α,β-D-CNA induced rigidity within oligonucleotides†

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Introduction of α,β-D-CNA featuring canonical values of the torsional angles α and β within oligonucleotides leads to an overall stabilization and improved rigidity of the duplex DNA as demonstrated by UV experiments, circular dichroism and corroborated by molecular dynamics simulations.

The challenging task in the field of oligonucleotide chemistry of bringing the α–ζ dihedrals along the sugar–phosphate backbone under control would help the chemist to prepare well-defined structures of nucleic acids and to better understand the complex interrelation that lies between these torsions and the overall solution structure of DNA. In structural terms, the most promising DNA analogues are usually expected to be those for which the backbone torsion angles closely match the geometry of torsion angles α–ζ in standard A- or B-type duplexes (Fig. 1).[1]

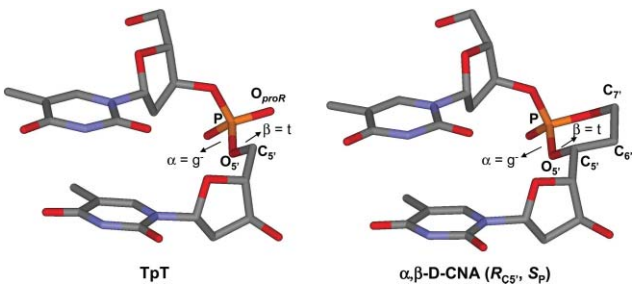

Fig. 1 Stick plot showing the half-chair conformation present in an unmodified TpT step in a B-type duplex and the chair conformation of the dioxaphosphorinane structure restricting the torsional angles α and β within ($R_{C5'}$,S_P) α,β-D-CNA.

The concept of conformational restriction has lead to nucleic acid analogues with promising antisense applications in therapy and diagnosis.[2] The most prominent recent examples of such analogues that leads to duplexes with an increased thermal stability are locked nucleic acids (LNA) in which the ribofuranose subunit is locked (*via* a covalent bridge between the 2′-oxygen and 4′-carbon atoms) into the 3′-*endo* conformation ($\delta = g^+/a^+$), so that the sugar–phosphate backbone of the modified strand closely resembles the A-form geometry adopted in RNA hybrids.[3,4]

Nevertheless, to date, there has been no successful approach to constraining into a canonical value any torsional angle other than δ with respect to the others.[5] Interestingly, when looking to a dinucleotide step extracted from a B-form duplex, one can see that the C5′, O5′, P and O_{proR} atoms are in the same geometry as the one defined in a six membered ring in a chair conformation (Fig. 1). Thus the canonical values (g^-,t) of the α and β torsional angle respectively, could be maintained in such a structure. Therefore our aim was to fix this canonical geometry by connecting the C5′ to the O_{proR} atom of the phosphate linkage by an ethylene group *i.e.* introducing them in a dioxaphosphorinane structure with an *R* and *S* absolute configuration of the 5′ carbon and phosphorus atom respectively.

With this in mind, we have undertaken an experimental program to explore the chemistry of DNA and RNA analogues with a modified backbone. Our approach is based on the introduction of the neutral 1,3,2-dioxaphosphorinane ring structure at key positions along the sugar–phosphate backbone (Fig. 2).[6]

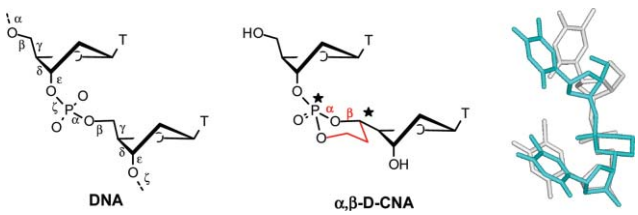

Fig. 2 Left: the six backbone torsion angles (labelled α to ζ) of nucleic acids. Middle: the α,β-D-CNA dinucleotide in which α and β are stereocontrolled by a dioxaphosphorinane ring structure. Right: superimposition of the X-ray structures of ($R_{C5'}$,S_P) α,β-D-CNA TT (blue)[7] and of unmodified TpT (grey).[8]

We reasoned that the resulting constrained nucleic acids (termed D-CNAs) could locally adopt either helical or non helical conformation depending on the spatial arrangement of the dioxaphosphorinane system and which backbone bonds are included in its ring structure. As an initial step in this direction, we have already reported on the synthesis and binding properties of oligonucleotides including α,β-D-CNA dinucleotide building units in which α and β torsions are locked either in a canonical (g^-,t) or in noncanonical (g^+,t) conformation.[9] We showed that the ($R_{C5'}$,S_P) configured α,β-D-CNA TT (g^-,t), fitted well at the dinucleotide level with unmodified TpT (Fig. 2) and increased DNA duplex formation ability (+5 °C/mod).

To better understand the impact of the incorporation of an α,β-D-CNA (g^-,t) within an oligonucleotide, we have measured melting temperatures of duplexes with various and relative positions of α,β-D-CNA (g^-,t) and simulated its dynamic behaviour within the duplex with AMBER 8 software.[10]

The molecular dynamics of two duplexes have been simulated, an unmodified dA_{10}–dT_{10} (**ODNref**) as reference and a dA_{10}–dT_4**TTT**$_4$ (**ODNgm**) in which the α,β-D-CNA (g^-,t) is located in

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the middle of the dT strand, for 1520 and 3100 ps, respectively. The resulting structures are shown with the axis average position of the double helix (Fig. 3) and the atomic fluctuations of each simulated duplex strand (Fig. 4).

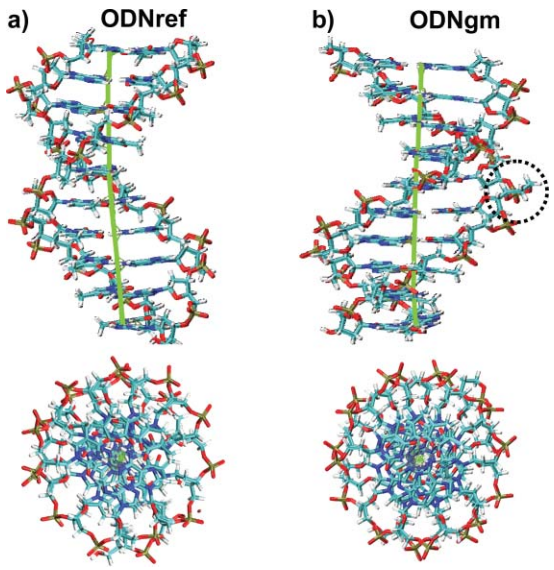

Fig. 3 Side and top views of the B-type duplex produced by molecular dynamics simulations (AMBER 8.0), the double helix axes are outlined in green. a) unmodified dA_{10}–dT_{10} (**ODNref**). b) dA_{10}–$dT_4\mathbf{TTT}_4$ (**ODNgm**), **TT** = ($R_{C5'}$,S_P) α,β-D-CNA TT.

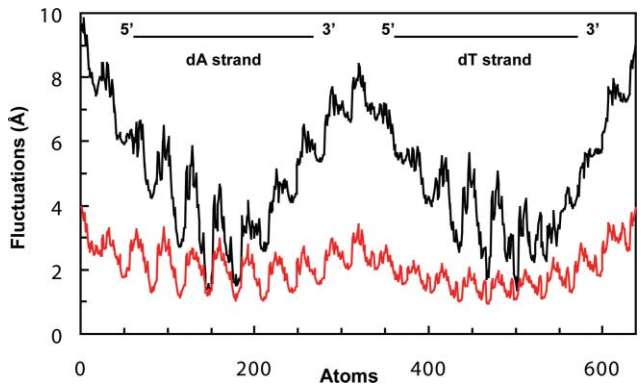

Fig. 4 Atomic fluctuations of each strand of simulated duplexes dA_{10}–dT_{10} (**ODNref**, black, 1400 ps) and dA_{10}–$dT_4\mathbf{TTT}_4$ with **TT** = ($R_{C5'}$,S_P) α,β-D-CNA TT (**ODNgm**, red, 2020 ps).

A first indication of the induced rigidity, is the lower RMSD (root mean square deviation) calculated for **ODNgm** (1.96) with respect to **ODNref** (2.18), which can be visualized by the straightness of the double helix axis of the modified duplex (Fig. 3b). But the most impressive result stood in the restricted atomic fluctuations observed in both strands of **ODNgm** compared to **ODNref** (Fig. 4). Whereas **ODNref** exhibits a maximum of fluctuation variation, ΔF_{max} of 8.53 and 7.69 Å for dA and dT strands respectively, ΔF_{max} are lowered to 2.95 and 2.91 Å, respectively for **ODNgm**.

It is clear that the α,β-D-CNA (g^-,t) induced a major effect in the middle of the sequence where fluctuations are usually minimized, with local fluctuations (ΔF_{mid}) of 1.01 Å in the dT strand but also in the complementary dA strand (ΔF_{mid} = 1.91 Å) to be compared with 3.70 and 3.35 Å respectively in **ODNref**.

Table 1 Sequences and melting temperatures of α,β-D-CNA containing duplexes

ODN	Sequence[a]	T_m[b]/°C	ΔT_m/°C
1	dA_{14}–dT_{10}	24.2	—
2	dA_{14}–$dT_4\mathbf{TTT}_4$	30.1	+5.9
3	dA_{14}–dT_{14}	35.0	—
4	dA_{14}–$dT_6\mathbf{TTT}_6$	38.1	+3.1
5	5′-GCAAAAACTTGC-3′ 3′-CGTTTTTGAACG-5′	48.0	—
6	5′-GCAAAAAC**TT**GC-3′ 3′-CGTTTTTGAACG-5′	53.2	+5.2
7	5′-GCAAAAACTTGC-3′ 3′-CGTTT**TT**GAACG-5′	52.2	+4.2
8	5′-GCAAAAACTTGC-3′ 3′-CGT**TTT**GAACG-5′	54.3	+6.3
9	5′-GCAAAAACTTGC-3′ 3′-CGT**TT**TTGAACG-5′	53.4	+5.4
10	5′-GCAAAAACTTGC-3′ 3′-CG**TT**TTTGAACG-5′	52.4	+4.4
11	5′-GCAAAAAC**TT**GC-3′ 3′-CGTTT**TT**GAACG-5′	55.9	+7.9
12	5′-GCAAAAAC**TT**GC-3′ 3′-CGTT**TT**GAACG-5′	56.4	+8.4
13	5′-GCAAAAAC**TT**GC-3′ 3′-CGT**TTT**GAACG-5′	57.6	+9.6
14	5′-GCAAAAAC**TT**GC-3′ 3′-CG**TT**TTTGAACG-5′	57.8	+9.8

[a] **TT** = ($R_{C5'}$,S_P) α,β-D-CNA TT within the strand. [b] UV melting experiments were carried out in sodium phosphate buffer (10 mM, pH 7.0) containing NaCl (100 mM) and EDTA (1 mM).

These results nicely illustrate the examination of the thermal melting temperature behavior of duplex **2** and **4** with ΔT_m of +5.9 and +3.1 °C, respectively (Table 1). It is noteworthy that one ($R_{C5'}$,S_P) α,β-D-CNA TT within an oligothymidilate strand increased the DNA duplex formation ability to the same extent as one LNA containing oligothymidilate does towards its RNA counterpart only.[4]

These results underscore the fact that α,β-D-CNA stands for a proper DNA mimic with both the *α* (*gauche*(−)) and *β* (*trans*) torsional angles fixed in the canonical B-type values, but also with a sugar puckering in favour of a south conformation as a consequence of the presence of a neutral phosphotriester linkage.[11]

A high average stabilization of +5.0 °C was observed for **ODN 6–10** regardless of the α,β-D-CNA position within the strand (Table 1, entries 6–10), which is in accordance with our preliminary published results.[9] Interestingly, a combination of two α,β-D-CNA positioned on each complementary strand reached the maximum overall duplex stabilization (ΔT_m = +9.8 °C) when the two modified nucleotides were located the furthest away from each other (Table 1, entry 14). In contrast, this stabilization was lowered by ~2 °C (ΔT_m = +7.9 °C) when the two modifications were positioned close by (Table 1, entry 11). Therefore, this suggests that a synergetic effect was obtained when the α,β-D-CNA are positioned near the 3′-end of the strand with a favorable propagation of the stabilization in the 5′end direction. These experiments also provide evidence that the neutrality brought by the phosphotriester moieties is not responsible for the observed improvement of duplex stabilization as exemplified by **ODN 12**, in which these two neutralized phosphates are opposite in the minor

groove of the duplex, which did not exhibit a particular behavior with a ΔT_m of +8.4 °C (Table 1, entry 12). This is also supported by the fact that the differences in T_m values between the duplexes (**ODN 11–14**) containing the α,β-D-CNAs and the natural control duplex (**ODN 5**) are largely insensitive to a 50-fold increase in the concentration of NaCl (in the 0.02–1.0 M range, ESI, Fig. S4†). Indeed the rigidity brought by the dioxaphosphorinane structure replacing the ionic phosphodiester linkage enables duplex stability to be strengthened instead of diminished as is often observed with a nonionic group.[12]

Further information on the structure of the DNA duplexes was obtained from CD spectroscopy. The CD spectra of the complementary duplexes **ODN 2** and **4** with $d(A_{14})$ are very similar to that of unmodified **ODN 1** and **3** (ESI, Fig. S5 and S6†). However, an increase of the positive Cotton effect at about 280 nm for both ODNs can be seen including the α,β-D-CNA analog that could be attributed in this particular case of oligothymidylate to a slight structural alteration in nucleobase organization within the duplex. On the other hand, CD spectra of the duplexes **ODN 6–14** which are also very similar to that of their native DNA counterpart **ODN 5** with a more classical B-DNA type shape (ESI, Fig. S7–S10†), showed a tightening of the positive Cotton effect by 2 nm that could reveal a diminishing in the overall conformational states of the duplex structure. Nevertheless, these results indicated that with neither one nor two modifications is there any significant change in binding mode or conformation with respect to the natural system.[13]

In summary, the present study showed that preorganized ($R_{C5'}$,S_P) α,β-D-CNA TT within an oligonucleotide allowed a high DNA duplex stabilization level either in oligothymidilate or in mixed sequences. Introduction of a dioxaphosphorinane structure in the internucleotidic linkage induced a conformational rigidity that seems to reduce the overall duplex flexibility and induced straightness of the B-type double helix structure. Therefore α,β-D-CNA dinucleotides constrained in canonical B-type form may become an interesting tool to probe the relative importance of local DNA duplex flexibility towards protein recognition.

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