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DUPLEX- AND TRIPLEX-FORMING PROPERTIES OF 4'-THIO-MODIFIED OLIGODEOXYNUCLEOTIDES

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Abstract: The melting temperatures (T_ms) of a series of 4'-thio-modified oligodeoxynucleotides bound to complementary ssDNA, ssRNA and dsDNA have been determined. The results demonstrate an increase in T_m for the duplexes formed with RNA, but a reduction when bound to DNA. When forming a triplex with dsDNA, the greatest increases in T_m are observed when the 4'-thio-modifications are placed in contiguous stretches.

The inhibition of gene expression mediated by the binding of an antisense oligonucleotide to complementary mRNA offers great potential for therapeutic intervention in many human diseases.[1-3] In the antisense approach, the oligonucleotide is targeted to either a cellular or viral mRNA and is thus expected to inhibit its translation to the corresponding protein. It has also become clear that oligonucleotides could target double-stranded DNA and thus inhibit replication or transcription, the first step of gene expression. The inhibition of transcription of dsDNA occurs *via* an oligonucleotide forming a triplex with the dsDNA, which is held together by Hoogsteen base-pairing. This approach has become known as the anti-gene strategy.[4] Triple-helix formation is mainly limited to only homopurine-homopyrimidine sequences within the target DNA, although recently some relaxation in the target sequence requirements for third-strand binding has been observed.[5] One beneficial reason for targeting dsDNA instead of RNA, is that there are always far fewer copies of the gene present in the cell than there are molecules of the transcribed mRNA.

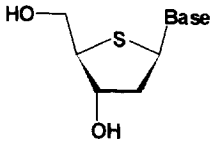

Figure 1. The structure of a 4'-thio-2'-deoxynucleoside.

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Unmodified oligonucleotides are limited in their usefulness for *in vivo* applications, primarily due to rapid degradation of the oligonucleotide by extra- and intracellular nucleases. In an attempt to impart nuclease resistance, while simultaneously minimizing deleterious effects on duplex stability and other properties, many chemically modified oligonucleotides have been synthesised.[6-8] Recently, we have published some properties of oligodeoxynucleotides containing 4′-thiothymidine,[9] in which the oxygen atom in the thymidine furanose ring is replaced by a sulfur atom (**figure 1**). For the first time, we report herein the synthesis of a series of oligodeoxynucleotides, in some of which both 2′-deoxycytidine and thymidine residues are replaced by their corresponding 4′-thio analogues. The triplex-forming properties of these modified oligodeoxynucleotides with dsDNA, and their duplex-forming properties with both ssDNA and ssRNA have been studied.

The sequences used for the duplex and triplex formation studies are shown in **table 1**. These sequences have been used by others on numerous occasions to study the effects of modification on an oligonucleotide on duplex and triplex formation, which therefore allows direct comparisons to be made.[10,11]

No.	Oligonucleotide Sequence (5′ - 3′)
1	d(TTTTTCTCTCTCT)
2	d(TTTT**T**C**T**C**T**C**T**CT)[a]
3	d(**T**T**T**T**T**C**T**C**T**C**T**CT)[a]
4	d(TTTTT**C**T**C**T**C**T**C**T)[b]
5	d(TTTT**TCTCTCTCTC**T)[a,b]
6	d(**TTTTTCTCTCTCTC**T)[a,b]
7	d(AGAGAGAGAGAAAAA)
8	r(AGAGAGAGAGAAAAA)

[a] **T** = 4′-thiothymidylate
[b] **C** = 4′-thio-2′-deoxycytidylate

Table 1. Oligonucleotide sequences used for the melting studies.

Melting temperatures of the 4′-thio-modified oligodeoxynucleotides (**2-6**) bound to complementary ssDNA (**7**) were measured (**table 2**). The presence of 4′-thio-modified nucleotides always resulted in a decrease in the T_m of the duplex when compared to the unmodified duplex (**1/7**). The greatest destabilization (**4/7**) was observed for the oligodeoxynucleotide in which all the 2′-deoxycytidine residues, which were in alternating positions, were replaced by 4′-thio-2′-deoxycytidine modifications.

duplex	modification	number of modifications	T_m /°C	ΔT_m /°C	ΔT_m / mod^{-1} °C
1/7	unmodified	0	42.2	-	-
2/7	4′-thioT	5	40.9	-1.3	-0.26
3/7	4′-thioT	7	38.9	-3.3	-0.47
4/7	4′-thio-2′-dC	5	35.7	-6.5	-1.30
5/7	4′-thioT/4′-thio-2′-dC	10	32.3	-9.9	-0.99
6/7	4′-thioT/4′-thio-2′-dC	14	30.9	-11.3	-0.81

Table 2. T_m results for DNA/**ssDNA** duplexes.[12]

Comparison of the results on the stability of modified DNA/RNA duplexes (**table 3**) suggests that the presence of 4'-thio-2'-deoxycytidine residues in the oligodeoxynucleotide results in a slight destabilizing effect (*e.g.* **4/8**) which is counterbalanced by the slight stabilizing effect of the 4'-thiothymidine modification (*e.g.* **5/8** and **6/8**). There also appears to be a correlation between stabilization of the duplex and the sequence of the neighbouring nucleotides. This correlation is exemplified by comparing the ΔT_m per modification of duplexes **2/8** and **3/8**, where a depression in ΔT_m per modification is observed in duplex **3/8** when an additional two 4'-thiothymidine modifications are positioned adjacent to thymidine residues. Therefore, the sequence of the oligonucleotide, and the position in which modifications are placed, can significantly affect the degree of stabilization or destabilization caused by a modification. The results suggest that whereas alternating 4'-thiothymidine residues and continuous stretches of 4'-thio-2'-deoxynucleosides can be tolerated, alternating 4'-thio-2'-deoxycytidine residues cause destabilization of both DNA/DNA and DNA/RNA duplexes.

duplex	modification	number of modifications	T_m /°C	ΔT_m /°C	ΔT_m / mod^{-1} °C
1/8	unmodified	0	52.5	-	-
2/8	4'-thioT	5	57.4	-4.9	+1.00
3/8	4'-thioT	7	56.7	-4.2	+0.60
4/8	4'-thio-2'-dC	5	50.5	-2.0	-0.40
5/8	4'-thioT/4'-thio-2'-dC	10	56.6	+4.1	+0.45
6/8	4'-thioT/4'-thio-2'-dC	14	58.1	+5.6	+0.40

Table 3. T_m results for DNA/**ssRNA** duplexes.[12]

It has been seen on previous occasions[13,14] that there is often no direct correlation between the effect on T_m of modified-DNA/DNA duplexes and the corresponding modified-DNA/RNA duplexes, the latter of which are more relevant for antisense purposes. Our observations suggest that the backbone torsion angles of a DNA/RNA duplex are better able to accommodate 4'-thio-substitutions than can a DNA/DNA duplex, which has smaller *gamma* and *delta* dihedral angles along the sugar-phosphate backbone.

triplex	modification	number of modifications	T_m /°C	ΔT_m /°C	ΔT_m / mod^{-1} °C
1/2/7	4'-thioT	5	17.7	+0.3	+0.06
1/3/7	4'-thioT	7	17.9	+0.5	+0.07
1/4/7	4'-thio-2'-dC	5	18.8	-0.3	-0.06
1/5/7	4'-thioT/4'-thio-2'-dC	10	22.4	+5.0	+0.50
1/6/7	4'-thioT/4'-thio-2'-dC	14	25.1	+6.1	+0.44

Table 4. T_m results for DNA/**dsDNA** triplex.[15]

Some studies into the triplex-forming properties of 4'-thio-modified oligodeoxynucleotides have been performed (**table 4**). The results demonstrate that the simultaneous incorporation of 4'-thiothymidine and 4'-thio-2'-deoxycytidine modifications leads to a significantly higher triplex stability than would be expected on

the basis of the effects observed for isolated modifications. There appears to exist some co-operative effect that occurs only for stretches of contiguous modifications.

The data reported herein for the binding of 4′-thio-modified oligodeoxynucleotides to ssRNA as well as dsDNA, together with previous data on the stability of oligodeoxynucleotides containing 4′-thio-modified nucleosides to degradation by endonucleases,[9] suggest that this modification may be of benefit for biological applications when present in contiguous stretches within a chimeric oligonucleotide.

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15. The triplex melting temperature experiments were conducted by adding the 4′-thio-modified oligodeoxynucleotide (*i.e.* **2-6**) to the pre-formed (intermolecular) target duplex (**1/7**) to form the triplex, which is composed of two strands of natural DNA plus the modified homopyrimidine strand. Salt conditions were 1 mM Mg^{2+}, 140 mM K^{+}, 10 mM Na^{+} in 10 mM cacodylate buffer, pH 7.7 (the counter ion was Cl^{-}). The solutions contained 2 μM of both target duplex and modified oligonucleotide, giving a total strand concentration of 6 μM, and they were equilibrated for 60 minutes at room temperature prior to heating. The T_m of the control triplex (**1/1/7**) was measured in parallel with each of the modified triplexes.

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