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Characterization of fully 2′-modified oligoribonucleotide hetero- and homoduplex hybridization and nuclease sensitivity

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ABSTRACT

The nuclease stability and melting temperatures (T_m) were compared for fully modified oligoribonucleotide sequences containing 2′-fluoro, 2′-*O*-methyl, 2′-*O*-propyl and 2′-*O*-pentyl nucleotides. Duplexes formed between 2′ modified oligoribonucleotides and RNA have typical A-form geometry as observed by circular dichroism spectroscopy. Modifications, with the exception of 2′-*O*-pentyl, were observed to increase the T_m of duplexes formed with complementary RNA. Modified homoduplexes showed significantly higher T_ms, with the following T_m order: 2′-fluoro:2′fluoro > 2′-*O*-propyl:2′-*O*-propyl > 2′-*O*-methyl:2′-*O*-methyl > RNA:RNA > DNA:DNA. The nuclease stability of 2′-modified oligoribonucleotides was examined using snake venom phosphodiesterase (SVPD) and nuclease S1. The stability imparted by 2′ modifications was observed to correlate with the size of the modification. An additional level of nuclease stability was present in oligoribonucleotides having the potential for forming secondary structure, but only for 2′ modified oligoribonucleotides and not for 2′-deoxy oligoribonucleotides.

INTRODUCTION

One cornerstone of 'antisense' technology lies in the specific binding of an oligoribonucleotide to its target sequence. The formation of a duplex between an antisense oligomer and its target sequence (usually mRNA) can prevent gene expression by interfering with subsequent processing, transport or translation, or by degradation of the RNA via RNase H (1). Exogenous unmodified oligoribonucleotides are limited in therapeutic applications, however, by their inherent sensitivity towards nucleases (2,3). Chemical strategies to improve oligoribonucleotide stability are being explored, including modifications of the deoxyribo/ribo sugar and the heterocyclic base, as well as modification or replacement of the internucleotide phosphodiester linkage (4,5). To date, the most commonly employed synthetic modification is the backbone phosphorothioate analog, created by replacing one of the non-bridging oxygen atoms of the internucleotide linkage by a sulfur. While this type of modification results in enhanced stability to nucleolytic hydrolysis, it also decreases the stability of duplexes formed with either complementary DNA or RNA sequences (6–8). Nucleic acid modifications which can simultaneously provide nuclease resistance while minimizing deleterious effects on duplex stability continues to drive research in this arena.

Characterization of fully 2′-modified oligoribonucleotides was carried out with respect to their duplex stability (as measured by T_m) and their nuclease resistance to snake venom phosphodiesterase. It was envisioned that 2′-modifications could both enhance the duplex stability of oligoribonucleotides containing phosphorothioate internucleotide linkages, and provide substantial nuclease resistance to oligoribonucleotides containing normal phosphodiester linkages. Knowledge of these characteristics should be useful for the design of more effective antisense molecules. These same properties are of interest to those involved in using 2′-*O*-alkyl modified oligoribonucleotides as sequence-specific probes or modified RNA therapeutics.

Previous studies (9) have shown the effects of 2′-*O*-propyl and 2′-*O*-pentyl point substitutions on duplex stability. We set out to extend these studies to include the stability between fully modified 2′-*O*-propyl and 2′-*O*-pentyl oligoribonucleotides and RNA. In addition, we examined the stability of 2′-modified homoduplexes (both strands being 2′-modified oligoribonucleotides).

The nuclease stability of fully modified 2′-*O*-propyl and 2′-*O*-pentyl oligoribonucleotides has not been previously reported. The stability of these modified oligoribonucleotides relative to their 2′-deoxy and 2′-*O*-methyl analogs was compared in order to carefully assess the contribution of these modifications to nuclease stability.

MATERIALS AND METHODS

Synthesis and radiolabeling of oligoribonucleotides

The 2′-*O*-methyl phosphoramidites used were purchased from Glen Research, Sterling, VA. The 2′-fluoro phosphoramidites were prepared as described by Kawasaki (6), while the 2′-*O*-propyl and

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2′-*O*-pentyl phosphoramidites were prepared as described (9–11). CPG derivatized with 2′-*O*-modified nucleosides was prepared as described by Damha (12). The other reagents for solid phase DNA synthesis were purchased from commercial sources. Oligomers were synthesized using solid-phase chemistries on an ABI model 380B DNA synthesizer. Oligomers were purified by polyacrylamide gel electrophoresis, followed by desalting with Poly-pak cartridges (Glen Research, Sterling, VA). Oligoribonucleotides were 5′ end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. After the labeling reaction, the T4 polynucleotide kinase was heat inactivated at 95°C for 3 min and oligomers used without any further purification.

Nuclease stability studies

Snake venom phosphodiesterase (USB, Cleveland, OH) assays were performed using 1 μM oligomer at 37°C in a buffer of 50 mM Tris–HCl, pH 8.5, 72 mM NaCl and 14 mM $MgCl_2$ at an enzyme concentration of 5×10^{-2} or 5×10^{-3} U/ml. The enzyme was shown to maintain its activity under these conditions for at least 24 h. Nuclease S1 (Gibco-BRL, Gaithersburg, MD) assays were performed using 1 μM oligomer at 37°C in 30 mM NaOAc pH 4.5, 50 mM NaCl, 1 mM $ZnCl_2$. Nuclease S1 was used at either 1.9 U/ml or 1.9×10^6 U/ml. Aliquots of the nuclease stability reactions were removed at the indicated times, quenched by addition to an equal volume of 80% formamide gel loading buffer containing tracking dyes, heated for 2 min at 95°C and then stored at –20°C until analysis by denaturing polyacrylamide electrophoresis. Quantitation was performed on a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Determination of hybridization stability

Absorbance versus temperature curves of duplexes were measured at 4 μM strand concentration (duplexes) or 6 μM strand concentration (single-stranded studies) in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA, pH 7 as described elsewhere (13). The melting temperature (T_ms) and free energies of duplex formation were obtained from fits of data to a two state model with linear sloping baselines (14). Free energies of duplex formation are a more valid measure of thermodynamic stability than T_m (9,15). Experimental errors, however, were larger for ΔG^o_{37} than for T_m. Therefore, we report T_m values in Table 2 and 3 and Figure 4. When Figure 4 was plotted using ΔG^o_{37}, the trends were identical, but the error bars were larger.

Circular dichroism spectra

CD spectra were recorded at ambient temperature in a JASCO J-600 spectropolarimeter. Salt concentrations were identical to those used for the melting curves; the oligoribonucleotide concentrations were 3 μM each strand. Ellipticities were converted to $\Delta\varepsilon$ and are reported per mol residue.

RESULTS

The oligoribonucleotide sequences synthesized for nuclease resistance studies are shown in Table 1. Incorporation of the different 2′-*O*-alkyl moieties into the 12mer series (Table 1) was verified by electrospray mass spectroscopy and the calculated and measured masses agreed to within 0.01%. The additional oligoribonucleotide sequences used for T_m studies are shown in Table 2.

Table 1. Modified oligoribonucleotide sequences used in nuclease studies

2′ Modification	Backbone	17mer series[a]	12mer series[b]
2′-deoxy	P=O	1592	5947
2′-*O*-methyl	P=O	3299	5946
2′-*O*-propyl	P=O	4140	7000
2′-*O*-pentyl	P=O	4250	7688
2′-deoxy	P=S	4893	7734

[a]17mer: 5′-GGA CCG GAA GGU ACG AG-3′.
[b]12mer: 5′-CUA AGC AUG UCA-3′.
underline = 2′-modified residues.

Table 2. T_ms of oligoribonucleotides containing uniform 2′-substitutions[a]

Modification	Phosphodiester[a] T_m (°C) 1343 series	Phosphodiester[a] T_m (°C) 1592 series	Single-stranded[b] T_m (°C) 1592 series	Phosphorothioate[a] T_m (°C) 1570 series
2′-deoxy	45.1	56.5	67.9(PO) 57.6(PS)	55.3
2′-*O*-methyl	62.8	79.6	73.4(PO)	80.9
2′-*O*-propyl	58.5	74.8	77.8(PO)	78.3
2′-*O*-pentyl	45.9	61.8	ND[c]	54.2
2′-fluoro	70.3	89.1	ND[c]	88.6
RNA	59.2	74.5	68.6(PO)	ND[c]

[a]T_ms were measured versus RNA complements in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA, pH 7.0 at 4 μM strand concentration (phosphodiester = PO, phosphorothioate = PS).
[b]T_ms were measured without a complementary strand in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA, pH 7.0 at 4 μM strand concentration.
[c]ND = not determined.
Sequences studied:
1343 series CoGoAoCoToAoToGoCoAoAoGoToAoC
1592 series GoGoAoCoCoGoGoAoAoGoGoToAoCoGoAoG
1570 series TsGsGsGsAsGsCsCsAsTsAsGsCsGsAsGsGsC
underline = 2′ modified residue; o = phosphodiester linkage; s = phosphorothioate linkage.

Table 3. T_ms of 2′-modified homoduplexes

Duplex	T_ms (°C) 1343[a] Analogs 1 × TMTB[b]	T_ms (°C) 1592[a] Analogs 1 × TMTB[b]	T_ms (°C) 1592[a] Analogs 0.1 × TMTB[b]
DNA:DNA	52.3	60.6	43.3
RNA:RNA	59.2	74.5	60.9
2′-*O*-methyl:2′-*O*-methyl	69.2	85.5	66.3
2′-*O*-propyl:2′-*O*-propyl	72.3	>95	81.5
2′-fluoro:2′-fluoro	84.9	>100	84.8

[a]Sequences are listed in Table 2.
[b]1 × TMTB is 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA, pH 7.0.

Hybridization of modified oligoribonucleotide to its RNA complement was evaluated spectrophotometrically. The T_ms for the various duplexes are listed in Table 2. Compared to the unmodified 2′-deoxy sequence (1343, 1592 or 1570), all 2′-substituted oligoribonucleotides had equal or greater duplex stability (as evaluated by higher T_ms) when hybridized to their RNA complement except the 2′-*O*-pentyl phosphorothioate molecule. The T_m of this single exception is only ~1 °C lower than that of the 2′-deoxy (1570) in the same phosphorothioate background. The rank order of duplex T_m is 2′-fluoro > 2′-*O*-methyl >RNA = 2′-*O*-propyl > 2′-*O*-pentyl > 2′ deoxy. The 2′-fluoro and 2′-*O*-methyl modifications increase the phosphodiester duplex T_m over even that of the RNA analog.

The duplex stabilization trend displayed by 2′-modified oligomers was further investigated by measuring duplex T_ms when both strands contained 2′-modifications. T_m measurements for phosphodiester 2′-modified homoduplexes are listed in Table 3. In 0.1 M NaCl, the duplexes formed with some of the 1592 analogs were exceedingly stable and the T_m was difficult to evaluate. For these sequences, the T_ms were re-measured in 0.01 M NaCl. When both strands are modified, the rank order of duplex T_m is: 2′-fluoro:2′fluoro > 2′-*O*-propyl:2′-*O*-propyl > 2′-*O*-methyl:2′-*O*-methyl > RNA:RNA > DNA:DNA.

Circular dichroism studies, with 17mer oligodeoxynucleotides containing from one to five 2′-*O*-modified adenosine residues, showed RNA:DNA hybrid duplexes with characteristics that were intermediate between B-form and A-form helices (9). The CD spectra of hybrids of the 2′-*O*-modified 1592 analogs with RNA complement obtained are very similar to that of the RNA:RNA duplex with a strong positive band near 260 nm and a negative band near 212 nm (Fig. 1). Ellipticity at 260 nm, for the 2′-substituted hybrids, is somewhat greater than that of the RNA:RNA duplex. Similar results were observed for the 1343 and 1570 sequences (data not shown). These results suggest that, not suprisingly, fully substituted 2′-*O*-modified hybrids adopt an A-form helix similar to an RNA:RNA duplex.

The nuclease stability of the 17mer series (Table 1) was examined using snake venom phosphodiesterase. The times required to reduce the quantity of full length material to 50% of its initial value ($t_{1/2}$) are listed in Table 4. Relative nuclease stability was calculated by dividing the observed $t_{1/2}$ value by the $t_{1/2}$ time of the unmodified compound. At an enzyme concentration of 5 × 10^{-3} U/ml, both the 2′-*O*-methyl and 2′-*O*-propyl analogs in the 17mer series were 74-fold more stable than the unmodified control. The enzyme concentration was increased in

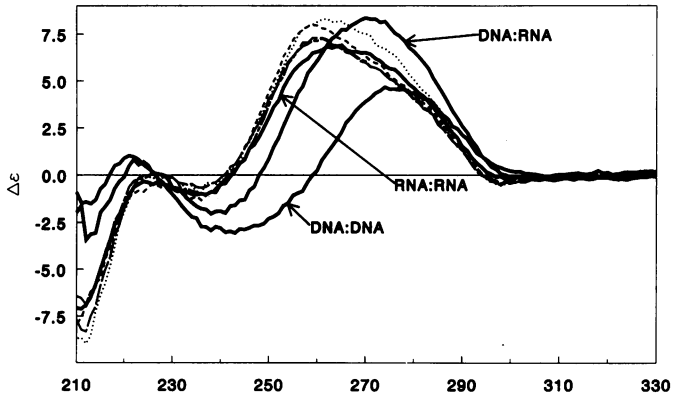


Figure 1. Circular dichroism spectra of 1592 and its 2′ modified analogs hybridizing to RNA. ..... 2′-*O*-methyl; — — — — 2′-*O*-propyl; — - — - — - 2′-*O*-pentyl; - - - - - - 2′-fluoro. The solid curves are RNA:RNA and DNA:DNA duplex controls.

an attempt to discriminate nuclease stability differences between the 2′-*O*-methyl and 2′-*O*-propyl analogs in this series. Performing the SVPD assay at an enzyme concentration of 5 × 10^{-2} U/ml resulted in 50% degradation of full-length oligomer 1592 in ~2.5 min. Under identical reaction conditions, the 2′-*O*-methyl phosphodiester, 2′-*O*-propyl phosphodiester and 2′ deoxy phosphorothioate analogs were 120-, 160- and 350-fold more stable than the 2′-deoxy phosphodiester control (Table 4). Denaturing polyacrylamide gel analysis of these analogs indicated that degradation of the fully modified oligoribonucleotides does not proceed significantly beyond the first few 3′ nucleotides while the unmodified oligomer showed a ladder of degradation products (Fig. 2). Qualitatively, our analyses suggest that the 2′-*O*-pentyl analog is even more resistant to exonuclease degradation than the 2′-*O*-methyl or 2′-*O*-propyl oligomers (data not shown). Quantitative analysis of the 2′-*O*-pentyl analog has been difficult for two reasons. First, the 2′-*O*-pentyl oligomers appear to be poor substrates for polynucleotide kinase resulting in inefficient labeling. Secondly, quite often the majority of the labeled 2′-*O*-pentyl oligomer is trapped at the top of the gel. For this series of 17mers, the order of stability appears to be: 2′-deoxy phosphorothioate ≥ 2′-*O*-pentyl phosphodiester > 2′-*O*-propyl phosphodiester > 2′-*O*-methyl phosphodiester > 2′-deoxy phosphodiester.

Table 4. 2′-*O*-modified oligoribonucleotide half lives with S1 nuclease and snake venom phosphodiesterase

Oligomer	$t_{1/2}$ (min) (5×10^{-3} U/ml)	Relative $t_{1/2}$	$t_{1/2}$ (min) (5×10^{-2} U/ml)	Relative $t_{1/2}$
Snake venom phosphodiesterase (12mer series[a])				
5947	12.0	1.0	1.5	1.0
5946	38.0	3.2	11.0	7.3
7000	68.0	5.7	15.0	10.0
8191[b]	>68.0	>5.7	ND[c]	–
7734	975	81.3	133	88.7
Snake venom phosphodiesterase (17mer series[a])				
1592	27.0	1.0	2.5	1.0
3299	2000	74	300	120
4140	2000	74	400	160
4893	no degradation	–	875	350
8312	ND[c]	–	21	8.4
8313	ND[c]	–	54	21.6
8314	ND[c]	–	53	21.2
S1 nuclease (12mer series)				
5947	2.0	1.0	<1.0	1.0
5946	no degradation	–	90	>90
7000	no degradation	–	no degradation	–
7734	13.0	6.5	<1.0	–

[a]Sequences and modifications are given in Table 1.
[b]This oligomer is a fully modified 2′-*O*-pentyl 12mer, except the 5′ residue is a 2′-deoxy C.
[c]ND = not determined.

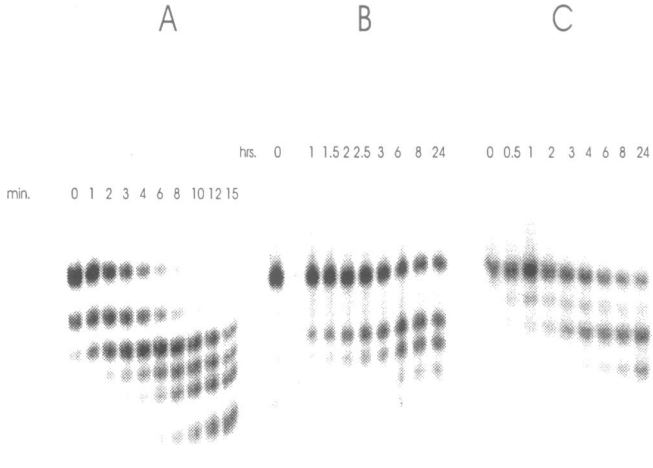
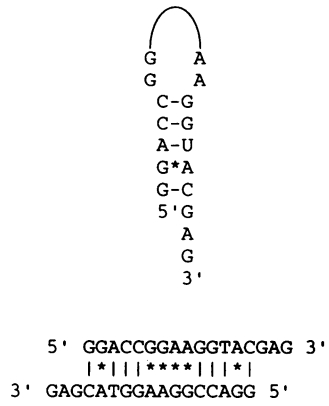


Figure 2. SVPD degradation of 17mer oligoribonucleotide series: (**A**) 1592 (2′-deoxy phosphodiester). (**B**) 3299 (2′-*O*-methyl phosphodiester). (**C**) 4140 (2′-*O*-propyl phosphodiester).

The relative nuclease stability of the 12mer series shown in Table 1 was determined using snake venom phosphodiesterase. This 12mer series contains 2′-*O*-alkyl sugar modifications at every position, including the 3′ terminal residue. The 2′-*O*-methyl phosphodiester, 2′-*O*-propyl phosphodiester and 2′ deoxy phosphorothioate analogs were 7-, 10- and 89-fold more stable than the unmodified control (Table 4). Again, the stability observed for the fully modified 2′-*O*-pentyl 12mer oligoribonucleotide is qualitatively the same as for the 17mer 2′-*O*-pentyl analog. The order of stability was the same as the 17mer series. However, in contrast to the abbreviated degradation pattern of the 17mer 2′-*O*-alkyl analogs, the 12mer series exhibited a ladder of degradation products (data not shown). The rank order of stability of the 12mers is identical to the 17mers, but quantitative relative stabilities differ in the two series. The 2′-*O*-methyl and 2′-*O*-propyl analogs in the 12mer series are 3–10-fold more resistant to snake venom phosphodiesterase than the unmodified control, and in the 17mer series, these analogs exhibit 70–160-fold increase in stability relative to the unmodified control.

The differences in nuclease stability and high homoduplex stability lead us to speculate that the greater stability associated with the 17mer series may be due to the formation of a stable secondary structure. Examination of the 17mer sequence indicated that this oligoribonucleotide has the potential to form the structures shown in Figure 3. Calculated free energies of the RNA versions of the hairpin and duplex shown in Figure 4 are –5.1 and –7.8 kcal/mol, respectively (16). Two observations suggest the 2′-*O*-methyl and 2′-*O*-propyl 17mers exist in one of these structures. First, thermal denaturation analysis showed hyperchromic shifts for the 2′-*O*-methyl and 2′-*O*-propyl oligomers with associated T_ms of 73.4 and 77.8°C, respectively (Table 2). Secondly, the SVPD degradation pattern observed for the 17mer 2′-*O*-methyl and 2′-*O*-propyl analogs is consistent with 3′-exonuclease degradation of the three base single-stranded overhang at the 3′-terminus of both structures (Figure 2).

To test the hypothesis that duplex or hairpin formation is contributing to the increased nuclease stability observed in the 17mer sequence, 2′-*O*-methyl oligomers were prepared which contained a 2 or 5 nt deletion at the 5′ terminus (Fig. 3). These deleted sequences (8313 and 8312) cannot form the structures hypothesized for oligomer 3299. In addition, the 17mer was prepared as an unstructured oligomer (oligomer #8314, Fig. 3) by



Oligomer	Sequence	
1592	5' GGA CCG GAA GGT ACG AG 3'	2'-deoxy
3299	5' GGA CCG GAA GGU ACG AG 3'	2'-O-Me
8314	5' AAA CCA AAA AAC CAG AG 3'	2'-O-Me
8313	5' A CCG GAA GGU ACG AG 3'	2'-O-Me
8312	5' G GAA GGU ACG AG 3'	2'-O-Me

Figure 3. Potential structures of 17mer series oligoribonucleotides and deletion sequences for the 17mer series. Dashes represent Watson–Crick base pairing, asterisks represent G–A mismatches. All oligoribonucleotides contain a 3′ terminal dG and a phosphodiester internucleotide linkage.

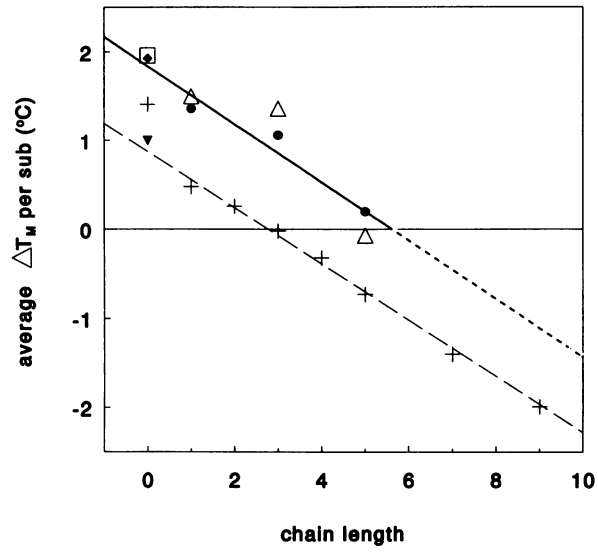


Figure 4. ΔT_m per substitution averaged over the sequences in Table 1 plotted versus the number of carbon atoms in the modification. ●, uniform 2′-*O*-alkyl substitution in phosphodiester backbone; ◆, uniform 2′-F substitution in phosphodiester backbone; Δ, uniform 2′-*O*-alkyl substitution in phosphorothioate backbone; □, uniform 2′-F substitution in phosphorothioate backbone; ▼, RNA in a phosphodiester backbone; +, oligoribonucleotides containing a few 2′ substituted adenosines in a background of unmodified deoxynucleotides [data from (9)].

replacing all the 2′-*O*-methyl G and 2′-*O*-methyl U residues with 2′-*O*-methyl A and 2′-*O*-methyl C residues, respectively. For the purpose of maintaining the same 3′ terminal sequence, the three 3′ terminal residues were not changed, and the 2′-*O*-methyl C and the 2′-*O*-methyl A residues at positions 13 and 14 were switched in order to further diminish the possibility of duplex formation. Oligomer 8313, a 15mer with 2 nt deletions (5′), exhibited an overall decrease in nuclease stability. The full length 2′-*O*-methyl 17mer was 120-fold more stable to snake venom phosphodiesterase relative to the unmodified control, while 8313 was only ~20-fold more stable (Table 4). A similar decrease in the relative $t_{1/2}$ was observed for the unstructured 17mer (oligomer 8314, 120 to ~20-fold). Oligomer 8312, in which the five 5′ terminal residues have been deleted showed an even greater loss of nuclease resistance, as its relative $t_{1/2}$ decreased from 120-fold greater than the unmodified control for the 17mer parent to 8-fold greater than the control, similar to the 2′-*O*-methyl results in the 12mer series.

The stability of the 12mer series of 2′-*O*-modified oligomers was also investigated using S1 nuclease, a single-stranded endonuclease. At an enzyme concentration of 1.9 U/ml, no degradation of the 2′-*O*-methyl phosphodiester or 2′-*O*-propyl phosphodiester 12mers is observed. Under the same conditions, the 2′ deoxy phosphodiester and the 2′ deoxy phosphorothioate analog have $t_{1/2}$s of 2 and 13 min, respectively (Table 4). Differences in the stability of 2′-*O*-methyl versus the 2′-*O*-propyl modification were observed at a 10^5-fold higher S1 nuclease concentration. At an enzyme concentration of 1.9×10^5 U/ml, the $t_{1/2}$ of the 2′-*O*-methyl analog is 90 min, while no degradation of the 2′-*O*-propyl oligomer was observed.

DISCUSSION

The ΔT_m is the increase or decrease in duplex T_m caused by substitution of 2′-modified nucleosides for deoxynucleosides. Figure 4 plots average ΔT_m per substitution for the sequences in Table 2. The ΔT_m per substitution is positive for 2′-fluoro, 2′-*O*-methyl, or 2′-*O*-propyl substitution, and is essentially zero for 2′-*O*-pentyl substitution. The effect in a phosphodiester background (Fig. 4, solid symbols) parallels that in a phosphorothioate background (Fig. 4, open symbols). A good correlation is observed between the decrease in T_m and increasing size of the alkyl group. This effect is similar to that reported by Lesnik (9) for oligoribonucleotides containing from one to five 2′-substituted adenosines in a background of unmodified deoxynucleotides (Fig. 4, + symbols). When 2′ modifications are substituted at every position, however, they have a significantly more positive effect on T_m than do 2′ substitution only at a few sites. Duplex stabilization by uniform 2′-*O*-methyl or 2′-fluoro substitution has been observed previously (6,17,18). Our results extend these findings to include fully modified 2′-*O*-propyl and 2′-*O*-pentyl oligoribonucleotides. Previous studies of partially modified oligoribonucleotides have shown a correlation between alkyl chain length and average ΔT_m, with 2′-*O*-propyl being the chain length having an average ΔT_m of ~0, the 'break even' point relative to the 2′-deoxy. For fully modified 2′-*O*-alkyl oligoribonucleotides, however, this study demonstrates that the alkyl chain length 'break even' point is the longer 2′-*O*-pentyl modification. This stabilization most likely results from the increase in the %N sugar pucker observed in the series 2′-fluoro > 2′-*O*-methyl > 2′-*O*-propyl > 2′-*O*-pentyl (19). CD spectra of hybrids suggest the conformation of the DNA:RNA heteroduplex is between the conformation of A-form RNA and that of B-form DNA, while

CD spectra of fully modified 2′ substituted hybrids exhibit a spectrum like that of A-form RNA. This suggests that 2′ substitution at every position does indeed shift the conformation to an RNA:RNA like structure, consistent with the sugar pucker predictions. Consecutive 2′ substitutions may be more stabilizing than isolated substitutions because the sugar pucker is uniform along the chain, avoiding multiple transitions produced by point substitutions.

Modifications at the 2′ position enhance nuclease stability, but effects upon duplex stability with RNA depend upon the nature of the substituent. Increased phosphodiester bond stability to purified nucleases has been observed for oligoribonucleotides containing 2′-*O*-methyl, 2′-*O*-allyl, 2′-*O*-(3,3-dimethylallyl) and 2′-*O*-butyl residues (17,20,21). We have extended these studies to include 2′-*O*-propyl and 2′-*O*-pentyl modifications. Larger, bulkier substituents, however, have deleterious effects upon hybridization stability. Uniformly modified 2′-*O*-(3,3-dimethylallyl) oligoribonucleotides do not bind to their complementary RNA sequence (20,21). Introduction of 2′-*O*-nonyl adenosine residues near the 3′ end of a 15mer DNA oligomer enhanced nuclease stability 64-fold over that of the unmodified oligomer (10), but the average ΔT_m of 2′-*O*-nonyl containing oligoribonucleotides is approximately –2°C/modification (9). These studies indicate that the nuclease stability imparted by a 2′-*O*-alkyl substituent is enhanced by longer or bulkier moieties, but that there is a trade-off between increased nuclease resistance and stability of 2′-*O*-modified oligomer:RNA duplexes. Our data suggests that fully modified 2′-*O*-methyl and 2′-*O*-propyl oligoribonucleotides possess a desirable mix of nuclease resistance and enhanced duplex stability. They are more nuclease resistant and have a higher affinity for RNA than the 2′-deoxy analog. Both 2′-*O*-methyl and 2′-*O*-propyl chimeric oligonucleotides have shown increased efficacy over the 2′-deoxy analog when tested as phosphorothioates (22).

Our results show that homoduplexes formed between two oligoribonucleotide strands that are fully 2′-modified have higher T_ms than both RNA:RNA and DNA:DNA duplexes. These observations and the greater nuclease stability associated with the 17mer series led us to speculate that these oligomers are more resistant to exonucleolytic degradation by snake venom phosphodiesterase because of stable secondary structure or duplex formation. These speculations are supported by experimental evidence showing a hyperchromic shift associated with the single-stranded oligoribonucleotides and by the decrease in nuclease stability that was observed when sequence deletions or replacements were introduced to disrupt the proposed duplex region. A stem–loop structure at the 3′ end of either an unmodified oligomer or the phosphorothioate analog has been shown to increase the observed oligomer half-life when incubated with snake venom phosphodiesterase, DNA polymerase I or 10% fetal calf serum (23). The observed nuclease stability of the 17mer most likely is a result of both the resistance imparted by the 2′-alkyl modification itself and the resistance imparted by the proposed secondary structure.

An understanding of the characteristics imparted by 2′-*O*-modifications is important for their subsequent consideration as therapeutics or diagnostic agents. The high affinity of 2′-modified homoduplexes may lead to structured antisense oligoribonucleotides, perhaps modulating their ability to bind to their target RNA. Although this is a concern, antisense activity has been demonstrated using 2′-modified oligoribonucleotides as chimeric molecules (22) and as uniformly modified compounds (24,25). The higher homoduplex stability associated with 2′-modified oligoribonucleotides may also prove useful in designing stem–loop therapeutic oligoribonucleotides, or in stabilizing helical regions of other structured therapeutics such as ribozymes.

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