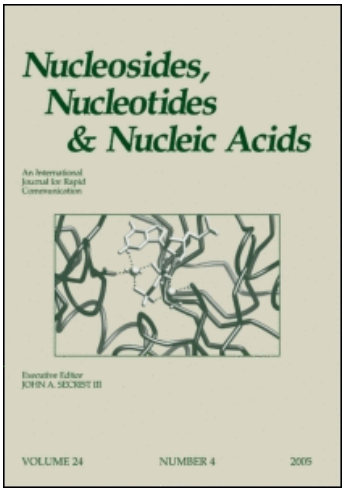

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INTERACTION OF LNA OLIGONUCLEOTIDES WITH MDR1 PROMOTER

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ABSTRACT

LNA oligonucleotides [1] can be used for targetting to double stranded DNA by the "strand invasion" mechanism. We used affinity modification by reactive oligonucleotide conjugates for investigation of oligonucleotides interaction with structured DNA. The tested LNAs and oligonucleotides of the same sequence were assayed as anti-mdr1 drugs in different cell cultures. One of the oligos, LNA79 strongly inhibited mdr1 induction in Hela cells and totally prevented activation of mdr1 in K-562.

We have investigated binding of oligonucleotides and strong binding oligonucleotide analogus, locked nucleic acids (LNA) to different regions of the mdr1 promoter sequences. The term "Locked Nucleic Acids" has been used to emphasize that the usual conformational freedom of the furanose ring in standard nucleosides is restricted in LNA by a methylene linker that connects the 2′-O position to the 4′-C position. LNA78 is complementary to the A:T rich low melting point region near the heat shock response element, LNA77 is complementary to the G:C rich region within important regulatory region of the mdr1 activator binding site and LNA79 is complementary to the region capable of alternative hairpin formation near the start of transcription.

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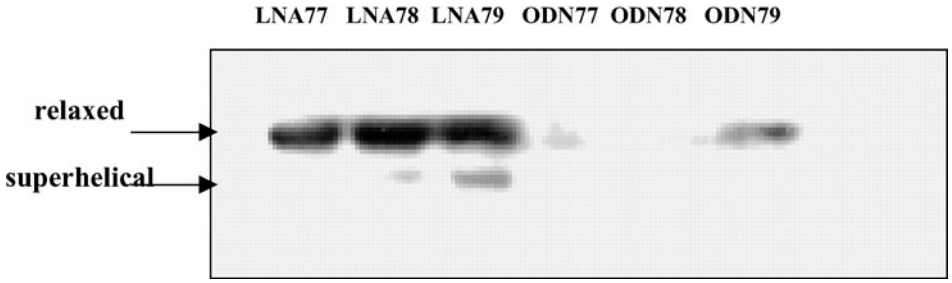

Figure 1. Gel-shift analysis of oligonucleotides binding to pMDRCAT5 plasmid.

LNA 77: pCGCCCCGGCGCdt
LNA78: pATTTCAACTTATGTAGda
LNA79: pTAGTGGAAAGAdc

A, **G**, **T and C** represent adenine, guanine, thymine and 5-methylcytosine LNA monomers, da, dc and dt – deoxyriboadenosine, cytidine and thymidine respectively.

We investigated the interaction of LNAs and ODNs of the same sequences with mdr1 promoter sequences in relaxed and supercoiled plasmid pMDRCAT5, containing $-258/+121$ region of mdr1 promoter sequence. This region was shown to be responsible for activation by heat shock, UV-irradiation and some therapeutics

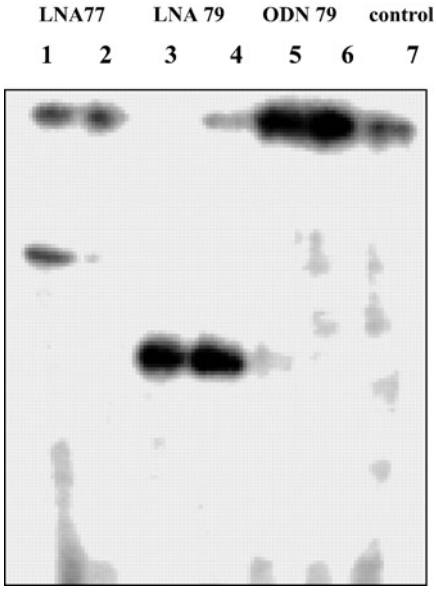

Figure 2. Primer extension analysis of chemical crosslinking of reactive derivatives to pMDRCAT5 plasmid. Lanes 1, 3, 5, 7 – plasmid cleaved by Pst I prior to alkylation, lanes 2, 4, 6 – circular plasmid. The plasmids were incubated with 10^{-6}M RCl-derivatives of LNAs or ODNs in TE buffer for 18 hours. Modified residues were converted to strand breaks by piperidine treatment. Cleavage positions were determined by primer extension.

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[2]. The results of gel-shift analysis (Fig. 1) indicate that all tested LNAs bind to the relaxed plasmide whereas ODNs of the same sequence do not demonstrate substantial binding to the DNA.

The specificity of LNAs binding was assayed using the affinity modification method, with reactive conjugates of the oligonucleotides, bearing the alkylating group tethered to the 5′-phosphate end. Results of the experiment shown in Figure 2 indicate no detectable non specific cleavage. The reactive derivative of LNA 79 efficiently cleaves the DNA both in linear and in circular plasmids. The reactive derivative of LNA 77 demonstrates less efficient cleavage especially in the undigested plasmid. The data correlate with binding efficiencies of the oligonucleotides (Fig. 2).

The data (Fig. 3) demonstrate, that the reactive derivative of LNA79 alkylates the third base (cytosine 73) from the end of the target sequence. This position specificity of the reaction suggests that LNA-DNA complex has A-form conformation.

We have assayed LNAs and ODNs as anti-mdr1 compounds in cell culture. As it is seen from the data shown in Figures 4,5, doxorubicine induces mdr1 RNA synthesis in K562 cells and all used cytostatics activate mdr1 synthesis in Hela cells. From the data, shown in Figure 3 it is seen, that LNA79 strongly inhibits mdr1 induction in Hela cells and totally prevents activation of mdr1 in K-562 cells

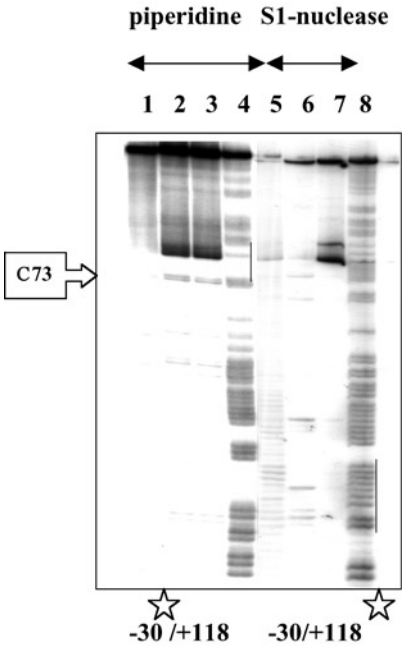

Figure 3. Cleavage of +30/−118 mdr1 fragment by alkylation with reactive derivatived of LNA79 and ODN 79 followed by piperidine treatment. Lanes 1, 5 – controls; 2, 6, the fragment incubated with LNA79-RCl in TE; 3, 7, the fragment incubated with LNA79-RCl in STE; 4, 8, A + G ladder. The target sequences are indicated by lines. [^{32}P]-label at the 5′ end on upper or lower strand of the fragment is marked by ☆.

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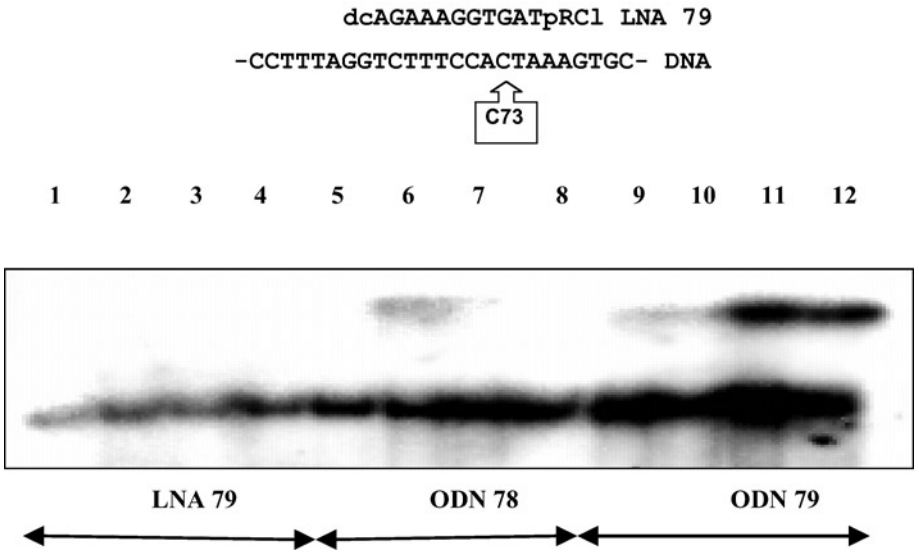

Figure 4. In vivo down-regulation of mdr1 expression by LNA79 (1×10^{-6}M) in Hela cells. Lanes 1, 5, 9, control cells; lanes 2, 5, 10, cells treated by cytarabine; lanes 3, 6, 11, cells treated by doxorubicine; lanes 4, 7, 12, cells treated by vinblastine.

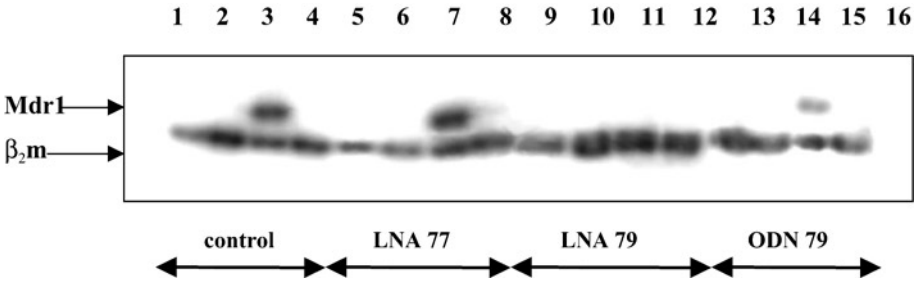

Figure 5. In vivo down-regulation of mdr1 expression by LNA79 in K562 cells. Lanes 1, 5, 9, 13 – control cells; lanes 2, 5, 10, 14 – cells treated by cytarabine; lanes 3, 6, 11, 15 – cells treated by doxorubicine; lanes 4, 7, 12, 16 – cells treated by vinblastine.

while LNA77 and ODNs 77 and 79 did not influence mdr1 activation. These data indicate, that LNA79 can be considered as a potential anti-mdr1 compound.

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