Rules Governing the Orientation of the 2′-Hydroxyl Group in RNA

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Molecular dynamics simulations reveal that, in C3′-endo sugar puckers, only three orientations are accessible to the 2′-hydroxyl groups distinctive of RNA molecules: towards (i) the O3′, (ii) the O2′ of the same sugar, and (iii) the shallow groove base atoms. In the rarer C2′-endo sugar puckers, orientations towards the O3′ atom of the same sugar are strongly favoured. Surprisingly, in helical regions, the frequently suggested intra-strand O2′-H(n)...O4′(n+1) interaction is not found. This observation led to the detection of an axial C-H...O interaction between the C2′-H2′(n) group and the O4′(n+1) atom contributing to the stabilization of RNA helical regions. Subsequent analysis of crystallographic structures of both RNA and A-DNA helices fully supports this finding. Specific hydration patterns are also thought to play a significant role in the stabilization of RNA structures. In the shallow groove of RNA, known as a favourable RNA or protein-binding region, three well-defined hydration sites are located around the O2′ atom. These hydration sites, occupied by water molecules exchanging with the bulk, constitute, after dehydration, anchor points for specific interactions between RNA and nucleic acids, proteins or drugs. Therefore, the fact that the 2′-hydroxyl group is not monopolised by axial stabilization, together with its water-like behaviour, facilitates complex formation involving RNA helical regions.

Keywords: molecular dynamics; tRNA; anticodon; hydration; tertiary interactions

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Introduction

The presence of the 2′-hydroxyl group on the RNA pentose sugar ring is at the origin of the profound structural and dynamical differences observed between RNA and DNA molecules. Structural and biophysical studies have led to the conclusion that the additional hydroxyl group stabilizes the C3′-endo sugar pucker, typical of RNA, compared to the C2′-endo sugar pucker, prevalent in DNA systems (Saenger, 1984). It is also frequently suggested that a recurrent intra-strand axial stabilization occurs in helical regions through the formation of an intra-molecular hydrogen bond between the 2′-hydroxyl group and the sugar ring O3′ atoms on their 3′ side (Jeffrey & Saenger, 1991). However, experimental methods are unable to locate unambiguously the positions of the mobile hydrogen atom of the 2′-hydroxyl group. By crystallography, the occurrence of specific hydrogen bonds can be inferred only from proximity criteria involving the positions of heavy atoms. Yet, a precise knowledge of the orientations and of the dynamics of the 2′-OH bond, which is an important RNA recognition element, is essential to clarify its specific structural implications in the stabilization of helical regions, complex RNA tertiary folds and RNA/protein interactions. Furthermore, this knowledge may be useful in the study of the mechanisms of catalytic RNA molecules that all involve direct or indirect participation of the 2′-hydroxyl group (Gesteland & Atkins, 1993; Eckstein & Lilley, 1996).

Below, we address the question of the orientation of the hydrogen atom in the 2′-hydroxyl group for sugars in the typical RNA C3′-endo pucker by analysing a set of 3 ns of molecular dynamics (MD) simulations of the 17 nucleotide long tRNAAsp anticodon hairpin for which general conformational (Auffinger & Westhof, 1996) and hydration features (Auffinger et al., 1996a; Auffinger & Westhof, 1997) have already been...
Orientation of the 2'-OH Group in RNA

reported. The same analysis is also performed for sugars adopting the rare C2'-endo pucker on a 500 ps MD simulation of the full tRNAAsp molecule (Auffinger et al., 1996a).

Results and Discussion

2'-OH orientations for C2'-endo sugars

From the simulated trajectories, the distribution of the H2-C2-O2-H dihedral angles has been calculated for all residues of the hairpin. This distribution reveals clearly three favoured domains of orientations for the 2'-OH bond (Figure 1). These three domains are (i) the O3 domain where the hydroxyl bond orientation is stabilized by favourable sugar-backbone or intra-ribose electrostatic interactions, and (iii) the "Base" domain where the hydroxyl bond points towards the N3(R) or O2(Y) atoms of the attached base (R stands for purines and Y for pyrimidines). Steric contacts between the hydroxyl group and the two H2 and H4 ribose hydrogen atoms, with average distances \( d(H_2...O_2) \approx 2.5 \AA \) and \( d(H_4...O_2) \approx 2.6 \AA \), delimit these domains. Energetically penalizing orientations, corresponding to a H2-C2-O2-H dihedral angle close to zero, are also avoided. Additionally, the H2 backbone atom, located at an average distance of 2.7 \( \AA \) from the O2 atom in a regular helical RNA conformation, induces steric hindrance, which sets the larger size of the H2 forbidden domain (Figure 1). These favoured orientations lead to specific hydration patterns around the 2'-hydroxyl group (see Figure 1c to f and below).

It should also be noted that for a 500 ps simulation of the full tRNAAsp molecule (Auffinger et al., 1996a), the calculated profile of the H2-C2-O2-H dihedral angle distribution for all the sugars in the C2'-endo conformation is exactly the same as the profile derived from the 3 ns of MD simulations on the anticodon hairpin (data not shown). This result emphasizes the generality of the above stereochemical rules, since they are verified for sugars in a great diversity of environments involving helical and non-helical structural contexts.

The present findings agree well with those of an MD simulation of an RNA hairpin where a strong preference for the O3 orientation was noted (Singh & Kollman, 1996) and with the available experimental data. By NMR, it has been observed that the rate of exchange of the 2'-hydroxyl protons in RNA duplexes is considerably slower than in monomers, suggesting that these protons are involved in hydrogen-bonding interactions either with RNA or water atoms (Bolton & Kearns, 1978; Leroy et al., 1985; Conte et al., 1996). The close proximity of the negatively charged O3 oxygen atom of the phosphate group (O3 domain) or of the O2 cyclic oxygen atom (O2 domain) could significantly hinder proton exchange for both electrostatic and steric reasons. Moreover, in our simulations, no water molecule making long-lived hydrogen bonding contacts to the 2'-OH hydrogen atoms was observed (Auffinger & Westhof, 1997). In a recent NMR study of an RNA dodecamer, NOEs were observed between the 2'-hydroxyl and the ribose H2 protons for all nucleotides except the terminal ones (Conte et al., 1996; for the hydroxyl group of the 3'-terminal residue, as a result of the absence of a 3'-phosphate group, a predominant O4 orientation instead of the generally favoured O3 orientation was found). These NOEs are compatible with the "Base" and O3 orientations. Additional weak NOEs between the 2'-OH and the H1 protons, indicating that the 2'-OH bond points away from the H1 atom, agree with the preferred O3 orientation observed in our MD simulations.

The occurrence of intra-strand O2-H(n)...O4(n+1) hydrogen bonds is also compatible with the above NMR observations (Conte et al., 1996). However, and most surprisingly, no such intra-strand interaction was observed during our simulations (Figure 1). Experimentally, by methylation of the 2'-OH group or its replacement by chlorine or fluorine atoms, it was inferred that the O2-H(n)...O4(n+1) hydrogen bond may not be crucial for the stabilization of RNA duplexes (for review, see Saenger, 1984). Besides, only rarely were such intra-strand hydrogen bonds proposed to occur in helical parts of high resolution RNA crystal structures, although, in non-helical regions, the occurrence of O2-H(n)...O4(n+1) hydrogen bonds has been sometimes suggested as, for example, in the active site of a freeze trapped catalytic hammerhead intermediate (Scott et al., 1996). It was also reported that, in the helical regions of tRNAthe, the full hydrogen-bonding capacity of the 2'-hydroxyl groups is rarely exploited (Quigley & Rich, 1976). Thus, in agreement with experimental results, our simulations indicate that RNA molecules are not stabilized by recurrent intra-strand O2-H(n)...O4(n+1) hydrogen bonds (Auffinger & Westhof, 1997).

Inter-ribose axial C-H...O interactions

Are there then other possible intra-strand axial stabilizing interactions in RNA? A close examination of our trajectories suggested that, for a regular helical backbone conformation, the ribose H2(n) atom is at hydrogen-bonding distance from the O4(n+1) atom on the 3' side (average distance around 2.6 \( \AA \); see Figure 1b). Hence, repetitive and intra-strand C-H...O hydrogen bonds may stabilize RNA helical regions. In order to gather experimental evidence for the occurrence of such weak C-H...O interactions, a statistical analysis of crystal data has been performed. The \( d(H...O) \) and \( \theta(C-H...O) \) distributions were calculated from 26 RNA and 12 RNA/protein structures deposited in the Nucleic Acid Data Base (NDB: Berman et al., 1992; Figure 2). The congruence between the computed and experimental distributions of the C2-H2(n)...O4(n+1) distances and angles supports strongly the occurrence of such intra-strand inter-
actions in helical RNA regions. For comparison, the distributions corresponding to the well-recog-
nized C6/8-H...O5₀ contact between the base and the O5₀ backbone atom of the same residue, occurring in A and B-DNA as well as in RNA molecules (Saenger, 1984; Jeffrey & Saenger, 1991; Wahl & Sundaralingam, 1997), have been plotted. All these distributions cluster in a region delimited by average H...O distances in the 2.5 to 2.7 Å range and average C-H...O angles in the 136 to 149° range. These values are within the limits proposed by Taylor & Kennard (1982) for C-H...O hydrogen bonds (C...O distances between 3.0 and 4.0 Å with C-H...O angles between 90 and 180°) and close to...
the average values found for C-H...O interactions in proteins (Derevena et al., 1995).

C-H...O hydrogen bonds are now widely recognized as belonging to key structuring interactions (Wahl & Sundaralingam, 1997). They have found numerous applications in the design of imaginative and elegant supramolecular structures (Desiraju, 1995, 1996). They occur repetitively in protein tertiary motifs (Derewenda et al., 1995), with resolutions in the 1.4 to 3.0 Å range (H-atom positions have been calculated using a C-H bond length of 1.09 Å). For all distributions, data from nucleotides in a non-helical conformation were excluded (see Figure 1b). A detailed analysis indicated that distributions calculated from high-resolution crystal structures are statistically similar to distributions including data from low-resolution structures. The areas in white contain more than 97% of the points. Points outside these areas correspond to residues in specific structural contexts.

![Figure 2. Geometrical characteristics of the C-H...O interactions.](image)

Figure 2. Geometrical characteristics of the C-H...O interactions. Left: Computed distributions of C-H...O angles versus H...O distances for the C-H...O interactions extracted from our 3 ns MD simulations set of the tRNA anticodon hairpin. Right: Experimental distributions of C-H...O angles versus H...O distances for 26 RNA and 12 RNA/protein crystal structures, deposited in the NDB (Berman et al., 1992), with resolutions in the 1.4 to 3.0 Å range (H-atom positions have been calculated using a C-H bond length of 1.09 Å). For all distributions, data from nucleotides in a non-helical conformation were excluded (see Figure 1b). A detailed analysis indicated that distributions calculated from high-resolution crystal structures are statistically similar to distributions including data from low-resolution structures. The areas in white contain more than 97% of the points. Points outside these areas correspond to residues in specific structural contexts.

Orientation of the 2'-OH Group in RNA

...atoms have been described in Z-DNA structures (Egli & Gessner, 1995) as well as between sugar C-H groups and O4 atoms in an intercalation motif adopted by cytosine-rich DNA molecules (Berger et al., 1996). In that structure, anomalous short phosphorus-phosphorus distances coincide with the occurrence of a systematic network of C-H...O hydrogen bonds involving deoxyribose donor (C1′-H, C4′-H) and acceptor (O4) groups. Hence, C-H...O interactions involving the acidic H2/6/8 base hydrogen atoms and the less acidic sugar C-H groups must be considered as structurally important.

Yet, intriguingly, in RNA molecules, an inter-ribose axial C-H...O interaction is preferred over a supposedly stronger O2-H(n)...O4(n + 1) hydrogen bond, although similar examples in which C-H...O interactions “disturb” networks of O-H...O and N-H...O hydrogen bonds are known (for example, see Desiraju, 1995, 1996; Engh et al., 1996; Wahl et al., 1996b). Several explanations for the occurrence of such a distinctive interaction scheme can be proposed. Within the hard and soft acid and base (HSAB) principle (see Desiraju, 1995), the soft O2 acceptor would prefer to form a hydrogen bond with the proximal C2′-H2′ group, while the harder O2-H hydrogen bond donor would rather bind to water and/or interact with the O2 atom of the same ribose or the backbone O5 atom (Figure 1). The assessment that hydroxyl groups prefer to interact with water molecules is supported by recent crystal structures of RNA duplexes where O2 atoms are observed to be rather heavily hydrated (Egli et al., 1996; Wahl et al., 1996a). MD simulations reveal further that, while an average of two water molecules are making hydrogen-bonded contacts to the 2′-OH group, almost no water molecules are observed in the vicinity of the O4 (Auffinger & Westhof, 1997) and the C2′-H2′ atoms (Auffinger et al., 1996a). Given the above described unavailability of the O2-H(n) donor, the C2′-H2′(n) group remains the only possible hydrogen-bonding partner for the cyclic O4(n + 1) oxygen atom. Thus, the notorious deficit of hydrogen bond donors in nucleic acids is compensated by the formation of C-H...O hydrogen bonds such as ribose-to-ribose C2′-H2′(n)...O4(n + 1) and base-to-backbone C6/8-H...O4′ interactions. Additionally, it can be noted that, although the enthalpic contribution of C-H...O interactions is weaker than those of the more classical O-H...O hydrogen bonds, the entropic part of the C-H...O interactions must be in favour of the C2′-H2′(n)...O4(n + 1) interactions compared to the O2-H(n)...O4(n + 1) hydrogen bonds, since in the latter the mobile 2′-OH group would be restrained in a sterically penalized orientation (H2-C′-O4-H dihedral angle close to zero).

Similarly, a screen of the NDB (Berman et al., 1992) for C-H...O interactions occurring in A-DNA structures, characterized by C5′-endo sugar puckers, revealed the presence of intra-strand C2′-H2′(n)...O4(n + 1) hydrogen bonds (Figure 3).
Thus, such intra-strand C-H...O hydrogen bonds are a distinctive feature of C3-endo sugar pucker in both A-DNA and RNA helices and contribute, besides specific hydration motifs and base-pair stacking, to the axial stabilization of A-form nucleic acid helical structures.

From an energetic point of view, such C-H...O interactions, which have to be considered as essentially electrostatic, represent clearly a small enthalpic contribution to the global free energy of the system, although this contribution is difficult to quantify. Several experimental and theoretical studies led to the consensus view that typical values for C-H...O interactions are comprised in the 0.5 to 2 kcal/mol range where they can compete with regular hydrogen bonding interactions (for a review, see Steiner, 1996). Yet, as noted in this and other studies, energetically weak interactions such as C-H...O, C-H...N, O-H...π and C-H...π, display in some contexts structure-determining influences, especially when locally a deficit of hydrogen bond donor or acceptor groups occurs (Desiraju, 1995; 1996; Steiner, 1996), as is the case with nucleic acids.

Hydration of 2'-OH groups

Besides direct intramolecular hydrogen bonding, hydroxyl groups contribute to the stability of RNA molecules by providing a scaffold for the formation of a water network in the shallow groove (Egli, 1996; Egli et al., 1996). From the calculated distribution of water molecules, three well-defined water clusters surrounding the 2'-hydroxyl groups could be localized. These water clusters can be linked to the three favoured orientations of the hydroxyl groups and are located over the O3′ and O5′ atoms of the same residue and in the “Base” domain (see Figure 1c to f). In the MD simulations, the average number of water molecules surrounding the O3′ atom is close to 2.5, the occupancy of the base domain being close to 1.1, that of the O5′ domain close to 0.8 and that of the O4′ domain close to 0.6. In each orientation, water molecules are found in a position where they can satisfy the acceptor character of the RNA hydroxyl group without disturbing its donor potential. Such a distribution of water molecules is energetically favourable, since a rotation of the hydroxyl group perturbs only slightly the network of the nearby solvent molecules. Water molecules occupying the “Base” domain and linking hydroxyl groups to the N3(R) or O2(Y) base atoms have been described in crystal structures of tRNA molecules (Westhof et al., 1988) and other RNA systems (Westhof & Beveridge, 1990; Egli et al., 1996). In a high-resolution structure of an RNA duplex where all water molecules in the first hydration shell could be located, an average number of two to three water molecules in the vicinity of the hydroxyl groups was observed (Egli et al., 1996). These water molecules were found to form four clusters, three of them being exactly superimposable on those observed in our simulations (see Figure 1 of Egli et al., 1996). The fourth and smallest cluster, located above the O4′(n + 1) oxygen atom, results mainly from crystal packing effects. The close agreement between these calculated and experimental water cluster positions confirms our findings concerning the three favoured orientations of the 2'-hydroxyl groups.

RNA/RNA and RNA/protein interactions

It is noteworthy that water molecules, especially those located in the “Base” domain, are often replaced by hydroxyl groups belonging to an adjacent helix in RNA/RNA packing contacts of crystal structures (Figure 4; Dock-Bregeon et al., 1989; Schindelin et al., 1995; Cate et al., 1996; Masquida & Westhof, 1998). In ribose zipper motifs (Dock-Bregeon et al., 1989; Schindelin et al., 1995; Cate et al., 1996), a 2'-hydroxyl group of a helical sugar replaces the water molecule located in the purine and pyrimidine “Base” domain of an adjacent helical sugar (Figures 4 and d). Although water molecules located in the “Base” domain do not generally form long-lived hydrogen-bonding patterns as a result of their rotational dynamics and exchange frequently with neighbouring solvent molecules (Auffinger & Westhof, 1997), tertiary contacts involving hydroxyl groups result in confinement of the orientation of the involved 2'-OH groups in the “Base” or OY domains with interacting groups located in the same domains (Figure 4d). Thus, such tertiary interactions display increased dynamic stability over interactions involving water molecules.
Figure 4. Inter-residue contacts involving 2'-OH groups. a, For riboses, the water molecule in the “Base” domain of the 2'-hydroxyl group (see Figure 1f) can be replaced by a hydroxyl group of a second ribose leading to a “ribose zipper like” motif. b, In the general case, the “Base” domain of sugars in the C3'-endo conformation can be occupied, besides water molecules, by amino groups forcing a O3' orientation for the 2'-OH bond (left) or a hydroxyl group forcing a “Base” orientation for the 2'-OH bond (right). c, Snapshot taken from a MD trajectory showing a water molecule located in the “Base” domain of a cytosine residue. The 2'-OH bond points towards the O3 atom. Such water molecules found in the “Base” domain are rapidly exchanging with neighbouring solvent molecules (Aufinger & Westhof, 1997). d, Snapshot extracted from a 500 ps MD simulation of the entire tRNAAsp molecule (Aufinger et al., 1996a) showing a dynamically stable tertiary contact between the 2'-OH groups of the U8 and A46 riboses (both in the C3'-endo conformation) and the N1 atom of base A21 (in the crystal structure, d(U8)O2...N1[A21]) = 3.4 Å and d(U8)O2...O2[A46] = 3.4 Å; Westhof et al., 1985). For both sugars, the 2'-OH bond lies in the “Base” domain (see Figure 1) and the atoms interacting with the 2'-hydroxyl groups are occupying the “Base” and O3 hydration sites. This view exemplifies the water-like behaviour of the 2'-hydroxyl groups, which interact here in a way analogous to the ribose zipper motifs described in numerous RNA crystal structures (Dock-Bregeon et al., 1989; Schindelin et al., 1995; Cate et al., 1996).
In short, when not participating in tertiary interactions, the hydroxyl group displays a water-like behaviour and helps to delineate a hydrophilic pocket often occupied by rapidly exchanging water molecules. It may thus be concluded that strong hydration sites, as observed around the 2'-hydroxyl group, point to possible RNA-RNA and RNA/protein interaction sites. More specifically, the "Base" domain may be considered as a possible strong binding site for hydroxyl and amino groups belonging to RNA, proteins or antibiotics, as observed for adenine amino groups (Figure 4b) in sheared G-A base-pairs between residues 13 and 22 in the crystal structures of tRNA<sup>A<sub>D</sub></sup> (Biou et al., 1994), tRNA<sup>G<sub>B</sub></sup> (Rould et al., 1991), as well as in the tandem G-A base-pairs of the hammerhead ribozyme (Pley et al., 1994).

### 2'-OH orientations for C<sub>2</sub>-endo sugars

In specific structural contexts and outside regular helices, RNA riboses adopt C<sub>2</sub>-endo conformations. In tRNA<sup>A<sub>D</sub></sup>, ten riboses (out of 75 nucleotides) involved in tertiary interactions or in intercalation motifs are found in such a conformation (Westhof et al., 1985). From a 500 ps MD simulation of the full tRNA<sup>A<sub>D</sub></sup> molecule (Auffinger et al., 1996a), the following stereochemical rules were derived for the 2'-hydroxyl bond orientation of sugars in the C<sub>2</sub>-endo conformation. The calculated distribution of the H<sub>2</sub>-C<sub>2</sub>-O<sub>2</sub>-H dihedral angle indicates that again the O<sub>2</sub>-O<sub>1</sub>-H orientation is strongly favoured (Figure 5). This O<sub>2</sub>-domain is, however, shifted from 100° to 160° as a result of the trans conformation adopted by the ribose C<sub>3</sub>-C<sub>4</sub> dihedral angle in a C<sub>2</sub>-endo sugar pucker. Orientations toward the H<sub>1</sub> and H<sub>2</sub> atoms are stericly hindered, given average H<sub>1</sub>-H<sub>2</sub> distances of 2.6 Å. All other possible orientations are not observed with the exception of those characterized by a bulge around 300° and a bulge close to 30° in the distribution profile (see Figure 5). The latter corresponds to the 2'-OH group of residue A7, which is hydrogen-bonded to the (m<sup>1</sup>C<sub>4</sub>)pro-R<sub>9</sub> atom (in the crystal, d[pro-R<sub>9</sub>...O<sub>2</sub>] = 2.8 Å, (Westhof et al., 1985)). For the other residues, orientations corresponding to H<sub>2</sub>-C<sub>2</sub>-O<sub>2</sub>-H dihedral angle close to zero are avoided as is the case for sugars adopting the C<sub>3</sub>-endo pucker.

### Summary and Biological Significance

The present results show that, for sugars adopting the typical RNA C<sub>3</sub>-endo ribose conformation in helical as well as non-helical regions, the 2'-OH bond points preferentially towards the O<sub>2</sub> or O<sub>1</sub> atoms of the same residue or towards the N<sub>9</sub>(R) or O<sub>4</sub>(Y) atoms of the attached base with a strong preference for the O<sub>2</sub>-orientation (Figure 1). Similarly, in the rarer RNA C<sub>2</sub>-endo ribose conformation, occurring in non-helical structural contexts, the 2'-OH bond points preferentially towards the O<sub>3</sub>-atom as a result of favourable intra-residue electrostatic interactions (Figure 5).

Yet, in helical conformations characterized by C<sub>3</sub>-endo sugar puckers, the ribose-to-ribose O<sub>2</sub>-H(n)...O<sub>4</sub>(n+1) intra-strand hydrogen bond is generally not formed. Instead, a recurrent C-H...O hydrogen bond between the C<sub>2</sub>-H<sub>2</sub>(n) group and the O<sub>2</sub>(n+1) oxygen atom is found to contribute to RNA axial stabilization. This contact fulfils the hydrogen-bonding acceptor potential of the ribose O<sub>4</sub> oxygen atom, which is not accessible to water in RNA helical conformations (Auffinger & Westhof, 1997). Consequently, the functionally important 2'-hydroxyl group is not monopolized by the tedious task of ensuring axial stabilisation in RNA as frequently proposed.

Instead, for each of the three orientations of the 2'-OH bond, the RNA conformation accommodates water molecules interacting with the lone pairs of
the hydroxyl group and with the hydroxyl proton. Thus, after water displacement or through water-mediated interactions, the shallow groove 2'-OH group becomes available for establishing new RNA/protein or RNA/RNA tertiary contacts, especially in the “Base” and O$_3$ domains.

The present stereochemical rules could be of help in the interpretation of X-ray or NMR data, as the hydrogen position of the 2'-OH group cannot be generally detected by any of these methods. It is noteworthy that NMR refinements are generally performed using in vacuo protocols that neglect solvation effects. Under such conditions O$_{2i}$-H$_2(n)...$O$_{4i}(n + 1)$ are almost systematically formed contrary to what is observed when explicit water molecules are included in the calculation (see also Miller & Kollman, 1997). NMR and crystal structure refinement procedures should also benefit from an explicit consideration of C-H...O and other weak interactions.

Furthermore, the hydroxyl groups are often the target of biochemical experiments where they are either removed or methylated in order to prove the existence of specific tertiary interactions or to modify the RNA hydrolytic and thermal stability (Gesteland & Atkins, 1993; Eckstein & Lilley, 1996). The knowledge of the allowed orientations of hydroxyl groups, together with the recognition of the importance of C-H...O and other “weak” interactions, could contribute to a finer interpretation of the results of such experiments as well as to a better understanding and design of RNA-binding molecules.

**Computational Methods**

Coordinates of the tRNA molecule and of the anticodon hairpin were extracted from the tRNA$_{Asp}$ crystallographic structure (NDB code tRNA07; Westhof et al., 1985) and were used to generate six 500 ps multiple molecular dynamics (MD) trajectories (Auffinger et al., 1995) totalling 3 ns of simulation of the anticodon hairpin (Auffinger & Westhof, 1996, 1997) and a 500 ps MD trajectory of the tRNA$_{Asp}$ molecule (Auffinger et al., 1996a). The anticodon hairpin was neutralized by 16 NH$_4^+$ counterions and solvated by 1143 SPC/E water molecules. The tRNA molecule was neutralized by 75 NH$_4^+$ counterions and solvated by 8055 SPC/E water molecules. The particle mesh Ewald summation method (Darden et al., 1993; Essmann et al., 1995), as implemented in the AMBER 4.1 suite of programs (Pearlman et al., 1994), was used for the treatment of the long-range electrostatic interactions (Louise-May et al., 1996), including an explicit representation of all RNA hydrogen atoms. The atomic partial charges were derived from low-temperature X-ray studies of isolated nucleotides (Pearlman & Kim, 1990). More details on the parameters and equilibration protocols can be found in the following references (Auffinger et al., 1996a; Auffinger & Westhof, 1996, 1997).

The tRNA$_{Asp}$ anticodon hairpin includes a five-base pair stem containing a ‘wobble’ GU pair and a seven-nucleotide loop with a U-turn at position 33, a recurrent structural motif of complex folded functional RNA molecules. For all MD simulations, no flip of the sugar puckers from C$_3$-endo to C$_2$-endo (or reverse) was observed (Auffinger & Westhof, 1996).

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