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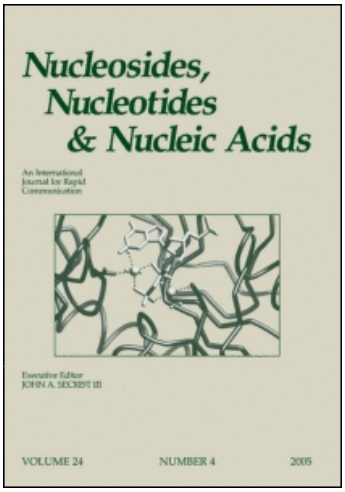

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PNA-DNA Chimeras Forming Quadruplex Structures

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PNA-DNA Chimeras Forming Quadruplex Structures

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ABSTRACT

^{1}H-NMR, CD, and UV spectroscopy have been used to investigate the structure of PNA/DNA chimeras forming quadruplex structures. In particular, we synthesized $^{5'}$TGGG$^{3'}$-t (1) and $^{5'}$TGG$^{3'}$-gt (2), where lower and upper case letters indicate PNA and DNA residues, respectively. CD spectrum and all NMR data of (1) are typical of quadruplexes involving four parallel strands. UV melting profile of (1) indicates that its thermal stability is quite similar to that observed for the reference structure $[d(TGGGT)]_4$. ^{1}H-NMR spectrum for $^{5'}$TGG$^{3'}$-gt (2) shows that this oligonucleotide is not able to fold into a single, well-defined species.

Key Words: PNA; DNA; Chimeras; Quadruplex structure; NMR.

Nucleic acids have the ability to fold into a variety of different structures. In particular, guanosine-rich DNA has been shown to form quadruplex structures. This structural *motif* has biological significance. In fact, genomic sequences from

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telomeric DNA, for an example, can form G-quartets and a multitude of proteins have been found to bind these DNA structures.[1] The design of modified oligonucleotides that, in principle, can recognize those target proteins is one of the major goal of our studies. Therefore, in an attempt to increase the stability of DNA quadruplexes, we decided to investigate the possibility for DNA/PNA chimeras to adopt these unusual structures. Peptide Nucleic Acid (PNA) is an analogue of DNA (Fig. 1) in which the pseudopeptide backbone replaces the canonical sugar-phosphate one. PNA mimics the behaviour of DNA and tightly binds complementary nucleic acid strands. We focused our attention on the analogues of the quadruplex $[d(TGGGT)]_4$. In particular, we synthesized $^{5'}$TGGG$^{3'}$-t (1) and $^{5'}$TGG$^{3'}$-gt (2), where lower and upper case letters indicate PNA and DNA residues, respectively. The DNA/PNA chimeras were synthesized by a recently proposed[2] synthetic approach. The two chimeras were completely characterized by spectroscopic methods and the quadruplex complexes were studied by ^{1}H-NMR, CD, and UV spectroscopy.

NMR samples were prepared at a concentration of 0.7 mM for (1) and 0.5 mM for (2) (0.5 ml, 90% H_2O/ 10% D_2O), having 10 mM potassium phosphate, 70 mM KCl, 0.2 mM EDTA (pH 7.0). ^{1}H-NMR spectra were recorded using pulsed-field gradient WATERGATE[3] for H_2O suppression. One-dimensional proton spectrum of $^{5'}$TGGG$^{3'}$-t (1) shows the presence of 5 signals from three G-H8 and two T-H6 protons in the aromatic region, and 3 imino peaks resonating between 11 and 12 ppm. These indicate the formation of a G-quadruplex structure containing three G-tetrads, and possessing a fourfold symmetry with all strands parallel to each other. Unfortunately ^{1}H-NMR spectrum for $^{5'}$TGG$^{3'}$-gt (2) shows that this oligonucleotide is not able to fold into a single, well-defined species. The proton spectrum of (1) has been partially assigned on the basis of NOESY and TOCSY data obtained at 500 MHz (T = 300 K). The NOESY data demonstrate that all three G residues are in an *anti* conformation by the weakness of the G-H8/H1' intraresidue NOEs. Moreover, CD spectrum (Fig. 2) is typical of quadruplexes involving four parallel strands (maximum and minimum Cotton effect at 263 nm and 243 nm, respectively). A thorough analysis of all NMR data allowed us to conclude that the quadruplex structure of (1) is very similar to that observed for the four stranded quadruplex $[d(TGGGT)]_4$.

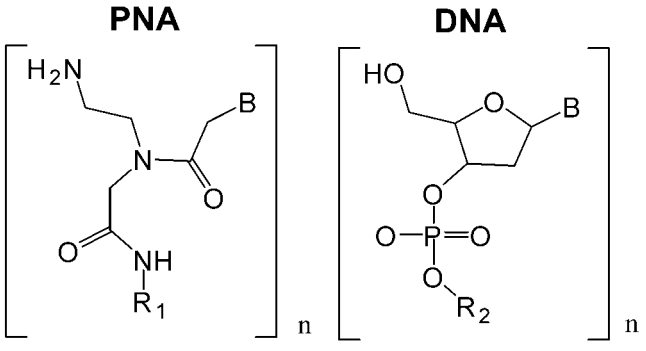

Figure 1.

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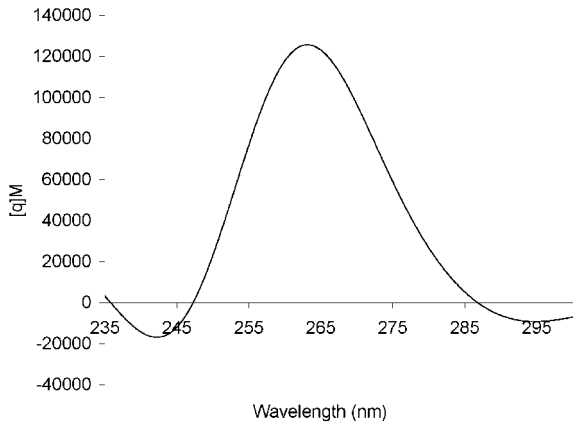

Figure 2.

The effect of the substitution of a regular DNA residue with a PNA residue was further analysed by UV melting experiments. Melting profile of (1) and of the corresponding unmodified $[d(TGGGT)]_4$ were recorded at 295 nm, giving a well-shaped sigmoid curves. As expected, no suitable melting curves could be obtained for (2). These data show that, in the case of (1), thermal stability is quite similar to that observed for the reference structure $[d(TGGGT)]_4$.

The usage of PNA moiety in quadruplex structures could, in principle, increase their nuclease resistance, and therefore improve the biological activity of molecules such as anti-thrombin[4] and anti-HIV integrase aptamers.[5]

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