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Enantiomeric deoxy-L-nucleotides stabilize a *Z*-forming DNA decanucleotide

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Abstract

Two contiguous pairs of enantiomeric 2′-deoxy-l-ribonucleotides have been incorporated in a 5-methyl-CG alternating decadeoxyribonucleotide duplex. Data from circular dichroism and UV melting experiments indicate that the two base-pair long enantio-domain within the duplex was able to stabilize a *Z*-DNA-like conformation.

Keywords: nucleic acids; conformation; enantiomeric nucleotides; circular dichroism.

The conformational heterogeneity of DNA has been well established. Local DNA conformations are thought to play a biological role in gene expression by altering DNA–protein interactions.[1,2] Left-handed *Z*-DNA represent a major conformational change which can exist in vivo although its precise role remains to be identified.[3] Properties of *Z*-DNA have been obtained through the use of poly[*d*(GC)] or DNA plasmids containing various alternating $(CG)_n$ inserts. Oligonucleotides can also adopt the *Z* conformation provided conditions are applied which can stabilize this left-handed conformation, i.e. use of high alcohol or salt concentrations[4–6] and introduction of chemical modifications such as C-5 methylation or C-5 bromination of cytosine.[7] More recently, C-8 methylation of guanine was shown to markedly stabilize the *Z* conformation of short oligodeoxynucleotides under physiological salt concentrations.[8,9]

A non-*Z* left-handed conformation, mirror-image of right-handed *B*-DNA, was evidenced by circular dichroism in enantio-DNA[10,11] which is constituted of enantiomeric 2′-deoxy-L-nucleotides. Additionally, it was concluded that DNA and enantio-DNA have, as expected, the same type and strength of hydrogen-binding and base–base stacking interactions. Although the mirror-image of *B*-DNA is markedly different from that of *Z*-DNA, we addressed the question as to whether the introduction into a short DNA duplex amenable to a *Z* conformation of a few unnatural 2′-deoxy-L-nucleotides, prone to induce a left-handed conformation, might contribute to stabilize the *Z* conformation.

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For that purpose, self-complementary decadeoxynucleotide d(^{Me}CG ^{Me}CG*CG* ^{Me}CG ^{Me}CG) (I, ^{Me}C stands for 5-methyl cytosine), having two enantiomeric deoxy-L-nucleotides (*C* and *G*) inserted in the middle of the sequence, was synthesized on a DNA synthesizer using the phosphoramidite method.[10,12] The location of the two L-nucleotides was chosen in a way that allows the formation of two contiguous *C*·*G* pairs upon self-hybridization of I, giving rise to a short enantio-DNA domain in the middle of the duplex.

Circular dichroism was used to monitor the conformational state at various sodium chloride concentrations and the results were compared with those obtained from corresponding stereoregular d(^{Me}CG ^{Me}CG ^{Me}CG ^{Me}CG ^{Me}CG) (II). The CD spectra for the NaCl titration of I and II at 13°C are shown in Fig. 1A and 1B, respectively. The low salt (0.1 M NaCl) spectrum of stereoregular II indicates the *B* conformation[13] with a trough at 255 nm and a peak at 284 nm and the high salt (3.5 or 4 M NaCl) spectrum indicates the *Z* conformation with a trough at 295 nm and a peak at 260 nm. Similar results were observed for L-nucleotide containing I although a deeper trough was observed at high salt concentrations. This is consistent with the existence for I of the *B* conformation at low salt concentrations and the *Z* conformation at high salt concentrations. Upon a gradual increase of the NaCl concentration, both I and II underwent a *B*-to-*Z* conformation transition. The NaCl concentration at the midpoint of the *B*-to-*Z* transition was determined by monitoring the formation of the 295 nm trough (Fig. 2A). Plots of $\Delta\varepsilon$ at 295 nm as a function of the NaCl concentration afford a sigmoidal curve for both I and II. The transition midpoint was 1.04 M NaCl for stereoregular II in agreement with previous results[14] obtained with d(^{Me}CG)$_4$, whereas, it was as low as 0.65 M for I. Moreover, the sharper transition observed in the latter case reflects a higher cooperativity during the *B*-to-*Z* transition.

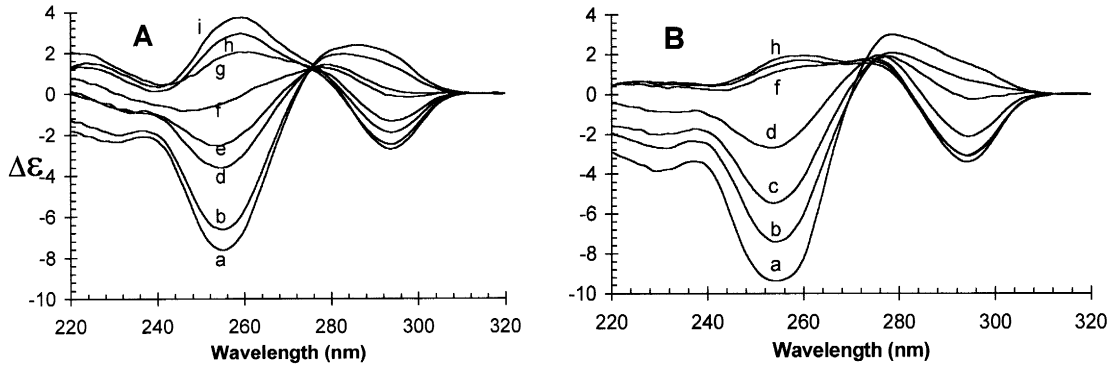

Fig. 1. The CD spectra for the NaCl titration of II (A) and I (B) in 0.01 M Tris–HCl (pH 7) at 13°C showing the *B*-to-*Z* transition. NaCl concentration was: 0.1 M (a); 0.5 M (b); 0.75 M (c); 1 M (d); 1.25 (e); 1.5 M (f); 2 M (g); 3 M (h); or 4 M (i). Oligonucleotide concentration was 14 μM

The effect of temperature on the conformation of I and II were studied by CD and UV spectroscopy. UV thermal denaturation studies of I were carried out at 260 nm. Each curve exhibited a single cooperative transition and corresponding melting temperature (T_m) values were obtained from the inflection point. At 2 M NaCl concentration, I has a linear $1/T_m$ versus ln[I] dependence over the 5–50 μM range of oligonucleotide concentrations (Fig. 2B). This rules out the formation of any hairpin duplex in equilibrium with a double-stranded duplex in the *Z* conformation.[15] The effects of temperature on the conformations of I and II have also been studied by circular dichroism. In 0.1 M NaCl, as the temperature increases (18, 44 and 54°C), the main changes are a decrease of the band amplitudes resulting from progressive melting of the *B*-form duplexes into single strands (data not shown). In 3 M NaCl and at 44°C, whereas the CD curve of II still exhibits a well at 295 nm, a large complex positive band centered

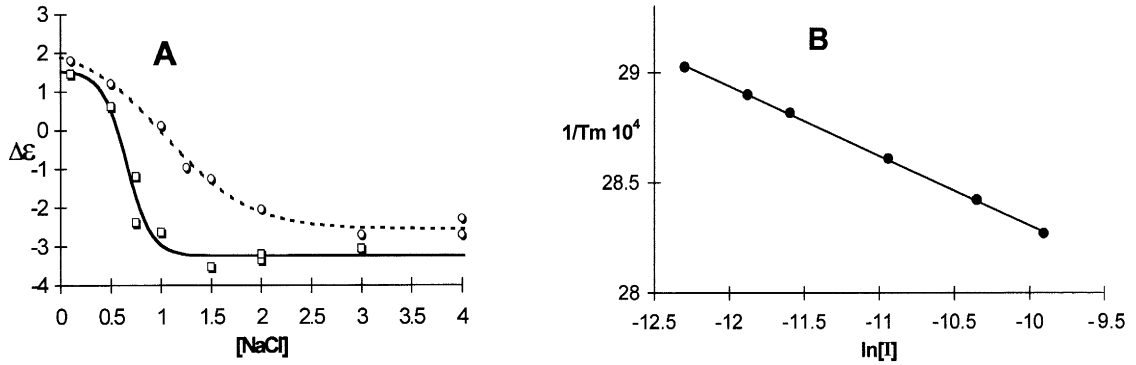

Fig. 2. (A) Salt concentration dependence of the *B*-to-*Z* transition for I (□) and II (⊙) at 13°C; $\Delta\varepsilon$ values at 295 nm were plotted for each salt concentration. (B) Plot of the reciprocal T_m as a function of the logarithm of oligonucleotide I concentration in 2 M NaCl, 0.01 M cacodylate (pH 7)

at 270 nm is present, reflecting dramatic conformational changes (Fig. 3A). At 54°C, the spectrum is similar to that observed at the same temperature and in 0.1 M NaCl. In contrast, the trough at 295 nm remains present for compound I at 54°C, revealing the persistence of the *Z* conformation in the duplex (Fig. 3B) in accordance with the high T_m value (T_m 76°C) observed in high salt conditions.

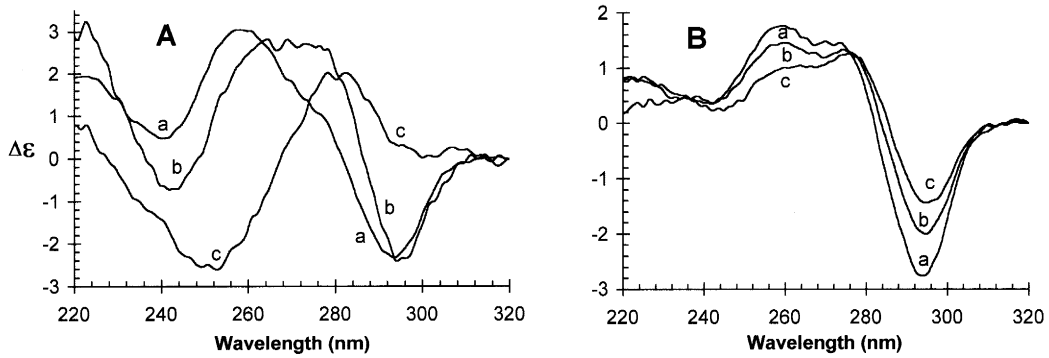

Fig. 3. CD spectra at: 18°C (a); 44°C (b); and 54°C (c) of I in the presence of 0.1 M NaCl (A) or 3 M NaCl (B)

In conclusion, we have found that substitution, within a short DNA duplex amenable to a *Z* conformation, of two contiguous nucleotide pairs for two pairs of enantiomeric L-nucleotides stabilize the left-handed *Z* conformation. This could be assigned to the ability of L-nucleotide pairs to induce, at low salt concentrations, a local left-handed conformation different from that existing in *Z*-DNA but which may contribute to lower the energy required for a *B*-to-*Z* transition in the DNA duplex. NMR studies which could confirm these findings and clarify the origin of the observed stabilizing effect are currently under way.

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