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Communications to the Editor

Adjacent G:A Mismatch Base Pairs Contain B_{II} Phosphodiesters in Solution

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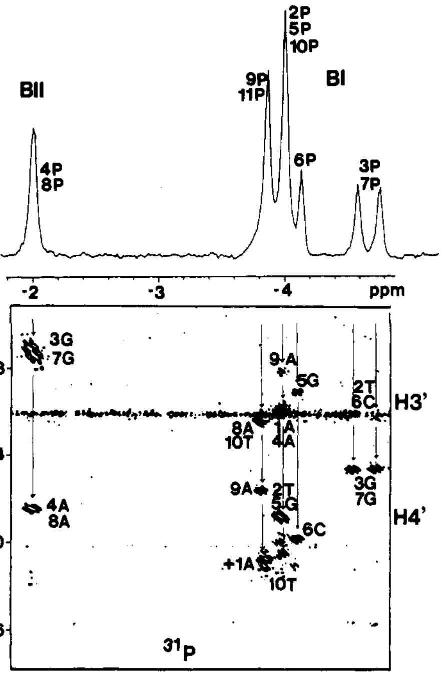
The C3′–O3′–P segment of the backbone chain in B-DNA oligomers usually has a B_I conformation in which the torsion angles ϵ (C3′–O3′) and ζ (O3′–P) are (t,g^-).[1] Although the B_{II} (g^-,t) conformation is proposed as an alternative structure for the backbone of B-DNA,[2,3] it is usually considered to be a higher energy form of B_I, and its occasional occurrence in DNA crystal structures is often attributed to crystal packing forces.[4,5]

Analysis in solution by NMR[6–9] and Raman spectroscopy[10] is ambiguous. An observed $J_{H3'-P}$ of say 5 Hz has four solutions for ϵ, namely, −75°, −10°, 130°, and 195°; the former range (g^-) constitutes B_{II} and the latter range (t) constitutes B_I phosphates. Often the backbone conformation in solution is arbitrarily constrained to the B_I conformation,[6,7,9] and clear evidence for B_{II} phosphates is lacking.

The unusual DNA undecamer $[d(ATGAGCGAATA)]_2$ forms a remarkably stable B-type duplex with four internal G:A mismatches, for which Li et al. have proposed a novel hydrogen-bonding scheme.[11] We have used several hundred NMR-derived proton distance constraints (many of them unexpected) to generate 22 distance geometry structures for this unusual duplex. In *every one* of these structures the phosphate linkers connecting G3 to A4 (4P) and G7 to A8 (8P) at the adjacent mismatch sites converged to a B_{II} $\epsilon(g^-)$ conformation. Concomitant with this backbone rearrangement, the ^{31}P resonances of these two phosphates shifted downfield by ca. 2 ppm from the other B_I phosphates. This is the first clear demonstration of a DNA B_{II} phosphate in solution, and it is confirmed by Monte Carlo methods.



1 2 3 4 5 6 7 8 9 10 +1
5′ A p T p G p A p G p C p G p A p A p T p A
A p T p A p A p G p C p G p A p G p T p A 5′

Figure 1. ^{31}P–^1H heteronuclear correlation spectrum of $[d(ATGAGCGAATA)]_2$ at 30 °C. The one-dimensional ^{31}P spectrum is shown at the top with H3′/H4′/H5′ chemical shifts on the vertical axis. The peak labeled B_{II} at −2 ppm (corresponding to two phosphates) is downfield-shifted by ca. 2 ppm from the other peaks. The (n − 1)H3′–(n)^{31}P crosspeaks (upper) are connected to the (n)^{31}P–(n)H4′ crosspeaks by vertical lines and are labeled with residue numbers. The combined (n − 1)H3′–(n)^{31}P–(n)H4′ correlations lead to unambiguous assignments for most of the phosphorus peaks. The downfield-shifted peaks are obviously 4P and 8P. As observed by others,[14] the 4-bond ^{31}P–H4′ crosspeaks are invariably more intense than the 3-bond ^{31}P–H5′(R)/H5′(S) crosspeaks.

Firstly, all nonexchangeable protons, including the stereospecific H5′(R)/H5′(S) protons of 3G/7G, were assigned. Many unusual NOEs around the adjacent G:A mismatch pairs were detected: these included strong 4A and 8A crosspeaks between (n)AH2–(m + 1)H1′, (n)AH2–(m + 1)H4′, and (n)AH2–(m + 1)H5′ protons, as well as several other unexpected intensities (Chou et al.). These atypical NOEs, combined with all other measuremable NOEs, were converted into distance constraints for the DSPACE program,[12] from which 22 independent structures were generated. Experimental lower-bound constraints were used to restrict backbone torsional angles to ranges consistent with the observed backbone coupling constants and NOEs, as described by Kim et al.,[13] and the DG structures were further refined by back-calculation against the experimental NOESY spectra.[12] The refined structures all exhibited respectable helicity and converged to a narrow family of structures with small pairwise RMSD values (0.3–0.9 Å). Values of −50° ± 4° for ϵ and +103° ± 13° for ζ were observed for 4P and 8P, compared to values of 175° ± 15° and −85° ± 15° (i.e., t,g^-) for the other phosphate steps. Furthermore, exceptionally large twist angles of 77° were found at the G3–A4

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and G7–A8 mismatch steps, while much smaller twist values of 16–30° were found for all other base pair steps.

Secondly, two phosphorus resonances shifted downfield from ca. −4 to −2 ppm in the one-dimensional ^{31}P spectrum. From a ^{31}P–^{1}H correlation experiment,[6,14] these markedly downfield-shifted ^{31}P peaks were assigned to 4P/8P (i.e., 3GpA4 and 7GpA8) via the already assigned H3′/H4′ protons, see Figure 1.

Thirdly, to search more widely for alternate structures, a Monte Carlo conformational search[15-17] incorporating the refined NOE distance constraints was performed on the dinucleotide steps 3G–4A and 5G–6C using a modified program for nucleic acid molecules (Chuprina). Twist, roll, and slide and *x*-, *y*-, and *z*-displacements were used as variables, the sugar conformation was varied throughout the *S* domain, and the χ angles were restricted to the broad anti range to reduce the search time. Twelve million different dinucleotide conformations were generated starting from a B-form model. Those conformations that did not fit the experimental NOE distance constraints were rejected, and only the remaining ~300 that fit all of the experimental data were used for statistical analysis. For the ~300 acceptable 3G–4A conformers, the ϵ angles were *all* in the −120° to −80° range and all ζ angles were ca. +170° (i.e., no t,g^- conformers), while the corresponding angles in the ~300 allowed G–C steps were +160° ± 30° and −120° ± 60°, respectively (i.e., all t,g^-).

Our observation of a 2 ppm ^{31}P downfield shift and a 77° twist at the 3GpA4 step agrees with previously proposed shift/twist correlations.[3,18,19] Furthermore, our refined values of ca. +105° for ζ and ca. −150° for α in the B_{II} phosphate are in qualitative agreement with Gorenstein's proposal[18] that $\zeta(t) - \alpha(t)$ phosphates should resonate downfield of $\zeta(g^-) - \alpha(g^-)$ conformations. However, the correlations between ^{31}P chemical shift and $J_{H3'-P}$ proposed by Gorenstein and co-workers[14,20] are not borne out by our results. These authors[14,20] predicted that a predominantly B_{II} conformation should have a phosphorus chemical shift of −3.0 ppm and a $J_{H3'-P}$ value of 10 Hz. The chemical shift of the 4P and 8P B_{II} phosphates ($\epsilon = -50°$) is −2.0 ppm (about twice the predicted downfield shift), and they have $J_{H3'-P}$ values of less than 5 Hz rather than the predicted 10 Hz, suggesting that the assumptions underlying the predictions need to be reexamined. While ζ and α appear to be correlated to the ^{31}P chemical shift, ϵ and $J_{H3'-P}$ do not.

In a recently published paper, Li et al.[11] reported the construction of an energy-minimized model of an ATGAGC/GCGAAT hexamer containing adjacent G:A mismatch base pairs that satisfied their partial NMR distance data. In constructing this model, all torsional angles were kept as close as possible to standard B-form DNA values (i.e., B_I phosphates were imposed). In contrast, the structure generated from our full data set of refined NMR distances (with no prior backbone assumptions), together with the anomalous chemical shift of the phosphodiester linking the adjacent GA pairs, strongly suggests that this step involves a B_{II} backbone conformation and that a B_I conformation at this step is incompatible with a complete set of experimental distance data. Finally, we note that 3P and 7P, preceding the B_{II} phosphates at the mismatch site, are shifted upfield to ca. −4.7 ppm from the ca. −4 ppm shift of the normal B_I phosphates.

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Registry No. Guanine, 73-40-5; adenine, 73-24-5; d(ATGAGCGAATA), 116338-86-4.

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Expedient Assembly of Carbocyclic, Heterocyclic, and Polycyclic Compounds by Me_3Sn Radical Mediated Carbocyclizations of Dienes and Trienes: A Novel Oxidative Cleavage of the C–Sn Bond

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The formation of carbocyclic and heterocyclic compounds from terminal dienes by free-radical processes has received considerable attention in recent years.[1-5] However, except for a single example involving a transannular reaction,[6] and excluding vinylic radicals,[7] the use of trialkylstannyl radicals for the carbocyclization of dienes has remained largely unexplored. This is probably due to early reports of mono- or bis-addition without cyclization[6,8] or due to the view that the stannyl alkyl radical adduct would revert to alkene rather than cyclize, especially in dilute solution.[9] The paucity of methods to efficiently cleave an unactivated C–Sn bond[10,11] has undoubtedly been an added deterrent in considering the obvious potential of this approach to ring formation. We now report that trimethylstannyl radicals[12] add to the unsubstituted terminal olefinic carbon atom of a variety of activated and

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