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Inclusion of methoxy groups inverts the thermodynamic stabilities of DNA–RNA hybrid duplexes: A molecular dynamics simulation study

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ABSTRACT

Modified nucleic acids have found profound applications in nucleic acid based technologies such as antisense and antiviral therapies. Previous studies on chemically modified nucleic acids have suggested that modifications incorporated in furanose sugar especially at 2′-position attribute special properties to nucleic acids when compared to other modifications. 2′-*O*-methyl modification to deoxyribose sugars of DNA–RNA hybrids is one such modification that increases nucleic acid stability and has become an attractive class of compounds for potential antisense applications. It has been reported that modification of DNA strands with 2′-*O*-methyl group reverses the thermodynamic stability of DNA–RNA hybrid duplexes. Molecular dynamics simulations have been performed on two hybrid duplexes (DR and RD) which differ from each other and 2′-*O*-methyl modified counterparts to investigate the effect of 2′-*O*-methyl modification on their duplex stability. The results obtained suggest that the modification drives the conformations of both the hybrid duplexes towards A-RNA like conformation. The modified hybrid duplexes exhibit significantly contrasting dynamics and hydration patterns compared to respective parent duplexes. In line with the experimental results, the relative binding free energies suggest that the introduced modifications stabilize the less stable DR hybrid, but destabilize the more stable RD duplex. Binding free energy calculations suggest that the increased hydrophobicity is primarily responsible for the reversal of thermodynamic stability of hybrid duplexes. Free energy component analysis further provides insights into the stability of modified duplexes.

1. Introduction

Chemically modified nucleic acids (AOs) are attractive compounds in biomedical treatments involving nucleic acids because of their special features compared to pure DNA and RNA nucleic acids [1]. Regulation of gene expression by introducing chemically modified nucleic acids offer several advantages over traditional methods [2,3]. The transfer of genetic information from DNA to protein can be blocked at several stages, including mRNA, ribosome and double stranded RNA (RNA interference) using AOs. In general, two mechanisms are accepted for the action of AOs in regulating gene expression: (a) binding of AOs to mRNA sequence to form a stable duplex, and (b) activation of ribonuclease H (RNase H) activity that involves cleavage of mRNA molecules [4,5]. RNase H is an endonuclease enzyme that specifically identifies and cleaves the RNA strand of the DNA–RNA hybrid duplex without affecting the DNA strand. This recognition is non-sequence specific and can be exploited for biomedical purposes known as antisense and antiviral therapies [6–8]. This approach has been shown to be a potential way to block the transfer of genetic information. Since the first generation antiviral drugs are already in market, several studies have been carried out on the AOs [9]. A number of chemical modifications have been studied in great detail [10–21]. Despite several available studies, search for suitable chemical modifications that can form stable duplexes with the mRNAs is an essential exercise.

Chemical modifications at 2′-position of the furanose sugar have been studied extensively [1,10,14–16]. Modifications at this position offer several advantages over other modifications. Their synthetic preparation is relatively easy compared to others and preorganizes the sugar conformation into C3′-endo similar to A-RNA duplex. These substitutions also modulate the duplex stability, affinity towards mRNA, nuclease resistance, uptake, hydrophobic interactions and hydration [1]. 2′-Methoxy modified nucleic acid is one such promising oligonucleotide that exhibits high affinities toward RNA targets [22]. It has been shown that the 2′-*O*-methyl modified nucleic acids are resistant to RNase H activity [23] and this modification shows a significant improvement in both nuclease activity and high affinity towards RNA target [24]. Previous

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studies have shown that the effect of 2′-*O*-alkyl and 2′-F modifications depends on the base composition present in duplexes [24,25]. DNA–RNA hybrids are hybridized molecules between pure DNA and pure RNA duplexes and are formed as key intermediates in many important biological processes like DNA replication, reverse transcription, etc. [26,27]. These are substrates for RNase H enzyme and are usually less stable than RNA duplexes [6–8]. They exhibit a conformation intermediate to A-form and B-form [8]. Previous studies have suggested that the hybrid properties depend on the deoxypyrimidine and GC content present in hybrids [28]. These molecules have extensively been studied by different experimental methods and observed that the hybrids with high deoxypyrimidine content are thermodynamically more stable than other hybrids and highly resistant to RNase H activity [28].

Duplex stability due to 2′-*O*-methyl modifications arise because of their ability to shift the conformational equilibrium of deoxyribose sugars toward C3′-endo (N-type) that results in A-RNA like conformation for the oligonucleotide and increases stacking interactions among nucleobases [1,24,25]. The resulting RNA-like conformation of oligonucleotides due to 2′-*O*-methyl modifications was confirmed by CD spectroscopy [24,29], NMR spectroscopy [30], and X-ray studies [31]. Modelling studies have also supported the A-like conformation to 2′-*O*-methyl modified nucleic acids in line with the experimental studies [32–34]. In addition to A-like conformation, the 2′-*O*-methyl modified duplexes have under-winding helical structure suggested by recent studies [34]. Previous studies have shown that the 2′-*O*-methyl modified DNA–RNA hybrids display higher stability than their RNA counterparts that may arise due to the steric interaction between the 2-carbonyl groups of pyrimidines and the 2′-*O*-methyl substitutions, and enhanced base stacking [35,36]. These putative hydrophobic interactions arise in modified DNA because of the consecutive 2′-*O*-methyl groups positioned on the surface of the minor groove [30]. Previous studies have suggested that inclusion of 2′-*O*-methyl modifications to the deoxyribose sugars of DNA–RNA hybrids reverses their thermodynamic stability [37]. This effect also depends on the AT/GC content present in the duplex and the modified duplexes display higher stability than RNA duplex. It has been suggested that there are two effects, conformational and hydrophobic, responsible for the total stabilization effect of the modified duplexes. The structural changes induced by 2′-substituent and the reasons for differential effects on the thermodynamic stability of hybrid duplexes with different base composition are yet to be explored. Molecular dynamics simulations can be efficiently used to understand the dynamic nature of biological macromolecules [38–43]. The aim of the present study is to understand the impact of 2′-*O*-methyl modification on two different hybrid duplexes and the factors responsible for the reversal in thermodynamic stability of hybrid duplexes upon modification.

2. Methods

2.1. Molecular dynamics simulation protocol

To investigate the impact of 2′-*O*-methyl modification on the stability of DR and RD hybrids, molecular dynamics (MD) simulations have been performed on two DNA–RNA hybrids by modifying their DNA strands with 2′-*O*-methyl groups and respective pure hybrid duplexes (Fig. 1). An additional simulation corresponding to A-RNA duplex was also run to compare the properties of modified hybrids with A-like conformation. The two hybrid duplexes are represented as DR and RD, and their respective modified duplexes are as mDR and mRD, and pure RNA duplex is denoted as RR. The starting structures for these hybrids (DR and RD) and RNA duplexes were taken from the available experimental data (pdb ids: 1DRR and 1RRD for hybrids, 1RRR for pure RNA) [44] and the selected sequences have equal AT/GC content. The modified duplexes mDR and mRD have been generated using the respective pure hybrid duplexes. All the simulations were performed using the NAMD simulation program [45] with the CHARMM36 all atom nucleic acid force field [46–48]. After collecting the coordinates for pure duplexes, the subsequent modifications for 2′-*O*-methyl groups on DNA strand were done using the CHARMM biomolecular simulation program [49]. The parameters for 2′-*O*-methyl modifications have been used from previous work of Macchion et al. [50]. Missing hydrogens were added using the HBUILD module. These systems were minimized for 500 steps using the steepest descent (SD) method by harmonically restraining the heavy atoms of the duplexes applying a force constant of 10 kcal mol^{-1} Å^{-2}. These minimized structures were then immersed in a water box with edge lengths 54 Å × 40 Å × 40 Å and TIP3P water model [51] was used. The box edge lengths were chosen in such a way that water molecules occupy 8 Å in all the dimensions beyond duplexes and the interacting water molecules within the region of 2.2 Å around the duplex were removed. The total charge of the systems was made to zero by placing sodium ions at random positions around the nucleic acids. The solvent and ions were then minimized for 500 steps each of SD and adopted basis Newton Rapson (ABNR) methods by applying harmonic restraints on heavy atoms of nucleic acid duplexes. Without removing the restraints, a 100 ps equilibration in NVT ensemble was performed. The covalent bonds involving hydrogens were constrained by applying SHAKE algorithm in all the calculations [52]. Long range interactions were treated by using particle mesh Ewald (PME) summation [53,54]. Periodic boundary conditions were applied by using the CRYSTAL module in CHARMM [55]. The simulations were done at 283 K temperature at which NMR experiments on hybrids have been performed. Temperature was controlled by Nose–Hover thermostat [56] and the Langevin piston algorithm [57] was used to maintain pressure of the systems. All the simulations were performed in the isothermal–isobaric (NPT) ensemble. Lennard–Jones (LJ) potential was truncated at 12 Å by applying a smoothing function from 10 Å to 12 Å [58]. The production simulations were extended up to 50 ns and an integration time step of 2 fs was employed. Leapfrog integrator was employed to integrate the Newton's equation of motion and the coordinates were saved for every 5 ps for further analysis. During the MD simulations, a weak harmonic constraint with a force constant 4.0 kcal mol^{-1} Å^{-2} was applied on the terminal hydrogen bonds in order to avoid the base pair opening [33,40–41]. VMD [59] and Curves+ [60] programs were used for analysis.

2.2. Free energy calculations

In experiments, binding free energies and melting temperatures have been used as characteristic features to understand the stability of nucleic acids [28,37,44]. The changes in nucleic acid helical structure and properties after modifications are explained based on the variations observed in the binding free energies or melting temperatures. Computational methods can alternatively be used to calculate the binding free energies. In the present study, molecular mechanics combined with generalised Born and surface area (MM-GBSA) method was used and in this method, the absolute free energy of duplex is equal to the sum of the molecular mechanical energy, the solvation energy, and the vibrational, rotational, and translational entropies [38–41].

$$G = E_{MM} + G_{Solv} - TS_{MM}$$

where G represents absolute free energy, E_{MM} represents the total molecular mechanical energy, G_{Solv} gives contribution from solvent, T is absolute temperature and S_{MM} represents the conformational entropy.

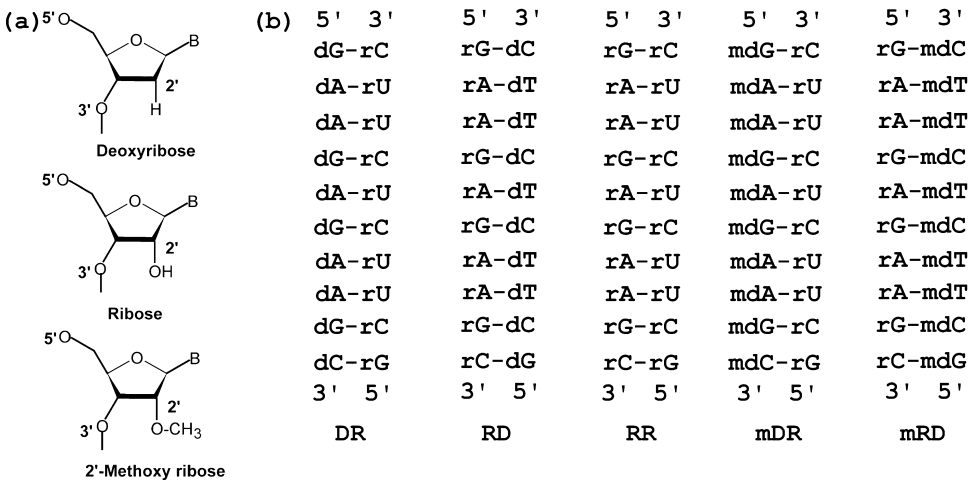

Fig. 1. (a) Representation of furanose sugars present in DNA, RNA and 2′-methoxy modified duplexes; (b) two hybrids, DR (PDB:1DRR) and RD (PDB:1RRD), one RNA, RR (PDB:1RRR) and modified hybrid duplexes, mDR and mRD considered in the present study. "md" in the sequences denote the 2′-OMe modified nucleotides.

The solvation free energy was estimated as the sum of the electrostatic solvation energy (G_{GB}) and the non-polar solvation energy ($G_{non\text{-}polar}$). $G_{Solv} = G_{GB} + G_{non\text{-}polar}$; where the electrostatic contribution to the solvation free energy was estimated by using the generalised Born molecular volume (GBMV) method. The nonpolar contribution towards solvation free energy was estimated as $G_{non\text{-}polar} = \gamma \times SASA$ where $\gamma = 0.0072$ kcal Å^{-2}, and SASA is the solvent accessible surface area. The solvent accessible surface area SASA was calculated by using a probe particle of radius 1.4 Å.

The binding free energy associated in the formation of double helix can be calculated using the following relation:

$$\Delta G_{Bind} = G_{duplex} - G_{strand1} - G_{strand2}$$

here the duplex represents the double helix and strands represent individual strands of duplex.

The change in absolute free energy after modification can be calculated as $\Delta G_{Hydrophobic} = \Delta G_{mDR} - \Delta G_{DR}$ and the total stabilization effect is the sum of two terms [37].

$$\Delta G_{Total\ effect} = \Delta G_{Conformational} + \Delta G_{Hydrophobic}$$

The conformational free energy associated with the hybrid to RNA duplex can be calculated as $\Delta G_{Conformational} = \Delta G_{RR} - \Delta G_{DR}$ and the hydrophobic free energy can be calculated during the modification as $\Delta G_{Hydrophobic} = \Delta G_{mDR} - \Delta G_{DR}$.

2.3. Analysis of the trajectories

Several structural and energetic analyses have been performed on these hybrids to characterize the changes due to the modifications at 2′-position in deoxyribose sugars. All the translational and rotational motions were removed by aligning the duplex on the initial structure and final 45 ns simulation trajectories were used for the analyses. The root mean square deviations of nucleic acid along the trajectory were calculated with respect to the initial structure. The atomic fluctuations corresponding to all the heavy atoms have been calculated by aligning the atoms over starting structure. The conformational entropies corresponding to duplex and individual strands were estimated at temperature 283 K via harmonic approximation [39]. The snapshots corresponding to duplexes and individual strands were extracted from the solvent box by considering the final 45 ns trajectories (9000 snapshots). The base pair interaction energies corresponding to Watson–Crick base pairs were calculated. The stacking interaction energies were calculated by following a protocol described in an earlier study [42].

Table 1
Average values of root mean square deviations (Å) of entire duplex, backbone and nucleobases corresponding to all the duplexes considered in the present study.

Duplex	Full	Backbone	Bases
DR	1.62 ± 0.02	1.87 ± 0.03	1.12 ± 0.02
mDR	1.71 ± 0.03	2.03 ± 0.03	1.04 ± 0.03
RD	1.28 ± 0.02	1.52 ± 0.03	0.86 ± 0.01
mRD	1.52 ± 0.03	1.83 ± 0.04	0.93 ± 0.02
RR	1.43 ± 0.02	1.72 ± 0.03	0.86 ± 0.01

3. Results and discussion

3.1. Structural deviations and fluctuations

To understand the structural changes in all the duplexes during MD simulations, root mean square deviations (RMSD) with respect to their initial structure were calculated. As shown in Fig. 2, time evolution of RMSD indicates that the structural changes in duplexes are small (RMSD: 1–2 Å range) and they are well equilibrated during the simulations. It was observed that the DR hybrid and its modified duplex mDR show high deviation values in comparison to other duplexes. The average values of RMSD corresponding to different regions of duplexes were calculated in order to investigate their contributions. The common atoms present in sugar and phosphate regions of all the duplexes were considered as backbone. These values indicate that the backbones of modified duplexes exhibit higher deviations than pure duplexes because of the presence of methyl group (Table 1). The DR hybrid exhibit higher deviations for all the regions, and the pure RNA has the smallest deviation values among all duplexes.

The flexibility of hybrid duplexes after chemical modification was assessed via calculation of root mean square fluctuations (RMSF). The fluctuations corresponding to entire duplex, backbone and bases of individual strands have been calculated and are shown in Table 2. The modified duplexes exhibit considerably small fluctuations compared to their respective pure nucleic acids (maximum difference is about 0.33 Å). The average values indicate that the introduced modifications decrease the atomic fluctuations of the backbone of DNA strands while the fluctuations of its complementary RNA strand have slightly increased. On the other hand, the modifications increase the fluctuations of nucleobases of the DNA and decrease the fluctuations of nucleobases of their complementary RNA strands. The variations in RMSD and RMSF together indicate that the induced structural changes in nucleic acids after

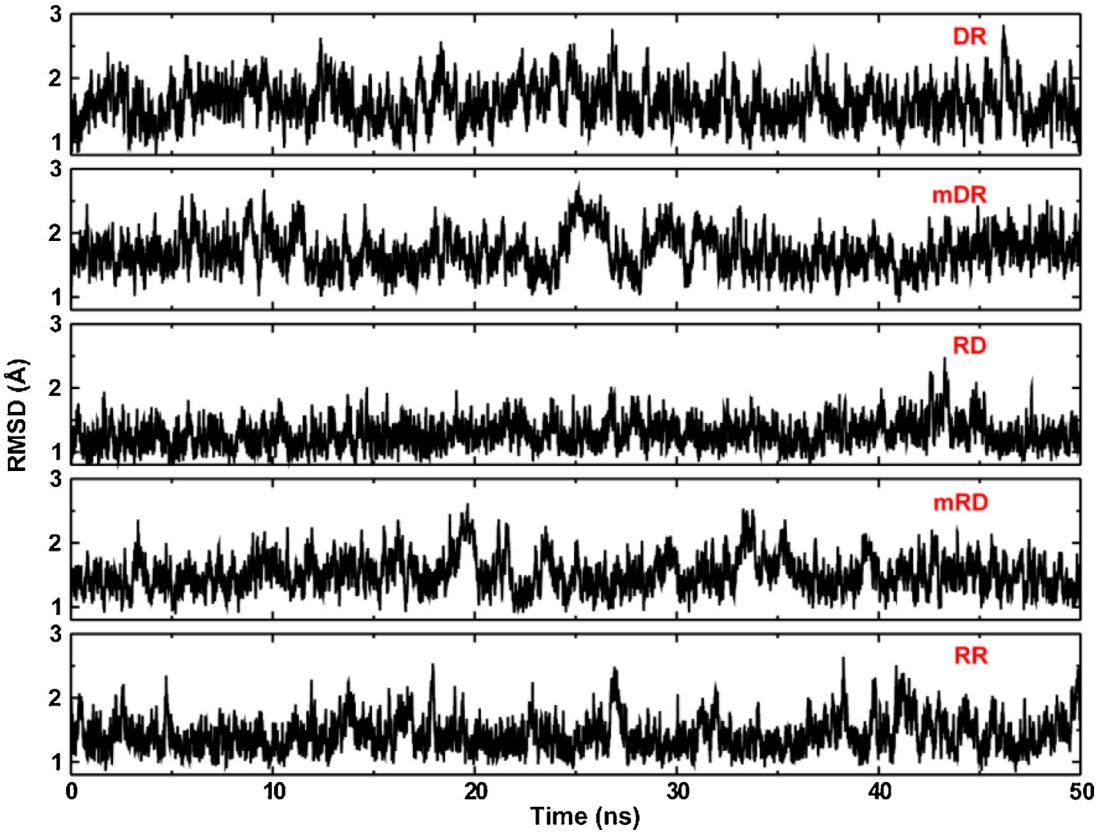

Fig. 2. Root mean square deviations of modified hybrid (mDR, mRD) and pure (DR, RD) duplexes along the simulation time. The RMSD corresponding to pure RNA duplex (RR) is also included here.

Table 2
Root mean square fluctuations (in Å) for full duplex, backbone and bases of all the duplexes considered in the present study.

Duplex	Full	Strand 1		Strand 2	
		Backbone	Bases	Backbone	Bases
DR	1.14	1.80	1.10	0.86	0.77
mDR	1.12	1.59	1.13	0.90	0.83
RD	1.04	1.55	1.04	0.79	0.71
mRD	0.93	1.22	0.93	0.82	0.70
RR	0.98	1.35	0.94	0.83	0.74

the introduction of 2′-*O*-methyl modifications are highly dependent on the sequence.

3.2. *Impact of 2′-O-methyl modifications on base pairing and stacking interactions*

Base pairing and stacking are very important for the stability of nucleic acid duplexes. Base pair interaction energies corresponding to all the base pairs of pure and modified duplexes were calculated and are shown in Table 3. The interaction energy values indicate that the incorporated modifications do not show significant improvement on the Watson–Crick base pair interactions. Inclusion of 2′-*O*-methyl modifications slightly improves interaction energy among base pairs of DR and RD hybrids (a maximum difference of about 0.7 kcal mol^{-1}) except for certain base pairs (G4–C7; G9–C2) of RD hybrid where considerable reduction in base pair interactions are observed. This indicates that the impact of 2′-*O*-methyl modifications on DNA–RNA hybrid duplexes is dependent on the sequence.

To understand the impact of modifications on the interactions of nucleobases with bases in the same strand and complementary strand, π-stacking interactions corresponding to all the nucleobases were calculated and are shown in Table 4 and Table S1. It has been shown earlier that the incorporation of 2′-*O*-methyl groups reduce the overlap area among nucleobases within the strand [34], thereby reducing the stacking interactions among bases. This is reflected in the interaction energy values (Table 1) which indicate the stacking among the bases in the same strand to become less favourable by up to 4.8 kcal mol^{-1}. Incorporation of 2′-*O*-methyl modifications decreases base stacking in the modified strand (2.4 kcal mol^{-1}), and the stacking among the bases of its complementary strand for DR hybrid increases slightly up to 0.6 kcal mol^{-1}. Interestingly, modified RD duplex has reduced base stacking interactions for both the strands when compared to its pure RD duplex (4.8 kcal mol^{-1}). The modified strand of RD hybrid has a sequence of pyrimidine bases and the interactions of the large methyl group with 5-carbonyl group causes the backbone of the nucleic acid to reorient in order to attain A-like conformation. The bases corresponding to modified nucleotides show better stacking interactions with the bases in their complementary strand for both the duplexes except for some nucleobases (Table S1). The variations observed in inter and intra nucleobases stacking interactions indicate that the 2′-*O*-methyl modifications decrease the base stacking among the bases of the modified strands and increase the base

Table 3
Interaction energies (kcal mol^{-1}) corresponding to Watson–Crick base pairs in all the modified and pure duplexes.

Base pair	DR	mDR	RD	mRD	RR
A2–T(U) 9	−11.29 ± 0.14	−11.58 ± 0.02	−10.83 ± 0.02	−11.14 ± 0.02	−11.54 ± 0.02
A3–T(U) 8	−11.39 ± 0.04	−11.47 ± 0.23	−11.24 ± 0.02	−11.32 ± 0.01	−11.70 ± 0.01
G4–C7	−23.13 ± 0.05	−23.19 ± 0.04	−23.20 ± 0.03	−23.05 ± 0.10	−23.15 ± 0.05
A5–T(U) 6	−11.59 ± 0.01	−11.69 ± 0.02	−11.35 ± 0.01	−11.39 ± 0.01	−11.73 ± 0.01
G6–C5	−23.20 ± 0.02	−23.25 ± 0.03	−23.16 ± 0.04	−23.21 ± 0.02	−23.22 ± 0.02
A7–T(U) 4	−11.46 ± 0.01	−11.62 ± 0.02	−10.92 ± 0.03	−11.30 ± 0.01	−11.62 ± 0.01
A8–T(U) 3	−11.57 ± 0.01	−11.65 ± 0.01	−11.22 ± 0.01	−11.35 ± 0.01	−11.64 ± 0.01
G9–C2	−22.49 ± 0.13	−23.14 ± 0.02	−23.18 ± 0.02	−22.95 ± 0.04	−23.16 ± 0.02

Table 4
Base–base stacking energies (in kcal mol^{-1}) corresponding to nucleotides within the same strand excluding terminal nucleotides.

Nucleotide	DR	mDR	RD	mRD	RR
Strand 1					
A2	−18.0 ± 0.1	−15.6 ± 0.1	−16.5 ± 0.1	−15.9 ± 0.0	−15.9 ± 0.1
A3	−14.1 ± 0.1	−13.8 ± 0.2	−15.5 ± 0.1	−14.3 ± 0.0	−14.3 ± 0.0
G4	−18.5 ± 0.1	−16.8 ± 0.1	−18.0 ± 0.1	−17.4 ± 0.0	−17.3 ± 0.1
A5	−18.9 ± 0.1	−17.1 ± 0.1	−18.1 ± 0.1	−17.4 ± 0.0	−17.4 ± 0.0
G6	−19.0 ± 0.1	−17.1 ± 0.1	−17.9 ± 0.0	−17.4 ± 0.0	−17.3 ± 0.1
A7	−17.7 ± 0.0	−15.9 ± 0.1	−16.3 ± 0.0	−16.3 ± 0.0	−16.1 ± 0.1
A8	−14.7 ± 0.0	−14.1 ± 0.0	−15.5 ± 0.1	−14.5 ± 0.0	−14.3 ± 0.0
G9	−22.9 ± 0.0	−21.8 ± 0.0	−22.4 ± 0.0	−17.6 ± 0.5	−22.5 ± 0.0
Strand 2					
C2	−9.3 ± 0.1	−9.4 ± 0.0	−8.8 ± 0.0	−9.0 ± 0.4	−9.4 ± 0.0
T(U) 3	−6.0 ± 0.0	−6.1 ± 0.0	−8.1 ± 0.1	−7.2 ± 0.0	−6.1 ± 0.0
T(U) 4	−5.0 ± 0.0	−4.5 ± 0.0	−7.3 ± 0.1	−6.6 ± 0.0	−4.6 ± 0.0
C5	−8.0 ± 0.0	−8.3 ± 0.0	−8.7 ± 0.0	−8.6 ± 0.0	−8.2 ± 0.0
T(U) 6	−8.1 ± 0.0	−8.3 ± 0.1	−8.8 ± 0.0	−8.7 ± 0.0	−8.3 ± 0.0
C7	−7.9 ± 0.0	−8.2 ± 0.1	−8.8 ± 0.0	−8.6 ± 0.0	−8.3 ± 0.0
T(U) 8	−5.7 ± 0.0	−6.3 ± 0.0	−8.0 ± 0.1	−7.3 ± 0.0	−6.2 ± 0.0
T(U) 9	−4.2 ± 0.1	−4.6 ± 0.0	−7.2 ± 0.1	−6.5 ± 0.0	−4.5 ± 0.0

stacking in its complementary strand while the stacking among the bases of the two strands of RD hybrids decreases upon 2′-*O*-methyl modification. Given the limitations in the current biomolecular force fields, estimations of the stacking interaction energies are approximate and hence only the qualitative comparison is possible.

3.3. Transition in sugar and backbone conformation after 2′-O-methyl modifications

The helical conformation of the nucleic acid duplexes depends highly on the pseudorotation angles of furanose sugars, glycosidic dihedral angles and phosphodiester backbone angles. It is known that the ribose and deoxyribose sugars exhibit conformation in C3′-endo and C2′-endo regions respectively that results in A-like and B-like conformations to RNA and DNA duplexes, respectively [33,40–42]. Since DNA–RNA hybrid has both DNA and RNA strands in the same duplex, the global conformation of the duplex is A/B-like or close to A-like depending on the sequence [28,41]. Probability distributions corresponding to pseudorotation angles of DNA and modified DNA strands were calculated and are shown in Fig. 3. In agreement with the previous studies, the modified DNA strands exhibit conformation similar to A-like for both the DR and RD duplexes where the starting conformation is intermediate A/B-like. The incorporation of chemical modifications in DNA strands does not show any kind of impact on their partner RNA strand's conformation (Fig. S1). It is also observed that the introduced 2′-*O*-methyl modifications increases the amplitude of deoxyribose sugars.

The glycosidic angle (χ) is defined as O4′–C1′–N9–C4 for purines and O4′–C1′–N1–C2 for pyrimidines. This angle indicates the orientation of nucleobases with respect to furanose sugar and two different conformations may be adopted: *syn* or *anti*. It is observed that the bases in canonical nucleic acids exhibit *anti* conformation. Probability distributions corresponding to individual strands have been calculated and the probabilities corresponding to DNA and modified DNA strands are shown in Fig. 4. The DNA strands of hybrid duplexes sample conformations in *anti* and high *anti* regions. Inclusion of 2′-*O*-methyl groups diminishes the conformations sampled in high *anti* region and samples only *anti* region observed in typical RNA duplexes. The shift in conformations indicates that the modified duplexes exhibit conformation similar to A-like. The orientation of duplex backbone can be explained by using their characteristic dihedral angles defined as α (O3′–P–O5′–C5′), β (P–O5′–C5′–C4′), γ (O5′–C5′–C4′–C3′), δ (C5′–C4′–C3′–O3′), ϵ (C4′–C3′–O3′–P), and ζ (C3′–O3′–P–O5′). Probability distributions corresponding to all these backbone angles were also calculated and are shown in Fig. S2. The dihedral angles corresponding to phosphodiester bonds α and ζ together suggest that the backbones of modified DNA strands are oriented. The regions sampled by ϵ angle in hybrid duplexes were not observed after modification. The minor changes observed in β angle and shift in δ and γ angles towards RNA value suggest that the backbone orients in order to adopt RNA-like conformation after introducing the chemical modifications.

3.4. Helical parameters and minor groove widths of 2′-O-methyl modified duplexes

The intrinsic stability of nucleic acids depends on the orientation of bases with respect to each other and base pairs with respect to helical axis. These are often referred to as helical axis, base pair and base pair step parameters (helical parameters). Most of these parameters are interrelated to one another. The probability distributions corresponding to all the helical parameters have been calculated. Previous studies have suggested that the incorporation of 2′-*O*-methyl modification in RNA duplex overwinds the helix and this phenomenon is reflected in the variations of slide and twist parameters [32,34]. Distributions of slide and twist parameters cor-

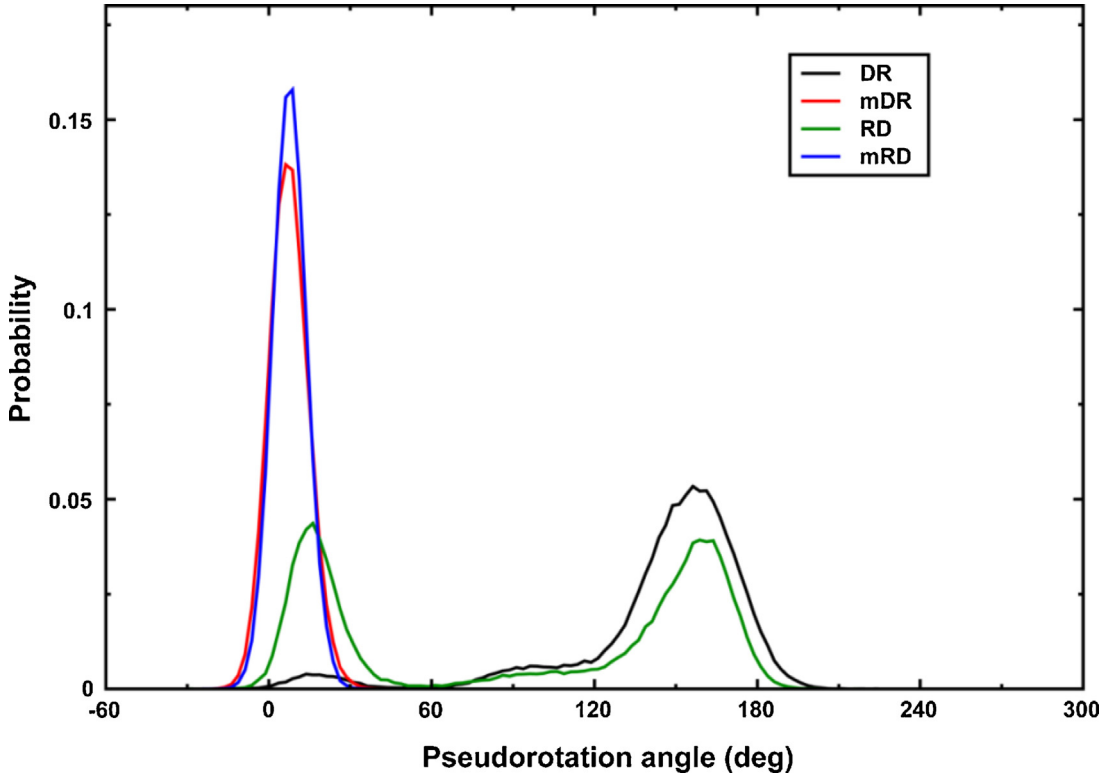

Fig. 3. Probability distributions of pseudorotation angles corresponding to DNA strands of modified hybrid (mDR, mRD) and pure (DR, RD) duplexes.

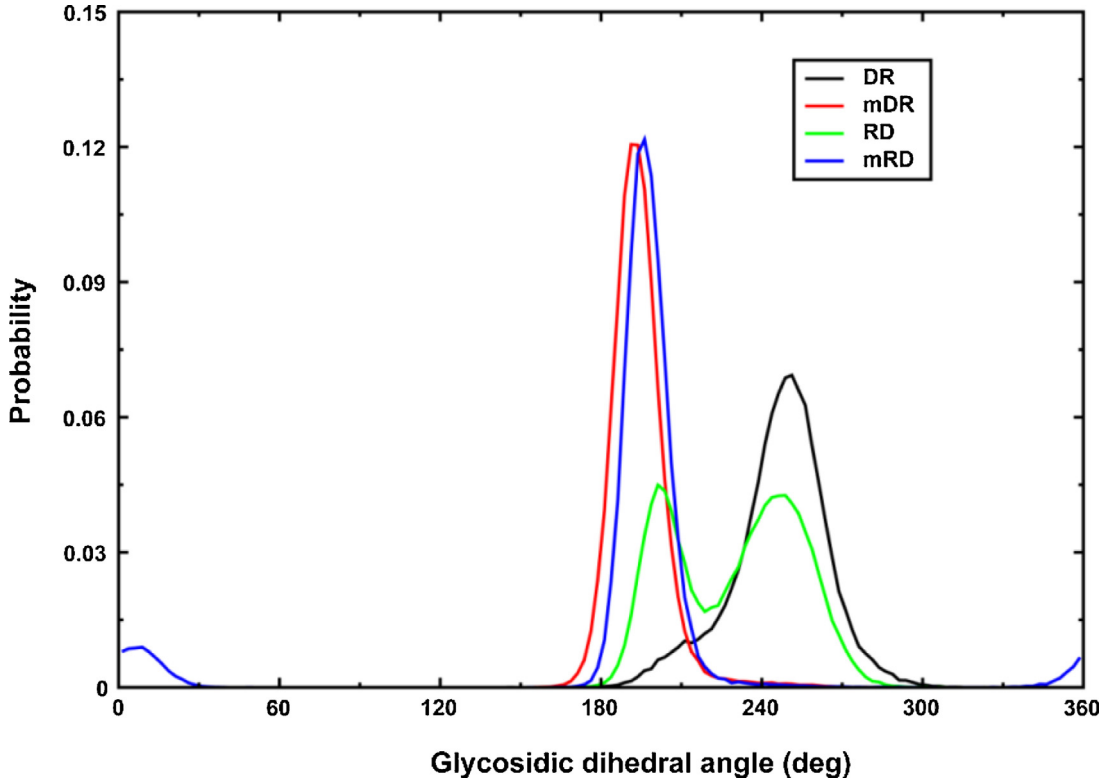

Fig. 4. Probability distributions of glycosidic angles corresponding to DNA strands of modified hybrid (mDR, mRD) and pure (DR, RD) duplexes.

responding to hybrids, modified hybrids and RNA duplexes were calculated and are shown in Fig. 5. These distributions show the decrease in twist and slide values after modifications thus indicating transition in hybrid conformation towards A-type. This suggests helical overwinding of the hybrid duplexes after the incorporation of chemical modification in line with previous studies [34]. Probability distributions corresponding to individual parameters are also provided in Figs. S3–S5. These probability distributions indicate

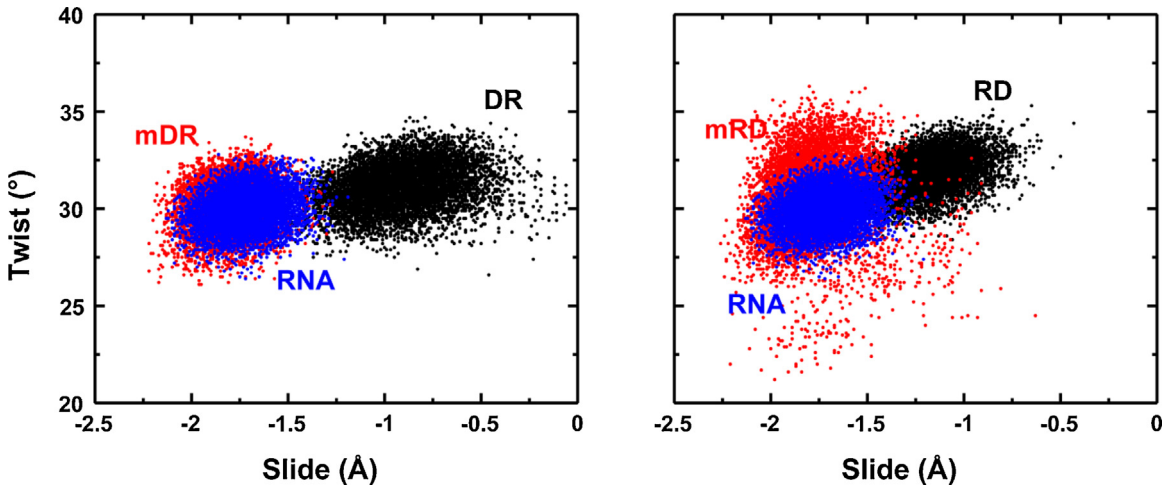

Fig. 5. Scatter plots corresponding to slide and twist parameters of pure (DR, RD) and modified hybrid (mDR, mRD) duplexes.

Table 5
Average values of hydration numbers and SASA values (Å^2) around the different regions of duplexes.

Region	DR	mDR	RD	mRD	RR
Hydration number					
Backbone	263.11 ± 0.66	278.69 ± 0.67	266.99 ± 0.56	265.79 ± 0.6	257.42 ± 0.62
Major G	50.47 ± 0.25	47.68 ± 0.25	46.44 ± 0.23	44.15 ± 0.22	51.96 ± 0.25
Minor G	55.09 ± 0.28	53.36 ± 0.29	50.66 ± 0.25	45.01 ± 0.26	54.65 ± 0.28
SASA					
Backbone	941.75 ± 66.8	884.65 ± 76.0	782.4 ± 44.8	841.96 ± 71.3	901.9 ± 58.8
Bases	291.84 ± 45.4	211.76 ± 39.2	204.74 ± 15.3	191.81 ± 29.5	271.97 ± 46.8

the modified duplexes to maintain helical geometry with the bases shifted towards the minor groove (Fig. S3). The translation and rotation with respect to helical axis (h-rise and h-twist) further suggest the conformational transformation towards A-RNA duplex (Fig. S4). The base pair translational (shear, shift and stagger) and rotational (buckle, opening and propeller) parameters with respect to their short axis were also calculated and zero values of these parameters indicate the Watson–Crick (WC) base pairing and non-zero values describe deviations from WC pairing. Probability distributions show that the modifications in DR hybrid reduce the WC deformations, and are closer (similar) to base pairs of RNA duplex. Significantly higher deformations are observed for RD duplex after incorporating chemical modification that could contribute towards its instability after modifications (Fig. S5). Minor groove width regions corresponding to pure and modified hybrid duplexes have been calculated and the corresponding probability distributions are shown in Fig. S6. The minor groove widths of modified duplexes are very similar to the values of RNA duplexes. This further supports the previous discussion that the 2′-*O*-methyl modifications to deoxyribose sugars of hybrid duplexes shift the conformation to A-like [34].

3.5. Chemical modifications leads to dehydration of the duplex

Water plays an important role in the stability of nucleic acids. Previous studies have shown that inclusion of chemical modifications in backbone alters the water behaviour around the nucleic acid, and this depends on the nature of the modification [32,34]. The number of water molecules around the backbone atoms and groove regions of hybrid duplexes were calculated to understand the change in nature of water after introducing the 2′-*O*-methyl modifications. The average values of number of water molecules indicate that less number of water molecules is present around the groove regions of the chemically modified duplexes (Table 5). This is because of the hydrophobic nature of methyl group that is incorporated into the duplex that make the modified duplexes more hydrophobic in nature than their parent ones (Fig. S7). Similar kind of changes in water network has been observed for other chemically modified nucleic acids in previous experimental studies [32]. The numbers of water molecules also indicate a slight increase in hydration around the DR hybrid backbone and decrease around the backbone of RD hybrid even though the effect around groove regions is same.

To further understand the local structural changes in duplexes, solvent accessible surface area (SASA) values around the backbone and nucleobases have been calculated (Table 5). SASA is a convenient measure for calculating the surface area that is exposed to the solvent thus indicating local solvent interactions [61]. It is expected that the introduced chemical modifications increase the solvent exposed surface area for entire duplex because of the presence of the large methyl group. The average values of SASA indicate that chemical modifications reduce the exposed surface area around the backbone of DR hybrid. However, the exposed surface area increases in case of the RD hybrid. But the change around the nucleobases is same for both the duplexes. The hydration numbers and SASA values together suggest contrasting hydration patterns around the two hybrid duplexes, DR and RD, upon 2′-*O*-methyl chemical modification.

3.6. Modifications reverse the thermodynamic stability of hybrid duplexes

Thermodynamic stabilities of nucleic acids can be examined by using binding free energies. In the present study, MM-GBSA method

Table 6
Relative binding free energies (kcal mol^{-1}) of the modified hybrid and hybrid duplexes with respect to A-RNA duplex.

Duplex	$\Delta\Delta G_1$	$\Delta\Delta G_2$
DR	7.42 ± 0.34	8.91 ± 0.35
mDR	−0.75 ± 0.56	1.04 ± 0.72
RD	2.43 ± 0.44	2.62 ± 0.51
mRD	25.24 ± 4.35	25.78 ± 5.61

$\Delta\Delta G_1$: free energy without entropic term; $\Delta\Delta G_2$: free energy including entropic term.

Table 7
Contribution from various factors on the reversal of thermodynamic stabilities of hybrid duplexes (all energies are in kcal mol^{-1}).

Term	DR	RD
Modification effect	−7.86	23.16
Conformational effect	−8.91	−2.62
Hydrophobic effect	1.05	25.78

has been employed to calculate the binding free energies of the duplexes and this method has been shown to provide reliable free energies to understand the relative thermodynamic stabilities of the nucleic acids [38,40,41]. Binding free energies were calculated for all the duplexes and the relative binding free energies with respect to RNA are presented in Table 6. These values indicate that the RNA duplex is more stable than others as observed in experimental studies [37]. However, mDR hybrid is more stable when the entropy contribution is neglected. It has been suggested that when 2′-*O*-methyl modifications are incorporated in DNA strand of the less thermodynamic stable hybrid (DR), its stability increases. But the duplex stability decreases when the 2′-H atoms of deoxyribose sugars of most thermodynamically stable hybrid (RD) duplex are substituted by 2′-*O*-methyl groups [37]. The relative binding free energies obtained from the MD simulations indicate that when the 2′-*O*-methyl groups are introduced into the DNA strand of the DR duplex, the duplex thermodynamic stability increases. But the stability of RD hybrid decreases after modifying its deoxyribose sugars with 2′-*O*-methyl groups. This is in agreement with results reported in previous studies [37]. The relative binding free energies further indicate that the RD duplex is more stable than DR duplex in accordance with the experimental studies [28]. However, the modifications reduce the gap between the binding free energy values of DR hybrid and RNA duplexes (−0.75 kcal mol^{-1}); increase the gap between the binding free energy values of RD hybrid and RNA duplex (25.24 kcal mol^{-1}). This further suggests that the modifications bring the conformation of the duplexes toward A-RNA but there is a considerable difference between the modified hybrids. In other words, the 2′-*O*-methyl modification reverses the thermodynamic stabilities of DR and RD hybrids and follows the order RR ∼ mDR > RD > DR > mRD.

The total effect for the methyl modification on duplex stability comes as the resultant of two factors, namely conformational and hydrophobic effects and the contribution of these two terms towards duplex conformation and internal structure after modification can be estimated (see Section 2). The effective free energy values are shown in Table 7 and indicate that the modifications to DR hybrid resulted in a more stable duplex whereas it is not the case for RD hybrid. This stability can be attributed to the large favourable conformational changes in hybrid DR compared to RD, in which the hydrophobic term dominates its modification. The changes associated with conformational effect rather than hydrophobic effect is high for DR hybrid whereas hydrophobic effect dominates in case of RD hybrid. The modification allows both the hybrids to change conformationally to A-form but the hybrid RD is very rigid internally compared to DR hybrid. This suggests that the modification converts the DR hybrid to a stable duplex but not the RD hybrid. The hybrid with high deoxypurine content (DR) has more pronounced structural changes as a result of 2′-*O*-methyl modifications compared to hybrids with high deoxypyrimidine content (RD). This is in good agreement with previous observations made in experiments that the less stable hybrid should have higher $\Delta G_{mDR} - \Delta G_{DR}$ values than the more stable duplex [37]. The present study show that the 2′-*O*-methyl modification fails to result in a stable duplex with the most thermodynamically stable duplex RD; however, the least stable duplex DR does give a stable duplex.

To understand the origin for this dissimilar effect of 2′-*O*-methyl modifications on the two hybrid DR and RD duplexes, the energies associated with various changes of duplexes were calculated and are given in Table 8. The van der Waals energy favours the conformational changes in hybrid DR but not in RD after modification. The molecular mechanical energy of the duplexes does not favour conformational rearrangement after modification. The interaction between two strands favours backbone rearrangement after modification in DR hybrid, owing to strong favourable backbone interactions. The impact of non-polar solvation energy on the backbone rearrangement due to chemical modification is negligible. However, the solvent electrostatics highly favours this backbone rearrangement in hybrid DR but not in RD duplex. The total electrostatic interactions completely oppose the duplex rearrangement after chemical modifications. The conformational rearrangement of modified duplexes is an entropically driven process. At the end, the absolute free energies indicate that the modification decreases their free energy values, and the values are close to that of RNA duplex (RR).

Table 8
Free energy component analysis performed using MM-GBSA method for all the duplexes studies here.

Term	DR	mDR	RD	mRD	RR
E_{vdw}	31.78 ± 0.10	30.61 ± 0.18	14.44 ± 0.25	21.72 ± 0.66	39.60 ± 0.11
E_{ele}	932.62 ± 5.05	1591.08 ± 17.54	1072.89 ± 5.66	1743.42 ± 7.34	1479.70 ± 5.97
E_{mm}	2327.82 ± 5.15	3077.06 ± 16.53	2477.57 ± 5.94	3239.10 ± 7.22	2956.18 ± 5.72
E_{int}	1295.31 ± 2.96	1248.31 ± 11.94	1267.99 ± 2.32	1255.39 ± 4.99	1248.41 ± 2.86
$E_{int,bkb}$	1430.26 ± 3.29	1407.04 ± 13.04	1424.40 ± 2.12	1425.51 ± 5.26	1403.04 ± 2.98
$E_{int,base}$	−180.22 ± 0.25	−187.14 ± 0.31	−184.47 ± 0.24	−181.37 ± 1.08	−185.99 ± 0.11
$E_{sol,np}$	29.26 ± 0.01	29.81 ± 0.04	29.18 ± 0.04	29.80 ± 0.08	28.99 ± 0.01
$E_{sol,polar}$	−4501.03 ± 4.91	−4703.11 ± 16.00	−4538.68 ± 6.18	−4682.93 ± 7.01	−4626.86 ± 5.41
$E_{ele,tot}$	−3568.34 ± 3.12	−3114.66 ± 4.98	−3467.52 ± 3.32	−2943.46 ± 4.95	−3148.46 ± 4.45
$-TS_{mm}$	−477.43 ± 0.85	−488.41 ± 2.53	−475.39 ± 0.64	−502.96 ± 3.3	−454.94 ± 1.05
ΔG	−2621.38	−2084.65	−2507.32	−1916.99	−2096.63

All the energies are in kcal mol^{-1}.
E_{vdw}: van der Waals energy; E_{ele}: electrostatic energy; E_{mm}: molecular mechanical energy; E_{int}: interaction energy between two strands; $E_{int,bkb}$: interaction energy between backbone atoms of two strands; $E_{int,base}$: interaction energy between bases of two strands; $E_{ele,tot}$: total electrostatic energy ($E_{ele} + E_{sol,polar}$); $E_{sol,np}$: non-polar contribution of solvation energy; $E_{sol,polar}$: polar contribution of solvation energy; TS_{mm}: absolute molecular mechanical entropy, ΔG: absolute free energy of the duplex.

4. Conclusions

Molecular dynamics (MD) simulations have been performed on two DNA–RNA hybrid duplexes and the complementary 2′-methoxy modified duplexes to investigate the effect of modification on duplex stability. These two hybrid duplexes contain different deoxynucleobase content and display dissimilar thermodynamic stability. It has been reported that inclusion of 2′-*O*-methyl modifications in DNA strand of hybrid duplexes results in reversal of their thermodynamic stability. Several structural and energetic analyses have been performed on the resultant MD trajectories in the present study and it is observed that the modified duplexes are quite stable during the simulations. The probability distributions of pseudorotation angles, glycosidic angles and backbone angles suggest that the introduced modifications drive the conformations of both the hybrid duplexes towards A-RNA like conformation. The modified duplexes exhibit high deviations and fluctuations when compared to respective unmodified duplexes. The variations observed in slide and twist suggests that the modified duplexes undergo overwinding compared to parent duplexes upon 2′-*O*-methyl modification. This is in good agreement with previous results [34]. The distributions of helical parameters have also indicated a similar conformation as A-RNA for the modified hybrid duplexes. Modified DNA–RNA hybrid duplexes have minor groove width regions that are very similar to A-RNA values. The hydrophobic nature of bulky methyl group leads to dehydration of the hybrid duplexes and shows contrasting hydration pattern for both the hybrids. MM-GBSA method was used to calculate the binding free energies and the relative binding free energies indicate that the introduced modifications stabilize the less stable DR hybrid and destabilize the more stable RD duplex. Binding free energy calculations further indicate that the increased hydrophobicity due to 2′-*O*-methyl group is solely responsible for the reversal of thermodynamic stability of hybrid duplexes. These results are in good agreement with previous experimental and computational studies [34,37]. Free energy component analysis has been performed to further understand the energetics involved in the stability of chemically modified duplexes. The total electrostatic interactions oppose the conformational rearrangement of chemically modified duplexes, and the rearrangement is an entropically driven process. The present MD simulation results also suggest that the thermodynamic stabilities of 2′-*O*-methyl incorporated hybrid duplexes depend on the base composition of their parent hybrids. A systematic study is required on hybrid duplexes with different base sequence combinations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2015.07.009

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