Locked Nucleic Acids and Intercalating Nucleic Acids in the Design of Easily Denaturing Nucleic Acids: Thermal Stability Studies

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Intercalating nucleic acids (INA®s) with insertions of (R)-1-O-(1-pyrenylmethyl)glycerol were hybridized with locked nucleic acids (LNAs). INA/LNA duplexes were found to be less stable than the corresponding DNA/LNA duplexes when the INA monomer was inserted as a bulge close to the LNA monomers in the opposite strand. This property was used to make "quenched" complements that possess LNA in hairpins and in duplexes and are consequently more accessible for targeting native DNA. The duplex between a fully modified 13-mer LNA sequence and a complementary INA with six pyrene residues inserted after every second base as a bulge was found to be very unstable ($T_m = 30.1$ °C) in comparison with the unmodified double-stranded DNA ($T_m = 48.7$ °C) and the corresponding duplexes of LNA/DNA ($T_m = 81.6$ °C) and INA/DNA ($T_m = 66.4$ °C). A thermal melting experiment of a mixture of an LNA hairpin, with five LNA nucleotides in the stem, and its complementary DNA sequence gave a transition with an extremely low increase in optical density (hyperchromicity). When two INA monomers were inserted into the stem of the LNA hairpin, the same experiment resulted in a significant hyperchromicity comparable with the one obtained for the corresponding DNA/DNA duplex.

Introduction

In the last two decades many attempts have been made to improve the characteristics of native nucleic acids, such as hybridization affinity, stability towards cellular nucleases and the ability to penetrate the cell membrane.[1] A variety of modified oligonucleotides are used now in biophysical and biochemical studies.[2] However, if the modified oligonucleotides have efficient hybridization properties towards single-stranded DNA (ssDNA) or ssRNA, they usually bind even more strongly to a complementary sequence of their own type, as is the case for peptide nucleic acids (PNA)[3] and locked nucleic acids (LNA).[4] The high self-affinity limits the number of sequences of DNA and RNA that can be targeted with the synthetic oligonucleotides, due to the formation of stable intra- and intermolecular secondary structures.

It has been reported that DNA possessing N^4-ethyl-cytosines or 2,6-diaminopurines and 2-thiouracils instead of cytosines, adenines and thymines forms considerably less stable base pairs than natural bases.[5] The lower level of secondary structure was shown to allow hybridization with complementary native DNA probes.[5a] Pseudo-complementary nucleic acids constructed from PNA with modified bases were used for sequence-specific targeting of double-stranded DNA (dsDNA) by double duplex invasion.[5d,e] A minimum content of A/T base pairs (40%) is a limiting factor for the invasion and excludes many gene sequences to be targeted. Therefore, the design of modified oligonucleotides for binding with native DNA and RNA without cross self-complexation is still a challenge in the chemical biology of nucleic acids.

Here we suggest an alternative approach that could overcome the problem of "quenched" complementary sequences of modified nucleic acids. During recent years, special attention has been paid to the design of oligonucleotide probes with the ability to discriminate between ssDNA and ssRNA, which has been explored in the development of nonradioactively labelled oligonucleotides, antisense and antigene technologies. We suppose that so-called easily denaturing nucleic acids could be constructed from DNA- and RNA-discriminating molecules. If these structures are placed opposite each other in the complementary regions, it would be thermodynamically more favourable to target native nucleic acids than each other due to different binding ability towards A-type and B-type helices.

Recently, we reported the synthesis and hybridization properties of two discriminating nucleic acids: LNA[6] and intercalating nucleic acids (INA®).[7] LNA is a class of oligonucleotide analogues containing one or more conformationally locked nucleotide monomers with a 2′-*O*,4′-*C*-methylene linkage (Scheme 1). LNA (both as fully modified and as LNA–DNA or LNA–RNA mix-mers) has shown unprecedented high-affinity hybridization towards complementary DNA and RNA. LNA binds better to RNA ($\Delta T_m = +2$ to $+10$ °C per modification)

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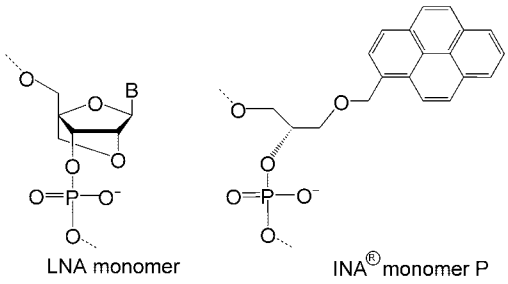


Scheme 1. *Chemical structures of LNA and INA P monomer. B = nucleobase.*

than to DNA ($\Delta T_m = +1$ to $+8$ °C per modification).[6c] However, LNA probes can "quench" each other and form very stable hairpins or duplexes.[4] Contrary to the LNA case, the bulge insertions of (*R*)-1-*O*-(pyren-1-ylmethyl)glycerol (monomer P, Scheme 1) into oligodeoxynucleotides (INA) induced increased affinity for complementary ssDNA (ΔT_m +1.0 to +11.0 °C), but reduced affinity for an identical sequence of ssRNA (ΔT_m −2.0 to −7.0 °C).[7a]

Combining LNA and INA thus seems a very promising approach to developing easily denaturing nucleic acids that could also be helpful in solving the problem of undesired self-hybridizations. Furthermore we think that INA is a good choice because it shows high sensitivity to mismatch when inserted into DNA duplexes.[7e]

Here we report the design and thermal-stability studies of single- and double-stranded oligodeoxynucleotide probes possessing LNA and INA targeting native DNA.

Results and Discussion

Duplexes possessing LNA and INA in opposite strands

NMR has been used to determine the structure of DNA/LNA duplexes, and it was found that one LNA monomer in the duplex was sufficient to induce a change in the furanose-ring conformation of the flanking 2′-deoxynucleotides from a South-type conformation, typically found in B-type DNA/DNA duplexes, to a North-type conformation, typically found in A-type RNA/RNA duplexes.[8] It was therefore expected that insertions of the INA monomer P as a bulge into regions with an A-type duplex structure would induce a decreased duplex stability, whereas the opposite should be observed for regions with B-type duplex structure. We studied the systematic insertions of monomer P as a bulge at all possible sites of an 11-mer DNA/LNA duplex (Table 1).

Target **I**, which does not contain any LNA monomers, was used as a reference for hybridizations with probes **2**–**12** (Table 1) which contain P monomers as a bulge at all possible positions, except at the 3′-end. Increased duplex stabilities were observed for target **I** with probes **2**–**12** in all cases when compared with unmodified probe **1**. In fact, probe **3** gave a remarkably stable duplex with an increase of 10.1 °C in the thermal melting temperature.

For target **II**, which possesses a single LNA molecule in the middle of the sequence, we observed that insertion of the INA monomer P in regions oriented one base-pair away from the position of the LNA monomer (probes **5**–**12**), induced an increase in duplex stability when compared with the unmodified dsDNA. In fact, the stabilizations observed were nearly identical with those detected for target **I**. This confirms that regions away from the LNA monomer still have a B-type structure.

Target **III**, which has three evenly placed LNA monomers, was supposed to induce A-type, RNA-like duplex structure in most parts of the duplex. This expectation is in agreement with the NMR structure determination on a similar duplex with three LNA monomers.[8] An increased melting temperature (+14.3 °C) was observed for target **III** towards DNA probe **1** when compared with unmodified dsDNA (**1**–**I**).

In comparison with LNA/DNA (targets **II** and **III** towards **1**), destabilisation of INA/LNA complexes was only detected when monomer P was placed as a bulged opposite neighbour to an LNA moiety in the complementary strand (**2** and **3** towards **II**, and **2**, **3**, **5**–**8** and **13** towards **III**). However, only in three cases of the INA/LNA duplexes (**2**/**II**, **3**/**II**, **13**/**III**) was the T_m lower than the T_m of both the corresponding INA/DNA duplexes (**2**, **3**, **13** towards **I**) and the corresponding LNA/DNA duplexes (**II** and **III** towards **1**).

From the study on targets **I**–**III**, it can be concluded that effective destabilization of INA/LNA duplexes can be achieved by insertion of INA monomer as an opposite neighbour to LNA. This result is in line with a recent finding that shows that intercalators exhibit significantly lower affinity for LNA-containing duplexes.[9] Increased stability is ob-

Table 1. *Thermal stabilities of oligodeoxynucleotides possessing INA and LNA in opposite strands.*[a]

Probes	Sequence, 3′→5′	Targets, 5′→3′ I TGTGATATGCT		II TGTGAT^{L}ATGCT		III TGT^{L}GAT^{L}AT^{L}GCT	
		T_m [°C]	ΔT_m [°C][b]	T_m [°C]	ΔT_m [°C]	T_m [°C]	ΔT_m [°C]
1	ACACTATACGA	42.4	–	47.4	–	56.7	–
2	ACACT**P**ATACGA	47.6	5.2	44.8	−2.6	52.9	−3.8
3	ACACTA**P**TACGA	52.5	10.1	46.5	−0.9	54.5	−2.2
4	ACAC**P**TATACGA	44.4	2.0	47.8	0.4	57.8	1.1
5	ACACTAT**P**ACGA	48.8	6.4	52.0	4.6	55.2	−1.5
6	ACA**P**CTATACGA	50.6	8.2	54.2	6.8	55.1	−1.6
7	ACACTATA**P**CGA	50.3	7.9	54.8	7.4	53.1	−3.6
8	AC**P**ACTATACGA	45.0	2.6	50.3	2.9	53.7	−3.0
9	ACACTATAC**P**GA	44.4	2.0	49.2	1.8	57.2	0.5
10	A**P**CACTATACGA	47.5	5.1	52.0	4.8	63.5	6.8
11	ACACTATACG**P**A	49.6	7.2	54.3	6.9	62.7	6.0
12	ACACTATACGA**P**	48.7	6.3	53.8	6.4	60.4	3.7
13	ACA**P**CTAT**P**ACGA	54.8	12.4	60.0	12.6	52.9	−3.8

[a] **P** denotes INA monomer, **T^L** denotes locked nucleotide of thymine; [b] The difference in T_m of duplexes with modified and corresponding unmodified probes.

served for duplexes with several LNAs; if not, all LNAs have INAs as opposite neighbours (see T_m of probes **2**–**6**, **8**–**12** towards **II** in comparison with **III**).

With the proper combination of INA monomers P and LNA monomers in the duplex, this method seems a promising tool for the design of easily denaturing nucleic acids which could be used for targeting native DNA.

Duplexes from LNA and INA mix-mers and fully modified LNA towards INA

The next step was to check the thermal stability of duplexes possessing LNA monomers and INA in the same strand (Table 2). To our surprise, the duplexes with probe **15** towards targets **V** and **VI** were even more stable than the corresponding reference duplexes **14**/**V**, **14**/**VI**, **15**/**IV**. In the latter case, all the INAs had an LNA as an opposite neighbour, but the neighbouring LNAs in the same strand as the INAs were detrimental to the denaturing effect. The significant stabilization is probably due to several LNAs as opposite neighbours in the duplex. However, a small destabilization was still observed for **15** against **V** (3.7 °C) and against **VI** (4.6 °C) when compared with LNA/LNA complexes without monomer P insertions (**16** towards **V** and **VI**). For duplexes with two to six pyrene insertions in one strand (probes **17**–**22**), there were only minor changes in duplex stabilities when the wild-type target **IV** was compared with target **V,** which has four LNAs.

The best results in our design of easily denaturated nucleic acids were obtained when the fully modified LNA sequence **VII** was used. Its duplexes with sequences **20**–**22**, which possess from four to six pyrene pseudonucleotides, had thermal melting temperatures that were even lower than that of the wild-type dsDNA (**14** towards **IV**). On the other hand, by using the same wild-type duplex as a reference, considerable increases in melting temperatures were observed when **VII** and **20**–**22** were hybridized with their complementary ssDNA (**14** and **IV**, respectively). The dramatic decrease in thermal stability of the complex formed by INA and fully modified LNA **VII** was observed when the number of P monomers in the INA strand was increased from three, in probe **18** (T_m 60.5 °C), to four, in **20** (T_m 36.7 °C). The position of the INA monomer is important. For example, when monomer P was moved from the nearest bulged position at the 5′-end, in probe **19**, to the middle of the sequence, in probe **20**, thermal stability of the INA/LNA complex changed from $T_m = 62.7$ °C (**19**/**VII**) to 36.7 °C (**20**/**VII**). It can be expected that very close insertions of the pyrene moiety P in the strand cause both synthetic problems during oligosynthesis, and difficulties of solubility in water for the final oligomer. On the other hand, destabilization of the complex formed by fully modified LNA and INA was achieved by repetitive insertion of P. Therefore, from our point of view, the INA monomer should be inserted after every second base as a bulge in deoxynucleotide strand in order to have an optimum design of easily denaturing nucleic acids when using fully modified LNA.

Based on the thermal stability studies, one can expect that the formation of LNA/DNA (**VII**/**14**) and INA/DNA (**22**/**IV**) duplexes is thermodynamically more favourable than formation of LNA/INA (**VII**/**22**) on mixing of all these strands together. It is considered appropriate that the T_m of the complex **VII**/**22** (30.1 °C) should be lower than the physiological temperature (37 °C). Such an easily denaturing nucleic acid duplex is therefore considered a good candidate for competing for duplex formation with wild-type dsDNA. It has been shown that double duplex invasion could be done without significant problems on dsDNA of the same length as the probes,[10] but there is still a problem with targeting and sequence-specific recognition of considerably long, nonsupercoiled dsDNA (50–150 base-pairs) under physiological conditions when using short invaders. The known successful attempts of targeting against dsDNA have been accomplished by PNA[5c,d] or LNA[11] on breathed plasmid DNA (more than 250 base-pairs) by using unnatural salt concentration. It is well known that thermally induced breathing of a long DNA duplex results, at physiological temperatures, in localized DNA melting of 10 base-pairs or more, which is similar in size to some transcriptional starting sites.[12] We suppose that these positions could be available for duplex invasion on nonsupercoiled dsDNA. However, studies involving the biologically relevant design of using easily denaturing nucleic acid duplexes are needed to establish the scope of the above-proposed approach.

Table 2. *Thermal stabilities, T_m [°C] of oligodeoxynucleotides possessing INA and LNA in the design of easily denaturing nucleic acid duplexes.*[a]

Probes	Sequence, 5′→3′	Targets, 3′→5′			
		IV TTTACTACCGACG	**V** TTT^{L}A^{Me}C^{L}TA^{L}CCGA^{L}CG	**VI** TTT^{L}PA^{Me}C^{L}TA^{L}PCCGA^{L}CG	**VII** (TTTA ^{Me}CTA^{Me}C^{Me}CGA^{Me}CG)^{L}
14	AAATGATGGCTGC	**48.7**	58.2	66.3	**81.6**
15	AAA^{L}TGPA^{L}TG^{L}G^{Me}C^{L}PTGC	64.9	78.4	77.8	>90
16	AAA^{L}TGA^{L}TG^{L}G^{Me}C^{L}TGC	63.1	82.1	82.4	n.d.[b]
17	AAATGPATGGCPTGC	57.8	58.5	66.4	66.4
18	AAAPTGATPGGCTPGC	61.1	65.1	58.5	60.5
19	APAATPGATPGGCTPGC	67.5	66.8	65.7	62.7
20	AAATPGATPGGPCTPGC	**59.7**	59.7	59.7	**36.7**
21	AAPATPGATPGGPCTPGC	**65.4**	61.4	59.3	**37.4**
22	APAAPTGPATPGGPCTPGC	**66.4**	68.0	61.7	**30.1**

[a] **P** denotes INA monomer. **T**^{L}, ^{Me}**C**^{L}, **A**^{L}, **G**^{L} denote locked nucleotides of thymine, 5-methylcytosine, adenosine and guanosine, respectively; [b] n.d., not determined.

In fluorescence in situ hybridization (FISH) experiments, the partial denaturation of chromosome is achieved by adding formamide.[13] The challenge in FISH is the detection of single copy sequence tracts. This requires both very high binding affinity and specificity of short oligonucleotides as well as extremely sensitive, non-time-consuming hybridization detection.[14] We believe that easily denaturing nucleic acid duplexes containing fully modified LNA together with its complementary INA-rich strand could be an attractive design for FISH.

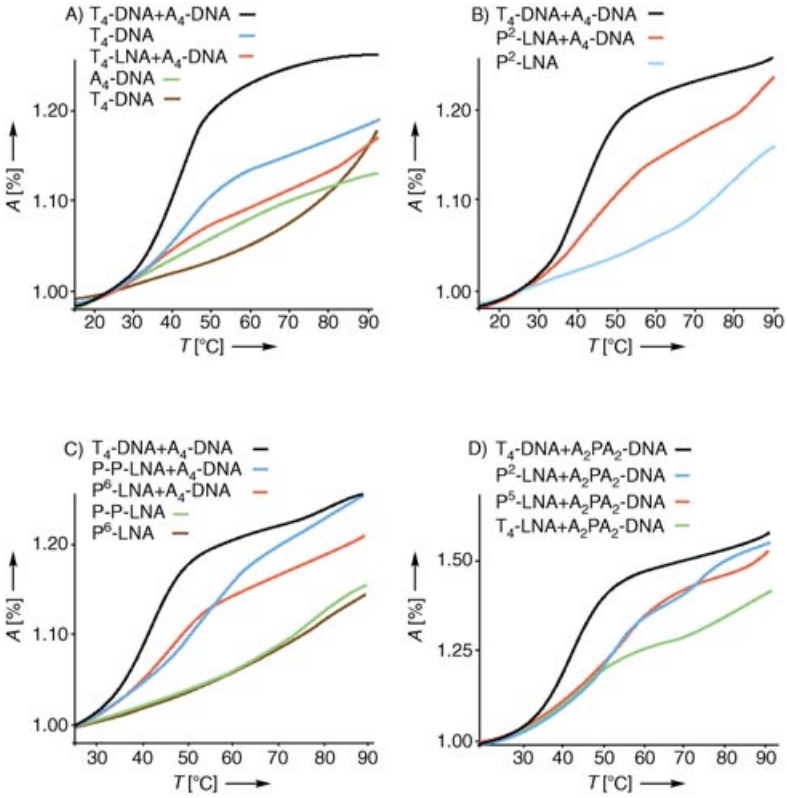


Figure 1. *Melting curves for hairpin probes with ssDNA targets detected at 260 nm. See Table 3 for a sequence key and Experimental Section for details. The hyperchromicity of T_4-DNA/A_4-DNA duplex in Figure 1A–C is used as a reference system.*

DNA hairpins

For "quenched" LNA, due to formation of stable hairpins, we decided to apply the findings of using LNA and INA for the design of easily denaturing nucleic acid duplexes to making LNA-hairpins that are more accessible for targeting native DNA. The hairpin-forming oligo T_4-DNA (see Table 3 for sequence key), which possesses either LNA or INA monomers, was used in the present investigation.

On heating the unmodified hairpin T_4-DNA alone in a thermal melting experiment, a clear transition was observed at 37.2 °C (Figure 1A, blue line, and Table 3), which was ascribed to opening of the hairpin to ssDNA. In a similar experiment with a hairpin containing LNA nucleotides in the stem, a higher transition temperature was expected due to increased stability, as judged from the reported elevated stabilities of LNA/DNA duplexes.[4,6] To test this hypothesis, T_4-LNAs (Table 3), analogous to T_4-DNA, were synthesized with five DNA nucleotides in the stem being replaced with the corresponding LNA monomers (T^L and $^{Me}C^L$). For this modified hairpin, only an incipient transition from hairpin to ssLNA could be observed above 80 °C (Figure 1A, brown line).

An equimolar mixture of T_4-DNA and A_4-DNA (Figure 1A, black line) gave a transition at the same temperature as the one observed for T_4-DNA alone (Figure 1A, blue line), but the increase in optical density (hyperchromicity) was much stronger for the transition of the T_4-DNA/A_4-DNA mixture. The increased hyperchromicity of the mixture could be best explained by the melting of a T_4-DNA/A_4-DNA duplex, because A_4-DNA had no transition above 20 °C (Figure 1A, green line). This is in agreement with earlier reports that adenine compared to thymine in the loop, destabilized a hairpin.[15] From melting curves it was impossible to estimate the ratio of T_4-DNA distribution between its hairpin structure and its duplex structure with A_4-DNA.

Due to the high stability of the T_4-LNA hairpin and the instability of the A_4-DNA hairpin, no transitions were expected to be found in the temperature range 20–80 °C for a mixture of these two oligomers. It was therefore puzzling to find a transition at 37.3 °C with a very low hyperchromicity (Figure 1A, red line). This extra transition for the T_4-LNA/A_4-DNA mixture can be best understood by comparison with the properties of palindromic sequences that have been extensively studied by NMR. For example, it has been shown that the self-complementary sequence 5′-CGCGTTAACGCG has two transition states, at 33 °C (duplex⇌hairpin) and at 48 °C (hairpin⇌random coil).[16] For the 5′-CGCGTTAACGCG sequence, complete conversion from hairpin to duplex has never been observed by NMR. This may indicate quenching

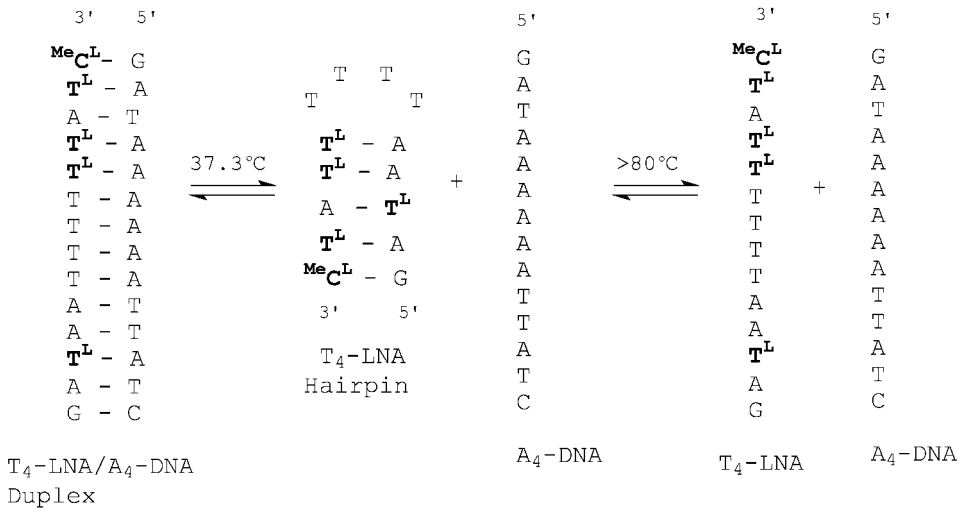
Table 3. *Transition temperatures, T_m [°C] for hairpin probes possessing INA and LNA with ssDNA targets.*[a]

Probes	Sequence, 5′→3′	Targets, 3′→5′		
		no target	A_4-DNA CTATTAAAAAATAG	A_2**P**A_2-DNA CTATTAA**P**AAAATAG
no probe		–	< 20	< 20
T_4-DNA	GATAATTTTTTATC	37.2	37.2	40.3
T_4-LNA	GA**T**^{L}AATTTT**T**^{L}**T**^{L}A**T**^{L} ^{Me}**C**^{L}	> 80	37.3	44.7
P^2-LNA	G**P**A**T**^{L}AATTTT**T**^{L}**T**^{L}A**T**^{L} ^{Me}**C**^{L}	(81.1)	46.4	49.9
P^5-LNA	GA**T**^{L}A**P**ATTTT**T**^{L}**T**^{L}A**T**^{L} ^{Me}**C**^{L}	(71.4)	43.3	51.9
P-P-LNA	G**P**A**T**^{L}A**P**ATTTT**T**^{L}**T**^{L}A**T**^{L} ^{Me}**C**^{L}	(69.1)	53.6	61.8

[a] **P** denotes INA monomer. **T**^{L} and ^{Me}**C**^{L} denote locked nucleotides of thymine and 5-methylcytosine, respectively.

of the equilibrium at temperatures lower than the first transition temperature.

By comparison with the nature of the palindromic sequences, the melting of the T_4-LNA/A_4-DNA mixture at 37.3 °C is best explained by a transition from LNA/DNA duplex to a mixture of T_4-LNA hairpin and A_4-DNA single strand (Scheme 2). The low hyperchromicity for the transition seems to indicate that the conversion from a mixture of A_4-DNA and T_4-LNA hairpin to DNA/LNA duplex was incomplete.



Scheme 2. *Proposed duplex resulting from hairpin/single-strand to single-strand/single-strand transformation.*

For palindromic oligomers, it has been suggested that the transition from duplex to hairpin took place through formation of a cruciform structure formed after creation of an initial bulge in the centre of the duplex upon melting.[17] Once the cruciform was formed, little energy was needed to propagate the mobile junction formed and to complete the separation of the two hairpins. We can argue for a similar mechanism in our case and also for the same type of mechanism operating in the opposite direction, because identical melting curves for up and down temperature modes were obtained.

As the duplex-hairpin transition depends on the nature of two complementary strands, it is assumed that the T_4-LNA hairpin is more easily transformed into a duplex if the DNA complement has a better binding affinity. This was indeed found to be the case when the INA monomer P was inserted into the middle of the A_4 region in the A_4-DNA (Table 3). For the oligo A_2PA_2-DNA in its duplex with T_4-LNA, melting was detected at 44.7 °C. Furthermore, a significant increase in the hyperchromicity was observed for this duplex when compared with the T_4-LNA/T_4-DNA duplex (Figure 1 D green line, and 1 A red line, respectively). The higher hyperchromicity indicates that the T_4-LNA/A_2PA_2-DNA duplex has a greater ability to be formed.

From the above finding (Tables 1 and 2) that insertions of monomer P opposite to the LNA monomer decreases the melting temperatures of LNA containing duplexes, it was deduced that proper insertions of monomer P in the stem of a T_4-LNA hairpin could reduce its thermal stability and make it prone to targeting A_4-DNA. We decided to incorporate the pyrene moiety into the DNA-rich and not into the LNA-rich stem of T_4-LNA hairpin, because this design gave the best result for easily denaturing nucleic acids.

As the transition temperature of T_4-LNA was too high to be determined, it was encouraging to observe thermal transitions for the monopyrene-modified oligomers P^2- and P^5-LNA at 81.1 °C and 71.4 °C, respectively, and for the double-pyrene-modified oligomer P-P-LNA at 69.1 °C. As the hyperchromicity was extremely low for these three transitions, one should be very cautious about the interpretation; this is symbolized by using parentheses for these transitions in Table 3. Irrespective of whether these transitions were due to opening of hairpins or to melting of undefined intermolecular complexes, to us they indicated that these pyrene-modified oligomers could be more accessible for duplex formation with their complementary ssDNA targets than T_4-LNA itself.

For the oligomer P^2-LNA, with the INA monomer P inserted after the first nucleotide in the stem, a considerably stronger hyperchromicity was observed on melting of the duplex with A_4-DNA (Figure 1 B, red line) than for T_4-LNA/A_4-DNA mixture (Figure 1 A, red line). The hyperchromicity was approximately half that observed for the T_4-DNA/A_4-DNA duplex which is shown in Figure 1 A–C as a reference. An increase in the transition temperature was also observed, as should be expected because of the stabilising effect of monomer P on hybridization to DNA (Table 2). With respect to both the transition temperature and hyperchromicity, a similar result was found for the oligomer P^5-LNA with a P monomer insertion close to the loop of its hairpin form when mixed with A_4-DNA (Figure 1 C, red line). With two P monomer insertions in the stem region, the resulting oligo P-P-LNA showed an even higher transition temperature for its corresponding duplex with A_4-DNA (Figure 1 C, blue line). More strikingly, the hyperchromicity was nearly the same as the one for the unmodified duplex. This is a clear demonstration that monomer P insertions into LNA with secondary structures can make this type of LNA more accessible to targeting and at the same time increase the duplex stability with the target. The latter was deduced from higher transition temperatures for the LNA probes with monomer P insertions (Table 3).

We attempted to further stabilize the duplexes with the LNA probes by inserting monomer P into the complementary DNA target. The pyrene moiety was inserted in the region of DNA corresponding to the loop in the LNA probes. As seen from Table 3, the highest transition temperature was found for two P monomer insertions in the LNA probe. Stabilizing the duplexes by extra P monomer insertion in the target also improved

the hyperchromicity as was observed for the A_2PA_2-DNA oligo when it formed complexes with P^2-LNA and P^5-LNA probes (Figure 1 D, blue and red lines).

Here we would like to mention that instead of synthesizing long, fully modified LNA, which could form hairpins, one should rather divide the strand into LNA-rich and INA-rich regions. This will allow such sequences to target native DNA.

Conclusion

The previous finding that bulged insertions of (*R*)-1-*O*-(pyren-1-ylmethyl)glycerol into deoxynucleotide strands reduces the transition temperatures of RNA/DNA duplexes was also found to be true for LNA/DNA duplexes. It was very effective when pyrene moiety P was inserted into oligodeoxynucleotide as a bulge opposite to an LNA monomer. This finding was used to make single-stranded and double-stranded modified complementary nucleic acids that are less stable than their corresponding complexes with native DNA. The best result was obtained with fully modified 13-mer LNA and the complementary INA possessing six P monomers as bulges after every second base pair. The hairpin-forming LNA was more accessible to targeting ssDNA if pyrene P moieties were inserted in the DNA-rich stem opposite LNA. The hyperchromicity of the thermal transition was considerably increased when compared with the mixture of LNA-hairpin with ssDNA. We think that our design of easily denaturing nucleic acid duplexes using locked and intercalating nucleic acids will be useful for targeting native DNA in biological studies.

Experimental Section

Oligonucleotide synthesis by using LNA and INA monomers: Incorporation of LNA and INA monomers into oligodeoxynucleotides (ODNs) were performed on a 0.2 μmol scale and was followed by purification of the ODNs with DMT-on by HPLC as previously described.[6b,7a] MALDI-TOF analysis was performed on a Voyager Elite Biospectrometry Research Station from PerSeptive Biosystems. All modified oligonucleotides gave satisfactory composition with a purity of over 93%, except for fully modified LNA (above 80%), which was verified by capillary gel electrophoresis.

Measurement of transition temperatures: Transition analyses were carried out on a Perkin Elmer UV/VIS spectrometer Lambda 2 with a PTP-6 (Peltier Temperature Programmer) device by using PETEMP rev. 5.1 software and PECSS software package v. 4.3. Melting temperatures (T_m, °C) were determined as a first derivative of melting curves, which were obtained by recording absorbance at 260 nm as a function of temperature at a rate of 1 °C min^{-1}. Thermal stability studies presented in Tables 1 and 3 and in Figure 1 were performed in hybridization buffer (10 mM Na-phosphate, 120 mM NaCl, 1 mM ethylenediaminetetraacetate (EDTA), adjusted to pH 7.0) at a concentration of 3.0 μM of each strand. A medium salt buffer (10 mM Na-phosphate, 100 mM NaCl, 0.1 mM EDTA, adjusted to pH 7.0) and 1.0 μM concentration of each strand were used in experiments presented in Table 2. The solutions were heated to 90 °C, maintained for 5 min at this temperature, and then gradually cooled before melting experiments. All melting temperatures are reported with an uncertainty ±0.5 °C, as determined from multiple experiments.

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