Structural basis for the RNA binding selectivity of oligonucleotide analogues containing alkylsulfide internucleoside linkages and 2'-substituted 3'-deoxyribonucleosides

Masad J. Damha*, Bin Meng, Daguang Wang, Constantin G. Yannopoulos and George Just

Department of Chemistry, McGill University, 801 Sherbrooke Street W., Montreal, QC, Canada H3A 2K6

Received May 24, 1995; Revised and Accepted August 30, 1995

ABSTRACT

In this report we describe the synthesis of oligonucleotides containing sulfide-linked dinucleoside units, namely $rT(2'OH)_s dT$, $rT(2'OMe)_s dT$, $dT_s rU(2'OMe)$ and $dT(2'OMe)_s rU(2'OMe)$. We also describe the interactions of such oligomers with complementary DNA and RNA targets, and provide the structural basis for their remarkable RNA binding selectivity. In all cases, the T_m values of the S/P-chimera duplexes were lower than those of the corresponding unmodified duplexes. We attribute this to steric interactions between the 5' sulfur and the atoms of the nearby base/sugar residues. The 2'-substituents (*i.e.*, 2'OH or 2'OMe) vicinal to the alkylsulfide internucleoside linkage significantly perturb the structure and stability of the duplexes formed with DNA, and more so than with RNA. The introduction of three $rT(2'OH)_s dT_p$ (or $rT(2'OMe)_s dT_p$) units into an oligodeoxynucleotide sequence was sufficient to abolish binding to complementary DNA but not RNA. The same three substitutions with $dT_s rU(2'OMe)_p$ and $dT(2'OMe)_s rU(2'OMe)_p$ did not abolish binding to DNA but the resulting complexes had poor thermal stability. The RNA-binding 'selectivity' exhibited by these oligomers is attributed to the tendency of the 2'-substituted (branched) furanoses to adopt the C3'-endo pucker, a conformation that is inconsistent with the B-form structure of helical DNA. The preference of these sugars to exist often *exclusively* in the C3'-endo form is attributed to stereoelectronic effects, namely *gauche* and *anomeric effects*. Our findings support the hypothesis that nucleoside analogues puckered exclusively in the C3'-endo form may result in them being especially good binders of targeted mRNA [S.H. Kawai (1991), Ph.D. Thesis, McGill University; Kawasaki *et al.* (1993) *J. Med. Chem.* 36, 831–841].

INTRODUCTION

There has been continuing interest in the synthesis and properties of analogues of DNA containing a sulfur atom at the internucleoside linkages, *e.g.* phosphorothioate or dialkylsulfide linkages (for a recent and comprehensive review, see ref. 1). So far, most of these modifications lead to a decrease in the affinity of the oligomer toward a complementary strand. A notable exception are oligonucleotides containing the 3'-thioformacetal linkage [3'-SCH₂OCH₂-5'] (2). Oligomers containing this linkage, alternating with phosphodiester, were found to bind to RNA better than the corresponding 'all'-phosphodiester oligomers. While high affinity and high sequence specificity are contradictory demands, high-affinity binding is desirable when an antisense oligonucleotide inhibits gene expression through a 'steric blocking' mechanism, *i.e.* by binding to a target mRNA and physically blocking the RNA from interacting with the ribosomes. Antisense oligomers with low binding affinity would result in [antisense]:[mRNA] duplexes with short lifetime, which should not substantially disrupt the expression of the targeted gene. In a few reported cases however [*e.g.* methylphosphonate/phosphate (3) and phosphorothioate/phosphate chimeras (4)], lower binding affinity can be more advantageous. This shows that 'more' (high affinity) is not always 'better' (5,6).

Our research in the antisense field has focused on oligonucleotide analogues in which some of the phosphodiester groups are replaced by the dialkyl sulfide linkage 3'-CH₂-CH₂-S-CH₂-5' (7–11). This linkage is non-hydrolyzable, non-ionic, and its length approximates that of a phosphodiester linkage reasonably well. More recently, we focused attention on the preparation of $rT(2'OH)_s dT$, $rT(2'OMe)_s dT$, $dT_s rU(2'OMe)$ and $rT(2'OMe)_s rU(2'OMe)$ dimer units as well as their 3'-*O*-phosphoramidite derivatives for incorporation into oligonucleotides (11,12). Our interest in these 2'-substituted derivatives was stimulated by our recent discovery that a dodecanucleotide incorporating a few $rT(2'OH)_s dT$ units display extremely poor binding to complementary DNA relative to RNA (10). While hybridization occurred for the sulfide

* To whom correspondence should be addressed

strand/RNA pair, no cooperative binding was observed between the sulfide ($rT(2'OH)_s dT$) strand and DNA over a wide range of salt concentrations. This manuscript addresses in detail the origin of this selectivity and demonstrate that the previously observed duplex stabilization by 2'-O-methyl groups (13–16) extends to oligonucleotides containing alkylsulfide linkages. Preliminary accounts of parts of this work have previously appeared (10,11).

MATERIALS AND METHODS

Preparation of oligonucleotides

All reagents and solid supports used in the solid-phase synthesis of oligonucleotides were purchased from Pharmacia. Sulfide-linked phosphoramidite dimers were used as a 0.11 M acetonitrile solutions. Synthesis of all oligomers was carried out on a Pharmacia GLB Gene Assembly Plus synthesizer. Sequences were prepared on a 0.2 μ mol scale and in the 'trityl off' mode employing the standard DNA syntheses cycle provided by Pharmacia. Coupling time for incorporation of sulfide dimers was extended to 9 min. RNA oligomers were synthesized by the silyl-phosphoramidite method as previously described (17).

Cleavage, deprotection and desalting of oligonucleotides

An oligo-support cassette removed from synthesizer was placed in a 2 ml Eppendorf tube and centrifuged to get rid of any trapped acetonitrile. This cassette was then transferred to a new Eppendorf tube and treated with 1 ml 29 % ammonium hydroxide solution (55°C, 16 h). The ammonia solution was collected by centrifuging the Eppendorf tube and then run through a Sephadex NAP-10 column which was pre-washed with 15 ml deionized water. The first eluting fractions (1.5 ml) were lyophilized to dryness and the resulting oligomer was redissolved in 1 ml of water. Purity of each oligomer was confirmed by PAGE (26% polyacrylamide–7 M urea) run at 4°C, at a current of 10 mA, for 6–8 h (9).

Melting experiments

Melting curves were measured in 10 mM NaH_2PO_4/Na_2HPO_4 , 1 M NaCl buffer (pH 7.0) and analyzed the same way as described in reference 9.

Circular dichroism

The CD spectrum of each sample was measured on a Jasco 500-C spectropolarimeter with a Jasco DP-J800/300 data processor. The cell temperature was kept at 5°C. The concentration of the oligonucleotides were $\sim 2.5 \mu$ M (each strand).

Molecular modeling

All calculations were performed on a IBM PC 486-33 MHz running Hypercube's HyperChem 3.0. Allinger's molecular mechanics method (MMX) was applied in all calculations with a cutoff function (switched: inner = 10 Å and outer = 14 Å). Convergence limits were kept constant at ~ 0.03 kcal/Å² for the dimers. The sulfide dimers T_sT , $rT(2'OH)_s dT$ and $rT(2'OMe)_s dT$ were minimized individually. The control was the unmodified dimer T_pT built in the B-form prior to minimization.

Scheme 1.

RESULTS AND DISCUSSION

Oligonucleotide synthesis

Sulfide/phosphate oligonucleotide chimeras were obtained by automated solid-phase synthesis using dimer blocks 1–4 (Scheme 1) and their phosphoramidite derivatives (12). The sequences prepared (5–17) are shown in Table 1. Average coupling yields for dimer incorporation were $\sim 90\%$ as assessed by the trityl assay method. Following chain assembly, the oligonucleotides were cleaved from the support, deprotected and purified as previously described (9). The single absorption band on polyacrylamide gel found for each oligomer indicated their high purity ($>95\%$) and the retarded mobility of the sulfide containing oligomers (6–17) relative to the unmodified sequence (5) was in agreement with their reduced charge/mass ratio (data not shown) (9).

Interaction of oligonucleotides containing $rT(2'OH)_s dT$, $rT(2'OMe)_s dT$ units with single-stranded DNA and RNA

Thermal Denaturation. Melting curves of the synthesized sulfide/phosphate oligomers 6–17 annealed to unmodified DNA 3'-d(CGCAAAAACGA)-5' (18) or RNA 3'-r(CGCAAAAACGA)-5' (19) were obtained in 1 M NaCl pH 7.0 buffer at a concentration of 2.5 μ M for each strand. Samples were heated to 70°C and then cooled to room temperature prior to melting. The rate of heating was 0.5°C/min. Control melts of the fully natural duplexes DNA/DNA 5/18 and DNA/RNA 5/19 gave melting temperature (T_m) values of 59.5 and 52.5°C respectively.

Introduction of $rT(2'OH)_s dT_p$ (1) or $rT(2'OMe)_s dT_p$ (2) units into the backbone of oligomer 5 decreased the thermal stability of duplexes formed with either the DNA and RNA target (Table 1). For one and two incorporations the destabilizing effect was additive, with 2 being slightly more destabilizing. Oligonucleotides containing three contiguous $T(2'-OR)_s T_p$ units (8, R = H; and 11, R = Me) had selective hybridization properties, binding only to target RNA (10). This 'selectivity' is a consequence of the 2'-OH or 2'-OMe substituent since a sulfide oligomer of the same sequence as 8 and 11 that incorporates $3 \times dT_s T_p$ dimers at the same positions was found to exhibit cooperative binding to both DNA 18 and RNA 19 targets (9). How do we account for: (i) the general destabilizing effect of (3'-CH₂-CH₂-S-CH₂-5') linkages, and (ii) for the discriminatory binding properties of sulfide strands containing 2'-substituted (OH and OMe) sugars?

Model building suggests that the destabilising effect of the dialkylsulfide (3'-CH₂-CH₂-S-CH₂-5') linkage is mainly steric in

Table 1. Effect of sulfide-linked dinucleoside substitution on melting temperatures (T_m)^a

Sequences	DNA 18 (°C)	$\Delta T_m/s$ - linkage (°C)	RNA 19 (°C)	$\Delta T_m/s$ - linkage (°C)	Selectivity T_m (RNA-DNA)
5 GCGT TTTTGCT	59.5	-	52.5	-	-7.0
6 GCGTTT ^{OH} _S TTTGCT	45.1	-14.4	45.2	-7.3	0.1
7 GCGT ^{OH} _S TTTT ^{OH} _S TGCT	31.3	-14.1	37.5	-7.5	6.2
8 GCGT ^{OH} _S TT ^{OH} _S TT ^{OH} _S TGCT	-	-	31.5	-7.0	>31.5
9 GCGTTT ^{OMe} _S TTTGCT	44.0	-15.5	44.0	-8.5	0
10 GCGT ^{OMe} _S TTTT ^{OMe} _S TGCT	30.1	-14.7	36.0	-8.3	6.0
11 GCGT ^{OMe} _S TT ^{OMe} _S TT ^{OMe} _S TGCT	-	-	30.5	-7.3	>30.5
12 GCGTTT _S U ^{OMe} TTGCT	47.8	11.7	45.7	6.8	-2.1
13 GCGT _S U ^{OMe} TTT _S U ^{OMe} GCT	37.7	10.9	40.8	5.8	3.1
14 GCGT _S U ^{OMe} T _S U ^{OMe} T _S U ^{OMe} GCT	26.2	11.1	35.5	5.7	9.3
15 GCGTTT ^{OMe} _S U ^{OMe} TTGCT	45.1	14.4	47.5	5.0	2.4
16 GCGT ^{OMe} _S U ^{OMe} TTT ^{OMe} _S U ^{OMe} GCT	32.9	13.3	45.5	3.5	12.6
17 GCGT ^{OMe} _S U ^{OMe} T ^{OMe} _S U ^{OMe} T ^{OMe} _S U ^{OMe} GCT	24.8	11.6	43.8	2.9	19.0

^a T_m 's are $\pm 0.5^\circ\text{C}$. Buffer: 10 mM NaH_2PO_4 , 1 M NaCl (pH 7.0)

nature. Model building of dTsdT dimer with a canonical B form type conformation revealed a close contact between the sulfur atom in the 5'-position with either the furanose oxygen and the H6 of the same thymidine (sdT) residue. Because of the difference in van der Waal's radii for sulfur and oxygen, these steric clashes are not significant in the case of an OS' atom. This kind of steric interaction has been proposed by Jones and co-workers (2), and more recently by Veal and Brown (18), to account for the differences between the binding characteristics of the 3'-thioformacetal (3'-S-CH₂-O-CH₂-5') and the (less favourable) 5'-thioformacetal (3'-O-CH₂-S-CH₂-5') substitution. Molecular mechanics calculations conducted in the present study indicates that this steric crowding can be largely alleviated through conformational adjustment about the C3'-O3' bond (5'-dT_s unit) without affecting all other torsional angles. However, this conformational adjustment results in significant unstacking and tilting of the bases, which we believe is the major cause of the destabilization observed for the 3'-CH₂-CH₂-S-CH₂-5' linkage (Fig. 1).

Selectivity for RNA versus DNA strands

Furanose sugars of branched (3'-deoxy)nucleosides adopt a C3'-endo pucker conformation. Inspection of the ¹H-NMR spectra of **20**, the known branched-chain thiosugar nucleosides **21–23**, dimers **1** and **2**, and several of their derivatives (e.g. **24**; Scheme 2) revealed that the anomeric (H1') proton of the 2'-substituted sugar appeared in all cases as a sharp singlet. This is exemplified by the spectrum of **24** whose enlargement is shown Figure 2. The coupling constant values $J_{H1'-H2'}$ (0 Hz), $J_{H2'-H3'}$ (~5–6 Hz) and $J_{H3'-H4'}$ (~10–11 Hz) for nucleosides **20–23**, and the 5'-nucleoside unit of dimer **24**, are very comparable and their magnitudes indicate that the furanose ring in these derivatives exist *exclusively* in the C3'-endo conformation, as observed in

Figure 1. Minimized structures of dTpdT (top) and dTsdT.

nucleic acid duplexes with A-conformation (Scheme 3a) (19). This preference is re-inforced by the X-ray crystal structure of bicyclic nucleoside **20** (7), the solution structure of branched-chain nucleosides (7,8), and the 5'-unit of dinucleosides

Figure 2. ^1H -NMR (500 MHz) spectrum of dTsdT **24** in CDCl_3 . Selected resonances of 5' and 3' residues are indicated.

Scheme 2.

$5'\text{-rT}(2'\text{OH})_s\text{dT-}3'$ and $5'\text{-rT}(2'\text{OMe})_s\text{dT-}3'$ (8–10,12). In addition, ^1H -NMR data reported on a number of 3'-deoxynucleosides are also consistent with a predominant C3'-endo pucker (20–22). β -D-3'-deoxycytidine derivatives, e.g. **25** (Scheme 2), are 'frozen' in the C3'-endo conformation (20).

The strong preference for the branched 3'-deoxynucleosides to adopt the C3'-endo pucker can be rationalized by stereoelectronic considerations. In natural ribonucleosides, the dynamic C3'–C2' endo equilibrium of the furanose moiety is controlled by the effects of the sugar (*gauche effect*; 23) and the heterocyclic base (*anomeric effect*; 24,25). The *gauche effect* of 3'-OH,

Scheme 3.

$[\text{O}4'\text{-C}4'\text{-C}3'\text{-O}3']$ drives the sugar equilibrium toward C2' endo, whereas that of 2'-OH $[\text{O}4'\text{-C}1'\text{-C}2'\text{-O}4']$ drives the equilibrium toward C3' endo (Scheme 3a). The anomeric effect also drives the C3'–C2' endo equilibrium toward C3' endo in order to place the base in the pseudoaxial orientation. Due to steric effects, the tendency for the base to occupy a pseudoaxial orientation is greater for a pyrimidine than for (the bulkier) purine (22). Elegant studies originating from the laboratory of Chattopadhyaya and co-workers (University of Uppsala, Sweden) have led to the quantification of these various effects (22,26).

The tendency of 2'-deoxynucleosides to adopt the C2'-endo conformation is a manifestation of the 3'-OH *gauche effect*, which overrides the anomeric effect. For example, 2'-deoxyadenosine exists predominantly in the C2'-endo conformation (66% C2'-endo at 291°K) in solution (27). This picture is quite different for 3'-deoxynucleosides. Inspection of Scheme 3b shows that the consequence of placing the hydroxyl group at the 2' position is that it becomes *gauche* oriented relative to the C1'–O4' bond

(2'-OH gauche effect). This conformation also places the thymine base in a pseudoaxial orientation which maximizes the 1,4-interaction of glycosidic nitrogen with one of the O4' lone pairs (anomeric effect). Without an opposing 3'-OH gauche effect, the 2'-OH gauche and anomeric effects re-inforce each other to drive the sugar equilibria of 3'-deoxynucleosides toward the C3'-endo form.

Preferred C3'-endo puckering of 3'-deoxythymidine ($\text{T}^{(2'-OR)}_s$) units and base sequence provides the structural basis for RNA selectivity. The ribose sugars in RNA also assume a C3'-endo conformation. The preference of ribonucleotides to exist as such results in RNA double helices and RNA-DNA hybrid duplexes assuming generally A-type helical structures, the more rigid RNA strand forcing DNA in this conformation in the latter case (28). In contrast, DNA is polymorphic (A, B, Z-DNA etc.) and can alter its conformation to accommodate many modes of binding to a complementary strand depending on environmental conditions. Because the B form of DNA is associated with high humidity in crystalline fibers or with aqueous solution of DNA, it is generally believed to represent the DNA structure occurring *in vivo*. This is particularly true for helices containing dA_n/dT_n tracks which resists B→A transitions even when water activity is low (29,30). Also, the sugars in B-DNA displays the C2'-endo puckering mode. Thus, two factors may be responsible for the RNA selectivity of oligomers **8** and **11**. The first factor is probably entropic in nature, i.e. the puckering of the branched-chain 5' sugars ($\text{rT}^{(2'OH)}_s\text{dT}$ and $\text{rT}^{(2'OMe)}_s\text{dT}$) exceeds the 3'-endo distribution of the ribonucleosides, and by far that of 2'-deoxynucleosides, a property that makes them more 'compatible binders' of targeted RNA (31). Cook and co-workers have also suggested that increased affinity of an antisense oligomer for a complementary RNA target can be achieved by decreasing the entropic motion of the sugar while maintaining a preorganized structure with an RNA-like conformation (21,32). The second factor is base sequence. dA/rU hybrids are known to require the least energy of any RNA-DNA hybrid to break the association between two strands (33). In fact, efficient transcription in living cells depends on oligomeric (dA/rU) regions to help release nascent precursor messenger RNAs from template DNA (34). The target DNA oligomer **18** is unique in that it contains a stretch of six consecutive dA residues, resulting in 'dA/rU'-like tracks, i.e. (dApdA)₃/(rTsOHdT)₃ or (dApdA)₃/(rTsOMe_sdT)₃, when it binds to sulfide oligomers **8** and **11**. rA/rU (RNA-RNA) hybrids are significantly more stable. Thus, the contrasting stability of dA/rU and rA/rU helices could explain in part why oligomers **8** and **11** show binding selectivity, with a significant preference for RNA 5'-r(AGCAAAAACGC) over DNA 5'-d(AGCAAAAACGC).

Effect of multiple 2'-O-methylations on interaction with single-stranded DNA and RNA

Given the above considerations it is perhaps surprising that we observed stable duplex formation between GCG[rT^(2'OMe)_srU^(2'OMe)]₃GCT **17** and the target DNA (*T_m* = 24.9°). Two distinct experiments suggest that the *contiguous* (six) methyl groups in this sequence are responsible for exerting stabilizing and perturbational effects on duplex formation. Firstly, the stabilization afforded by consecutive methyl substituents is evident from the *T_m* data (Table 1), i.e. ΔT_m per

Figure 3. Circular dichroism spectra of (A) DNA-DNA (**5/18**) (---) and DNA-RNA **5/19** (—); (B) DNA/DNA (**5/18**) (—); DNA **18** complexed with sulfide oligomer **12** (---), **13** (—•—), and **14** (—); (C) DNA/DNA (**5/18**) (—); DNA **18** complexed with sulfide oligomer **15** (---), **16** (—•—), and **17** (—) in 1 M NaCl, 10 mM phosphate buffer (pH 7.0). Refer to Table 1 for sequence number.

$\text{rT}^{(2'OMe)}_s\text{rU}^{(2'OMe)}$ was least negative for the most substituted oligomer **17** (−11.6°) [compared with **16** (−13.3°) and **15** (−14.4°)]. Secondly, unique structural effects of $\text{T}^{(2'OMe)}_s\text{rU}^{(2'OMe)}$ substitutions on duplex structure were revealed by circular dichroism (CD) spectroscopy (Fig. 3). The CD dichroism (CD) spectrum of the control duplex DNA-DNA (**5/18**) detected a B-like structure (Fig. 3A) (16,28). The DNA-RNA hybrid **5/19** control adopted a structure of the A-family, or intermediate between B-form and A-form, as exemplified by its CD spectrum (Fig. 3A). Substitution with $\text{dT}_s\text{rU}^{(2'OMe)}$ units in one of the strand does not substantially affect the CD spectrum of the DNA-DNA duplex (Fig. 3B). This finding is consistent with the notion that dA/dT duplexes resist

Table 2. Effect of 2'-O-methylation on duplex stability^a

Sequences	$\Delta T_m/s$ - linkage (°C) (DNA target)	$\Delta T_m/s$ - linkage (°C) (RNA target)
GCGT _S TT _S TT _S TGCT	9.4	7.7
GCGT _S ^{OMe} TT ^{OMe} TT _S ^{OMe} TGCT	no binding	7.3
GCGT _S U ^{OMe} T _S U ^{OMe} T _S U ^{OMe} GCT	11.1	5.7
GCGT _S ^{OMe} U ^{OMe} T _S ^{OMe} U ^{OMe} T _S ^{OMe} U ^{OMe} GCT	11.6	2.9

^a T_m 's are $\pm 0.5^\circ\text{C}$. Buffer: 10 mM NaH₂PO₄, 1 M NaCl (pH 7.0).

B→A conformational changes (28–30). However, increasing numbers of rT(2'-OMe)_srU(2'-OMe) substitutions in one of the strand causes the DNA–DNA duplex to become increasingly A-form (Fig. 3C). The maximum of the positive and negative bands in the CD spectrum of the (3×) rT(2'-OMe)_srU(2'-OMe) duplex **17/18** are at 274 and 243 nm, respectively, and are typical of DNA–RNA hybrids (16). It appears therefore that in the case of oligomer **17**, interaction with target DNA **18** is forced by the contiguous methyl substituents since it does not occur with oligomer **11**. The stabilizing effect of contiguous alkyl groups has been reported previously for 2'-OR-DNA–RNA duplexes (16), and is attributed to the displacement of water by the hydrophobic 2'-O-alkyl groups in the minor group which pushes the conformation toward the A form (28).

Multiple methylation has a positive effect on interaction with single-stranded RNA. This is evident when the oligonucleotide has at least three methyl substitutions (Table 2). ΔT_m per substitution is most negative in dTsT (7.7°) and rT(2'-OMe)_sdT (7.3°), followed by rTsU(2'-OMe) (5.7°) and rT(2'-OMe)_srU(2'-OMe) (2.9°). The favourable hybridization properties of GCG[rT(2'-OMe)_srU(2'-OMe)]₃GCT towards RNA is also likely due to consecutive methylation (16). This configuration leads to a sulfide-DNA–RNA hybrid with a virtually pure A-like structure (CD studies, data not shown) (35).

CONCLUSIONS

Our findings support the hypothesis that analogues of DNA that adopt the C3'-puckered conformation may result in them being especially good binders of RNA (21,30,31). The selectivity of phosphate/sulfide oligonucleotides **8** and **11** is attributed to the tendency of the 2'-substituted, 3'-deoxyfuranoses to adopt the C3'-endo pucker, a conformation that is inconsistent with the B form structure of helical DNA. The preference of these sugars to exist often exclusively in the C3'-endo form is attributed to stereoelectronic effects, namely gauche and anomeric effects exerted by the base and 2'-O-residues.

Recently, a number of synthetic oligonucleotides has been reported to exhibit binding selectivity to RNA over DNA (20,36–38). The oligomers discussed in the present study and 2',5'-linked RNA (36) are among the most selective. 2',5'-Linked DNA has recently been synthesized by the groups of Breslow (39) and Switzer (40) and their data support duplex formation with complementary 2',5'-linked DNA. However, binding experiments with natural (3',5')-DNA or (3',5')-RNA has not been reported, thus it is unknown whether this analogue is 'RNA selective'. The present study predicts that the furanoses of

2',5'-DNA, similar to those described here, will pucker in the C3'-endo conformation. It also predicts that 2'-5'-DNA will display RNA binding selectivity. The recent finding that 2',5'-linked duplex DNA adopts an A-type conformation supports our hypothesis (41).

Finally, our study on oligonucleotides containing 2'-O-methylated sugars show that the stabilizing effect of methyl groups previously observed in 2'-OMe-RNA–RNA duplexes extends to oligomers containing the 3'-CH₂CH₂SCH₂-5' linkages. However, the negative steric interaction between 2'-OMe groups and sulfur atoms overrides the stabilizing effect of the methyl substituents resulting in duplexes of lower thermal stability.

The development of oligonucleotides with selective hybridization properties may prove useful in the design of more selective antisense systems and novel hybridization probes for nucleic acids biosensors (42,43).

ACKNOWLEDGEMENTS

M.J.D. and G.J. gratefully acknowledge financial support by the Natural Sciences and Engineering Research Council of Canada (NSERC) and Le Ministère de l'éducation du Québec (FCAR). M.J.D. also thanks the Canadian Foundation for AIDS Research (CANFAR) for financial support.

REFERENCES

- Y.S. Sanghvi and P.D. Cook (eds) (1994) *Carbohydrate modifications in antisense research*. ACS Symposium Series 580.
- Jones, R.J., Lin, K.-Y., Milligan, J.F., Wadwani, S. and Matteucci M.D. (1993) *J. Org. Chem.* **58**, 2983–2991.
- Giles, R.V., and Tidd, D.M. (1992) *Nucleic Acids Res.* **20**, 763–770.
- Stein, C.A., Subasinghe, C., Shinokazu, K. and Cohen, J.S. (1988) *Nucleic Acids Res.* **16**, 3209–3221.
- Herschlag, D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6921–6925.
- Moulds, C., Lewis, J.G., Froehler, B.C., Grant, D., Huang, T., Milligan, J.F., Matteucci, M.D. and Wagner, R.W. (1995) *Biochemistry* **34**, 5044–5053.
- Kawai, S.H., Chin, J. and G. Just (1990) *Nucleosides Nucleotides* **9**, 1045–1060.
- Kawai, S.H., Wang, D. and Just, G. (1992) *Can. J. Chem.* **70**, 1573–1580.
- Kawai, S.H., Wang, D., Giannaris, P.A., Damha, M.J. and Just, G. (1993) *Nucleic Acids Res.* **21**, 1473–1479.
- Meng, B., Kawai, S.H., Wang, D., Just, G., Giannaris, P.A. and Damha, M.J. (1993) *Angew. Chem. Int. Ed. Engl.* **32**, 729–731.
- Just, G. and Kawai, S.H. (1994) In Y.S. Sanghvi and P. Dan Cook (eds) *Carbohydrate modifications in antisense research*. American Chemical Society Symposium Series, vol. 580, pp.52–65.
- Wang, D., Meng, B., Damha, M.J. and Just, G. (1995) *Nucleosides Nucleotides*, in press.
- Inoue, H., Hayase, Y., Imura, A., Iwai, S., Miura, K. and Ohtsuka, E. (1987) *Nucleic Acids Res.* **15**, 6131–6149.

- 14 Iribarren, A.M., Sproat, B.S., Neuner, P., Sulston, I., Ryder, U. and Lamond, A.I. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7747-7751.
- 15 Ecker, D.J., Vickers, T.A., Bruce, T.W., Freir, S.M., Jenison, R.D., Manoharan, M. and Zoundes, M. (1992) *Science* **257**, 958-961.
- 16 Lesnik, E.A., Guinasso, C.J., Kawasaki, A.M., Sasmor, H., Zounes, M., Cummins, L.L., Ecker, K.J., Cook, P.D. and Freier, S.M. (1993) *Biochemistry* **32**, 7832-7838.
- 17 Damha, M.J. and Ogilvie, K.K. (1993) In Agrawal, S. (ed.), *Protocols for Oligonucleotides and Analogs: Synthesis and Properties*. Methods in Molecular Biology, The Humana Press, Inc. Totowa, N.J., Vol. 20, pp. 81-114.
- 18 Veal, J.M. and Brown, F.K. (1995) *J. Am. Chem. Soc.* **117**, 1873-1880.
- 19 Altona, C. and Sundaralingam, M. (1972) *J. Am. Chem. Soc.* **94**, 8205.
- 20 Sheppard, T.L., Rosenblatt, A.T. and Breslow, R. (1994) *J. Org. Chem.* **59**, 7243-7248.
- 21 Griffey, R.H., Lesnik, E., Freier, S., Sanghvi, Y.S., Teng, K., Kawasaki, A., Guinasso, C., Wheeler, P., Mohan, V. and Cook, P.D. (1994) In Y.S. Sanghvi and P. Dan Cook (eds) *Carbohydrate modifications in antisense research*, American Chemical Society Symposium Series, vol. 580, pp. 212-224.
- 22 Plavec, J., Thibaudeau, C. and Chattopadhyaya, J. (1994) *J. Am. Chem. Soc.* **116**, 6558-6560.
- 23 Wolfe, S. (1972) *Acc. Chem. Res.* **5**, 102, and references therein.
- 24 Edward, J.T. (1955) *Chem. Ind.* 1102.
- 25 Lemieux, R.U., Chu, N.J. (1958) Abstr. Papers Amer. Chem. Soc. Meetings 133, 31N.
- 26 Thibaudeau, C., Plavec, J. and Chattopadhyaya, J. (1994) *J. Am. Chem. Soc.* **116**, 8033-8037.
- 27 Hruska, F.E., Wood, D.J., McCaig, T.N. and Smith, A.A (1974) *Can. J. Chem.* **52**, 497.
- 28 Saenger, W. (1984) *Principles of Nucleic Acid Structure*. Springer-Verlag, New York.
- 29 Peck, L.J. and Wang, J.C. (1981) *Nature* **292**, 375-378.
- 30 Rhodes, D. and Klug, A. (1981) *Nature* **292**, 378-380.
- 31 Kawai, S.H. (1991) Ph.D. Thesis, McGill University, Montreal, Quebec, Canada, pp. 82.
- 32 Kawasaki, A.M., Casper, M.D., Freier, S.M., Lesnik, E.A., Zounes, M.C., Cummins, L.L., Gonzalez, C. and P.D. Cook (1993) *J. Med. Chem.* **36**, 831-841.
- 33 Martin, F.H. and Tinoco, I. Jr. (1980) *Nucleic Acids Res.* **8**, 2295-2299.
- 34 Stryer, L. (1988) *Biochemistry*, Freeman Co., New York.
- 35 B. Meng (1994) Ph. D. thesis, McGill University, Montreal, Quebec, Canada.
- 36 Giannaris, P.A. and Damha, M.J. (1993) *Nucleic Acids Res.* **21**, 4742-4749.
- 37 Wang, S. and Kool, E.T. (1995) *Nucleic Acids Res.* **23**, 1157-1164.
- 38 Adams, A.D., Petrie, C.R. and Meyer, R.B. (1991) *Nucleic Acids Res.* **19**, 3647-3651.
- 39 Jung, K.-E. and Switzer, C. (1992) *J. Am. Chem. Soc.* **114**, 6255-6256.
- 40 Dougherty, J.P., Rizzo, C.J., and Breslow, R. (1992) *J. Am. Chem. Soc.* **114**, 6254-6255.
- 41 Robinson, H., Jung, K.-E., Switzer, C. and Wang, A. H.-J. (1995) *J. Am. Chem. Soc.* **117**, 837-838.
- 42 Piuino, P.A.E., Krull, U.J., Hudson, R.H.E., Damha, M.J. and Cohen, H. (1994) *Anal. Chim. Acta* **288**, 205-214.
- 43 Piuino, P.A.E., Krull, U.J., Hudson, R.H.E. and Damha, M.J. (1995) *Anal. Chem.* **67**, 2635-2646.