RNA Structures

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Calculation of the Dependence of Homo- and Heteronuclear ^{3}J and ^{2}J Scalar Couplings for the Determination of the 2′-Hydroxy Conformation in RNA**

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The presence or absence of the 2′-hydroxy group within the pentose sugar accounts for the major difference between RNA and DNA. In canonical environments, the pentose sugars of RNA and DNA adopt C3′-*endo* and C2′-*endo* conformations, respectively, leading to distinct differences in the hydration and structural stability.[1] In addition to the conformational preferences imposed by the 2′-OH group, its vital role in RNA catalysis has been studied extensively for self-splicing viral ribozymes, type II introns and spliceosomal RNAs.[2–4] Thus, conformational studies of 2′-hydroxy groups provide unique insights into the structural features of RNA and shed light on the structure–activity relationship of RNA catalytic processes.

Recently, we reported the assignments and conformational analysis of the 2′-hydroxy groups in the 30-mer HIV-2 transactivation response element (TAR) RNA[5] in solution at low temperature,[6,7] while another study from Ying and Bax focuses on the conformational analysis of the 2′-hydroxy groups in the RNA hairpin derived from the helix 35 of the 23S ribosomal RNA.[8] Molecular dynamics (MD) simulations of an RNA hairpin previously identified three preferred orientations for the 2′-hydroxy proton when the ribose sugar adopts the C3′-*endo* pucker:[9] a) toward O3′, stabilized by attractive electrostatic interaction with the phosphate backbone (O3′ domain), b) toward O4′, stabilized by intra-ribose electrostatic interactions (O4′ domain) and c) toward the base, stabilized by interactions with the N3 or O2 atom of the attached base (base domain). In our previous study we reported preferences for the 2′-OH groups of canonical A-

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form helical regions to occupy the O3′ or base domains,[7] while the work of Ying and Bax places all 2′-OH groups of the hairpin RNA in the base domain.[8] Both findings support a stabilizing network of hydrogen bonds that spans the minor groove of the RNA helix and includes two water molecules in the base plane of nucleotides belonging to opposite strands in a base pair step. Such network of hydrogen bonds has been proposed in a high-resolution X-ray study of an RNA duplex.[10] Although ^3J(C1′,OH2) and ^3J(C3′,OH2) couplings could be obtained for several residues of the RNAs under investigation at low temperature, thus far the absence of a Karplus-like parametrization of the heteronuclear couplings impeded the quantitative analysis of the ^3J(C1′,OH2) and ^3J(C3′,OH2).

Here we present ab initio calculations, using density functional theory (DFT), to derive the Karplus-like relations for the heteronuclear and homonuclear ^3J(C1′/C3′/H2′,OH2) and ^2J(C2′,OH2) couplings involving the 2′-hydroxy group. Establishing the dependence of the ^3J(C1′/C3′/H2′,OH2) on the intervening dihedral angle H2′-C2′-O2′-OH is essential for a quantitative analysis of the measured scalar couplings and thus allows the accurate determination of the 2′-hydroxy group conformation.

The DFT calculations of the ^3J(C1′/C3′/H2′,OH2) and ^2J(C2′,OH2) couplings were performed using Gaussian 03 Revision C.02[11] on a ribose molecule bearing an amino group at C1′ along with a phosphodiester attached to the 3′-hydroxy group. A geometry optimization was carried out with the ribose in the C3′-*endo* conformation using a b3Lyp/6-31g(d) basis set as implemented in Gaussian.[12,13] The dihedral angles of the ribose ring remained within the typical ranges for the C3′-*endo* sugar pucker during the geometry optimization. The ^3J and ^2J couplings related to the orientation of the 2′-hydroxy group were calculated using a b3Lyp/6-311g(2df, 2pd) basis set. The dependence of the three vicinal ^3J(C1′/C3′/H2′,OH2) and the two-bond ^2J(C2′,OH2) scalar couplings on the intervening H2′-C2′-O2′-OH dihedral angle θ is shown in Figure 1 (see also Table S1 in the Supporting Information for the theoretical coupling values at each angle θ).

The calculated Karplus-like curve for the homonuclear ^3J(H2′,OH2) coupling compares well to the Karplus-like curve determined from experimental data on model compounds reported by Fraser et al.[14] and confirms the good quality of the model system used in the DFT calculations. The two curves for the heteronuclear ^3J(C1′/C3′,OH2) couplings are asymmetric and differ substantially from each other. In Figure 1 we compare the dependence of the ^3J(C1′/C3′,OH2) couplings on the H2′-C2′-O2′-OH dihedral angle calculated here (continuous lines) with a crude approximation of the Karplus-like curves used in our previous work.[7] This approximated dependence was obtained by scaling the coefficients of the ^3J(C,H) Karplus relation in alkenes to reflect the averaged value of the ^3J(C,OH) coupling in ethanol (dashed lines). In the theoretically calculated curve, the ^3J(C3′,OH2) coupling reaches a maximum of 6.5 Hz at $\theta =$ 302° (Figure 1b, green continuous line). The averaged coupling, assuming equal populations of the *gauche* +, *gauche* −, and *trans* conformations, is 2.3 Hz, in good qualitative agreement with the average coupling of 2.9 Hz

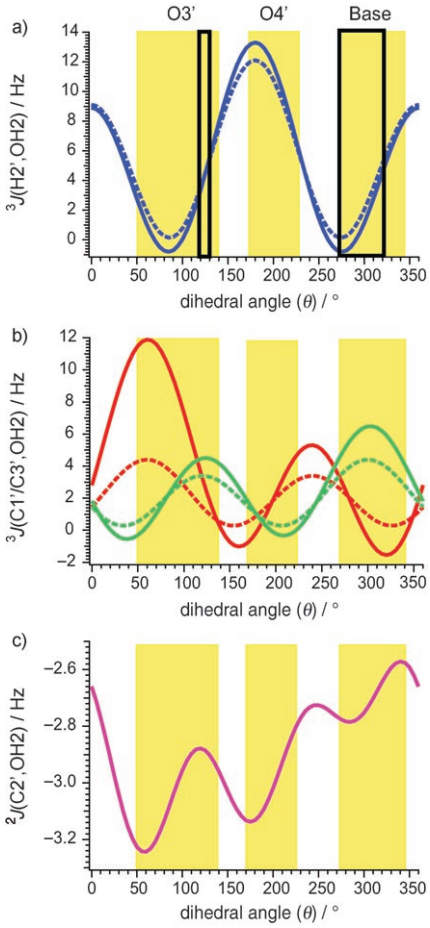

Figure 1. a) DFT-derived Karplus relations for the dependence of the ^3J(H2′,OH2) coupling on the dihedral angle H2′-C2′-O2′-OH, θ. The Karplus-like curve $^3J(H2',OH2) = 11.8\cos^2\theta - 2.2\cos\theta - 0.7$ derived by the DFT calculations is represented by the blue continuous line, while the Karplus-like curve determined by Fraser et al.[14] using two experimental values for 3β-acetoxy-5β,6β-oxidocholestan-19-ol, is shown by the dashed blue line for comparison. b) DFT-derived Karplus relations for the dependence of the ^3J(C1′,OH2) and ^3J(C3′,OH2) couplings on the dihedral angle H2′-C2′-O2′-OH. $^3J(C1',OH2) = 9.6\sin^2(\theta+30) + 3.3\sin(\theta+25.3) - 1.0$ (in red) and $^3J(C3',OH2) = 5.9\sin^2(\theta-33.2) - 1.0\sin(\theta-27.0) - 0.4$ (in green). The dashed lines represent the crude approximations derived in Reference [7] by scaling the coefficients of the ^3J(C,H) Karplus relation in alkenes to reflect the averaged value of the ^3J(C,OH) coupling in ethanol. c) DFT-derived relation for the dependence of the ^2J(C2′,OH2) coupling on the dihedral angle H2′-C2′-O2′-OH: $^2J(C2',OH2) = 0.6\cos^3(\theta+5.9) - 0.2\cos^2(\theta-46.1) - 0.4\cos(\theta-21.2) - 2.8$. Preferred conformational regions for the 2′-hydroxy proton identified by MD simulations are in yellow.[9] The black rectangles show the conformational regions in agreement with averaged and maximum scalar coupling values.

observed for the ^3J(C,OH) coupling in ethanol.[15] On the other hand, the ^3J(C1′,OH2) coupling adopts the maximum value of 11.6 Hz at $\theta = 62°$ (Figure 1b, red continuous line), which largely deviates from the crude approximation used previously (Figure 1b, red dashed line).[7] The ^2J(C2′,OH2) coupling assumes quite uniform values ranging from −3.3 to −2.6 Hz and shows only a moderate dependence on θ. These values are in very good agreement with those measured by Ying and Bax.[8]

To understand the reasons for the strong asymmetry of the two ^3J(C1′/C3′,OH2) couplings, we performed DFT calculations to obtain the ^3J(C1′/C3′,OH) value for the linear 1-(dimethylamino)-2-hydroxy-1-methoxybutane-3-methylphosphate at dihedral angles θ(H-C-O-H) = 60° and 300° (Table S1 in the Supporting Information). The very similar values obtained for the ^3J(C1′,OH2) and the ^3J(C3′,OH2) couplings at $\theta = 60°$ and 300°, respectively, indicate that the strong asymmetry of the couplings in the ribose results from the cyclic structure of the sugar. Additionally, we verified the possibility that the high value observed at 62° for the ^3J(C1′,OH2) coupling could be significantly reduced by the involvement of the 2′-OH in a hydrogen bond either as a proton donor or acceptor. However, the presence of a water molecule that forms a hydrogen bond with the 2′-OH reduces the maximum value of the ^3J(C1′,OH2) coupling by 0.8 Hz only.

The effect of the ribose conformation on the Karplus-like relationship of the ^3J(C1′/C3′/H2′,OH2) couplings was verified by calculating the three couplings at the diagnostic θ angles of 60° and 300° for a ribose assuming the C2′-*endo* conformation. The values of the ^3J(C1′,OH2) and the ^3J(H2′,OH2) couplings are not significantly affected by the ribose conformation (< 0.5 Hz), while the ^3J(C3′,OH2) and the ^2J(C2′,OH2) couplings increase at $\theta = 300°$ by 1.6 Hz and −0.8 Hz, respectively.

The pronounced difference of both the ^3J(C1′,OH2) and the ^3J(H2′,OH2) couplings associated with the three distinct, preferred conformational regions (Figure 1) readily allows for distinguishing the conformational preferences around the dihedral angle H2′-C2′-O2′-OH when these couplings are available. In our previous work we found that the ^3J(H2′,OH2) and the ^3J(C1′,OH2) couplings did not exceed 5.5 and 4.0 Hz, respectively, for each RNA residue, while Ying and Bax measure uniform values of the ^3J(C1′,OH2) couplings of the order of 1.5 Hz.[8] Furthermore, in both studies the ^3J(C3′,OH2) coupling is found to be on average larger than the ^3J(C1′,OH2) coupling. These observations, in combination with the Karplus-like dependence calculated here, limit the conformational space accessible to the 2′-hydroxy group to a very small region of the O3′ domain ($\theta = 120$–$130°$) or to a large portion of the base domain ($\theta = 270$–$320°$; Figure 1). However, the observation that the H4′,OH2 NOE is always smaller than the H1′,OH2 NOE, as reported by Ying and Bax[8] for the RNA hairpin derived from the helix 35 of the 23S ribosomal RNA and measured by us for the G nucleotides in the helical regions of the TAR-RNA (Table S3 in the Supporting Information) seems to rule out population of the O3′ domain with $\theta > 90°$ (Figure S2 in the Supporting Information). In contrast, the approximated version of the Karplus dependence for the ^3J(C1′/C3′,OH2) couplings used in our previous work together with the measured heteronuclear couplings allowed for population of a large portion of the O3′ domain.

Thus far, the values of the ^3J(C1′/C3′/H2′,OH2) scalar couplings have been interpreted assuming one single conformation of the 2′-OH group. However, the ratio of the NOEs involving the 2′-OH reported by Ying and Bax[8] and measured here for the G nucleotides of the TAR-RNA (Table S3 in the Supporting Information) do not fully reconcile with the 2′-hydroxy groups occupying the base domain. While the H4′,OH2 NOE is always smaller than the H1′,OH2 NOE in the base domain, population of this domain exclusively would imply that the H4′,OH2:H1′,OH2 NOE ratio is approximately 0.1 while the H2′,OH2:H1′,OH2 NOE ratio is less than 2 or that the H4′,OH2:H1′,OH2 NOE ratio is greater than 0.1 while the H2′,OH2:H1′,OH2 NOE ratio is greater than 2 for a ribose molecule with $\tau_c = 5$ ns and in the presence of spin diffusion effects. The average ratios of 0.24 for the H4′,OH2:H1′,OH2 NOEs and of 1.25 for the H2′,OH2:H1′,OH2 NOEs suggest that conformational averaging is in effect. The absence of high values for all the measured couplings together with the mean values of each coupling and of the NOE ratios would reconcile with conformational averaging of the 2′-OH group between the O3′ domain with θ close to 120° and the base domain. This picture would still support the involvement of the 2′-OH in a network of hydrogen bonds spanning the minor groove.[7,10] The 2′-hydroxy group of one of the two nucleotides would switch between the O3′ domain, where it can accept a hydrogen bond from a water molecule, and the base domain, where it can function as a proton donor in a hydrogen bond with a water molecule, while the second nucleotide would remain in the base domain. Both situations, where the two 2′-hydroxy groups of opposite nucleotides in base pair steps are either alternatively in the O3′ and base domain or both in the base domain, support the hydration model of the minor groove proposed by Egli et al.[10] Conformational averaging between the O3′ domain and base domain for one of the two nucleotides would imply that one 2′-hydroxy and one water molecule concertedly switch their acceptor–donor roles.

The hypothesis of a two-state model for the conformation of the 2′-hydroxy group has to be verified by a quantitative interpretation of precisely measured ^3J(C1′/C3′/H2′,OH2) couplings together with diagnostic NOEs between the 2′-OH and the ribose protons. The Karplus parametrization reported here is an essential tool for this analysis and thus for an accurate understanding of the structural and functional role of the 2′-hydroxy group in RNA.

Experimental Section

The ^2J and the ^3J couplings involving the 2′-hydroxy groups were calculated using the DFT approach as implemented in Gaussian 03 Revision C.02.[11] The total nuclear spin–spin coupling was determined using the b3Lyp/6-311g(2df,2pd) basis set calculating the contributing Fermi contact (FC), spin dipolar (SD), paramagnetic spin orbit (PSO), and diamagnetic spin orbit (DSO) terms. The NOEs of the 2′-OH groups were measured in a ^{13}C-edited HSQC NOESY spectrum with a mixing time of 50 ms for a 1.5 mM sample of ^{13}C/^{15}N G-labeled TAR-RNA dissolved in H_2O/D_2O (90:10) at pH 6.4 (10 mM phosphate buffer, 50 mM sodium chloride).

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