/°C
1	5′-d(GTGATATGC)/3′-d(CACTATACG)	28	—
2	5′-d(GTGATATGC)/3′-r(CACUAUACG)	28	—
3	5′-r(GUGAUAUGC)/3′-d(CACTATACG)	27	—
4	5′-r(GUGAUAUGC)/3′-r(CACUAUACG)	38	—
5[1]	5′-d(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-d(CACTATACG)	44	+5.3[b]
6[1]	5′-d(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-r(CACUAUACG)	50	+7.3[c]
7[2]	5′-(**G^{L}T^{L}G^{L}A^{L}T^{L}A^{L}T^{L}G^L ^{Me}C^L**)/3′-d(CACTATACG)	64	+4.0[b]/+4.1[d]
8[2]	5′-(**G^{L}T^{L}G^{L}A^{L}T^{L}A^{L}T^{L}G^L ^{Me}C^L**)/3′-r(CACUAUACG)	74	+5.1[c]/+4.0[c]
9	5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-d(CACTATACG)	55	+9.3[d]
10	5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-d(———T———)	38	—
11	5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-d(———G———)	37	—
12	5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-d(———C———)	34	—
13	5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′-r(CACUAUACG)	63	+8.3[e]
14	5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)/3′- r(———C———)	45	—

[a] A = adenosine monomer, C = cytidine monomer, G = guanosine monomer, U = uridine monomer, T = thymidine monomer, ^{Me}C = 5-methylcytidine monomer, $\mathbf{X^L}$ = LNA monomer.[1,2] Oligo-2′-deoxynucleotide sequences are depicted as d(sequence) and oligoribonucleotide sequences as r(sequence). 5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC) = **LNA-1**. The melting temperatures (T_m values) were obtained as the maxima of the first derivatives of the melting curves (A_{260} *vs.* temperature) recorded as described earlier.[1,2] ΔT_m values are the increases in the thermal stability per LNA monomer incorporated compared to the corresponding reference duplex. [b] Compared to entry 1. [c] Compared to entry 2. [d] Compared to entry 3. [e] Compared to entry 4.

amidite approach[5] using ribonucleoside 3′-phosphoramidites and thymine LNA monomer 3′-phosphoramidite.[1,2]§

The results of thermal melting studies are shown in Table 1 (entries 9–14). Sharp monophasic transitions were obtained in all experiments. It is convincingly demonstrated that **LNA-1** displays hitherto unseen increases in thermal stability (towards DNA: ΔT_m = +9.3 °C, entry 9, compared to entry 3; towards RNA: ΔT_m = +8.3 °C, entry 13, compared to entry 4). From experiments targeting singly mis-matched complements (entries 10–12 and 14) it is revealed that recognition by **LNA-1** is sequence selective. These results indicate that the chimeric ribo-LNA oligomers (or possibly oligomers consisting of LNA monomers and 2′-*O*-alkylribonucleotide monomers[6]) could prove very useful, *e.g.* for optimizing the properties of antisense oligonucleotides.

Because of the known preference for ribonucleotide monomers in A-type duplexes to adopt an N-type conformation,[7] and the established preorganization of LNA monomers into an N-type conformation,[1,2]‡ we had anticipated an additive effect (though levelling off when increasing the number of LNA monomers) on the thermal stability of duplexes involving ribo-LNA. However, comparison of the T_m values obtained for the fully modified LNA (entries 7 and 8) with the T_m values obtained for **LNA-1** reveals an interesting point. Thus, while introduction of only three LNA monomers leads to increased T_m values of +28 °C (towards DNA, entry 9) and +25 °C (towards RNA, entry 13), the corresponding increases in T_m obtained for the fully modified LNA were comparatively lower, namely +37 and +36 °C, respectively. In other words, the effect of introducing six additional LNA monomers in the nonamer amounts to a total increase in T_m of only +9 and +11 °C, respectively. It seems from these results that an LNA monomer profoundly effects the neighbouring ribonucleotide monomers, probably by inducing an overall preorganization, *e.g.* through increased intra-strand base stacking. A similar trend, albeit less pronounced, can be observed from the data shown in entries 5 and 6 for the deoxy-LNA containing three $\mathbf{T^L}$ monomers. By comparing the T_m values of entries 5 and 9 it is revealed that the thermal affinity towards DNA is larger for **LNA-1** than for the corresponding deoxy-LNA. This fact cannot be explained by the shift from an RNA- to a DNA-like oligomer as the corresponding reference T_m values are similar (28 and 27 °C, entries 1 and 2). Instead, the above suggested effect of the LNA monomers on the surrounding ribonucleotide monomers could be an explanation.

Through the results reported herein and the results reported recently,[1,2] the compatibility of LNA monomers with both ribo- and deoxyribo-nucleotide monomers (as well as the effectiveness of fully modified LNA) for high-affinity recognition of both DNA and RNA targets has been established. The structural variants of LNA evaluated demonstrate the universality of LNA-mediated nucleic acid targeting and indicate superior nucleic acid recognition for ribo-LNA. In addition, the pivotal role of the pentofuranose-phosphate backbone in nucleic acid recognition processes has been stressed. Further studies on thermodynamics and the structure of the duplexes have been initiated in order to fully understand the remarkable properties of LNA.

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Notes and References

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‡ Synthesis of the uracil and cytosine LNA monomers has been independently published by another group (S. Obika, D. Nanbu, Y. Hari, K. Morio, Y. In, T. Ishida and T. Imanishi, *Tetrahedron Lett.*, 1997, **38**, 8735). Oligomerization of these monomers was not reported.

§ **LNA-1** was synthesized on a 0.2 μmol scale on a polystyrene solid support (Pharmacia) derivatized by a cytidine monomer using a Biosearch 8750 DNA Synthesizer. The stepwise coupling yield of the thymine LNA monomer 3′-phosphoramidite {(1*R*,3*R*,4*R*,7*S*)-7-[2-cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane} was >99% (20 min couplings) which was equivalent to the coupling yields obtained (6 min couplings) for commercial ribonucleoside 3′-phosphoramidites {2′-*O*-*tert*-butyldimethylsilyl-3′-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)-2-*N*-(*tert*-butylphenoxyacetyl)guanosine (Perseptive Biosystems) and -6-*N*-(phenoxyacetyl)adenine (Biogenex)} as determined spectrophotometrically by the release of the DMT cation after each coupling step. After detritylation, the oligomer was cleaved from the solid support and partly deprotected using 40% aqueous methylamine (10 min, 55 °C). After cooling to −18 °C, the solid support was removed (centrifugation) and washed [2 × 0.25 cm^3; EtOH–MeCN–H_2O (3 : 1 : 1, v/v/v)]. The solvents were evaporated, and the residue was desilylated using a method described earlier.[8] Capillary gel electrophoresis[9] was used to document the purity of **LNA-1** (>90%). The composition of **LNA-1** [5′-r(G$\mathbf{T^L}$GA$\mathbf{T^L}$A$\mathbf{T^L}$GC)] was verified by MALDI-MS:[9] $[M - H]^-$ found 2932.9; calculated 2932.9.

1 S. K. Singh, P. Nielsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455.
2 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.
3 D. G. Schultz and S. M. Gryaznov, *Nucleic Acids Res.*, 1996, **24**, 2966.
4 E. T. Cool, *Chem. Rev.*, 1997, **97**, 1473.
5 M. H. Caruthers, *Acc. Chem. Res.*, 1991, **24**, 278.
6 L. L. Cummins, S. R. Owens, L. M. Risen, E. A. Lesnik, S. M. Freier, D. McGee, C. J. Guinosso and P. D. Cook, *Nucleic Acids Res.*, 1995, **23**, 2019.
7 P. Herdewijn, *Liebigs Ann.*, 1996, 1337.
8 F. Wincott, A. DiRenzo, C. Schaffer, S. Grimm, D. Tracz, C. Workman, D. Sweedler, C. Gonzalez, S. Scaringe and N. Usman, *Nucleic Acids Res.*, 1995, **23**, 2677.
9 M. Meldgaard, N. K. Nielsen, M. Bremner, O. S. Pedersen, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1951.

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