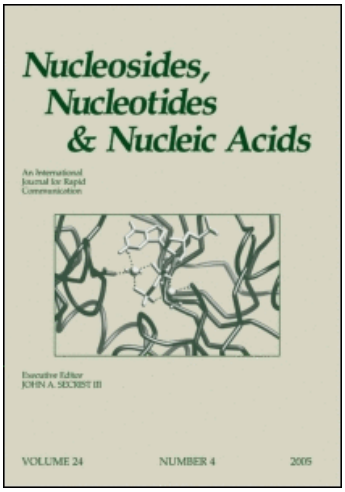

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Nuclease Stability of LNA Oligonucleotides and LNA-DNA Chimeras

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ABSTRACT

The stabilizing properties of LNA and α-L-LNA oligonucleotides against endo- and 3′-exonucleases have been evaluated.

Key Words: LNA; α-L-LNA; Nuclease stability.

LNA (Locked Nucleic Acids)[1–3] oligonucleotides and LNA-DNA chimeras present very attractive properties that reveal them as ideal candidates in antisense therapy.[4] However, no systematic study on the optimal design of LNA-containing antisense molecules has been carried out. This prompted us to undergo an evaluation of the nuclease stability of oligonucleotides containing LNA.

First, we tested the stability of LNA-containing oligothymidylates against 3′-exonuclease (Snake Venom Phosphodiesterase, SVPD) by quantifying the amount of full-length oligonucleotide by RP-HPLC analysis and confirming it by ESI, and compared it to natural DNA. A fully modified oligonucleotide remained completely stable towards SVPD after 2 h. Moreover, a significant increase in stability can be appreciated by blocking the 3′-end with just two LNAs (83% of full length oligonucleotide remained after 2 h digestion, Fig. 1).

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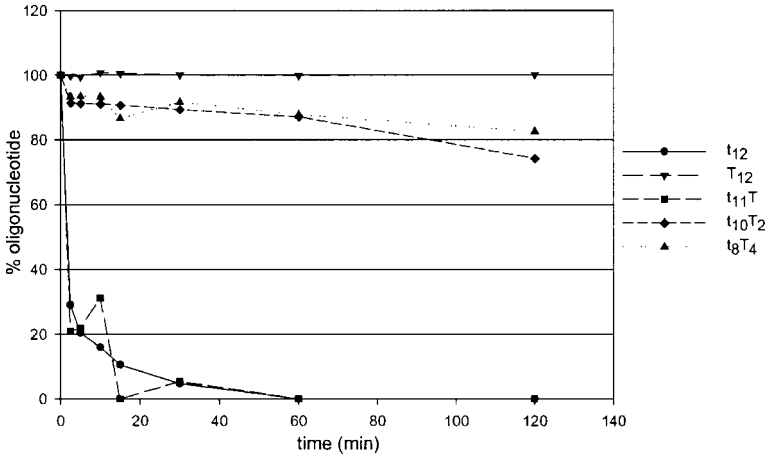

Figure 1. Stability against SVPD of oligonucleotides containing LNA and the corresponding controls. The experimental conditions were: 26 μg/mL oligonucleotide; 0.3 μg/mL SVPD; in 50 mM Tris-HC1 (pH 8.0), 10 mM $MgCl_2$ at 37°C. Lower case bases correspond to DNA and upper case to LNA.

Furthermore, we investigated the effect on stability of a fully modified LNA against S1-endonuclease (Fig. 1). The increased stability of these modified oligonucleotides relative to their deoxynucleotide and phosphorothioate backbone analogs was compared in order to carefully assess the contribution of the LNA modification. After 2 h digestion, most of the modified oligonucleotide remained (85%), while neither the oligodeoxynucleotide nor the phosphorothioate analog could be detected after 30 min digestion.

Next, we evaluated the effect on stability of an increasing deoxynucleoside gap against S1-endonuclease using 16mers with LNA containing flanks. Hydrolysis rates

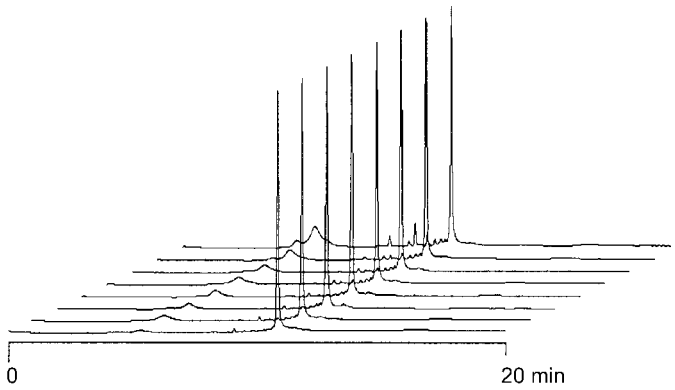

Figure 2. RP-HPLC analysis of the SVPD digestion of $t_9T^{\alpha}{}_2t$ as a function of time (0, 2.5, 5, 10, 30 min, 1 h and 2 h).

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were determined, and the digestion products were characterized. It is important to point out that no fragmentation of the LNA flanks was identified (data not shown).

Furthermore, we successfully prepared different oligonucleotides containing α-L-LNA, which were confirmed by mass spectrometry (ESI) and their purity was assessed by RP-HPLC. These constructs were tested against SVPD (Fig. 2) and S1-endonuclease, and the stability was assessed by quantifying the amount of full-length oligonucleotide by RP-HPLC analysis and confirming it by ESI. It is interesting to stress that a fully modified α-L-LNA oligonucleotide of 16 nt in length presents a remarkable stability against S1-endonuclease.

In conclusion, we have clearly demonstrated the striking stabilizing properties of the LNA and α-L-LNA oligonucleotides against endo- and 3′-exonucleases. Further work is in progress in order to evaluate the enzymatic recognition of oligonucleotides containing other LNA analogs.

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