What Affects the Effect of 2′-Alkoxy Modifications? 1. Stabilization Effect of 2′-Methoxy Substitutions in Uniformly Modified DNA Oligonucleotides

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ABSTRACT: The thermostability of hybrid duplexes with uniformly 2′-methoxy modified DNA strands (D′R and RD′), their unmodified DNA:RNA counterparts (DR and RD), and corresponded RNA:RNA (RR) duplexes for six sequences with different GC and deoxypyrimidine (dPy) content was measured. The linear correlation between the total stabilization effect of 2′-methoxy modifications ($\Delta\Delta G°_{37}$(D′R-DR)) and the relative stability of corresponding unmodified hybrids compared to the RR counterparts ($\Delta\Delta G°_{37}$(RR-DR)) suggests that the initial conformational and the thermodynamic state of the "parent" unmodified hybrid governs the effect of 2′-methoxy (and may be other 2′-alkoxy) modifications whose mechanism of action includes an S → N conformational shift resulting in an RNA-like A-form duplex. We also found a correlation between the "hydrophobic" part of the total effect ($\Delta\Delta G°_{37}$ (D′R-RR)) and the dA fraction in the modified DNA strand, suggesting that the "hydrophobic" effect of the 2′-methoxy groups results mainly from intraresidue steric effects increasing rigidity of the modified sugar rings. The correlations observed enabled us to predict the stability of hybrids with 2′-methoxy modified DNA strands for any sequence except for sequences with $(dU)_{10}$ and $(dA)_{10}$ strings.

The potential use of short oligodeoxyribonucleotides (ODNs)[1] as antisense drugs targeting cellular mRNAs has resulted in increased interest in the structure and thermodynamic properties of DNA:RNA hybrid duplexes and their modified analogues (*1*, *2*). Uniformly 2′-alkoxy modified ODNs have been found to be effective inhibitors of gene expression acting by RNase H-independent mechanisms (*3*−*7*). The best 2′-alkoxy modifications have been shown to significantly improve both nuclease stability and the affinity of ODNs to RNA targets (*8*−*12*). However, results reported indicate that the effect of 2′-alkoxy as well as 2′-fluoro modifications varies significantly from sequence to sequence (*8*, *9*, *11*, *13*, *14*). Therefore the selection of a sequence for testing the effect of 2′-modifications affects the result. To date, the source of this difference is still unclear and is an object of interest.

The stabilization effect of 2′-alkoxy modifications results mainly from an ability of electronegative 2′-substitutions such as fluorine and alkoxy groups to shift the conformational equilibrium of the DNA sugar rings from the C2′-endo (S) toward the C3′-endo (N) conformation, resulting in an RNA mimic A-conformation of oligonucleotides (*15*−*17*). Hybridization of uniformly 2′-modified ODNs to RNA targets results in an RNA-like A-form duplex formation as judged by CD (*9*, *13*), NMR (*18*), X-ray crystallography, and modeling (*19*) data. Thus the conformation of uniformly modified hybrid duplexes is always close to the A-form of the corresponding RR duplexes. The extra stability of 2′-methoxy modified DNA:RNA hybrid duplexes (D′R/RD′) compared to their RNA:RNA counterparts (RR) has been suggested to be a result of steric repulsion between the 2-carbonyl groups of pyrimidines and the 2′-methoxy substitutions (*20*, *21*), of enhanced base stacking (*22*), and of putative hydrophobic interactions between consecutive 2′-methoxy groups positioned at the surface of the minor groove of uniformly modified DNA (*18*, *19*).

In contrast to A-form which is similar for all modified hybrids, unmodified hybrids with a different deoxypyrimidine (dPy) fraction in the DNA strands have intermediate conformations with parameters varying between those for their DNA and RNA counterparts (*23*−*30*). As a consequence, the relative thermodynamic stabilities of unmodified hybrids compared to their RR counterparts ($\Delta\Delta G°_{37}$(RR-DR/RD) and ΔT_m(RR-DR/RD)) also are different for hybrids with different dPy fractions and different GC content (*28*, *31*−*33*). Therefore the free energy gained under a transition from the various intermediate conformational states of unmodified hybrids to the A-form of uniformly modified ones should vary from sequence to sequence. On the basis of this consideration, we suggested that the effect of 2′-methoxy modifications on the affinity of modified DNA strands to RNA targets calculated as $\Delta\Delta G°_{37}$(D′R-DR) and ΔT_m(D′R-DR) should be highest for the least stable hybrids and vice versa. In this paper we demonstrate that there is a linear correlation between the total effect of 2′-methoxy modifications and the difference in stability of unmodified hybrids and their RR counterparts, indicating that the

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[1] Abbreviations: ODN, oligodeoxyribonucleotides; DR/RD, hybrid duplexes DNA:RNA or RNA:DNA; RR, duplex RNA:RNA; D′R/RD′, hybrid duplexes with uniformly 2′-methoxy modified DNA strand; dPy, dPu, deoxypyrimidine and deoxypurine residues respectively; dA, deoxyadenylyl residue; f_{dA}, a fraction of deoxyadenylyl residue (dA); CD, circular dichroism; NMR, nuclear magnetic resonance; EDTA, ethylenediaminetetraacetic acid; f_S, a fraction of S conformation of sugar moiety of nucleotide residues.

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Table 1: Thermodynamic Parameters for 2′-Methoxy Modified (D′R/RD′), Unmodified (DR/RD) Hybrids, and Their RNA (RR) Counterparts

					T_m (°C)/$-\Delta G°_{37}$ (kcal/mol)[a]				
duplex	length (bp)	dA (%)	dGC (%)	dPy (%)	RR	DR[b]	D′R	RD[b]	RD′
5′-CUCGUACCUUCCGGUCC (1)	17	6	65	76	75.2	62.6	82.2	56.5	80.4
3′-GAGCAUGGAAGGCCAGG		29		24	22.9	16.0	27.2	12.7	24.6
5′-GCUCUCUGGC (2)	10	0	70	70	62.4	51.7	67.0	40.8	65.2
3′-CGAGAGACCG		30		30	16.1	12.3	17.6	8.9	16.8
5′-GAGCUCCCAGGC (3)	12	17	75	50	73.8	59.9	77.3	56.7	79.7
3′-CUCGAGGGUCCG		8		50	22.0	14.9	23.9	14.0	23.4
5′-UCCAGGUGUCCGCAUC (4)	16	13	62	62	76.1	61.1	82.1	61.8	81.1
3′-AGGUCCACAGGCGUAG		25		38	24.2	16.2	27.9	18.0	25.9
5′-GUACUUGCAUAGUCG (5)	15	20	47	53	60.3	49.2	65.6	46.2	64.5
3′-CAUGAACGUAUCAGC		33		47	18.7	12.8	20.5	10.9	19.7
5′-GCGUUUUUUUUUUGCG (6)	16	0	38	75	51.1	46.2	60.0	28.7	51.1
3′-CGCAAAAAAAAAACGC		63		25	13.6	11.2	17.6	5.9	13.8

[a] For each duplex, the first line denotes T_m (°C) values and the second line denotes $-\Delta G°_{37}$ (kcal/mol) values. [b] DR denotes a hybrid with an upper strand as a DNA and a lower strand as an RNA; RD denotes a hybrid with an upper strand as an RNA and a lower strand as a DNA.

stabilization effect of 2′ substitutions increases with the decrease in the stability of the unmodified "parents". The thermodynamic approach developed in this paper sheds light on the source of the difference in the magnitude of 2′-methoxy stabilization for different sequences as well the "hydrophobic" effect of 2′-methoxy groups in modified DNA strands. The correlations observed enabled us to predict an unusually large effect of the modification in a homopurine sequence (+4.2°/mod) and to calculate the stability of 2′-methoxy modified duplexes using RNA nearest-neighbors parameters (34) and a set of equations obtained from our data.

MATERIALS AND METHODS

Oligonucleotide Synthesis. 2′-Methoxy phosphophoroamidates were purchased from Glen Research (Sterling, VA). Oligonucleotides were synthesized using an Applied Biosystems 3804B automated DNA synthesizer and 5′-*O*-(dimethoxytrityl)-3′-*O*-phosphoroamidates. Standard phosphoroamidite coupling chemistry was utilized as described previously (*9*, *13*). Due to the procedure used, 2′-methoxy modified oligonucleotides had single 2′-deoxy residues at their 3′ ends. Alternating 2′-*O*-methyl, 2′-hydroxy chimeras (Table 3, sequence 6′, 6″) were purchased from Cruachem (Bearsden Bio Inc.).

Determination of Hybridization Thermodynamics. Absorbance versus temperature profiles were measured on a Gilford Response spectrophotometer in a buffer containing 100 mM Na^+, 10 mM phosphate (pH 7.1), and 0.1 mM EDTA. Oligonucleotides and their complements were combined at 4 μM each strand, heated 5 min at 90 °C, and cooled slowly to allow the formation of perfect duplexes. Oligonucleotide concentrations were calculated from the oligonucleotide absorption at 260 nm at 85 °C using extinction coefficients estimated according to the method of Puglisi and Tinoco (*35*). Oligonucleotide solutions were heated at a rate of 0.7 °C/min in 1 cm path length cells and then cooled to confirm reversibility and lack of evaporation. T_m values and free energies of duplex formation were obtained from fits of absorbance versus temperature curves to a two-state model with linear sloping baselines (*36*). Each value of T_m and free energy was the average of at least three experiments. Standard deviations did not exceed ±0.5° for T_m values and ±4% for $\Delta G°_{37}$ values in repeated independent experiments. Error bars smaller than the symbol were omitted from the plots.

Circular Dichroism Spectra. CD spectra were recorded in melting buffer using a JASCO J-600 spectropolarimeter at 20 °C in a 1 cm path length cell. The duplexes for CD experiments were prepared in the same manner as that for melting experiments. The ellipticities of duplexes were converted to $\Delta\epsilon$ ($L \cdot mol^{-1} \cdot cm^{-1}$) and are reported per mole of residue.

RESULTS

Six sequences (1−6) of different length with different GC and pyrimidine content and consequently with different relative stability (relative to RR counterparts) (*28*) were chosen for this study (Table 1). Hybrids of the special sequence 6 contained $(dU)_{10}$ or $(dA)_{10}$ strings comprising 62% of the hybrid length. All sequences and their complements were synthesized as DNA, RNA, and uniformly 2′-methoxy modified DNA (D′). For correct comparison with RNA duplexes, we used dU and 2′-methoxy-dU instead of T and 2′-methoxy-T in all DNA sequences. Measured T_m and $\Delta G°_{37}$ values for RNA:RNA (RR), modified hybrids (D′R and RD′), and their unmodified counterparts (DR and RD) are listed in Table 1. The increase in T_m values correlated well with the increase in $\Delta G°_{37}$ values for all duplexes studied (Figure 1), providing the basis for the consideration of duplex stability using both $\Delta G°_{37}$ and T_m data. $\Delta G°_{37}$ values derived from fitting melting curves are sensitive to the method of analysis (*36*−*38*) and can vary by 1−2 kcal/mol, increasing error estimates. In contrast, T_m values are insensitive to the method of analysis and do not depend on the two-state nature of the transition (*36*−*38*). As a result, T_m data are more reproducible and more easily lend themselves to quantitative evaluation.

The first two columns of Table 2 report ΔT_m and $\Delta\Delta G°_{37}$ for the modified hybrids compared to their unmodified counterparts. For sequences 1−5 (Table 2) values of ΔT_m(D′R-DR) varied from +1.17°/mod for D′R of sequence 5 up to +2.71°/mod for RD′ of sequence 2. Corresponding $\Delta\Delta G°_{37}$(D′R-DR) values varied from −0.51 up to −0.88 kcal/mol modifications. Similar variations were observed in the relative stability of unmodified parent hybrids DR versus RR duplexes (Table 2, columns 3, 4). Comparison of the results indicated that a linear correlation does exist

Table 2: Conformational and "Hydrophobic" Contributions in the Total Stabilization Effect of 2′-Methoxy Substitutions in DNA Strands of Hybrid Duplexes

	ΔT_m (°C)/$-\Delta\Delta G°_{37}$ (kcal/mol)[a]					
	total 2′Ome effect[b] (per mod)		conformational effect (per bp)		"hydrophobic" effect (per mod)	
duplex	(D′R-DR)	(RD′-RD)	(RR-DR)	(RR-RD)	(D′R-RR)	(RD′-RR)
5′-CUCGUACCUUCCGGUCC (1)	1.23	1.49	0.74	1.10	0.44	0.33
3′-GAGCAUGGAAGGCCAGG	0.70	0.74	0.41	0.60	0.27	0.11
5′-GCUCUCUGGC (2)	1.70	2.71	1.07	2.16	0.51	0.31
3′-CGAGAGACCG	0.59	0.88	0.38	0.72	0.17	0.08
5′-GAGCUCCCAGGC (3)	1.58	2.09	1.16	1.43	0.32	0.54
3′-CUCGAGGGUCCG	0.82	0.85	0.59	0.67	0.17	0.13
5′-UCCAGGUGUCCGCAUC (4)	1.40	1.29	0.94	0.89	0.40	0.34
3′-AGGUCCACAGGCGUAG	0.78	0.52	0.50	0.39	0.24	0.11
5′-GUACUUGCAUAGUCG (5)	1.17	1.31	0.74	0.94	0.38	0.30
3′-CAUGAACGUAUCAGC	0.55	0.63	0.39	0.52	0.13	0.08
5′-GCGUUUUUUUUUUGCG (6)	0.92	1.49	0.31	1.40	0.59	0.00
3′-CGCAAAAAAAAAACGC	0.43	0.53	0.15	0.48	0.27	0.01

[a] For each duplex, the first line denotes ΔT_m (°C); the second line denotes $-\Delta\Delta G°_{37}$ (kcal/mol). [b] Note that the total effect of 2′-methoxy modification for each hybrid expressed as ΔT_m/mod or $\Delta\Delta G°_{37}$/mod is not exactly the sum of the conformational (calculated per bp) and "hydrophobic" (calculated per mod) contributions because each sequence contained (N − 1) modified residues. The total effect is the sum of both contributions calculated per duplex. For example, the total effect for the 17-mer D′R of sequence 1 with sixteen 2′-methoxy substitutions expressed in ΔT_m values is (1.23 × 16) = (0.74 × 17) + (0.44 × 16), where the first term is the conformational part and the second one is the "hydrophobic" contribution calculated duplex.

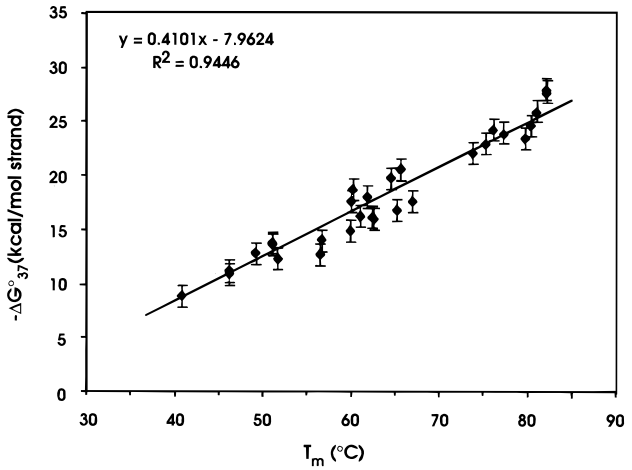

FIGURE 1: Correlation between the temperatures of melting (T_m) and the free energy of duplex formation ($-\Delta G°_{37}$) derived from fits of absorbance versus temperature curves to a two-state model with sloping baselines (27) in a buffer containing 100 mM Na^+, 10 mM Na phosphate (pH 7.1), and 0.1 mM EDTA.

between these parameters: the larger the difference between RR and unmodified hybrid stabilities, the greater the effect of the 2′-methoxy modifications in the modified D′R analogues (Figure 2a,b). As we expected, significantly lower deviations from the line were observed for T_m data compared to free-energy data.

DISCUSSION

Conformational Part of 2′-Methoxy Stabilization Effect. It is now generally accepted that purine and pyrimidine DNA strands in hybrid duplexes adopt different averaged conformations (*29*, *33*) with different widths of the major and minor grooves (*30*). The model based on NMR experiments (*23*−*27*, *29*) establishes an S → N conformational equilibrium for the DNA sugar moieties, suggesting a different fraction of S sugar conformation (f_S) in purine- and pyrimidine-rich DNA strands in hybrids (*29*, *30*). A smooth correlation between the conformational characteristics and the dPy content in the DNA strand suggests the existence of a

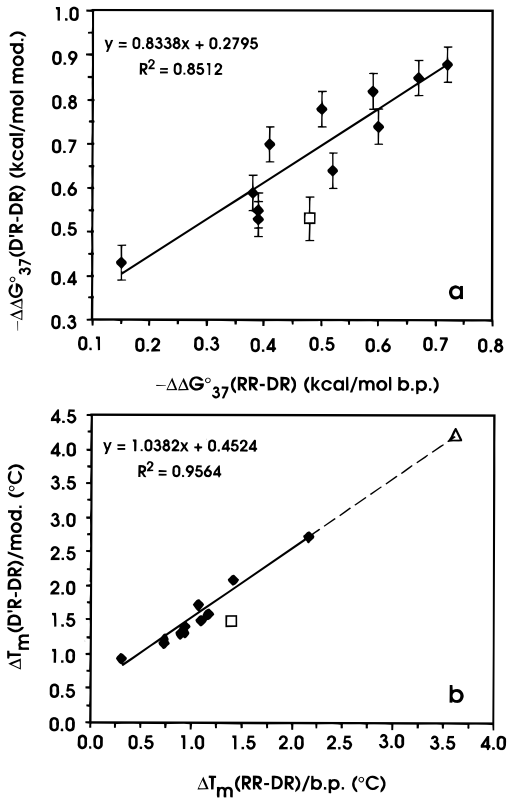

FIGURE 2: Correlation between the total stabilization effect of 2′-methoxy modifications and the relative stability of unmodified hybrids (compared to the stability of their RR counterparts) expressed as (a) a difference in free energy of duplex formation; and (b) a difference in duplex melting temperatures. Linear fits were calculated using data for the sequence 1−5 (solid symbols) ((□) effect of 2′-modifications in RD, sequence 6; (△) predicted stabilization effect for 2′-methoxy modifications in homopurine DNA strand, see text).

continuum of intermediate conformers for hybrids with different base compositions (*28*). The conformational diversity results in a significant variation in hybrid stability compared to RR counterparts ($\Delta\Delta G°_{37}$(RR-DR/RD) (Figure 3), depending on dPy content in the hybrid DNA strand as

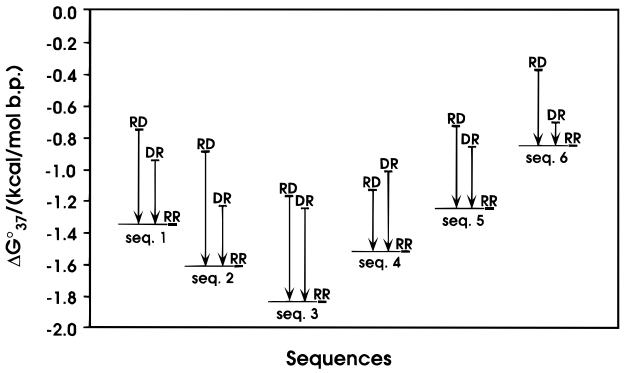

FIGURE 3: The difference in stability of unmodified DR and RD hybrids and corresponded RR duplexes.

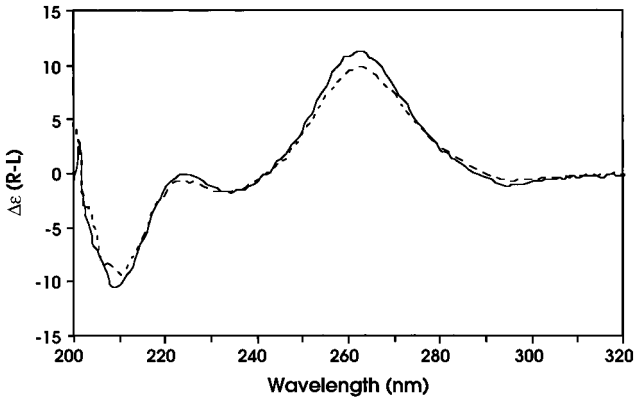

FIGURE 4: CD spectra of 2′-methoxy modified D′R (—) and RR (- - -) duplexes of sequence 4 (Table 1) at 20°.

well as GC base pair content (*28*). The data in Table 2 (columns 3, 4) demonstrate that DR hybrids with high dPy content in the DNA strand were more stable compared to their RR counterpart than corresponding RD hybrids with high dPu content. On the other hand, hybrids with similar dPy but different GC bp content revealed different relative stability compared to their RR counterparts (sequences 3 and 5) because the increase in GC bp content affects RR and hybrid stability differently (*28*).

Incorporation of 2′-methoxy modifications in DNA strands results in a shift of the sugar moiety conformation to predominantly N-type (*18*, *19*, *39*). CD spectra of hybrid duplexes with uniformly 2′-methoxy modified DNA strands of different pyrimidine content (24−78%) reveal typical A-form features suggesting that 2′-methoxy substitutions shift the S → N equilibrium in fully modified DNAs to a similar fraction of N-type independent of their initial conformational states (*9*). Therefore, the conformational part of the 2′-methoxy stabilization effect due to the S → N equilibrium shift of the DNA strand conformation is assumed to be equal to the difference between the stability of unmodified hybrids and their RR counterparts ($\Delta\Delta G°_{37}$(RR-DR/RD)/mol of bp or ΔT_m(RR-DR/RD)/bp) and will be different for hybrids with different base composition (Figure 3).

"Hydrophobic" Part of the Stabilization Effect and the Origin of "Hydrophobic" Effect of 2′-Methoxy Substitutions. The stabilization effect of 2′-methoxy modifications is not limited to the S → N conformational shift. The data listed in Table 1 and other data reported (*9*, *40-43*) demonstrate that the stability of 2′-methoxy modified hybrids exceeds the stability of their corresponding RR duplexes. The origin of the excess in stability of 2′-methoxy modified hybrids compared to the corresponding RR duplexes (Table 2, columns 5, 6) is not clearly understood.

NMR data indicate that 2′-*O*-methylation of pyrimidine residues in UpU and CpC dinucleosides stabilizes the N sugar conformation due to an intraresidue steric repulsion between 2-carbonyl and 2′-methoxy groups (*20*, *21*) and that 2′-methylation of guanylyl residues in GpG enhances base stacking interactions (*22*). Increased intensity of the positive band at 260 nm and the negative band at 210 nm in CD spectra of modified oligonucleotide duplexes (Figure 4) has been suggested to reflect increased base stacking and an increased fraction of N conformers in 2′-methoxy modified hybrids compared to RR counterparts (*17*, *44*, *45*). The interaction between consecutive 2′-methoxy groups on the surface of a minor groove has also been suggested as a source of additional stabilization (*19*, *43*). To elucidate what kind of interactions are the major source of the extra stability in 2′-methoxy modified oligonucleotide duplexes, we designed two RNA analogues of sequence 6 with alternating 2′-methoxy and 2′-hydroxy residues: rG**C**G**U**U**U**U**U**U**U**-**U**U**G**CG (6′) with seven 2′-methoxy residues and rGCGU-**U**U**U**U**U**U**U**GCG (6″) with five 2′-methoxy-dU in the middle of the sequence (bold face denotes 2′-methoxy residues). Comparison of the stabilization effect of alternating 2′-methoxy substitutions inserted in RNA analogues of sequence 6′ (Table 3) with the "hydrophobic" part of the total stabilization effect for the D′R hybrid of sequence 6 (Table 2, column 5) indicates that the hydrophobic effect for both consecutive and alternate substitutions was similar. In fact, for sequence 6″, the effect of alternating 2′-*O*-methyl-U substitutions was even greater than that observed for the uniform modification in sequence 6. Therefore interactions between adjacent 2′-methoxy groups play a minor role in the extra stabilization effect of 2′-methoxy substitutions.

Additional support for the hypothesis of an intraresidue origin of the "hydrophobic" effect was derived from the analysis of the data for the modified RD′ hybrid of sequence 6 with a $(dA)_{10}$ string and for other hybrids with different fractions of randomly located dA residues. In contrast to pyrimidine and guanine dimers, the 2′-*O*-methylation in ApA dimer has been shown to reduce base stacking and does not stabilize the N sugar conformation, suggesting that no additional stabilization occurs for 2′-*O*-methyl-A residues (*46*). Our observation that the RD′ hybrid of sequence 6 showed no hydrophobic contribution to the stability of the modified duplex (Table 2, column 6) supports this hypothesis. Moreover, the magnitude of the hydrophobic effect for hybrids decreased linearly with the increase of the dA fraction in the modified DNA strand (Figure 5).

Total Stabilization Effect of 2′-Methoxy Substitutions. The total effect of 2′-methoxy substitutions can be represented as a sum of two components where the first, conformational part depends on the relative stability of the unmodified parent hybrid and the second, "hydrophobic" part is due mainly to intraresidue interactions of 2′-methoxy groups:

$$\underset{\text{total effect}}{\Delta\Delta G°_{37}(\text{D′R-DR})} = \underset{\text{conformational effect}}{\Delta\Delta G°_{37}(\text{RR-DR})} + \underset{\text{"hydrophobic" effect}}{\Delta\Delta G°_{37}(\text{D′R-RR})} \quad (1)$$

Table 3: "Hydrophobic" Effect of Alternating 2′-*O*-Methyl Substitutions in RNA Analogs of Sequence 6

duplex[a]	T_m (°C)	$-\Delta G°_{37}$ (kcal/mol)	ΔT_m/mod	$-\Delta\Delta G°_{37}$/mod
5′-rGCGUUUUUUUUUGCG (6) 3′-rCGCAAAAAAAAAACGC	51.1[b]	13.6[b]		
5′-rG**C**G**U**U**U**U**U**U**U**U**G**CG (6′) 3′-rCGCAAAAAAAAAACGC	55.1	16.0	0.58	0.36
5′-rGCGU**U**U**U**U**U**U**U**GCG (6″) 3′-rCGCAAAAAAAAAACGC	54.5	15.8	0.69	0.32

[a] Bold letters indicate 2′-*O*-methyl ribonucleoside residues. [b] Data from Table 2.

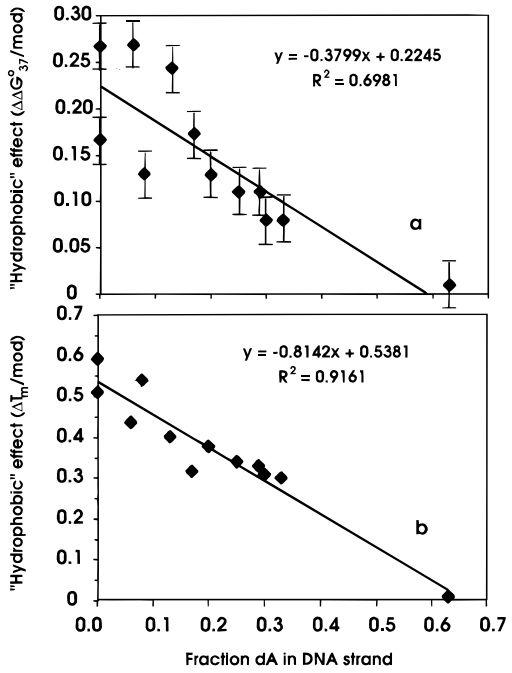

FIGURE 5: Effect of the dA content in a 2′-methoxy modified DNA strand on the "hydrophobic" part of the total stabilization effect in modified D′R and RD′ duplexes calculated from experimental data (Table 2, columns 5 and 6) and expressed as (a) a difference in free energy of duplex formation; and (b) a difference in duplex melting temperatures.

A similar equation can be written for T_m values:

$$\Delta T_m(\text{D′R-DR}) = \Delta T_m(\text{RR-DR}) + \Delta T_m(\text{D′R-RR}) \quad (1′)$$

For most sequences with mixed purine-pyrimidine base composition and moderate dA content, the conformational effect was 64−80% of the total effect, that is, it was 2−4 times higher than the "hydrophobic" stabilization (Figure 6). However, for some sequences, the relative contribution of each component was outside of this range. For example, the relative contribution of the conformational effect was 37% for the D′R hybrid of sequence 6 and almost 90% and 100% for RD′ hybrids of sequences 2 and 6, respectively (Figure 6).

Prediction of 2′-Methoxy Stabilization Effect for Sequences. In this study we report that the initial conformational and thermodynamic states of the unmodified parent DNA:RNA duplex govern the stabilization effect of 2′-methoxy (and maybe other 2′-alkoxy) modifications whose mechanism of action include an S → N conformational shift resulting in A-form duplex formation. The total stabilization effect correlated linearly with the difference in the thermodynamic stability of the parent hybrids and their RNA:RNA counterparts (Figure 2). To test our hypothesis, we calculated the difference in stability of one of the least stable homopurine-homopyrimidine hybrids 5′-dAAGGGAAGG·3′-rUUCCCUUCC (T_m = 20.5°) and its RR counterpart (T_m = 52.9°) from published data (*28*) and found ΔT_m(RR-DR) = +3.60°/bp. Using the equation from Figure 2b ($y = 1.0382x + 0.4524$), we predicted an unusually high stabilization effect for 2′-methoxy modifications in the homopurine DNA strand: +4.19°/mod. We synthesized the uniformly modified homopurine DNA strand and melted the hybrid (T_m = 54.3°). The total effect of the 2′-methoxy modifications in this homopurine sequence was +4.22°/mod, which was in very good agreement with the predicted value (Figure 2b). The hydrophobic part of the total effect calculated as ΔT_m(D′R-RR)/mod was 0.18°/mod, which was exactly the same as that calculated from the equation in Figure 5 for a DNA strand with 44% dA.

Equations 1 and 1′ suggest that the stability of modified hybrid duplexes can be predicted if the stabilities of the corresponded RR duplexes and extra "hydrophobic" components are known. Nearest-neighbor parameters can effectively calculate RR and hybrid duplex stability in 1 M NaCl (*34*, *47*). To convert these predictions in 0.1 Na^+, we plotted measured $\Delta G°_{37}$ and T_m in 0.1 Na^+ versus predicted $\Delta G°_{37}$ and T_m in 1 Na^+ for fifteen RR duplexes used in this study (Table 1) and reported previously (*28*) (data not shown). Linear relationships were obtained for RNA:RNA (RR) duplexes:[2]

$$\Delta G°_{37}(\text{RR, 0.1 M Na}^+) = 0.5433\,(\Delta G°_{37}(\text{RR, 1 M Na}^+)) + 4.9575 \quad (R^2 = 0.81) \quad (2)$$

$$T_m(\text{RR, 0.1 M Na}^+) = 0.9043(T_m(\text{RR, 1 M Na}^+)) - 3.1138 \quad (R^2 = 0.96) \quad (2′)$$

According to equations 1 and 1′, $\Delta G°_{37}$ and T_m for the 2′-methoxy modified hybrid are the sum of $\Delta G°_{37}$ or T_m values for the RR counterpart and the "hydrophobic" contribution:

$$\Delta G°_{37}(\text{D′R}) = \Delta G°_{37}(\text{RR}) + \Delta\Delta G°_{37}(\text{"hydrophobic"}) \quad (3)$$

$$T_m(\text{D′R}) = T_m(\text{RR}) + \Delta T_m(\text{"hydrophobic"}) \quad (3′)$$

where $\Delta\Delta G°_{37}$("hydrophobic") and ΔT_m("hydrophobic") are calculated using the linear relationships in Figure 5:

[2] Analogous equations were obtained for unmodified hybrids: $\Delta G°_{37}$ (0.1 M Na^+) = 0.6724($\Delta G°_{37}$ (1 M Na^+)) + 0.9792 (where R^2 = 0.92) and T_m (0.1 M Na^+) = 0.9142(T_m (1 M Na^+)) − 6.28852 (where R^2 = 0.94).

Table 4: Comparison of Predicted and Experimental Melting Temperatures (T_m, °C) for 2′-Methoxy Modified Hybrid Duplexes

duplex[a]	$T_m(RR)_{calc}$[b] (1 M Na^+)	$T_m(RR)_{pred}$[c] (0.1 M Na^+)	ΔT_m(hydroph)[d] (0.1 M Na^+)	$T_m(D'R/RD')_{pred}$ (0.1 M Na^+)	$\Delta T_m(RR_{exp} - RR_{pred})$[e] (0.1 M Na^+)	$\Delta T_m(D'R_{exp} - D'R_{pred})$[f] (0.1 M Na^+)
DR (1)	87.1	75.7	7.8	83.5	−0.5	−1.3
RD			4.8	80.5		−0.1
DR (2)	70.6	60.7	4.8	65.2	1.7	1.4
RD			2.6	63.4		1.8
DR (3)	82.0	71.0	4.4	75.4	2.8	1.9
RD			5.2	76.2		3.5
DR (4)	85.6	74.3	6.5	80.8	1.8	1.3
RD			5.0	79.3		1.8
DR (5)	72.9	62.8	5.3	68.1	−2.5	−2.5
RD			3.8	66.6		−2.1
DR (6)	68.3	58.6	8.1	66.7	−7.5	−6.7
RD			0.4	59.0		−7.9

[a] The number of the duplex corresponds to the number of the duplex in Table 1. [b] T_m values calculated for RR duplexes in 1 M Na^+ using nearest-neighbor parameters (*34*). [c] T_m values predicted for RR duplexes in 0.1 Na^+ using eq 2′. [d] ΔT_m(hydroph) denotes the "hydrophobic" part of the total stabilization effect in 0.1 M Na^+ calculated using eq 4′. [e] Deviation between T_m predicted and T_m experimental (Table 1, column 5) for RR duplexes in 0.1 M Na^+. [f] Deviation between T_m predicted and T_m experimental (Table 1, columns 7, 9) for D′R and RD′ duplexes in 0.1 M Na^+.

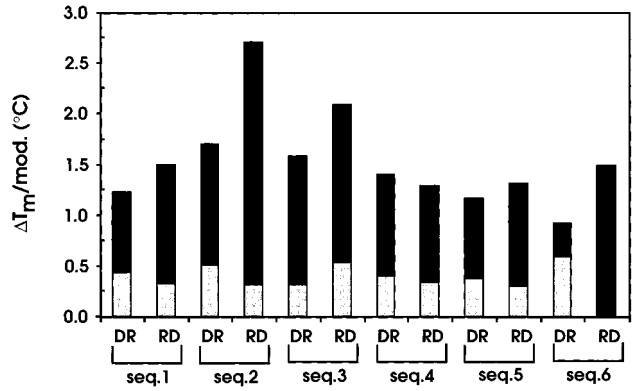

FIGURE 6: Relative contribution of conformational (RR-DR) (■) and "hydrophobic" (D′R-RR) (□) parts of the total stabilization effect of 2′-methoxy modifications in uniformly modified DNA strands of hybrid duplexes.

$$\Delta\Delta G^\circ_{37}(\text{"hydrophobic"}) = (-0.3799(f_{dA}) + 0.2245)N \qquad (R^2 = 0.70) \quad (4)$$

$$\Delta T_m(\text{"hydrophobic"}) = (-0.8142(f_{dA}) + 0.5381)N \qquad (R^2 = 0.92) \quad (4')$$

and f_{dA} is the fraction of dA in the unmodified DNA strand and N is the number of modifications. Thus ΔG°_{37} and T_m values for 2′-methoxy modified hybrid duplexes can be predicted using nearest-neighbor parameters (*34*) and equations 2−4, though it is obvious that the predictions for T_m can be done more accurately than those for ΔG°_{37}. This procedure was used to calculate ΔG°_{37} and T_m for modified hybrids of sequences 1−6. For sequences 1−5, the average deviation between predicted and experimental T_m values was 1.8 °C for both RR and 2′-methoxy modified hybrids (Table 4). The corresponding deviation for ΔG°_{37} was 2.3 kcal/mol of duplex (data not shown). For sequence 6, predicted T_m values for RR and DR′/RD′ hybrids exceeded the experimental T_m values by nearly 7 °C (Table 4), suggesting that conformational peculiarities of duplexes with $(rA)_n \cdot (rU)_n$ strings result in higher sensitivity to salt concentration than for other sequences.

SUMMARY

The total stabilization effect of 2′-methoxy substitutions is a result of two contributions. The first is a conformational effect resulting from the S → N conformational shift in the modified DNA strand of the hybrid. The magnitude of this contribution depended on the initial fraction of S conformation (f_S) in the unmodified DNA strand of hybrids. For relatively unstable hybrids with high dPu content and "high" f_S, 2′-methoxy substitution resulted in a large conformational effect. Conversely, for relatively stable hybrids with high dPy content and "low" f_S, the conformational effect of 2′-methoxy substitutions was small. The second contribution ("hydrophobic" effect) is an effect of 2′-methoxy substitutions above and beyond the shift to RNA-like geometry. This effect was due mainly to intraresidue stabilization in all residues except dA and did not require 2′-methoxy modification at adjacent residues. Quantitative analysis of these contributions allowed the prediction of stabilities for any 2′-methoxy substituted hybrid duplexes except the duplexes with long $(dA)_n$ and $(dU/T)_n$ strings.

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