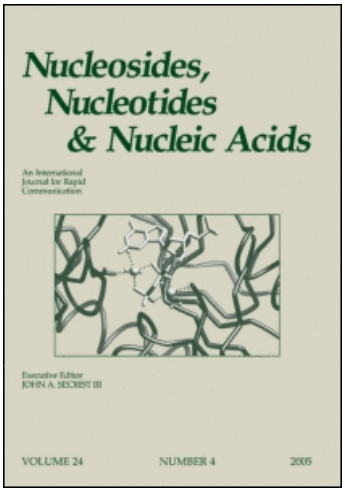

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Comparative Stability of Triple Helices Containing Modified DNA or RNA Pyrimidine Strands

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COMPARATIVE STABILITY OF TRIPLE HELICES CONTAINING MODIFIED DNA OR RNA PYRIMIDINE STRANDS

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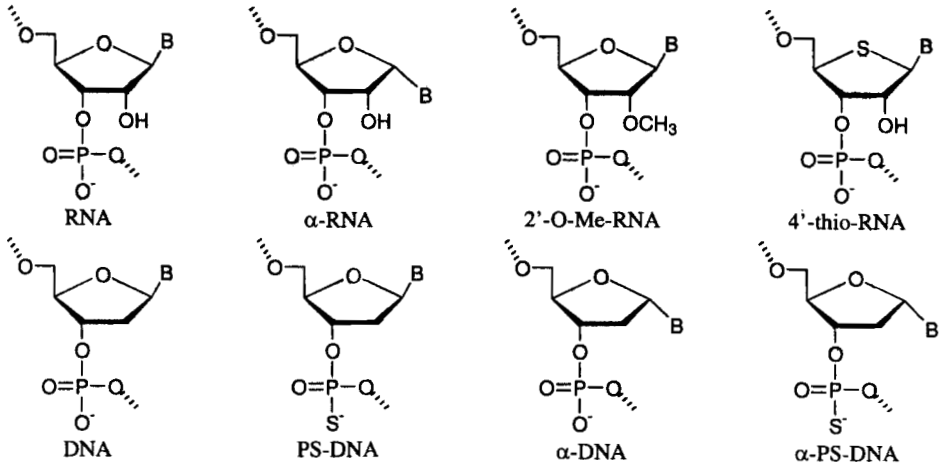
INTRODUCTION

Control of gene expression by oligodeoxyribonucleotide-directed triplex formation, known as antigene therapy (1), is widely studied since works have demonstrated the sequence-specific recognition of double helical DNA by a third strand (2,3). Triplex formation occurs when an oligopyrimidine strand binds in the major groove with a parallel orientation to the purine strand of polypurine polypyrimidine DNA duplex. Binding specificity is obtained from recognition of thymidine and AT base pair and N3 protonated cytosine and GC base pairs to form respectively the isomorphous T:AT and C^+:GC triplets via Hoogsteen hydrogen bonding (2,3). Triple helix formation involving cytosine is pH dependent. In order to use oligos for antigene therapy, they must fulfill at least two requirements such as nucleases resistance, since wild type DNA and RNA are rapidly degraded by nucleases present in cells and sera (4), and high binding affinity with the complementary double-stranded DNA in order to block the transcription or the replication of the corresponding gene. To accomplish the former point, several modifications of the phosphate or of the sugar have been proposed (5).

Here, we report the comparative stability, studied by UV melting curve analysis, of eight different triplexes constituted with 16-mer pyrimidine modified oligodeoxynucleotides (wild type DNA, PS-DNA, α-DNA or α-PS-DNA) or



β third strand (5'-3' orientation)

RNA	**5'-r(UUUUCUUUUCCCCCCU)-3'**
DNA	**5'-d(TTTTCTTTTCCCCCCT)-3'**

α third strand (3'-5' orientation)

RNA	**3'-r(UUUUCUUUUCCCCCCU)-5'**
DNA	**3'-d(TTTTCTTTTCCCCCCT)-5'**

H36

5'-d(AAAAGAAAAGGGGGGA-┐
T4
3'- (TTTTCTTTTCCCCCCT-┘

Figure: Top: Schematic structure of the height different series studied. Bottom: Schematic representation of triplex structure with RNA or DNA strands (16 mer) with a DNA hairpin (36 mer) containing a 4-T loop. Noteworthy that α-strands are oriented antiparallel to purine strand while β-strand are parallel (6).

oligoribonucleotides (wild type RNA, α-RNA, 4'-thio-RNA or 2'-O-Me-RNA) and a DNA hairpin (H36) (FIG.), in five different conditions. The composition of buffers varied in pH (5.5 and 6.5), salt concentration (100 mM and 1 M Na^+), and in the presence or absence of divalent cation (0 or 3 mM Mg^{2+}) or spermine (0 or 1 mM).

RESULTS AND DISCUSSION

At pH 5.5, the eight triplexes are formed with Tm values ranging from 24.7 to 50.9°C, the most stable being with RNA and 2'-O-Me-RNA. α-RNA showed low

Table: Tm values of triplexes formed between H36 in 100 mM NaAc, 1.0 mM EDTA at pH 5.5 and pH 6.5 ; in 1.0 M NaAc, 1.0 mM EDTA at pH 6.5 ; . in 3 mM $MgCl_2$, 10 mM Na cacodylate, 100 mM NaCl, 1.0 mM EDTA at pH 6.5 and in 1 mM spermine, 10 mM Na cacodylate, 100 mM NaCl, 1.0 mM EDTA at pH 6.5.

Third strand	Tm (± 0.5°C)				
	pH 5.5	pH 6.5			
	0.1 M Na^+	0.1 M Na^+	1M Na^+	3 mM Mg^{2+}	1 mM spermine
RNA	50.9	20.6	22.7	17.4	30.8
α-RNA	26.4	5.7	13.5	6.4	8.2
2'-O-Me-RNA	50.9	12.3	22.1	6.8	26.7
4'-thio-RNA	37.2	≈ 8*	≈ 4*	NT	≈ 10*
DNA	39.5	14.6	24.3	12.1	24.5
PS-DNA	24.7	≈ 8*	≈ 5*	NT	≈ 10*
α-DNA	36.6	9.4#	24.2	8.0#	21.1
α-PS-DNA	32.8	6.4	21.2	< 2#	20.6

NT : no transition, * broad transition, #same value for the self-association

stability (ΔTm -13.1°C) which could be explained by steric hindrance between the 2'-hydroxyl and the base. 4'-thio-RNA and α-DNA displayed a slight destabilization with respect to DNA (ΔTm -2.3 and -2.9°C respectively). Finally introduction of phosphorothioate led to a stronger decrease of Tm in β-series (PS-DNA - 1.0°C/mod.) than in α-series (α-PS-DNA - 0.25 °C/mod), with the result that α-PS-DNA forms a more stable triplex than PS-DNA does.

The increase of pH led to a dramatic decrease of Tm due to a lesser extent of N3 cytosine protonation. The triple helices constituted with RNA analogs are more destabilized (ΔTm -29.2 to -38.6°C) than those with DNA analogs (ΔTm -16.7 to -24.9°C). Noteworthy that at pH 6.5, 4'-thio-RNA and PS-DNA form only broad transition and will not discuss further.

At 1.0 M Na^+ concentration, triplexes are stabilized by 2.1 to 14.8 °C. In contrast, the presence of 3.0 mM Mg^{2+} bring a destabilization of the triplexes (except for α-RNA). Furthermore, the formation of triple helix with α-DNA and α-PS-DNA could not be ascertained since the observed value of Tm correspond to that resulting from self-association.

Finally with exception of α-RNA (ΔTm + 2.5°C), 1.0 mM spermine is sufficient to induce a strong stabilization of the triplexes (ΔTm 9.9 to 14.4°C).

CONCLUSION

Though RNA and RNA form high stability triple helices their poor resistance to nucleases (4) prevents their use as antigene agents. The use of α-DNA could be hampered by its propensity to self-associate. Finally α-RNA, 4'-thio-RNA and PS-DNA could not be retained on the basis of their low binding properties. In contrast, 2'-O-Me-RNA seems to be the best candidate with high binding capability and resistance to nucleases. Nevertheless, an alternative could be the use of α-PS-DNA which exhibits good binding ability in presence of spermine, is less prone to self-associate and has shown a very high nuclease resistance (7).

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