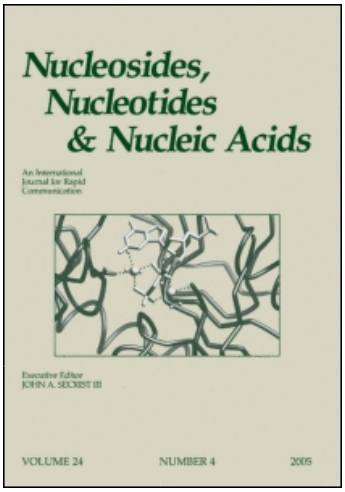

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Antisense Knockdown of PKC-α Using LNA-Oligos

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ABSTRACT

Full-length and 4 nucleotides truncated Locked Nucleic Acid (LNA) modifications of ISIS 3521 were compared for antisense properties in a cellular assay. ISIS 3521 is a 20-mer phosphorothioate designed to hybridise to human protein kinase C-α (PKC-α) mRNA and is currently submitted to clinical trials against cancer. We report that LNA can potentate this antisense oligo and retain the antisense potential with shorter oligos.

Key Words: LNA; Antisense; Phosphorothioates; ISIS 3521; Protein kinase C-α; A549 cells.

Locked Nucleic Acids (LNA) with their unprecedented high binding affinities for complementary RNA and DNA[1] are a class of molecules that are thought to be potential building blocks for development of therapeutics based on e.g., the principles of antisense.

Previously it has been reported that LNA oligos are potential therapeutic compounds in a number of different systems in vitro and in vivo.[2,3] We suggest the existence of a correlation between increasing affinity of a phosphorothioate through

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Table 1. Sequence, chemistry, antisense knockdown, and melting temperature (T_m).

Antisense oligo	Sequence (5′- 3′)	PKC-α transcript % mock ± (s.d.) n ≥ 4	T_m/ °C
ISIS 3521	gttctcgctggtgagtttca	55 ± 1.0	53
CUR2051	GTTCTCgctggtgaGTTTCA	38 ± 0.3	74
CUR2116	gttctcgctggtgagt	83 ± 1.7	50
CUR2055	GTTCtcgctggtGAGT	44 ± 4.9	59
ISIS 4559	ggttttaccatcggttctgg	114 ± 14.8	< 25
CUR2052	GGTTTTaccatcggTTCTGG	94 ± 14.7	< 25
CUR2056	GGTTtacctggtCTGG	92 ± 8.1	< 25
CUR2060	ggtttacctggtctgg	87 ± 15.1	< 25

LNA residues are indicated in capital letters and DNA residues are indicated with lowercase letters. All antisense oligos have a full phosphorothioate backbone. All cytosine residues are methylated. Melting point analysis was performed with 1500 nM concentration of antisense and complementary DNA oligo in buffer (100 mM NaCl, 0.1 mM EDTA, 10 mM NaP, pH 7.0).

modification with LNA and its potential as a specific antisense compound. This opens the possibility to improve antisense therapeutics through the use of shorter oligos by retaining the high affinity of these with LNA.

The clinically relevant 20-mer phosphorothioate ISIS 3521 is designed to hybridise to a part of human PKC-α mRNA and has shown to be effective in down-regulating PKC-α in the human lung carcinoma cell-line A549 relatively to the scrambled control ISIS 4559.[4]

We designed 4 LNA phosphorothioate oligos as either full length (20-mers) or truncated by 4 nucleotides (16-mers) with varying numbers of LNA nucleotides in the backbone by using ISIS 3521 or ISIS 4559 as parents and synthesised them on an ABI 8909 Expedite. Sequences and chemistry of the oligos are shown in Table 1. The 20-mer CUR 2051 and the 16-mer CUR 2055 are gap-mers that contains affinity flanks of 6 or 4 LNAs respectively from both 5′ and 3′ ends. The 20-mer CUR 2052 and the 16-mer CUR 2056 are sequence scrambled LNA gap-mers deriving from the sequence of ISIS 4559. Furthermore the 16-mer phosphorothioate CUR 2116 was prepared iso-sequential to CUR 2055.

Evaluation of the effects of the prepared oligos on the transcript for human PKC-alpha was assessed in A549 cells through transfections with 5 nM oligo or PBS for mock control, followed by 20 hours of culturing before harvesting the total RNA. The PKC-α transcript level was assessed with Real-time PCR.

Corresponding to the increase in affinity CUR 2051 showed a more effective knockdown on PKC-α than its parent ISIS 3521 by down-regulating the transcript to 38%/40% of the expression of mock/scrambled control treated as opposed to 55%/48% of the expression of mock/scrambled control treated. CUR 2055 was shown to retain or slightly improve the effectiveness of the parent ISIS 3521 by knockdown to 44%/51 % of the expression of mock/scrambled control treated where the corresponding shortened PS showed little or no effect on down-regulation of PKC-α. Results are presented in Table 1.

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