LNA (Locked Nucleic Acid): An RNA Mimic Forming Exceedingly Stable LNA:LNA Duplexes

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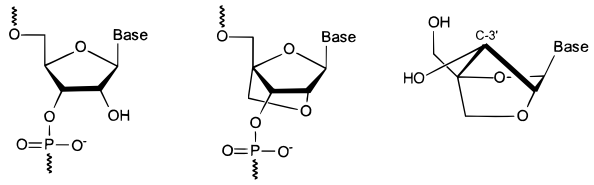
In the search for nucleic acid mimics capable of binding strongly to complementary DNA and/or RNA, synthesis of conformationally restricted oligonucleotide (ON) analogues has been intensified recently.[1] Among others, ONs containing monomeric nucleotides with bicyclic and tricyclic carbohydrate moieties have been examined.[2] We have recently introduced the novel ON analogue LNA (Locked Nucleic Acid; Figure 1)[3−5] containing 2′-*O*,4′-*C*-methylene linked bicyclic ribofuranosyl nucleosides locked in an N-type [3′-*endo*/^3E] conformation. In this paper, LNA:LNA hybridization is introduced as the most thermally stable nucleic acid type duplex system yet discovered, and the RNA-mimicking character of LNA is established at the duplex level.

Several examples of the formation of very stable nucleic acid type duplexes resulting from the pairing of two chemically modified ON strands have been described in the literature, e.g., involving pyranosyl-RNA,[6,7] 1′,5′-anhydrohexitol nucleic acid (HNA),[7,8] bicyclo-DNA,[2a,2b,7] and tricyclo-DNA.[2e,7] Stimulated by the unprecedented thermal stabilities and the very satisfactory base-pairing selectivities observed for duplexes between LNA and unmodified DNA or RNA,[3] we decided to evaluate the stability of LNA:LNA duplexes.[9] The results from thermal denaturation studies are depicted in Table 1. From entries 1, 3, and 4 it can be extracted that introduction of three LNA monomers ($\mathbf{T^L}$ or $\mathbf{A^L}$) induces significantly increased melting temperatures ($\Delta T_m = +15$ °C/+11 °C) toward the DNA complements. Remarkably, the duplex formed between the two complementary deoxy-LNAs[10] each containing three LNA monomers (Table 1, entry 6; three $\mathbf{T^L}$:$\mathbf{A^L}$ base pairs) exhibited a ΔT_m value of +34 °C indicating a more than additive effect of LNA:LNA base pairing on the thermal stability (entries 3, 4, and 6). An even more dramatic stabilizing effect can be induced by complex formation between ribo-LNA[10] and deoxy-LNA. Thus, an increased thermal stability corresponding to a ΔT_m value of +47 °C was obtained (Table 1, entry 7 compared to entry 2; three $\mathbf{T^L}$:$\mathbf{A^L}$ base pairs) reflecting the pronounced effect of LNA monomers on the base-pairing properties of oligoribonucleotides.[3d]



Characteristics of LNA:[3,4]

- Unprecedented thermal stabilities of duplexes towards complementary DNA and RNA (ΔT_m / LNA monomer = +3 to +9 °C)
- Stability towards 3′-exonucleolytic degradation
- Efficient automated oligomerization
- Good aqueous solubility

Figure 1. Structure of RNA and LNA (base = nucleobase) and characteristics of LNA. Also shown is the locked N-type conformation of an LNA nucleoside. ΔT_m/LNA monomer = change in melting temperature (T_m value) per LNA monomer incorporated compared to the T_m value of the corresponding unmodified duplex.

As the next step, all-modified LNAs were evaluated. In line with the results described above, a more than additive increase in the thermal stability ($\Delta T_m = +56$ °C) of the duplex between an all-modified LNA and the deoxy-LNA containing three $\mathbf{A^L}$ monomers was observed (Table 1, entry 8 compared with entries 1, 4, and 5). Furthermore, LNA:LNA complex formation between two all-modified LNAs was evaluated (Table 1, entries 9 and 10). For the complementary LNAs (entry 9) we were unable to detect a duplex dissociation within the range of measurement in the standard medium salt buffer (T_m estimated >93 °C). However, in a low salt buffer (1 mM sodium phosphate) a transition (ΔT_m ~93 °C) was evident. It is noteworthy that we were unable to detect a transition for the corresponding reference DNA:DNA duplex when using the low salt conditions (Table 1, entry 1; T_m estimated <10 °C). The excellent ability of LNA to discriminate mismatched nucleotides in unmodified ONs has been demonstrated earlier.[3] To allow for a preliminary evaluation of the selectivity of LNA:LNA base pairing, a duplex between two all-modified LNAs with a single mismatch (Table 1, entry 10; one $\mathbf{A^L}$:$\mathbf{A^L}$ mismatch) was examined. Whereas only the initiation of a transition could be detected in the medium salt buffer, a T_m value of 76 °C was determined in the low salt buffer. Thus, compared to the fully matched LNA:LNA duplex, the introduction of one $\mathbf{A^L}$:$\mathbf{A^L}$ mismatch induced a decrease in the thermal stability of 17 °C,[11] indicating a satisfactory base-pairing selectivity despite the very high thermal stabilities. The melting results shown in Table 1 reveal that LNA:LNA hybridization constitutes the most stable nucleic acid type duplex system hitherto discovered.[12] This fact not only underlines the importance of conformational restriction in molecular recognition events but also points to a

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(9) The LNAs were synthesized and analyzed as described earlier.[3b]

(10) 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.[3d]

(11) Introduction of one $\mathbf{A^L}$:A mismatch in an LNA:DNA duplex induced a decrease in the thermal stability of 19 °C compared to an $\mathbf{A^L}$:T base pair.[3b]

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Table 1. Melting Experiments on Reference Duplexes and LNA:LNA Duplexes

entry	duplex[a]	T_m (°C)[b]	ΔT_m (°C)[c]
	References		
1[3b]	5′-d(GTGATATGC)/3′-d(CACTATACG)	29[d]/<10[e]	ref
2[3d]	5′-r(GUGAUAUGC)/3′-d(CACTATACG)	27[f]	ref
	LNA vs DNA		
3[3b]	5′-d(G**T**^{L}GA**T**^{L}A**T**^{L}GC)/3′-d(CACTATACG)	44[f]	+15
4	5′-d(GTGATATGC)/3′-d(C**A**^{L}CT**A**^{L}T**A**^{L}CG)	40[f]	+11
5[3b]	5′-(**G**^{L}**T**^{L}**G**^{L}**A**^{L}**T**^{L}**A**^{L}**T**^{L}**G**$^{L\ Me}$**C**^{L})/3′-d(CACTATACG)	64[f]	+35
	LNA vs LNA		
6	5′-d(G**T**^{L}GA**T**^{L}A**T**^{L}GC)/3′-d(C**A**^{L}CT**A**^{L}T**A**^{L}CG)	63[d]	+34
7	5′-r(G**T**^{L}GA**T**^{L}A**T**^{L}GC)/3′-d(C**A**^{L}CT**A**^{L}T**A**^{L}CG)	74[d]	+47[g]
8	5′-(**G**^{L}**T**^{L}**G**^{L}**A**^{L}**T**^{L}**A**^{L}**T**^{L}**G**$^{L\ Me}$**C**^{L})/3′-d(C**A**^{L}CT**A**^{L}T**A**^{L}CG)	85[d]	+56
9	5′-(**G**^{L}**T**^{L}**G**^{L}**A**^{L}**T**^{L}**A**^{L}**T**^{L}**G**$^{L\ Me}$**C**^{L})/ 3′-(**^{Me}C**^{L}**A**$^{L\ Me}$**C**^{L}**T**^{L}**A**^{L}**T**^{L}**A**$^{L\ Me}$**C**^{L}**G**^{L})	>93[d]/93[e]	>+64[d]/>+83[e]
10	5′-(**G**^{L}**T**^{L}**G**^{L}**A**^{L}**A**^{L}**T**^{L}**G**$^{L\ Me}$**C**^{L})/ 3′-(**^{Me}C**^{L}**A**$^{L\ Me}$**C**^{L}**T**^{L}**A**^{L}**T**^{L}**A**$^{L\ Me}$**C**^{L}**G**^{L})	>93[d]/76[e]	>+64[d]/>+66[e]

[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. Oligo-2′-deoxynucleotide sequences are depicted as d(sequence) and oligoribonucleotide sequences as r(sequence). [b] 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[3b] using 1.5 μM concentrations of the two complementary strands (assuming identical extinction coefficients for LNA and the corresponding unmodified strands). All transitions were monophasic. [c] ΔT_m values are the overall increases in the thermal stability compared to the corresponding reference duplex. [d] Medium salt buffer: 10 mM sodium phosphate, pH 7.0, 100 mM sodium chloride. [e] Low salt buffer: 1 mM sodium phosphate, pH 7.0. [f] Measured in medium salt buffer containing EDTA: 10 mM sodium phosphate, pH 7.0, 100 mM sodium chloride, 0.1 mM EDTA. In our experience, the effect of the added EDTA on the T_m values is within ±1 °C. [g] Calculated relative to entry 2; All other ΔT_m values are calculated relative to entry 1.

Table 2. Thermodynamic Data[a]

duplex	$-\Delta H$ (kJ/mol)	$-\Delta S$ (kJ/(mol K))	$-\Delta G_{37\ °C}$ (kJ/mol)
Table 1; entry 1	317	0.94	27
Table 1; entry 2	287	0.85	25
Table 1; entry 3	294	0.81	42
Table 1; entry 5	423	1.13	73

[a] Determined from the linear relations between $1/T_M$ and $\ln[c]$.[14] Measured in medium salt buffer: 10 mM sodium phosphate, pH 7.0, 100 mM sodium chloride. Duplex concentration c = 1.5−35 μM (see footnote to Table 1 for further details).

general applicability of high-affinity nucleic acid analogues in supramolecular chemistry.

CD spectra of different complexes involving all-modified LNA are depicted as Supporting Information together with a CD spectrum of the corresponding RNA:RNA duplex. As anticipated from the restriction of LNA monomers into an N-type conformation,[3] the CD spectra show that the LNA:DNA, LNA:RNA, and LNA:LNA complexes all structurally resemble the A-form duplex of the RNA:RNA reference,[1a,13] Especially the LNA:RNA complex appears to be an excellent mimic of the reference RNA:RNA A-form duplex. The structure of the LNA:DNA and LNA:LNA complexes deviate to some extent from the structure of the RNA:RNA duplex, and elements in the CD spectrum characteristic of a B-form duplex are apparent.

Preliminary thermodynamic evaluation of LNA-mediated nucleic acid recognition has been performed from the concentration dependencies of T_m (Table 2).[14] As expected from the strong preorganization of LNA, the introduction of three LNA monomers ($\mathbf{T}_L$) (Table 1; entry 3) seems to induce an entropically favored duplex formation toward DNA, compared to the unmodified duplexes (Table 1; entries 1 and 2).[15] However, formation of the duplex between the all-modified LNA and complementary DNA (Table 1; entry 5) appears to be entropically disfavored and strongly enthalpically favored. The low melting temperature (<10 °C under low salt conditions) of the DNA:DNA reference duplex (Table 1; entry 1) and the very high melting temperatures of the LNA:LNA duplexes (Table 1; entries 8 and 9) preclude a similar analysis of these complexes. Enthalpically favored duplex formation has been reported for conformationally restricted *N*3′-*P*5′-phosphoramidate oligonucleotides[16] and explained by extensive hydration of the all-modified duplex.[17] Whether similar reasoning can be applied to duplex formation involving all-modified LNA awaits further investigations.

The universality of LNA-mediated hybridization has been stressed by the formation of the exceedingly stable LNA:LNA duplexes. The RNA-mimicking character of LNA is reflected not only with regard to the N-type conformational restriction of the monomeric nucleosides[3,5] but also convincingly with regard to the secondary structure of the LNA:RNA duplex. Importantly, the general and very stable LNA hybridization proceeds by duplex formation via natural Watson−Crick hydrogen bonding in a predictable manner.[18] These results establish the development of LNA as an important and illustrative example of biomimetic chemistry.

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Supporting Information Available: Melting curves for the duplexes shown in Table 1 and CD curves (11 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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