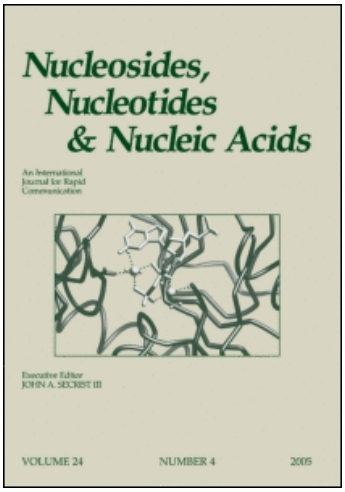

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Synthesis and DNA Binding Properties of DNA-PNA Chimeras

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ABSTRACT

A systematic study to evaluate the ability of 5′-DNA-3′-p-(N)-PNA-(C) chimeras to form triple helix structures has been undertaken. Preliminary results carried out on a 16-mer chimera with three PNA monomers at the 3′-end showed the formation of a stable DNA-PNA/DNA/DNA triplex, having similar conformational behaviour to a canonical DNA/DNA/DNA triplex.

Key Words: DNA-PNA chimera; Triplex; UV- and CD-monitored thermal stability.

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Peptide Nucleic Acids (PNA) constitute a very promising class of DNA mimics which are very stable to enzymatic degradation and have excellent DNA binding properties.[1] Their poor water solubility and inability to activate RNase-H in PNA-RNA heteroduplexes, which limit their potential therapeutic applications, can be overcome by using PNA-DNA chimeras which possess unaltered binding affinity towards complementary nucleic acids, are highly water-soluble and resistant to the exonuclease degradation.[2]

A number of papers recently appeared in the literature dealing with the synthesis and the hybridization properties of these chimeras. Most efforts have been devoted to study the interactions of DNA-PNA chimeras with single stranded DNA, specifically addressing these molecules as antisense agents. Not much is known about their ability to sequence specifically bind to duplex DNA, and therefore on the possibility to exploit these molecules as efficient triple helix forming oligonucleotides (TFOs). On the other hand, as far as homopyrimidine PNAs are concerned, these oligomers are well known to recognise double stranded DNA by a kinetically slow mechanism involving displacement of the pyrimidine DNA strand, resulting in very stable PNA-DNA-PNA triplexes.[3] It is therefore of interest to investigate to what extent the hybridization properties of DNA-PNA chimeras towards duplex DNA are dictated by the DNA domain, as would be desirable for any in vivo application within an antigene approach, still maintaining some advantages associated with the use of PNAs, as the high chemical and enzymatic stability.

In this context, we have undertaken the synthesis of a certain number of 16-mer 5′-DNA-3′-p-(N)-PNA-(C) chimeras, differing in the number of PNA monomers within the same C,T-alternating base sequence. Studies on their ability to form triplex complexes with the complementary duplex $(AG)_8/(CT)_8$, carried out by UV melting, CD and DSC analysis studies, together with molecular mechanics and dynamics calculations, are currently underway in our laboratories.

As a preliminary result, we here describe the synthesis and the hybridization properties of the 16-mer chimera $^{5'}$TCTCTCTCTCTCTctc (the DNA monomers are in capital letters and the PNA residues are in small letters; the junction between the two domains is assured by a stable phosphoramidate bond linking the 3′-phosphoester group of a thymidine with the backbone-amino group of the first PNA monomer), in comparison with the same all-DNA TFO $^{5'}$TCTCTCTCTCT-CTCTC$^{3'}$. The chimera was prepared by exploiting an efficient solid phase strategy, based on the usage of commercially available Bhoc/Fmoc PNA monomers for the assembly of the PNA tract, followed by a deprotection/reprotection procedure of the nucleobase protecting groups.[4]

Triplex formation experiments were then carried out by mixing equimolecular amounts of each oligomer (1×10^{-6} M concentration each strand) at 85°C for 10 min in a 5 mM NaH_2PO_4, 140 mM KCl and 5 mM $MgCl_2$buffer at pH = 6.6, then slowly cooled to r.t. and equilibrated for 24 h at 4°C before performing the analyses. UV-monitored thermal denaturation analysis showed almost superimposable curves for the two triplexes, with a Tm = 21.3°C for the canonical triplex and a Tm = 21.5°C for the PNA-containing triplex.

CD studies, performed in the same buffer at pH = 7.0 at the final triplex concentration of 1.3×10^{-5} M, showed that the complex formed by the DNA-PNA chimera

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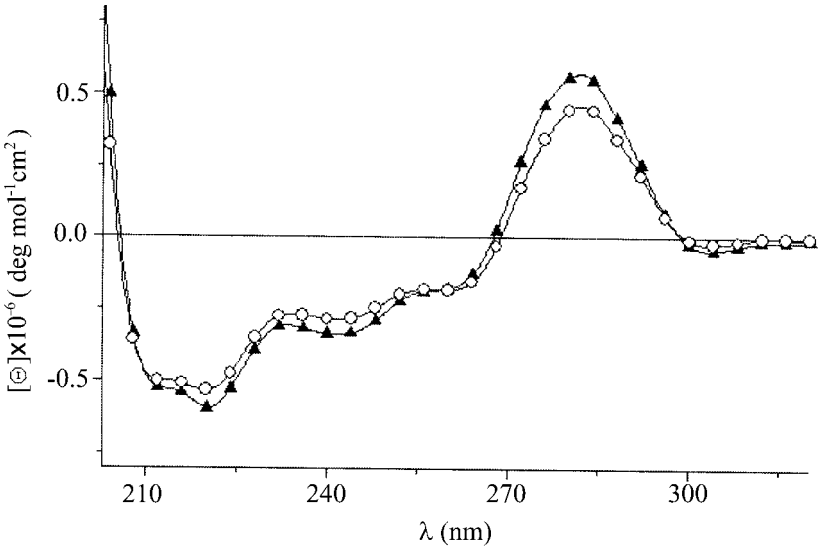

Figure 1. Superimposed CD spectra of all-DNA triplex (◯) and PNA-containing triplex (▲).

and the target duplex had a conformational behaviour absolutely similar to that of the unmodified triplex (see Fig. 1), thus showing that this kind of DNA-PNA chimera is perfectly able to recognise a duplex by a canonical triplex formation process. Further investigations are in progress to study the stability of this class of triplexes, aiming at elucidating the role of the PNA tail in the hybridisation of the third strand on the target duplex.

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