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Boundary Between DNA and Enantio-DNA as a Mimic of B-Z Junction.

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Abstract: A chimeric 16 b.p. long DNA fragment presenting two enantio-domains has been synthesized. The first domain constituted of base-paired D-nucleotides has a righ-handed B conformation while the second domain includes base-paired L-nucleotides and has a left-handed conformation, mirror image of B-DNA. Data from U.V. melting experiments indicate that the jonction between these two domains mimics to some extent B-Z junctions.

The conformational heterogeneity of DNA has been established. Left-handed Z-DNA represents a major conformational change which can exist *in vivo*[1,2] although its precise biological role remains to be identified[3]. The existence of short stretches of Z-DNA within a long right-handed B-DNA helix implies the formation of B-Z junctions. The properties of these junctions in plasmids and polynucleotides have been examined by a variety of methods[4,5]. It was concluded that the B-Z junction is short, probably less than five base pairs (b.p.) long, forms with an energetic cost in a sequence dependent manner and perturbs the structure of the flanking right-handed and left-handed-helix regions. More recently, oligodeoxynucleotides have been designed that are amenable to the formation of B-Z junctions provided conditions were applied which stabilized the left-handed part of the duplex, i.e. alternating purine-pyrimidine sequences, inclusion of 5-methylcytosine and use of high salt concentrations while the second part of the duplex remained the right-handed conformation[6-8].

A non-Z left-handed conformation, mirror image of right-handed B-DNA, was evidenced by circular dichroism in enantio-DNA[9,10] which is constituted of enantiomeric deoxy-2'-L-nucleotides. Moreover, it was concluded that DNA and enantio-DNA have the same type and strength of hydrogen-bonding and base-base staking interaction. Although the mirror image of B-DNA (thereafter called B*-DNA) is definitely different from Z-DNA, we addressed the question whether a B-B* junction between right-handed B-DNA and left-handed enantio-DNA might mimic a B-Z junction in terms of energy and size of the junction. If the answer is yes, designing and studying such a junction as a model of B-Z junction will avoid sequence limitations, use of base modifications and high salt conditions required to stabilize the left-handed part of the duplex[11].

In figure 1 are shown the different duplexes used in the present study[12]. Each duplex is formed of two blunt-ended domains of eight b.p. and linked together by natural 3'-5' phosphodiesters linkages.

Figure 1. Sequences of oligodeoxynucleotides used in this study.

Duplexes	Sequences	Comments
D1L2	$^{5'}$d(CAGTCGGT**CAAGTAGT**)$^{3'}$ $^{3'}$d(GTCAGCCA**GTTCATCA**)$^{5'}$	(D)DNA-(L)DNA junction containing duplex
mD1L2	$^{5'}$d(CAGTCGGT**CAAGTAGT**)$^{3'}$ $^{3'}$d(GTCA**T**CCA**GTTCATCA**)$^{5'}$	C•T mismatch in domain D1 of D1L2
D1mL2	$^{5'}$d(CAGTCGGT**CAA**T**TAGT**)$^{3'}$ $^{3'}$d(GTCAGCCA**GTTCATCA**)$^{5'}$	C•T mismatch in domain L2 of D1L2
mJ	$^{5'}$d(CAGTCGGT**CAAGTAGT**)$^{3'}$ $^{3'}$d(GTCAGCCA**T**TTCATCA)$^{5'}$	C•T mismatch in the junction of D1L2
D1D2	$^{5'}$d(CAGTCGGTCAAGTAGT)$^{3'}$ $^{3'}$d(GTCAGCCAGTTCATCA)$^{5'}$	(all D)DNA with same sequence as in D1L2
D1mD2	$^{5'}$d(CAGTCGGTCAA**T**TAGT)$^{3'}$ $^{3'}$d(GTCAGCCAGTTCATCA)$^{5'}$	C•T mismatch in domain D2 of D1D2
mD1D2	$^{5'}$d(CAGTCGGTCAAGTAGT)$^{3'}$ $^{3'}$d(GTCA**T**CCAGTTCATCA)$^{5'}$	C•T mismatch in domain D1 of D1D2

N or **N** denotes deoxy-2-D (or L)-ribonucleotides respectively. Underlined character indicates the position of the mismatch.

The left domain D1 in duplex D1L2 is a short natural DNA fragment whereas the right domain L2 is a short enantio-DNA. With the exception of the few base pairs included in the B-B* junction, it is assumed that the two domains D1 and L2 present the same conformation that the one they would have when not linked, i.e. B and B* respectively[13]. Since presence of a single mismatch is able to strongly decrease the thermal stability of perfectly base-paired duplexes, a C•T mismatch was introduced either in domain D1 or L2 four b.p. far from the boudary between the two domains, leading to mD1L2 and D1mL2 respectively. In contrast, introduction of a mismatch within the destabilized B-B* junction, as in mJ, is expected to produce a smaller effect. Comparison of thermal stability of these mismatched duplexes with that of D1L2 would allow to conclude on the extent of the B-B* junction in D1L2. An all natural duplex having the same sequence as that of D1L2, designed D1D2, was used as reference, as well as mD1D2 and D1mD2 having the same mismatch as those introduced in mD1L2 and D1mL2 respectively.

UV melting profiles of duplexes formed at 2 μM concentration in low salt conditions (0.1 M NaCl, 0.01 M sodium cacodylate, pH 7) are presented in Fig. 2. Each curve exhibits a single cooperative transition from which the melting temperature and thermodynamic parameters were calculated (Table). Introduction of a single C•T mismatch in the middle of either domain D1 or L2 in D1L2 produced a drop in Tm of 9.4 and 15.9 °C respectively. The average value of these ΔTm is comparable to the decreases observed when the same C•T mismatch was introduced in equivalent positions in D1D2 (ΔTm 11.5 and 12.4 °C for D1mD2 and mD1D2 respectively as compared to D1D2) and is markedly higher than that obtained when a C•T mismatch was adjacent to the boundary between the two domains in D1L2 (ΔTm 3.1 °C).

Taking in account the previously made assessment that both DNA and enantio-DNA possess the same conformation, base pairing and dynamic properties except for chirality[9,10], these results suggest that both

domains D1 and L2 are largely in the Watson-Crick duplex form and that B-B* junction does not encompass more than two or three b.p. on each side of the boundary. Furthermore, the drop of 15 °C observed in Tm values between D1D2 and D1L2 may be considered as a result of the sole presence of a B-B* junction in the later duplex. This B-B* junction is associated with a decrease in free energy of 6.1 kcal/mol which is enthalpic in origin.

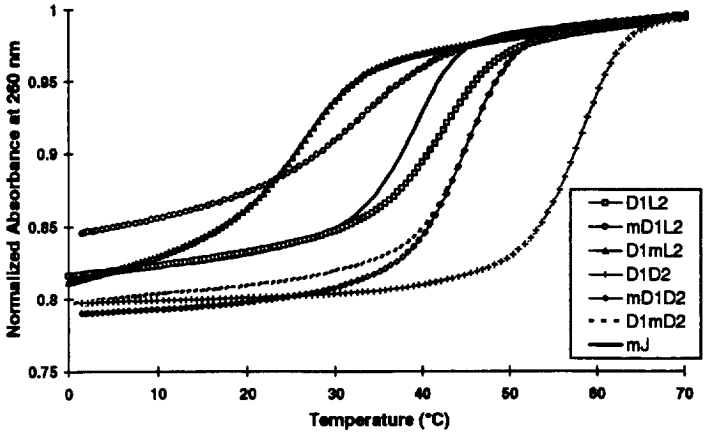

Figure 2. U.V. melting curves of duplexes formed at 2 µM for each strand in low salt conditions (0.1 M NaCl, 0.01 M sodium cacodylate, pH 7).

Table. Thermodynamic parameters[a] obtained from U.V. melting experiments.

	D1L2	mD1L2	D1mL2	mJ	D1D2	mD1D2	D1mD2
Tm (°C)	41.8	32.4	25.9	38.7	56.8	44.4	45.3
$-\Delta H°$ (kcal/mol)	84.3	54.3	69.4	94.4	109.5	99.4	101.2
$-\Delta S°$ (eu)	240	150	205	275	304	286	290
$-\Delta G°_{25°C}$ (kcal/mol)	12.7	9.5	8.4	12.4	18.8	14.3	14.7

[a] A non linear least-square method was used to fit the melting curves[14], applying a two state model with a bimolecular reaction[15]. The root mean square difference between the data and calculated curve was less than 0.4%. The Tm values given are the temperature for α equals 0.5 where α is the fraction of strand in double helix.

This later value falls in 4 to 7.7 kcal/mol range reported by Dai *et al* [16] for the estimated decrease in free energy associated with true B-Z junction and is 1.4 kcal/mol higher than that reported by Doktycz *et al*[17]. Although comparison with free energy values found for true B-Z junction should be taken with care due to the salt concentration[17,18] and sequence[8] dependence of B-Z junction free energy, our results supports the capacity of B-B* junction to mimic true B-Z junctions to some extent.

Finally, difference of destabilization observed when a C•T mismatch was introduced in domain D1 ($\Delta\Delta G°_{25°C}$ + 3.2 kcal/mol) or in domain L2 ($\Delta\Delta G°_{25°C}$ + 4.3 kcal/mol) of D1L2 deserves comments. Difference in sequence of b.p. flanking the B-B* junction in D1L2 may induce a non equal spanning of D1 and L2 domains by the B-B* junction which in turn could affect differently the stability of the base pairs located four b.p. far from the boundary between domains D1 and L2.

In conclusion, during this work, was synthesized the first short DNA fragment presenting two domains of opposite handedness and stable at physiological salt concentration without the assistance of any base

modification or peculiar sequence. Our results indicate that the B-B* junction is comparable to a B-Z junction in terms of free energy and size. A more precise knowledge of its structure in relation with its reactivity towards intercalating drugs will be obtained by NMR spectroscopy. Work along this line is in progress.

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11. While this work was in progress, 8-methylguanine was shown to stabilize Z-DNA under physiological salt conditions[19].
12. The oligodeoxynucleotide synthesis was carried out on a ABI 381A DNA synthesizer using the phosphoramidite method. Substituting 2'-deoxy-L-nucleoside phosphoramidites[10,20] for 2'-deoxy-D-nucleoside phosphoramidites allowed assembling of L-enantiomeric domains. Deoxy-2'-L-thymine-loaded CPG was used for the synthesis of oligodeoxynucleotides having a L-domain at their 3'-end.
13. The circular dichroism spectrum of D1L2 at 13 °C exhibits very weak signals between 220 and 320 nm which could result from the presence of two enantio-domains having opposite handedness and producing opposite signals. A non-zero C.D. spectrum may arise from absence of true symmetry in terms of sequence and orientation of each domain.
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