How does the 3'-Phosphate Drive the Sugar Conformation in DNA?

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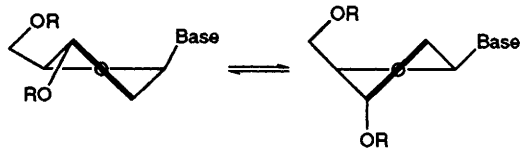
While the $^3J_{C(4')P}$, $^3J_{C(2')P}$, $^3J_{H(3')P}$, $^3J_{CH_3P}$ and $^3J_{CH_2P}$ coupling constants in simple model systems **13–16** remain unchanged over 278–358 K, considerable changes of endocyclic $^3J_{HH}$ coupling constants have been found to take place, which, for the first time, unequivocally show that the change of the North (3'-*endo*-2'-*exo*) ⇄ South (3'-*exo*-2'-*endo*) sugar pseudorotamer equilibrium is independent of the change of the phosphate backbone torsion, it is also found that the gauche effects between O(4') and 3'-OPO_3H^- in **9–12** and between O(4') and 3'-OPO_3Et^- in **13–16** are responsible for the stabilization of the South sugar conformer by $\Delta H^\circ = -1.5$ kJ mol^{-1} compared to 3'-OH in **5–8**.

There are three essential components in the molecular construction of polynucleotides: the pentofuranose, the heterocycle and the phosphodiester.[1] The pentofuranose moiety reduces its energy by becoming puckered in a preferential manner.[1,2] We have recently shown that various stereoelectronic gauche and anomeric effects[3] energetically steer the North (N) ⇄ South (S) conformational equilibrium. Some of the most important questions that have been repeatedly addressed in the conformational studies of nucleic acid, albeit unsuccessfully, are how does the sugar conformation dictate the phosphate backbone torsions, or is there any preferred phosphate torsion that steers the sugar conformation in a certain manner, or are there any correlated interdependencies of endocyclic sugar torsion with the preferred phosphate torsions?[4] Here we have studied simple temperature-dependent conformational changes in 2',3'-dideoxyribonucleosides **1–4**, 2'-deoxyribonucleosides **5–8** and their corresponding 3'-monophosphates **9–12** and 3'-ethylphosphates **13–16**, the latter have been chosen as simple model systems for di-(2'-deoxyribonucleoside)monophosphate in which the effect of intramolecular base–base stacking is completely eliminated. These studies for the first time clearly resolve the above questions and unequivocally show that the 3'-phosphate stereoelectronically (*i.e.* through gauche effect[3,5]) steers the constituent 2'-deoxyribofuranose conformation, while the changes in the sugar conformation do not have any influence on the rotamer distribution of two NMR measurable phosphate torsions (*i.e.* ε and β).



North sugar ($C_{3'}$-*endo*-$C_{2'}$-*exo*) Phase angle (P) = $0° \leq P \leq 36°$; Puckering amplitude (Ψ_m) = 38.6° ± 3°

South sugar ($C_{2'}$-*endo*-$C_{3'}$-*exo*) Phase angle (P) = $144° \leq P \leq 190°$; Puckering amplitude (Ψ_m) = 38.6° ± 3°

Base = adenine-9-yl (A), guanine-9-yl (G), cytosine-1-yl (C), thymine-1-yl (T)
R = H or an internucleotidyl-(3' → 5')-phosphodiester moiety in DNA

Vicinal proton–proton coupling constants ($^3J_{HH}$) of β-D-pentofuranose moieties in 2',3'-dideoxyribonucleosides (ddN)[3] **1–4**, 2'-deoxyribonucleosides (dN)[3] **5–8**, 2'-deoxy-3'-monophosphates (dNMP) **9–12** and 2'-deoxy-3'-ethylphosphates (dNEtMP) **13–16** were measured (^{1}H NMR at 500 MHz) in D_2O (error ± 0.1 Hz). The computer program PSEUROT (ver. 5.4)[6] has been used to translate the experimental coupling constants measured in the range 278–358 K in 10 K steps† into the two-state N ⇄ S conformational equilibrium‡ of constituent sugar moieties in **1–16**. The populations of N and S conformers for **1–16** at various temperatures were used to calculate the enthalpy ($\Delta H°$) and the entropy ($\Delta S°$) of the N ⇄ S pseudorotational equilibria through van't Hoff plots of [ln (X_S/X_N)] *vs.* $1/T$ (Table 1). Table 1 shows the temperature dependency of the populations of the N and S conformers at 278 and 358 K for the sugar moieties in **1–16** and their respective $\Delta H°$ and $\Delta S°$ values. The subtraction of $\Delta H°$ for ddN **1–4** from dN **5–8**, respectively, gives the relative strength of gauche effect of O(4)–C(4')–C(3')–3'OH fragment: −9.0 kJ mol^{-1} for dA, −7.3

Table 1 Thermodynamic data of the N ⇄ S conformational equilibria‡ for **1–16**

Compound	$\Delta H°$/ kJ mol^{-1}[a]	$\Delta S°$/ J K^{-1} mol^{-1}[a]	$-T\Delta S°$/ kJ mol^{-1}[b]	ΔG^{298}/ kJ mol^{-1}	%S[c] (278 K)	%S[c] (358 K)	Δ%S (358–278 K)
ddA **1**	4.8 (0.2)	7.4 (2.1)	−2.2	2.6	24	33	+9
ddG **2**	4.8 (0.2)	7.2 (2.1)	−2.1	2.7	23	32	+9
ddC **3**	8.0 (0.4)	15.9 (1.4)	−4.7	3.3	18	32	+14
ddT **4**	5.4 (0.2)	6.0 (0.8)	−1.8	3.6	17	25	+8
dA **5**	−4.2 (0.1)	−6.9 (0.7)	2.1	−2.1	73	64	−9
dG **6**	−2.5 (0.1)	−2.6 (0.9)	0.8	−1.7	68	63	−5
dC **7**	−1.2 (0.1)	0.1 (1.0)	0.0	−1.2	63	60	−3
T **8**	−1.8 (0.3)	−0.9 (0.5)	0.3	−1.5	66	62	−4
dAMP **9**	−5.4 (0.3)	−9.9 (0.7)	3.0	−2.4	76	65	−11
dGMP **10**	−4.1 (0.2)	−6.5 (0.7)	1.9	−2.2	73	64	−9
dCMP **11**	−3.2 (0.1)	−6.8 (0.5)	2.0	−1.2	64	56	−8
TMP **12**	−2.6 (0.1)	−4.3 (0.4)	1.3	−1.3	65	59	−6
dA-3'-OPO_3Et^- **13**	−5.0 (0.3)	−6.2 (0.6)	1.8	−3.2	81	72	−9
dG-3'-OPO_3Et^- **14**	−4.2 (0.2)	−5.0 (0.5)	1.5	−2.7	77	69	−8
dC-3'-OPO_3Et^- **15**	−1.9 (0.2)	−0.3 (0.6)	0.1	−1.8	69	65	−4
T-3'-OPO_3Et^- **16**	−2.6 (0.1)	−1.9 (1.1)	0.6	−2.0	71	66	−5

[a] $\Delta H°$ and $\Delta S°$ are the average values (their respective standard deviations are given in brackets) and were calculated from individual van't Hoff plots using populations of N and S conformers from several individual PSEUROT analyses.† [b] $-T\Delta S°$ term is given at 298 K. [c] The population of the S conformer was calculated through the relation: %S (T) = 100* [exp ($-\Delta G^T/RT$)]/ [exp ($-\Delta G^T/RT$) + 1]. R is the gas constant (8.314 J K^{-1} mol^{-1}).

kJ mol^{-1} for dG, −9.2 kJ mol^{-1} for dC and −7.2 kJ mol^{-1} for thymidine.

In a similar manner, the subtraction of $\Delta H°$ for ddN **1–4** from dNMP **9–12**, respectively, gives the relative strength of gauche effect of O(4')–C(4')–C(3')–3'OPO_3H^- fragment: −10.2 kJ mol^{-1} for dAMP, −8.9 kJ mol^{-1} for dGMP, −11.2 kJ mol^{-1} for dCMP and −8.0 kJ mol^{-1} for TMP. Therefore, the enhanced gauche effect of 3'-OPO_3H^- by −0.8 to −2.0 kJ mol^{-1} compared to that of the 3'-OH steers the sugar N ⇄ S equilibrium towards the S conformation more effectively. Similarly, the relative strength of the gauche effect of 3'-OPO_3Et^- which drives the sugar conformation towards S is obtained by subtraction of $\Delta H°$ values in ddN **1–4** from dNEtMP **13–16**, respectively: −9.8 kJ mol^{-1} for dA-3'-OPO_3Et^-, −9.0 kJ mol^{-1} for dG-3'-OPO_3Et^-, −9.9 kJ mol^{-1} for dC-3'-OPO_3Et^- and −8.0 kJ mol^{-1} for T-3'-OPO_3Et^-. Therefore, 3'-OPO_3H^- and 3'-OPO_3Et^- drive the sugar conformation towards S with comparable strengths.

The question of the correlation of the sugar pucker with the rotamer distribution of two NMR measurable phosphate torsions (*i.e.* ε and β) has been addressed through the conformational analysis of phosphodiester moieties in dNEtMP in **13–16**. The values of $^3J_{C(4')P}$, $^3J_{C(2')P}$, $^3J_{H(3')P}$, $^3J_{CH_3P}$ and $^3J_{CH_2P}$ (±0.4 Hz) for **13–16** show almost insignificant change (< ±0.4 Hz) in the temperature range 278–358 K measured at 10 K step.§ This suggests that as the N ⇄ S equilibria changes with the temperature, the populations of various ε (ε^t *vs.* ε$^-$ *ca.*, 1 : 1) and β (*ca.* 50% β^t) rotamers[10] do not change.

While the endocyclic $^3J_{HH}$ coupling constants show dynamic change over a temperature range of 278–358 K, the exocyclic $^3J_{C(4')P}$, $^3J_{C(2')P}$, $^3J_{H(3')P}$, $^3J_{CH_3P}$ and $^3J_{CH_2P}$ coupling constants remain unchanged, which suggests that the N ⇄ S sugar pseudorotamer equilibrium is neither steered by the preferential phosphate backbone torsion nor the preferred geometry of the sugar pseudorotamer drives the phosphate backbone to any specific ε or/and β torsion. This means that any observed phosphate folding, as evident from the change of $^3J_{HP}$ and $^3J_{CP}$ found in the NMR studies of oligo-DNA is due to the direct net result of intermolecular and intramolecular stacking ⇄ destacking and H-bonding interactions. This also means that any conformational change of the sugar moiety due to nucleobase interactions is not expected to be transmitted through the sugar to steer the phosphate backbone torsions to any preferred conformation. On the other hand, the experimentally found $\Delta\Delta H°$ of *ca.* 1 kJ mol^{-1} for the gauche effect between O(4') and 3'-OPO_3Et^- in **13–16** is responsible for the shift of the N ⇄ S sugar equilibrium to more S compared to 3'-OH in **5–8**, which suggests that the preference of the S sugar conformer in the native B-DNA in fact originates from the inherent chemical nature of being unique as the phosphodiester.

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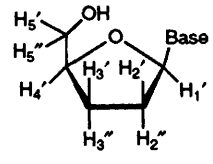
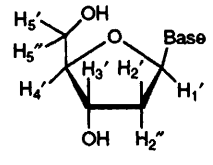
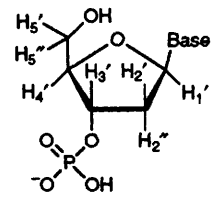


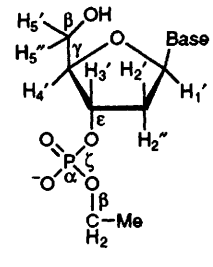
1: ddA
2: ddG
3: ddC
4: ddT



5: dA
6: dG
7: dC
8: T



9: dAMP
10: dGMP
11: dCMP
12: TMP



13: dA-3'OPO_3Et^-
14: dG-3'OPO_3Et^-
15: dC-3'OPO_3Et^-
16: T-3'OPO_3Et^-

Base = adenine-9-yl (A), guanine-9-yl (G), cytosine-1-yl (C), thymine-1-yl (T)

Footnotes

† $^3J_{HH}$ have been extracted from 500 MHz ^{1}H NMR spectra measured at 278–358 K in 10 K steps at *ca.* 20–30 mmol dm^{-3} for **9–12** and *ca.* 50 mmol dm^{-3} for **13–16**. Almost negligible change in the chemical shift (<0.1 ppm) of all protons over the whole temperature range suggests the absence of aggregation. $^3J_{HH}$ for **12** were also recorded in 5 K steps at 274–368 K which resulted in the same conformational picture for its sugar residue. $^3J_{HH}$ were analysed by the PSEUROT[6] program which calculates their best fit to the five conformational parameters (P and Ψ_m for both N and S conformers and corresponding mole fractions). The following λ electronegativities[6] were used for the substituents on H–C–C–H fragments in **9–16**: H 0.00, O(4') and phosphate 1.27; N of the bases 0.58; C(1'), C(3'), C(4') 0.62; C(2') 0.67 and C(5') 0.68. Several PSEUROT analyses were performed for **9–16** in which the geometries of minor N conformers were fixed in the range −36° < P_N < +36° in 18° steps and alternatively Ψ_m of both N and S type pseudorotamers were fixed in the range 30° < Ψ_m < 40° in 1° steps (rms < 0.3 Hz, ΔJ^{max} <0.5 Hz). The resulting populations from individual PSEUROT analyses were used to make van't Hoff plots. Individual $\Delta H°$ and $\Delta S°$ values were derived from each van't Hoff plot and used to calculate the average $\Delta H°$ and $\Delta S°$ of N ⇄ S equilibrium in **1–16** and their associated standard deviations presented in Table 1. The major S type pseudorotamers, which were optimised freely in the above PSEUROT analyses were characterised by: 145° < P_S < 160° and 30° < Ψ_m < 34° for **9**, 145° < P_S < 165° and 30° < Ψ_m < 36° for **10**, 133° < P_S < 145° and 30° < Ψ_m < 35° for **11**, 135° < P_S < 150° and 30° < Ψ_m < 36° for **12**, 148° < P_S < 160° and 30° < Ψ_m < 37° for **13**, 143° < P_S < 163° and 32° < Ψ_m < 37° for **14**, 133° < P_S < 150° and 30° < Ψ_m < 37° for **15**, 135° < P_S < 155° and 32° < Ψ_m < 36° for **16**.

‡ Previous NMR studies have clearly shown the presence of two distinctly identifiable dynamically interconverting N and S conformations of some sugar moieties in B ⇄ Z DNA[7] or A ⇄ Z RNA[8] or A-form ⇄ B-form lariat RNA[9] transformations as a result of change of the salt or alcohol concentration in the buffer or as a result of change of temperature. These dynamically interconverting N and S sugar pseudorotamers are characterized by only two distinct sets of resonances owing to their different stereochemical environments, and these resonances are characterized by the typical $^3J_{1'2'}$ coupling constants which are 0.5 Hz for the N and *ca.* 8 Hz for the S conformers. Note that under no known condition of NMR measurement, a third set of chemical shifts and $^3J_{1'2'}$ has been yet observed for any hypothetical third conformational state in the dynamic equilibrium of pentose sugar pseudorotamers. These are the reasons why we have considered a two-state N ⇄ S equilibrium in the conformational analysis of the sugar moieties in nucleosides and nucleotides.

§ $^3J_{CP}$ and $^3J_{HP}$ (error ±0.4 Hz) for **13–16** are given at 298 K. **13**: [$^3J_{C(4')P}$ = 5.6 Hz, $^3J_{C(2')P}$ = 3.8 Hz, $J_{H(3')P}$ = 7.3 Hz, $^3J_{CH_3P}$ = 6.8 Hz, $^3J_{CH_2P}$ = 7.2 Hz]; **14**: [$^3J_{C(4')P}$ = 5.8 Hz, $^3J_{C(2')P}$ = 3.8 Hz, $J_{H(3')P}$ = 7.4 Hz, $^3J_{CH_3P}$ = 6.6 Hz, $^3J_{CH_2P}$ = 7.2 Hz]; **15**: ($^3J_{C(4')P}$ = 6.2 Hz, $^3J_{C(2')P}$ = 3.6 Hz, $J_{H(3')P}$ = 7.2 Hz, $^3J_{CH_2P}$ = 7.1 Hz); **16**: [$^3J_{C(4')P}$ = 6.3 Hz, $^3J_{C(2')P}$ = 3.6 Hz, $J_{H(3')P}$ = 7.2 Hz, $^3J_{CH_3P}$ = 5.8 Hz, $^3J_{CH_2P}$ = 7.2 Hz].

The CH_2 protons of OEt group are isochronous and appear as 'quintet like' multiplets in ^1H NMR spectra.

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