RNA Conformations

DOI: 10.1002/anie.201007844

Determination of the Conformation of the 2′OH Group in RNA by NMR Spectroscopy and DFT Calculations**

Senada Nozinovic, Puneet Gupta, Boris Fürtig, Christian Richter, Sandor Tüllmann, Elke Duchardt-Ferner, Max C. Holthausen, and Harald Schwalbe**

In contrast to DNA, RNA exhibits pronounced functional, structural, and chemical diversity, which has been considered essential for the evolution of the RNA world.[1] The presence of the 2′OH group induces a change in the predominant sugar conformation and provides catalytic activity, but at the same time it reduces the chemical stability of RNA. 2′OH groups are involved in hydrogen-bonding interactions in noncanonical regions and are responsible for the formation of a well-ordered water network spanning the minor groove of the RNA helix.[2] It is therefore of considerable interest to determine experimentally the conformation of the 2′OH group.

Molecular dynamics (MD) simulations suggest three preferred orientations for the 2′OH group in the C3′-endo sugar conformation (Figure 1), while for the C2′-endo sugar conformation exclusively O3′-domain orientations are predicted.[3] Experimental determinations of this exocyclic torsion angle pose a serious challenge in RNA structure analyses, because the hydrogen atoms of 2′OH groups are only visible in electron density maps derived by X-ray crystallography at resolutions <1 Å and therefore their positions generally remain undetectable. Neutron diffraction is an alternative method for proton detection but reports are sparse, especially for large RNAs. Recent low-temperature NMR spectroscopic studies enabled conformational analyses involving the 2′OH proton.[4] Such studies rely on limited chemical exchange of the hydroxy group with the solvent. 2′OH groups of nucleotides in noncanonical regions of RNA are, however, in rapid exchange with hydrogen atoms of the solvent and the corresponding signals cannot be detected. Therefore the direct measurement of structural parameters restraining the exocyclic torsion angle remains impossible.

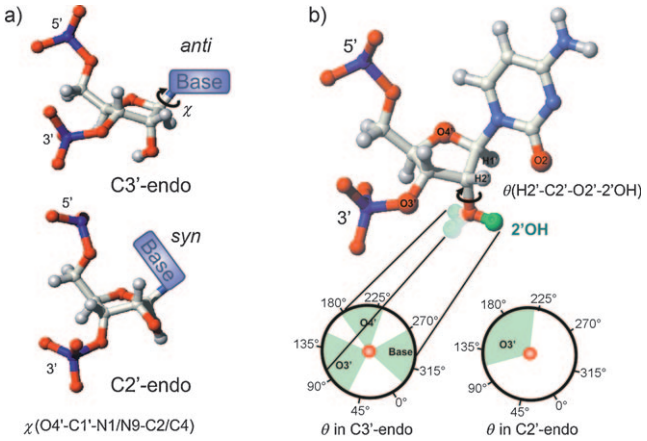

Figure 1. a) Representation of the sugar and nucleobase conformations by two exemplary structures with C3′-endo/*anti* and C2′-endo/*syn* conformations. b) The 2′OH orientation. The structure represents the three favored orientations of the 2′OH group (highlighted in green) in which it points towards O3′, O4′, or the nucleobase domain. Conformational wheels represent the distribution of the torsion angles θ(H2′-C2′-O2′-2′OH) for sugars identified in MD simulations.[3]

Based on the interpretation of ^1J(C,H) coupling constants we suggest here a general method for the conformational analysis of sugar pucker modes and nucleobase orientations as well as for the determination of 2′OH group conformations in large RNAs. In contrast to ^3J(H,H) coupling constants, which are typically well approximated by a conformational dependence on a single torsion angle (Karplus relationship), ^1J(C,H) coupling constants are sensitive to C–H bond lengths,[5] which in turn are influenced by local conformations and thus depend on multiple torsion angles.[5b,6] As a consequence, ^1J couplings are generally difficult to translate into conformational information.

The present work was inspired by reports from several groups, in which the influence of hyperconjugative effects ($n\rightarrow\sigma^*/\sigma\rightarrow\sigma^*$ donation) on ^1J(C,H) coupling constants in ethanol and methylamine was demonstrated.[7] Based on these findings we anticipated that the lone electron pairs of the 2′OH group in RNA should exert a corresponding influence on the ^1J(C,H) coupling constants of the adjacent bonds at C1′ and C2′ ring positions in a systematic and

[*] Dipl.-Chem. S. Nozinovic, Dr. B. Fürtig, Dr. C. Richter, Prof. Dr. H. Schwalbe
Johann Wolfgang Goethe-Universität
Zentrum für biomolekulare magnetische Resonanzspektroskopie
Max-von-Laue Strasse 7, 60439 Frankfurt (Germany)
Fax: (+49) 69-7982-9515
E-mail: schwalbe@nmr.uni-frankfurt.de
Dr. E. Duchardt-Ferner
Johann Wolfgang Goethe-Universität, Institut für Biochemie
Max-von-Laue-Strasse 7, 60438 Frankfurt (Germany)
M. Sc. P. Gupta, Dipl.-Chem. S. Tüllmann, Prof. Dr. M. C. Holthausen
Johann Wolfgang Goethe-Universität
Institut für anorganische und analytische Chemie
Max-von-Laue-Strasse 7, 60438 Frankfurt (Germany)
Fax: (+49) 69-798-29417
E-mail: max.holthausen@chemie.uni-frankfurt.de

[**] We thank Beatrix Suess and Julia E. Weigand for providing the 27-mer riboswitch RNA and Jens Wöhnert for helpful discussions. This work was supported by the state of Hesse (BMRZ), the DFG (SFB579: "RNA-Ligand-Interaction"), the Studienstiftung des Deutschen Volkes (grants to S.N. and B.F.), and CitySolar AG (grant to P.G.). H.S. is member of the DFG-funded Cluster of Excellence: Macromolecular Complexes.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201007844.

predictable way. By mapping this dependence it should be possible to determine the conformation of the 2′OH group.

In order to dissect the multiple conformational dependencies of ^1J(C,H) in RNA, we compared experimental NMR data with predictions from DFT calculations. Firstly, DFT calculations of ^1J(C,H) scalar coupling constants in nucleotide-like model structures were performed to confirm their dependence on different sugar and nucleobase conformations. Secondly, the influence of 2′OH group orientations for fixed sugar and nucleobase conformations was investigated by systematic scans of the 2′OH group orientation. The results revealed in fact that the sugar puckers and the nucleobase conformations, as well as the 2′OH group orientations all have an appreciable influence on ^1J(C,H) coupling constants. However, for known sugar and nucleobase conformations, 2′OH group orientations can be determined precisely solely by interpretation of ^1J(C,H) coupling constants. This is possible even for residues whose 2′OH proton cannot be detected by NMR spectroscopy owing to exchange with solvent H_2O or D_2O. ^1J(C,H) coupling constants can generally be measured at high resolution with high sensitivity so that the procedure suggested here is applicable also to large RNAs.

For DFT calculations the B3LYP/TZVP(+PCM:water) level of theory was chosen after a careful series of benchmark calculations of the NMR spin–spin coupling constants for relevant model systems (see Tables S1 and S2 in the Supporting Information). Then, for each nucleotide type, four representative molecular models with the main conformations characteristic for RNA (C3′-endo/*anti* or *syn*, C2′-endo/*anti* or *syn*) were constructed from the ribosome crystal structure (pdb code: 2ffk)[8] and the previously solved high-resolution 14-mer RNA solution structure (pdb code: 2koc)[4e] for calculations of the ^1J(C,H) coupling constants (see Table S6 in the Supporting Information). Orientations of the individual 2′OH groups were constrained in these calculations to the O3′ domain with torsion angles θ fixed at 100° for the C3′-endo and 160° for the C2′-endo conformation, respectively. The construction of the molecular models and the constraints introduced for partial geometry optimizations are described in detail in the Supporting Information.

Figure 2a shows a map of the resulting ^1J(C,H) coupling constants predicted for the four nucleobases assuming either a *syn* or an *anti* orientation for both sugar conformations (C3′-endo or C2′-endo), respectively. The calculated data show clearly that the sugar conformation has a significant effect on both coupling constants, while the nucleobase conformation (*syn* or *anti*) mainly influences ^1J(C1′,H1′). With computed coupling constants spanning a range of 14 Hz, a reliable differentiation of the four conformations is possible by comparison to measured ^1J(C,H) coupling constants. We therefore suggest the chart in Figure 2a as a simple means for determining RNA conformation and we tested it by analysis of a 27-mer riboswitch RNA, the structure and dynamic features of which were recently reported in an NMR study.[9a,b] The detailed results of this cross-validation are presented in the Supporting Information (Figure S8). The conformations of a total of 21 residues can be assigned unambiguously by comparison with Figure 2a. The remaining

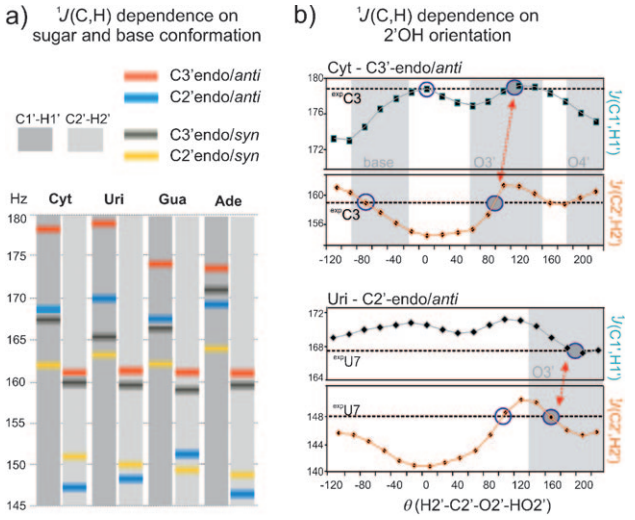

Figure 2. a) DFT-based ^1J(C1′,H1′) and ^1J(C2′,H2′) coupling constants calculated for each nucleotide (cytosine, uridine, guanosine, adenosine) adopting four mainly populated conformations defined by the individual sugar puckers (C3′- and C2′-endo) and nucleobase orientations (*anti* and *syn*). b) Computed ^1J(C,H) coupling constants for residues C3 and U7 as a function of the torsion angle θ(H2′-C2′-O2′-2′OH). Experimental values for C3 and U7 of the 14-mer RNA are indicated by dashed lines. Best fit between experimental and calculated values is indicated by the colored circles. 2′OH orientations most populated according to MD simulations are shadowed in gray.

five residues are characterized experimentally by averaged coupling constants (Figure S9 in the Supporting Information), which cannot be assigned to a single conformation. Indeed, those residues are placed within the bulge region and at the 3′-terminus of the RNA, and it is known from the original NMR study that these residues exhibit higher flexibility.[9b]

In a second set of DFT calculations, the dihedral angle θ(H2′-C2′-O2′-2′OH), which describes the orientation of the exocyclic 2′OH group, was varied from 0° to 340° in steps of 20°. Figure 2b shows the results obtained for a cytosine and for a uridine model constructed from C3 and U7 in the 14-mer solution structure (Figure 3a), with the sugar in C3′- and C2′-endo conformation, respectively, while the bases adopt *anti* conformations. We observed that the orientation of the 2′OH group exerts considerable influence on the ^1J(C,H) coupling constants: ^1J(C1′,H1′) couplings vary by 6 Hz (Cyt) and 3 Hz (Uri); ^1J(C2′,H2′) couplings, with differences of up to 7 Hz (Cyt) and 9 Hz (Uri), are even more sensitive. Agreement between the experimental and computed ^1J(C,H) couplings for these residues in the 14-mer is found only for one respective 2′OH group orientation (Figure 2b); for both nucleotides the same orientations had been derived from MD simulations.[4e]

Previously, comprehensive NMR measurements allowed us to determine the high-resolution solution structure (0.3 Å) of a 14-mer UUCG hairpin RNA.[4e] Analysis of homonuclear couplings and cross-correlated relaxation rates revealed that the sugar moieties of the stem nucleotides and of the hydrogen-bonded loop nucleotides U6 and G9 adopt C3′-endo conformations. The two loop nucleotides U7 and C8 adopt C2′-endo conformations and do not undergo conformational averaging on the NMR time scale (Figure 3a).[4e] The

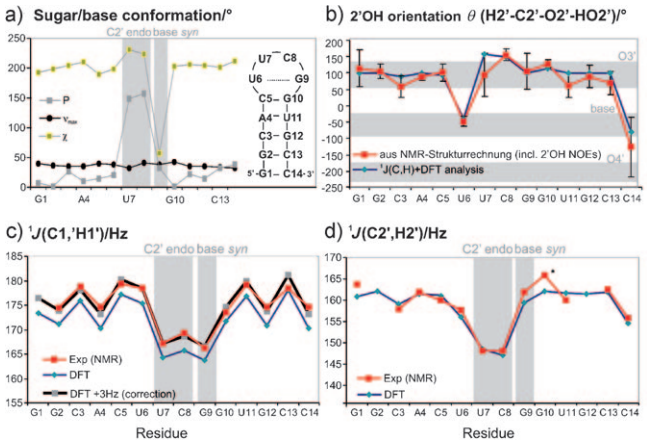

Figure 3. a, b) Secondary structure and conformation of the 14-mer RNA: nucleobase orientations described by the torsion angle χ, sugar puckers described by P and ν_{max},[14] and 2′OH group orientations described by the torsion angle θ were extracted from 20 lowest-energy structures. Loop residues with the unusual *syn* nucleobase orientation for G9 and C2′-endo sugar conformation for U7 and C8 are highlighted with gray shadows in (a), (c), and (d). Orientations toward O3′, nucleobase, and O4′ domains as predicted by MD simulations (Figure 1) are shadowed in gray (b). c, d) Experimental (NMR) and DFT computed ^1J(H1′,C1′)/^1J(H2′,C2′) coupling constants. DFT computed data for ^1J(C1′,H1′) are shown with and without a linear shift of +3 Hz. The experimental ^1J(C2′,H2′) coupling of G10 is marked by an asterisk (see text).

nucleobase of residue G9 adopts an unusual *syn* conformation.[10] In our previous work, NOE contacts of the 2′OH group were analyzed to determine the preferred orientation of the ribose moiety. The final structure bundle, which is consistent with the NOE analysis, indicates that the 2′OH groups of the stem mainly adopt the O3' domain orientation and that the 2′OH group of U6, which is located in the loop region, is oriented towards the nucleobase domain (Figure 3b). For loop residues U7, C8, and G9 and the terminal residues G1 and C14, NOE analysis could not be performed since no NMR signals of the 2′OH protons could be recorded owing to fast exchange with water. Hence, the 2′OH group orientations suggested for these residues in previous work resulted solely from the ARIA force field refinement[11] without experimental constraints and are thus only weakly defined.

The experimentally derived ^1J(C,H) coupling constants of the 14-mer RNA structure measured in H_2O are depicted in Figure 3c,d (Measurements in D_2O resulted in identical values; Figure S1 in the Supporting Information). ^1J(C,H) coupling constants cluster systematically around larger values for C3′-endo and lower values for C2′-endo conformations. As a notable exception, ^1J(C1′,H1′) for residue G9 does not follow the trend for C3′-endo conformations. This deviation is caused by the *syn* orientation of the nucleobase for G9.

We computed all coupling constants of the 14-mer RNA by DFT employing molecular models for the individual residues constructed from the high-resolution solution structure, constraining the 2′OH groups to the experimentally derived orientations (Figure 3; see the Supporting Information for details). Figure 3c and d show that the computed ^1J(C,H) coupling constants reproduce the experimental data very well, both in terms of the absolute values and in terms of conformational dependence, with the only exception of G10 (marked with an asterisk in Figure 3d). We note, however, that other data hint at the presence of additional conformational exchange phenomena of G10.[12] In the observed range of coupling constants (140–180 Hz), the average deviations from experimental data amount to 4 Hz and 1.7 Hz for ^1J(C1′,H1′) and ^1J(C2′,H2′) couplings, respectively. A reduced average error of only 1 Hz in ^1J(C1′,H1′) couplings is obtained by application of an empirical constant linear shift of +3 Hz, such that the maximum error for all coupling constants is 2.9 (G10 excluded).[13] The combined DFT and NMR analysis provides accurate information on 2′OH orientations of the 14-mer RNA and makes it possible to determine the conformation of the 2′OH protons that remain undetectable experimentally. The results presented above (Figure 2b) demonstrate that ^1J(C,H) calculations based on systematic scans of the torsion angle θ in appropriately constructed molecular models permit the unambiguous characterization of the 2′OH group.

In summary, we have investigated the conformational dependence of ^1J(C,H) coupling constants in RNAs as readily measureable NMR parameters, and we have demonstrated a marked dependence of ^1J(C1′,H1′) and ^1J(C2′,H2′) on the conformation of the sugar and the nucleobase, and the 2′OH group orientation. Experimental and theoretical results are in very good agreement, which confirms the high accuracy of the structure as well as the quantum chemical approach chosen. We have shown that ^1J(C1′,H1′) and ^1J(C2′,H2′) coupling constants can serve as valuable probes for ribose and nucleobase conformations in RNA molecules, and suggest their use as a new tool for conformational analysis. For the majority of nucleotides within an RNA molecule, the sugars assume either a C3′-endo or a C2′-endo conformation, while nucleobases can adopt *anti* and *syn* orientations.[15] The qualitative interpretation of the ^1J(C,H) couplings constitutes a reliable method to discriminate between the four conformational extremes with an accuracy sufficient for structure calculation. Our procedure is particularly useful for the structure determination of larger RNAs, since ^1J(C,H) coupling constants can be measured readily even without the use of isotope-labeling techniques. The utility of this approach is further demonstrated for the 27-mer riboswitch RNA in an unambiguous conformational analysis of rigid parts of the structure and identification of flexible regions. So far, the conformational characterization of 2′OH groups—which determine the unique conformational properties of RNA compared to DNA—was possible only in rare cases. The marked dependence of ^1J(C,H) coupling constants on 2′OH group orientations demonstrated here facilitates reliable indirect determination of this elusive structural parameter even under rapid-exchange conditions.

Received: December 13, 2010
Published online: April 20, 2011

Keywords: conformation analysis · density functional calculations · NMR spectroscopy · RNA

[1] W. Gilbert, *Nature* **1986**, *319*, 618.
[2] M. Egli, S. Portmann, N. Usman, *Biochemistry* **1996**, *35*, 8489.
[3] P. Auffinger, E. Westhof, *J. Mol. Biol.* **1997**, *274*, 54.
[4] a) M. Hennig, J. Fohrer, T. Carlomagno, *J. Am. Chem. Soc.* **2005**, *127*, 2028; b) J. Ying, A. Bax, *J. Am. Chem. Soc.* **2006**, *128*, 8372; c) J. Fohrer, M. Hennig, T. Carlomagno, *J. Mol. Biol.* **2006**, *356*, 280; d) J. Fohrer, U. Reinscheid, M. Hennig, T. Carlomagno, *Angew. Chem.* **2006**, *118*, 7191; *Angew. Chem. Int. Ed.* **2006**, *45*, 7033; e) S. Nozinovic, B. Fürtig, H. R. A. Jonker, C. Richter, H. Schwalbe, *Nucleic Acids Res.* **2010**, *38*, 683.
[5] a) C. Thibaudeau, R. Stenutz, B. Hertz, T. Klepach, S. Zhao, Q. Wu, I. Carmichael, A. S. Serianni, *J. Am. Chem. Soc.* **2004**, *126*, 15668; b) F. Cloran, I. Carmichael, A. S. Serianni, *J. Phys. Chem. A* **1999**, *103*, 3783.
[6] a) D. B. Davies, P. Rajani, M. MacCoss, S. S. Danyluk, *Magn. Reson. Chem.* **1985**, *23*, 72; b) M. L. Munzarová, V. Sklenar, *J. Am. Chem. Soc.* **2003**, *125*, 3649; c) J. T. Fischer, U. M. Reinscheid, *Eur. J. Org. Chem.* **2006**, 2074; d) Z. Vokáčová, F. M. Bickelhaupt, J. Šponer, V. r. Sychrovský, *J. Phys. Chem. A* **2009**, *113*, 8379; e) P. C. Kline, A. S. Serianni, *J. Org. Chem.* **1992**, *57*, 1772; f) C. A. Podlasek, W. A. Stripe, I. Carmichael, M. Shang, B. Basu, A. S. Serianni, *J. Am. Chem. Soc.* **1996**, *118*, 1413; g) A. S. Serianni, J. Wu, I. Carmichael, *J. Am. Chem. Soc.* **1995**, *117*, 8645; h) J. M. Duker, A. S. Serianni, *Carbohydr. Res.* **1993**, *249*, 281; i) J. H. Ippel, S. S. Wijmenga, R. de Jong, H. A. Heus, C. W. Hilbers, E. de Vroom, G. A. van der Marel, J. H. van Boom, *Magn. Reson. Chem.* **1996**, *34*, S156.
[7] a) S. Watanabe, I. Ando, Y. Sakamoto, *J. Mol. Struct.* **1982**, *82*, 237; b) R. H. Contreras, J. E. Peralta, *Prog. Nucl. Magn. Reson. Spectrosc.* **2000**, *37*, 321; c) N. C. Maiti, Y. Zhu, I. Carmichael, A. S. Serianni, V. E. Anderson, *J. Org. Chem.* **2006**, *71*, 2878.
[8] N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 905.
[9] a) J. E. Weigand, M. Sanchez, E.-B. Gunnesch, S. Zeiher, R. Schroeder, B. Suess, *RNA* **2008**, *14*, 89; b) E. Duchardt-Ferner, J. Weigand, O. Ohlenschläger, S. Schmidtke, B. Suess, J. Wöhnert, *Angew. Chem.* **2010**, *122*, 6352; *Angew. Chem. Int. Ed.* **2010**, *49*, 6216.
[10] a) E. Duchardt, C. Richter, O. Ohlenschläger, M. Görlach, J. Wöhnert, H. Schwalbe, *J. Am. Chem. Soc.* **2004**, *126*, 1962; b) J. Rinnenthal, C. Richter, J. Ferner, E. Duchardt, H. Schwalbe, *J. Biomol. NMR* **2007**, *39*, 17.
[11] a) M. Nilges, M. J. Macias, S. I. O'Donoghue, H. Oschkinat, *J. Mol. Biol.* **1997**, *269*, 408; b) J. P. Linge, S. I. O'Donoghue, M. Nilges, *Methods Enzymol.* **2001**, *339*, 71.
[12] A. Cherepanov, C. Glaubitz, H. Schwalbe, *Angew. Chem.* **2010**, *122*, 4855; *Angew. Chem. Int. Ed.* **2010**, *49*, 4747.
[13] A. Bagno, F. Rastrelli, G. Saielli, *Magn. Reson. Chem.* **2008**, *46*, 518.
[14] C. Altona, M. Sundaralingam, *J. Am. Chem. Soc.* **1972**, *94*, 8205.
[15] B. Schneider, Z. Moravek, H. M. Berman, *Nucleic Acids Res.* **2004**, *32*, 1666.

