Chemical Shift and Structure Relationship in Nucleic Acids: Correlation of Backbone Torsion Angles γ and α with ^{13}C Chemical Shifts

Xiao-Ping Xu, Wing-Lok A. K. Chiu, and Steve C. F. Au-Yeung*

Department of Chemistry
The Chinese University of Hong Kong
Shatin, New Territories, Hong Kong

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Knowledge of the backbone structure of nucleic acids is important, not only for the understanding of structural distortion in active biological processes such as the influence of drug binding on DNA bending and its recognition by damage recognition proteins containing the HMG-binding domain,[1,2] but also because of its influence on the overall helical twist of the DNA duplex. However, in the determination of nucleic acid structure by ^{1}H NMR, it is well-known that the backbone is a region with low density of structural constraints because of the limitations in obtaining conformational parameters by *J*-coupling or NOE information within crowded spectral regions, particularly for large nucleic acids. For example, the determination of the backbone torsion angle γ(O5′−C5′−C4′−C3′) from $^3J_{HH}$ through the measurement of $J_{H4'-H5'}$ and $J_{H4'-H5''}$ is often impractical because of the severe spectral overlapping of H5′ and H5″, the difficulty in their stereoassignment, and the poor detection because of the close proximity to the water peak. The ^{13}C chemical shift method, with high sensitivity to local conformation and favorable spectral resolution, has been successfully applied to the prediction of protein structure.[3] However, a similar endeavor has yet to be successful when applied to nucleic acids because the understanding of different conformational contributions to the ^{13}C shifts remains incomplete although experimental[4−7] and theoretical computation[8,9] attempts have been made to increase their understanding. Here we report the novel dependence of ^{13}C chemical shifts of the sugar ring upon backbone torsion angles γ, α(P−O5′−C5′−C4′), and δ(C5′−C4′−C3′−O3′) as well as the sugar pucker *P* through a study of the correlation of the ^{13}C chemical shifts with conformational parameters using crystalline nucleosides, nucleotides, and solution nucleic acid data. The correlations are further demonstrated by quantum chemical calculation of ^{13}C chemical shifts.

A total of 31 nucleosides and nucleotides were purchased from Sigma Chemical Co. and were used directly for this study.[10] Except for 3-deazauridine,[11] all X-ray structure parameters were taken from a data handbook.[12] Solid-state ^{13}C NMR spectra were recorded on a Bruker ASX 300 NMR spectrometer operated at 7.1 T using the CPMAS method. All shifts were referenced to external tetramethylsilane (TMS). The assignments of the spectra were made in the same way as described by Harbison et al.[4] and the references therein. All solution data for nucleic acids were collected from the literature.[13] The chemical shifts of all C3′ carbons for the compounds/residues with a phosphate ester at the 3′-position were corrected for the phosphate substitution effect.[14] The same correction was made in the C5′ chemical shift for 5′-phosphoester compounds or residues.

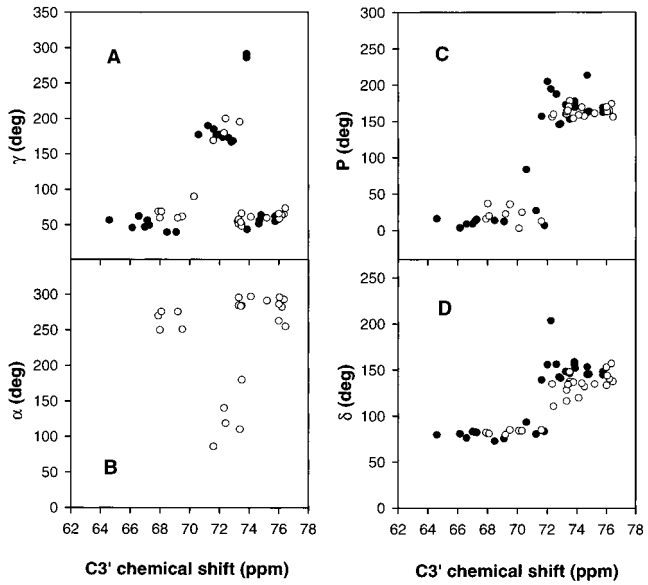

Figure 1. Structure parameters (deg), (A) torsion angle γ, (B) torsion angle α, (C) sugar pucker *P*, and (D) torsion angle δ, versus ^{13}C chemical shifts (ppm) for crystalline samples (●) and solution data (○).

The isotropic chemical shifts were calculated using the hybrid DFT-SCF version (using Becke's three-parameter hybrid method and employing the LYP correlation functions, B3LYP)[15] of the GIAO[16] method as implemented in the Gaussian 94 package[17] on a SGI workstation. The locally dense basis set 6-311G**/3-

* To whom correspondence should be addressed.

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Table 1. Calculated and Experimental Trends for the Dependence of ^{13}C Chemical Shifts on Torsion Angle γ (g = *gauche* and t = *trans*) and Sugar Pucker *P* (S = S-type and N = N-type) (ppm)

	ribose[a]						deoxyribose[b]						$\Delta\delta_{S-N/g}$[c]		$\Delta\delta_{S-N/t}$[c]	
carbon	$\delta_{N/g}$	$\delta_{S/g}$	$\Delta\delta_{S-N/g}$	$\delta_{N/t}$	$\delta_{S/t}$	$\Delta\delta_{S-N/t}$	$\delta_{N/g}$	$\delta_{S/g}$	$\Delta\delta_{S-N/g}$	$\delta_{N/t}$	$\delta_{S/t}$	$\Delta\delta_{S-N/t}$	calc	expt	calc	expt
C3′	68.82	77.90	9.08	74.40	76.72	2.32	69.36	78.57	9.21	75.05	76.62	1.57	9.15	6.67	1.95	1.48
C4′	87.30	93.47	6.17	87.03	90.19	3.16	87.82	89.11	1.29	86.48	88.99	2.51	3.73	4.61	2.67	3.68
C5′	63.88	68.54	4.66	69.02	67.89	−1.13	62.65	67.55	4.90	68.88	68.01	−0.87	4.78	3.63	−1.00	−1.66

[a] For cytidine with geometry parameters based on the statistical data.[20] [b] For deoxythymidine with geometry parameters based on the statistical data.[20] [c] The calculated average for cytidine and deoxythymidine and the experimental average for all available data.

21G,[18] with a 6-311G** basis for the carbon of interest and its immediate neighbors and a 3-21G basis for the remaining atoms, was adopted. Cytidine and deoxythymidine were used as the model compounds for ribose and deoxyribose groups, respectively. Energy-minimized conformers of cytidine and deoxythymidine were obtained using SYBYL 6.2.[19] Their bond length and torsion angle constraints were based on the statistical geometry parameters.[20] ^{13}C chemical shifts were obtained in parts per million relative to the absolute shielding constant (σ) of TMS (T_d, B3LYP/6-311+G**).

The plots of backbone torsion angle γ and sugar pucker *P* versus C3′ chemical shifts for crystalline nucleosides and nucleotides (●) as well as nucleic acids in solution (○) are presented in Figure 1A,C. These data show that the C3′ chemical shift is not solely dependent on sugar pucker *P* but also on the backbone torsion angle γ. There are three distinct regions for the C3′ chemical shift: the N-type/*gauche* region (<70 ppm), the N-type/*trans* and S-type/*trans* regions (71−73 ppm), and the S-type/*gauche* region (>74 ppm). That is, when γ is in the *gauche* conformation, the C3′ chemical shift for S-type puckering is downshifted by about 6.67 ppm on average compared to the N-type puckering. But for γ in the *trans* form, the C3′ chemical shift is located in the middle region. This may be rationalized by the change in the electronic structure arising from (i) the torsion angle effect and (ii) the space polarization effect because of the distance change (∼0.075 Å) in C3′ to O5′ when γ changes from the *gauche* to *trans* form. The dependence on the C3′ chemical shift of α (Figure 1B) and δ (Figure 1D) is similar to that of γ and *P* on the C3′ shift, respectively. This is an expected result because of the interdependence between α and γ as well as δ and *P*.[20,21]

We also observed that the trends of the dependence of the sugar pucker *P* on the C4′ and C5′ chemical shifts are similar to that of the sugar pucker *P* on the C3′ chemical shift (see the Supporting Information, Figure 1A,C) for γ in the *gauche* form. When γ is in the *trans* form, no obvious change is found in the trend for the dependence of the sugar pucker *P* on the C4′ chemical shift (see the Supporting Information, Figure 1B), but a quite different trend was observed for the C5′ shift (see the Supporting Information, Figure 1D). The change of γ from *gauche* to *trans* results in a downfield shift of 4−5 ppm for C5′ in the N-type sugar conformation.

To confirm the experimental shift−structure correlations, ^{13}C chemical shift calculations at the DFT level were carried out for cytidine and deoxythymidine. As shown in Table 1, the calculated results are in good agreement with the experimental trends. To our knowledge, this is the first successful calculation of ^{13}C chemical shifts in nucleic acids which gives good features of the experimental spectra. Therefore, according to the above ^{13}C chemical shift−structure relationship, the backbone torsion angles γ, α, and δ as well as the sugar pucker *P* may be determined from the chemical shifts of C3′, C4′, and C5′ in most cases. Note that a combined consideration of these chemical shifts is very important for a reliable structure conclusion, especially when the chemical shift is located close to the boundary of the regions.

In conclusion, we have demonstrated the correlations between the torsion angles γ, α, and δ as well as *P* and the chemical shifts of C3′, C4′, and C5′ in nucleic acids. The excellent agreement between experimental and calculation results and the similar shift−structure correlation patterns observed for nucleosides, nucleotides, and nucleic acids imply that ^{13}C chemical shifts of nucleic acids are a promising tool for the determination of nucleic acid structure because of the good local nature of sugar ring carbons. Further investigations of the ^{13}C chemical shift−structure relationship in nucleic acids are in progress.

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Supporting Information Available: A list of ^{13}C chemical shifts and structural parameters of crystal samples and solution data, plots of the sugar pucker *P* versus C4′ and C5′ chemical shifts with different torsion angles γ, and plots of the torsion angle α versus γ and δ versus the sugar pucker *P* (6 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(21) Supporting Information Figure 2.