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## DNA TRIPLE HELIX FORMATION BY N-ALKYLPHOSPHORAMIDATE α-OLIGONUCLEOSIDES

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ABSTRACT: Triplex formation of pyrimidine N-alkylphosphoramidate α-oligonucleosides (12-mer) containing either dC or 5-Me-dC with their phosphodiester oligonucleoside homopurine target was evaluated by UV melting experiments.

Recently, we have demonstrated that oligodeoxynucleotides (ODNs) combining two structural modifications, i.e. the inversion of the anomeric configuration in the sugar moieties and the substitution of phosphate diester by non-ionic N-alkylphosphoramidate linkages, form stable double helices with complementary RNA and DNA single strands<sup>1</sup>. Among these backbone-modifications, N-(2-methoxyethyl)phosphoramidate  $\alpha$ -ODNs hybridized more tightly to their targets than the corresponding natural phosphodiester  $\beta$ -oligonucleoside.

These results prompted us to investigate the ability of the phosphoramidate  $\alpha$ -ODNs to form triple helices with double-stranded DNA targets. For this purpose, pyrimidine N-alkyl-phosphoramidate  $\alpha$ -ODNs (12-mer) containing either cytosine or 5-methylcytosine were synthesized. Their thermal stability with their phosphodiester oligonucleoside homopurine target was evaluated at various pH (5.5, 6.2 and 7.0) by UV melting experiments. Data are presented in Table 1.

$$\alpha^{3'} - (TpTpTpCpTpTpCpCpTpCpTpTpT)^{5'} \quad p = O = P - N \qquad O \qquad O = P - N \qquad O$$
or
$$O = P - N \qquad O$$

 $\beta^{S^{*}}\text{-}(GpCpApApApGpApApGpApApGpApApC}\underset{^{3'}(CpGpTpTpTpCpCpTpTpCpCpTpTpCp}{} \text{ds DNA target phosphodiester}$ 

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TABLE 1. Tm (°C) data of triple helices at several pH

Oligomer	Phosphodiester β-ODN	N-(2-methoxyethyl)- phosphoramidate α-ODN		Phosphoromorpholidate α-ODN	
	cytosine	cytosine	5-Me-dC	cytosine	5-Me-dC
pH 5.5	29.0	55.0	61.0	46.0	53.5
pH 6.2	19.0	39.5	44.0	30.0	36.5
pH 7.0	<5	23.0	28.5	13.5	22.0

Experimental conditions: 3  $\mu$ M oligonucleotide concentration in 10 mM sodium cacodylate, 100 mM NaCl at pH 5.5, 6.2 and 7.0. Heating rate 20°C/min.

We have shown that N-alkylphosphoramidate  $\alpha$ -ODNs formed anti-parallel (relative to purine strand of the ds DNA target) triple helices much more stable than natural phosphodiester  $\beta$ -ODNs did ( $\Delta$ Tm +20°C for N-(2-methoxyethyl)phosphoramidate and  $\Delta$ Tm +10°C for phosphoromorpholidate  $\alpha$ -ODNs at pH 7). Stability of triple helices depends on pH conditions whatever the third strand is (phosphoramidate or phosphodiester). Furthermore the replacement of cytosine residues by 5-methyl-cytosine in N-alkylphosphoramidate  $\alpha$ -ODN enhanced the thermal stability of the triple helices ( $\Delta$ Tm +25.5°C for N-(2-methoxyethyl)phosphoramidate and  $\Delta$ Tm +18.5°C for phosphoromorpholidate  $\alpha$ -oligonucleosides at pH 7).

In conclusion, as already observed in the case of methylphosphonate  $\alpha$ -ODN<sup>2</sup>, the combination of modifications (up to three) in the phosphodiester backbone, sugar and nucleobase produces new non-ionic analogues exhibiting high affinity for single- or double-stranded nucleic acid targets. These properties make these new oligonucleotides attractive for antisense and antigene applications.

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