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Hydrophobic Groups Stabilize the Hydration Shell of 2′-*O*-Methylated RNA Duplexes

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In memoriam Peter A. Kollman

The higher thermodynamic stability of RNA compared to DNA helices of similar sequence is often ascribed to the 2′-OH group present in RNA and absent in DNA nucleotides. Indeed, the 2′-OH group is involved in specific solute–solvent hydrogen bond acceptor and donor interactions that participate in the stabilization of the RNA hydration shell.[1, 2] Yet, RNA duplexes containing 2′-*O*-methylated nucleotides are structurally more stable than their unmodified counterparts,[1, 3, 4] indicating that the replacement of a hydrophilic 2′-OH by a hydrophobic 2′-OMe group results in additional stabilization of RNA helices. This effect, also observed for DNA and hybrid duplexes containing methylated nucleotides, is commonly explained either by a stiffening of the C3′-*endo* form of the ribose induced by the methyl group, increased stacking of the bases,[5, 6] or favorable lipophilic interactions between consecutive hydrophobic groups.[7] For RNA duplexes, however, explanations based on conformational changes induced by the modified nucleotides are not convincing since NMR studies report that native and entirely methylated duplexes are structurally very similar.[8] It has also been proposed that 2′-*O*-methylations induce a reduction of the hydration of the shallow groove[9] associated with a decrease of the hydration enthalpy.[1] Here, on the basis of two 4.4-ns molecular dynamics (MD) simulations of the natural $r(CpG)_{12}$ and the fully modified $2'$-*O*-$Me(CpG)_{12}$ duplexes, we provide computational evidence indicating that, on the contrary, by creating optimal water binding pockets, the 2′-*O*-methylations lead to a significant stabilization of specific nucleotide–water interactions, which could favorably contribute to the enthalpy of hydration.

During the course of the MD simulations, in agreement with crystallographic,[7, 10] NMR,[8] and previous simulation data,[9] it is observed that the 2′-OMe groups are locked into a single orientation (toward the shallow groove, Figure 1), one of the three distinct orientations between which the more mobile 2′-OH groups switch frequently.[2] Despite these local differences the ribose groups of both duplexes retain their initial C3′-*endo* conformation. Thus, conformational restraints imposed by the 2′-OMe groups, which are of importance for flexible mono- and dinucleotides[6] or for methylated DNA strands, do not significantly displace the conformational

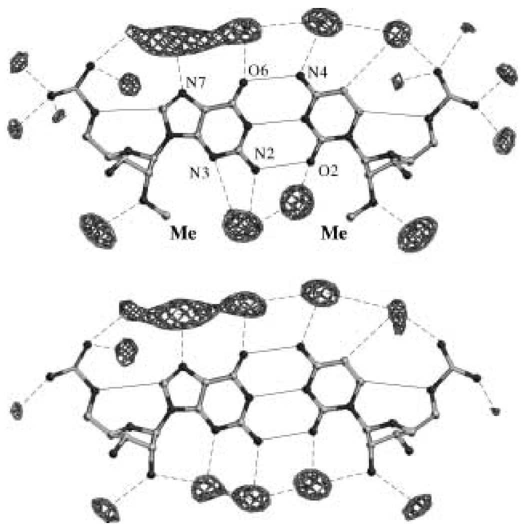

Figure 1. Solvent densities (water molecules and K^+ ions) surrounding 2′-*O*-Me(GC) (top) and r(GC) (bottom) base pairs drawn at a similar contour level.

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equilibria observed for RNA helices. Similarly, the stacking patterns determined for CpG and GpC steps are identical for the native and modified duplexes and should not contribute to the observed differences in stability. Thus, the present simulations support the proposal that conformational effects induced by the methylation of the 2′-OH groups are negligible for RNA helices.[8]

Instead, the methyl groups in the modified RNA significantly alter the hydration pattern from that of the native duplex. For the shallow groove of the methylated pairs, two "out-of-plane" hydration sites are observed (Figure 1). Since these hydration sites overlap, a single row of water molecules, similar to that observed in the X-ray structure analysis,[10] connect the base atoms. In the shallow groove of the native duplex, however, three nonoverlapping "in-plane" hydration sites (Figure 1) are seen, which form a row of three water molecules connecting the base atoms and the 2′-OH groups. Overall, it appears that in the modified duplex fewer water molecules contact the shallow-groove hydrophilic atoms than in the native duplex (1.8 vs 2.4; Table 1). Surprisingly, the number of hydrogen bonds formed between shallow-groove hydrophilic atoms and water molecules is similar for the (G)NH_2 and (C)O2 atoms, and slightly reduced for the (G)N3 atoms (the latter are involved, as described below, in asymmetrical hydration patterns). Thus, although the methyl groups do alter the shallow-groove hydration patterns, they do not lead to a significant shielding of the adjacent (C)O2 atoms and the overall accessibility of the (G)N3 atoms is only slightly reduced (see HB %; Table 1).

Table 1. Average number of H_2O/RNA contacts as well as hydrogen bonding percentages (HB %) calculated over the last 3.0 ns of the MD simulation and the twelve central base pairs (for details, see ref. [11]).

	Shallow-groove atoms			
	(G)N3	(G)NH_2	(C)O2	Total[a]
Average contacts				
2′-*O*-Me(GC)	0.4	1.5	0.6	1.8
r(GC)	0.8	1.5	0.9	2.4
Hydrogen bonding percentages (HB %)[b]				
2′-*O*-Me(GC)	40 (18)	84 (2)	85 (4)	
r(GC)	56 (5)	79 (3)	83 (3)	

[a] Due to overlaps between adjacent hydration sites, these numbers do not correspond to the sums of the individual atomic contributions. [b] Numbers in parentheses correspond to standard deviations from the average values.

To obtain information on the solvent dynamics, a comparative analysis of the residence times of the water molecules in the first hydration shell of both duplexes has been performed as described elsewhere.[11] It is observed that for the modified duplex the residence times of shallow-groove water molecules bound to the (G)N3, (G)NH_2, and (C)O2 are much longer than for the native duplex (Figure 2). This effect is most dramatic for the (C)O2 atoms: For the modified duplex residence times exceeding 1 ns are determined, whereas they do not exceed 500 ps for the native duplex. A detailed analysis of the trajectories reveals that, for 2′-*O*-Me(GpC) steps, long-lived water bridges (ca. 1 ns) connect the (C)O2 atoms belonging to adjacent base pairs (Figure 3), while no such long-lived hydration patterns have been observed for the native or similar duplexes.[11] For 2′-*O*-Me(CpG) steps, as a result of different stacking interactions, long-lived water bridges (ca. 500 ps) connecting the (G)N3 to the (G)NH_2

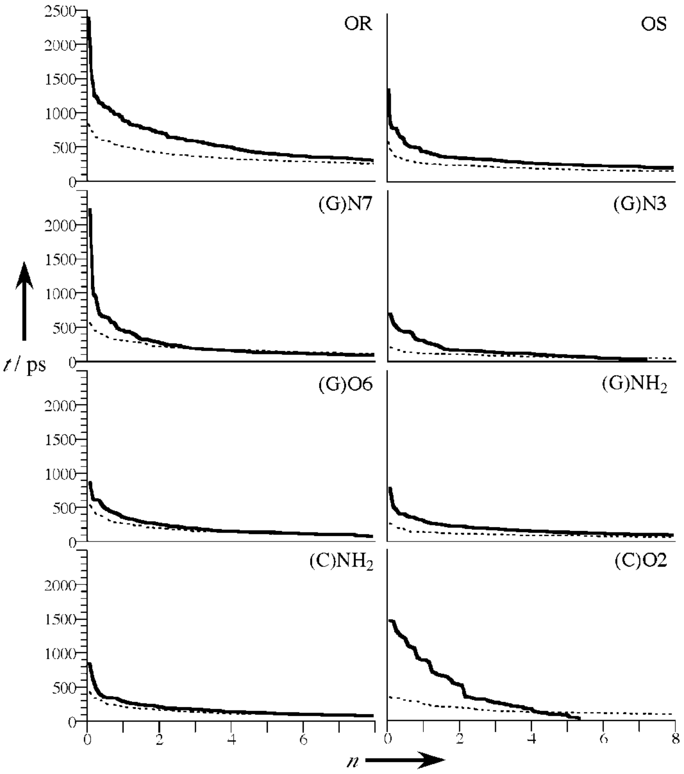

Figure 2. Residence time profiles for water molecules in $n(CpG)_{12}$ (----) and fully modified 2′-*O*-Me$(CpG)_{12}$ (——). The number (*n*) of atoms bound to selected hydrophilic atoms of the central GC pairs of the native and methylated duplexes is plotted as a function of their hydrogen bonding contact times (*t*).

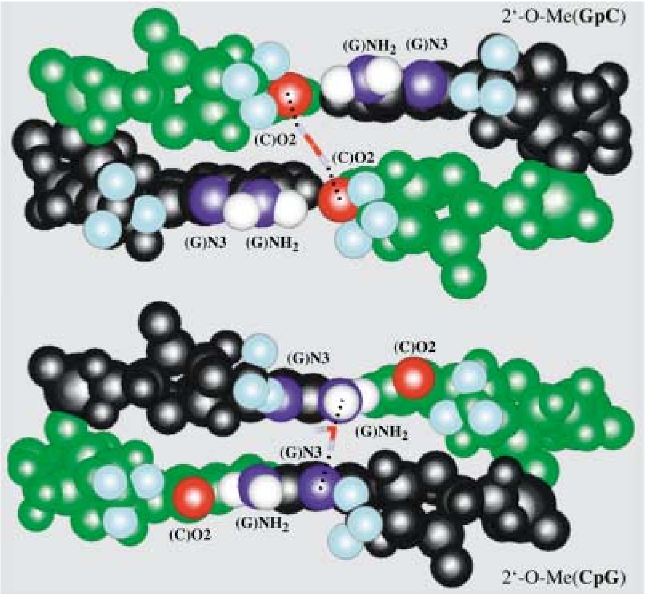

Figure 3. Snapshots extracted from the MD simulations of the methylated RNA duplex. Long-lived shallow-groove water molecules (stick model) bridging adjacent base pairs are shown for GpC (top) and CpG (bottom) steps. Green = skeleton of C, black = skeleton of G, red = shallow groove oxygen atoms and oxygen atoms of water, blue = shallow groove nitrogen atoms, cyan = hydrogen atoms of OMe groups.

group of the adjacent base pair are observed (Figure 3; note that only one of the two N3 atoms participates in this asymmetrical hydration pattern; the other one is shielded by a methyl group). Consequently, long-lived shallow-groove water bridges linking adjacent base pairs are distinctive of the methylated and not the native duplex. Thus, the methyl groups help to delineate, through steric contacts, sequence-dependent hydration pockets in which water molecules optimize their interactions with the hydrophilic (G)N3, (G)NH_2, and C(O2) atoms of the shallow groove.

Interestingly, although no modifications alter the shape of the deep groove, the calculated residence times for bound water molecules in the modified duplex are significantly longer than for those in the native duplex. This is related to the reduced rigidity of the modified duplex induced by both intrinsic conformational factors and cooperative effects associated with the longer lifetimes of the hydration patterns in the shallow groove. Indeed, for the native duplex the average B-factors calculated for all the heavy atoms of the 12 central base pairs are significantly higher ((56 ± 19) $Å^2$) than those of the methylated duplex ((34 ± 7) $Å^2$). The presence of methyl groups, therefore, leads to an ordering of the hydration shell, even at a distance from the added hydrophobic groups. It is also observed that ion (K^+) binding is enhanced in the methylated duplex since the longest calculated residence times of an ion in contact with the methylated and native duplex are 2.0 and 1.3 ns, respectively. Indeed, the cooperative effect leading to an increase of the residence times of bound water molecules is linked with that seen for the K^+ ions.

The present MD simulations shed new light on the stabilization mechanisms associated with 2′-*O*-methylation of RNA duplexes. As strong structural effects induced by the modified nucleotides have been ruled out, the effects associated with hydration changes appear central. Here, a combination of steric (OMe), attractive, and repulsive interactions (O2, N3, NH_2) delineates notches in which water molecules fit ideally. As an outcome of the dynamically more stable hydration patterns observed in the shallow groove, the entire structure of the duplex is more rigid and the hydration patterns on the deep groove side are more stable. Although it is very difficult to deconvolute enthalpic and entropic contributions, it is proposed that the reduced mobility of the methylated duplex and of its hydration shell leads to an entropy loss that is overcompensated by a more favorable enthalpy of hydration. Although multiply methylated fragments rarely occur in natural systems, it is inferred that the observed hydration changes are also locally significant for single modifications. Thus, hydration patterns and their dynamics should be considered in the evaluation of the biophysical effects associated with methylation of RNA structures,[12] which are so important for antisense technologies.[1, 3, 13]

More generally, the results give insight into the complexity of effects associated with the insertion of hydrophobic chemical groups into a hydrophilic structure. Usually it is assumed that the addition of hydrophobic groups to a solute in a hydrophilic solvent leads to weakened solute–solvent interactions, resulting in an increase of the solvent entropy and a decrease in the enthalpy associated with solute–solvent interactions. We showed here that the introduction of hydrophobic modifications does not systematically result in a reduction of the binding affinity of the solute for water when the geometrical disposition and surface patterns generated by the hydrophobic and hydrophilic groups are appropriate and such that water molecules can form structured hydrogen bonding networks around the solute.

Computational Details

The simulation protocols and analysis methods are identical to those described in previous work.[11] The AMBER 5.0 simulation package[14] and the Cornell force field[15] have been used for generating the two 4.4-ns MD trajectories of $r(CpG)_{12}$ and 2′-*O*-Me$(CpG)_{12}$ (note that the 4.4-ns MD trajectory of $r(CpG)_{12}$ is an extension of a 2.4-ns trajectory described earlier[11]). The charges of the modified residues have been adapted from those of the native nucleotides. The starting structures are derived from fiber diffraction data.[16] In agreement with X-ray and NMR studies,[8, 10] the 2′-OMe groups have been placed initially so as to point toward the shallow groove. The duplexes were placed in a box containing 5690 (RNA) and 5952 (methylated RNA) water molecules (single point charge/extended (SPC/E)) as well as 72 K^+ and 26 Cl^- ions, resulting in a concentration of added KCl of approximately 0.25 M. The target temperature and pressure were set to 25 °C and 1 atm, respectively. During the course of the simulations, both duplexes remain close to each other and to their initial A-form.

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