Assignment of Z DNA NMR Spectra of Poly d(Gm^5C) by Two-Dimensional Multinuclear Spectroscopy

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B and Z DNA structures differ significantly in the configuration of the sugar–phosphate backbone.[1] This difference is reflected in characteristic changes of their NMR spectra. The change in chemical shift of the ^{31}P resonances[2] and the change of NOE crosspeak intensity between G8 and G1′ protons[3–9] are the most pronounced effects observed so far. In previous ^{13}C NMR studies, the changes of chemical shifts in sugar regions of poly d(Gm^5C)[10] and d$(CG)_3$ and d$(CG)_4$[11] were reported, but the signals were not assigned.

Here we show that 2D ^1H-detected multinuclear NMR spectroscopy allows unambiguous assignment of ^1H and ^{13}C chemical shifts originating from the DNA backbone in a fragment d-(Gm^5C)$_{20}$ (40 base pairs) in the Z form, revealing significant differences in chemical shifts between B and Z DNA. The values of proton and carbon chemical shifts and their changes during the B to Z transition are important for the recognition of Z structure in solution and will provide insight into the effects of structure on magnetic shielding in double-stranded DNA. The very sensitive approach demonstrated here avoids the need for isotopic enrichment, chemical modifications, or large quantities of material.

The self-complementary DNA 40-mer d(Gm^5C)$_{20}$ was synthesized by the automated version of the phosphoramidite coupling method.[12] This material, being of uniform size, yields spectra of much higher resolution than those of the sonicated DNA polymers used in previous studies.[3,6–8,10] Conversion to the Z form was achieved by addition of Mg^{2+} ions.[13] For the NMR measurements 62 ODU (3 mg) of the sodium salt of d(Gm^5C)$_{20}$ were dissolved in 0.35 mL of D_2O, containing 10 mM $MgCl_2$ and 5 mM phosphate buffer, p^{2}H = 7.2. All measurements were performed at 60 °C, at which temperature 85% conversion to the Z form was observed.[14]

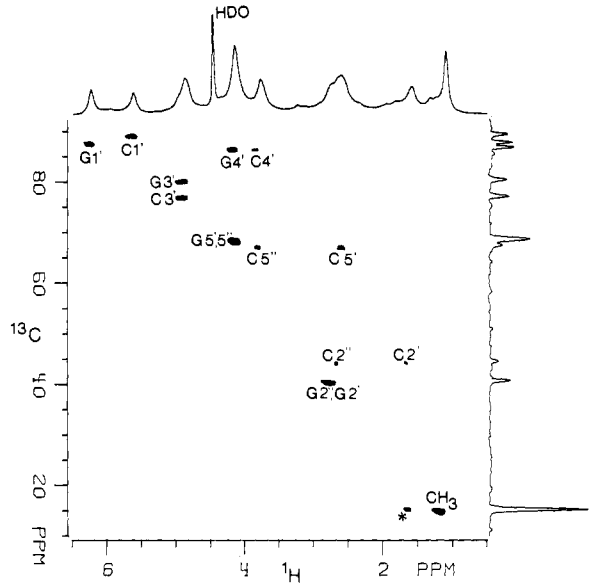

Figure 1. 500-MHz 2D absorption mode ^1H–^{13}C correlation spectrum of d(Gm^5C)$_{20}$ in the Z form recorded by using the ^1H-detected experiment.[15] Data were obtained from a 2 × 180 × 1024 matrix. Acquisition times in the t_1 and t_2 dimensions were 17 and 102 ms, respectively. 256 scans were recorded for each t_1 value and a relaxation delay between scans of 1.3 s was used. The total measuring time was 18.5 h using 62 ODU (3 mg) of d(Gm^5C)$_{20}$ in 10 mM $MgCl_2$, with 5 mM phosphate buffer in D_2O at 60 °C. ^1H and ^{13}C chemical shifts are relative to internal TSP. The regular ^1H spectrum and the projection of the matrix on the ^{13}C axis are shown along the sides of the 2D spectrum. The asterisk marks a low-molecular-weight impurity in the sample.

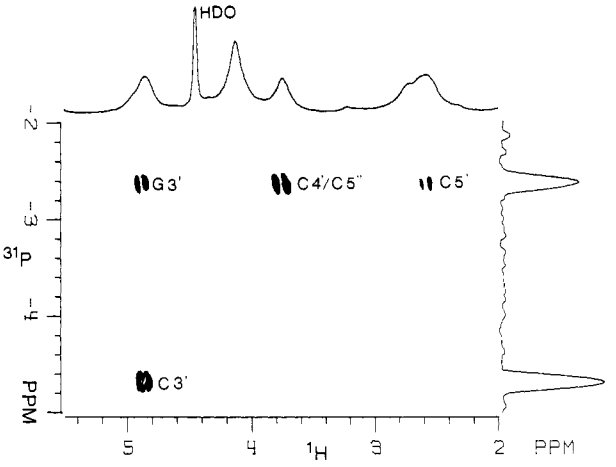

Figure 2. 500-MHz 2D ^1H–^{31}P correlation spectrum of the d(Gm^5C)$_{20}$ in Z DNA conformation recorded by using the ^1H-detected experiment.[11] Data were obtained from a 2 × 50 × 512 matrix. Acquisition times from −25 to +25 ms in t_1 and 170 ms in t_2 were used. The total measuring time was 10.5 h. ^1H and ^{31}P chemical shifts are relative to internal TSP and phosphate buffer (Na_2HPO_4), respectively. The regular ^1H spectrum and the projection of the matrix on the ^{31}P axis are shown along the sides. Only the ^1H and ^{31}P nuclei having substantial J coupling show correlation peaks. The splitting of the peaks in the ^1H dimension arises from the detection of proton antiphase magnetization. Absorptive and absolute value line shapes are obtained in the ^1H and ^{31}P dimensions, respectively.

The ^1H–^{13}C chemical shift correlation spectrum (Figure 1) was obtained by applying the same strategy of ^1H multiple quantum detection as described recently for measurements of proteins.[15]

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(14) The degree of the B to Z transition (which strongly depends on temperature, DNA molecular weight, the relative amounts of DNA, and monovalent and divalent cations) was measured by integrating ^{31}P NMR spectra. The peaks of B and Z structure are clearly separated because of slow exchange. The optimal temperature for the given solution conditions was 60 °C.

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Table I. Backbone ^1H, ^{13}C, and ^{31}P Chemical Shifts of $d(Gm^5C)_{20}$ in the Z Form and Differences in Shifts, Δ_{B-Z}, Relative to the B Form

	^1H		^{13}C	
	Z DNA	Δ_{B-Z}[a]	Z DNA	Δ_{B-Z}[a]
G1′	6.20	−0.28	87.53	−2.57
C1′	5.58	+0.05	88.95	−2.26
G2′	2.65	−0.13	40.47	+0.43
G2″	2.70	+0.07		
C2′	1.57	+0.42	44.25	−3.83
G2″	2.56	−0.24		
G3′	4.86	+0.09	80.02	+0.12
C3′	4.83	−0.04	76.70	+0.39
G4′	4.11	+0.23	86.47	+1.26
C4′	3.74	+0.40	86.40	−0.76
G5′	4.11	−0.01	68.14	+0.52
G5″	4.11	−0.01		
C5′	3.74	+0.40	67.11	+0.26
C5″	2.54	+1.60		

	^{31}P	
	Z DNA	Δ_{B-Z}[a]
Gpm^5C	−2.62	−1.19
m^5CpG	−4.68	+1.18

[a] The chemical shifts in the B form (100 mM NaCl, 5 mM phosphate buffer, 45 °C) were measured and assigned by the same procedure as described for the Z structure; $\Delta_{B-Z} = \delta_B - \delta_Z$; chemical shifts are given in ppm.

The Cl′ and Gl′ proton signals were assigned previously[3] and Figure 1 identifies the corresponding carbon resonances. Figure 1 also shows two pairs of correlations in the 2′/2″ region, one pair of correlations in the 5′/5″ region, and a correlation to a 5′-carbon for which the two protons are not resolved. The G2′, G2″, C2′, and C2″ protons are assigned from a 2D NOE spectrum (supplementary material) thus identifying the corresponding ^{13}C resonances. The 3′-protons show a small difference in chemical shift and are assigned through correlation to 2′-, 2″-, and 1′-protons in the 2D NOE spectrum. The G3′ resonance is shifted slightly downfield (≈0.03 ppm), which permits unambiguous assignment of the 3′-^{13}C signals. The crowded 4′–5′/5″ region in the 2D NOE spectrum between 3.7 and 4.1 ppm does not allow assignment of two 5′/5″ geminal pairs to a particular sugar moiety directly. However, an intense NOE 5′/5″ crosspeak at 2.54 and 3.74 ppm is clearly separated and was observed, although unidentified, in previous studies.[6,7]

The sugar specific assignment of the 5′/5″ signals is provided unambiguously by 2D ^1H–^{31}P chemical shift correlation (Figure 2). The proton-detected ^1H–^{31}P experiment with a constant time evolution period was applied as described previously.[12] The small difference of the chemical shifts between the two 3′-protons confirms the ^{31}P resonance assigment made by comparison of two phosphorothioate analogues.[15] Moreover, the correlations of the low-field ^{31}P Gpm^5C resonance (2.62 ppm) to the protons at 3.74 and 2.54 ppm can originate only from the interactions with 4′- and 5′/5″-protons in the O5′ part of the phosphodiester linkage, assigning these signals to the m^5C residue. The 4′ ^1H and ^{13}C signals separated by 0.07 and 0.37 ppm, respectively, are identified by comparing the crosspeak intensities with C3′, G3′, and C5″ protons in the 2D NOE spectrum. The ^1H, ^{13}C and ^{31}P chemical shifts obtained for the Z form of $d(Gm^5C)_{20}$ and the differences, Δ_{B-Z}, between the B and Z forms are summarized in Table I.

Although the values and comparison with those in B DNA will be discussed in detail elsewhere[17] their surprising nature deserves some comment. In the Z structure the configuration of the guanosine residue is changed from 2′-endo to 3′-endo and the glycosidic torsion angle from anti to syn compared to B DNA. This would suggest that the largest changes in the chemical shifts would be observed for signals originating from this sugar moiety. However, the assignment presented above shows that the most pronounced effects are observed in the m^5C nucleotide. In the proton spectrum the C2′, C4′, and C5′ signals are shifted substantially upfield (>0.40 ppm) with the largest change observed for the C5′ proton. The chemical shift difference (1.20 ppm) between C5′ and C5″ protons in Z DNA is unusual as is the large separation of C2′ and C2″ signals (0.99 ppm). The upfield shifts of the C2′ and the C5′ protons are most likely due to ring current effects; C2′H is located on top of the six-membered ring of the preceding guanine base and C5′H is located below the five-membered ring of the following guanine base. The change in the chemical shifts of C2′ and C2″ protons is accompanied by the largest Δ_{B-Z} in the ^{13}C spectrum (+3.83 ppm). Differences in ^{13}C chemical shifts greater than 1 ppm for Cl′, Gl′, and G4′ signals are also observed. These changes in ^{13}C chemical shift cannot be fully accounted for by ring current effects and may be caused in part by the change in orientation of the bases (and their dipole moments) with respect to the sugar carbons.[18] Comparison of the difference values (Table I) shows no correlation in the direction of changes in ^1H and ^{13}C chemical shifts during the B to Z transition. The observed values in the Z form are substantially different from those of B DNA and allow direct identification of the Z structure by NMR.

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Supplementary Material Available: 2D phase-sensitive NOE spectrum of $d(Gm^5C)_{20}$ in the Z DNA conformation (2 pages). Ordering information is given on any current masthead page.

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Reversal of Substrate Charge Specificity by Site-Directed Mutagenesis of Aspartate Aminotransferase

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The alteration of enzymes by site-directed mutagenesis to produce proteins with improved catalytic properties toward refractory substrates is an important goal of modern DNA technology. The most prominent example to date is found in the recent studies on subtilisin where changes at position 166 in the substrate binding pocket have produced a number of enzymes with increased catalytic efficiencies toward substrates that are less reactive with the wild-type enzyme.[1]

Aspartate aminotransferase (AATase; EC 2.6.1.1), a pyridoxal phosphate (PLP) dependent enzyme, catalyzes the reversible transamination of α-amino to α-keto acids, with the concomitant conversion of enzyme-bound PLP to pyridoxamine phosphate (PMP) (Scheme I).[2] The preferred substrates for this reaction are the dicarboxylic amino acids L-aspartate (I) and L-glutamate (II). We report here the results of a site-directed mutagenesis experiment that generates an inversion of the substrate charge specificity of *E. coli* AATase from the anionic I and II to the cationic amino acids L-lysine (III) and L-arginine (IV).

Crystallographic data[3] indicate that, in eukaryotic AATases,

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