interact with their target enzymes (adenylate cyclase or phosphodiesterase) and are converted to inactive GDP-bound forms by intrinsic GTPase activity. GTP-bound forms are regenerated by interaction with activated receptors. It is likely that, by analogy with EF-Tu and G proteins, the GTP- and GDP-bound forms of p21 represent active and inactive conformations that are interconverted by intrinsic GTPase and catalytic GTP-GDP exchange. Identification of the role of the active form of p21 and knowledge of the mechanism by which it is regenerated after hydrolysis would greatly accelerate understanding of the transformation process.

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Z-DNA Forms Without an Alternating Purine-Pyrimidine Sequence in Solution

Abstract. *Nuclear magnetic resonance spectra (proton and phosphorus-31) and ultraviolet absorption spectra of the DNA decamer d(br^{5}CGbr^{5}CGATbr^{5}CGbr^{5}CG), in which the central two adenine-thymine base pairs are out of order with the rest of the purine-pyrimidine alternation sequence, indicate that under appropriate solvent conditions (high salt and methanol) the molecule undergoes a structural transition from a right-handed B-DNA conformation to a left-handed Z-DNA conformation. Measurements of the two-dimensional nuclear Overhauser effect on the decamer indicate that all of the guanines as well as the two equivalent thymines adopt the* syn *conformation.*

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There is a dynamic equilibrium between right-handed B-DNA and left-handed Z-DNA, and the former is usually the major species (*1*). In B-DNA, all bases are in the *anti* conformation, whereas in Z-DNA, consecutive bases alternate between the *anti* and *syn* conformations. Purines adopt the *syn* conformation more readily than pyrimidines; thus the B to Z equilibrium has, until recently, been studied in solution only in polymers with alternating purine-pyrimidine sequences such as poly(dG-dC) (*2, 3*), and poly [d(A-C) · d(G-T)] as well as other modified polymers (*4, 5*), or in oligomers such as (m^{5}dC-dG)$_3$ (*6, 7*). Indirect evidence has suggested that naturally occurring sequences of DNA that are not strictly alternating purine-pyrimidine can be stabilized in the Z conformation by negative supercoiling (*8*). To investigate the structure of the putative Z-DNA in such sequences, we designed a series of DNA oligomers that were brominated at the 5-position of cytosine, since previous studies had shown that this modification strongly favors the Z-DNA conformation (*9*). A recently examined crystal structure of the DNA oligomer d(br^{5}CGATbr^{5}CG) showed that it adopts the Z conformation with its thymines in the *syn* conformation (*10*), but its stability as Z-DNA in solution remains questionable.

We now show that in solution the modified DNA decamer, d(br^{5}CGbr^5-CGATbr^{5}CGbr^{5}CG), which has a nonalternating purine-pyrimidine sequence, undergoes a structural transition from the B to Z conformation under appropriate solvent conditions. Large changes in the proton (^{1}H) and phosphorus (^{31}P) nuclear magnetic resonance (NMR) spectra are observed when the solvent is changed from an aqueous solution with low salt concentration to one that contains a high salt concentration and methanol. Measurements of the two-dimensional nuclear Overhauser effect (2D-nOe) (*10, 11*) indicate that all of the guanines as well as the two equivalent thymines are in the *syn* conformation in a methanol-water solution with a high concentration of salt. The differences between the absorption spectra of the molecule in the two solvent systems are also

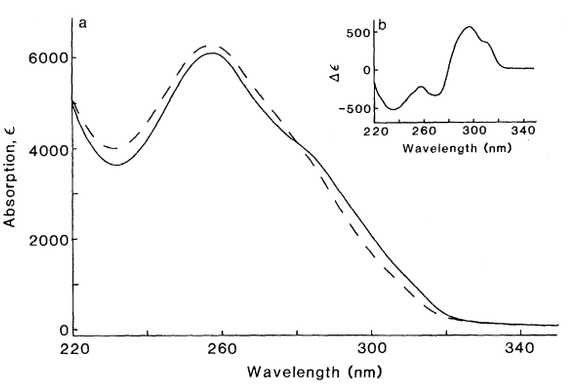

Fig. 1. (a) Ultraviolet absorption spectra of d(br^{5}CGbr^5-CGATbr^{5}CGbr^{5}CG) in aqueous solutions containing 0.1*M* NaCl (dashed line) and 3*M* NaCl and 33 percent methanol (solid line). (b) The difference spectrum (Z − B). There is an isosbestic point at about 279 nm. We also found that the absorption spectrum of the decamer in a 0.1*M* NaCl solution with 33 percent methanol is nearly identical to that in 0.1*M* NaCl alone. The DNA was prepared by improved phosphotriester synthesis (*22*). The starting material for the modified cytosines was 5-bromodeoxycytidine. The DNA concentration for these ultraviolet absorption experiments is 100 times less than for the NMR experiments.

consistent with those previously observed for B and Z DNA.

A distinct change in the absorption spectrum of d(br^{5}CGbr^{5}CGATbr^{5}CGbr^{5}CG) is observed when the solvent is changed from 0.1*M* NaCl in H_2O (low salt) to 3*M* NaCl, 33 percent methanol, and 67 percent H_2O (high salt and methanol) (Fig. 1a). The spectrum in the latter solution shows the characteristic hyperchromic effect in the 320- to 270-nm range and a hypochromic effect in the 270- to 240-nm range compared to the spectrum of the Z form of poly(dG-dC) in a low salt solution (*13*). The difference spectrum (Fig. 1b) shows a peak at 295 nm with a shoulder at 310 nm.

Evidence for two different conformations of the DNA decamer in the different solvents is also seen in the ^{31}P-NMR spectra of the two solutions (Fig. 2). In 1*M* salt solution all of the resonances are clustered in a region about 1 part per million (ppm) wide; this has also been observed at lower salt concentrations. In contrast, the ^{31}P-NMR spectrum of the high salt and methanol solution extends over a region of about 3 ppm. Four of the expected nine phosphorus resonances are shifted downfield in two pairs from the main cluster, by about 2.1, 2.0, 1.3, and 1.1 ppm from the largest peak, respectively.

Phosphorus-NMR studies of poly(dG-dC) (*2, 3*) and poly(dG-m^{5}dC) (*14, 15*) have shown that the transition from B-DNA to Z-DNA leads to a splitting of the phosphorous resonances into two groups with half the resonances shifting downfield by about 1.2 ppm. These spectral changes have been attributed to the different chemical and magnetic environments around the GpC and CpG phosphates in the zigzag alternating backbone of the Z-DNA structure (*16*). The low-field resonances have been assigned to the purine-3′, 5′-pyrimidine phosphates of GpC on the basis of the results obtained with (m^{5}dC-dG)$_3$ (*6*) and thiophosphate derivatives of poly(dG-dC) (*4, 5*). The data in Fig. 2 are consistent with conversion of the decamer from the B-DNA form in the low salt solution to the Z-DNA form in the high salt and methanol solution. If the entire molecule adopts a Z-DNA conformation, there should be large changes in the phosphodiester torsion angles and in the chemical and magnetic environments of alternate phosphates in the decamer. From the results obtained with (m^{5}dC-dG)$_3$ (*6, 7*), we expect two of the downfield-shifted resonances are from the Gpbr^{5}C phosphates and the other two from GpA and Tpbr^{5}C. Two of these resonances shift significantly further downfield (to about 2 ppm) than the previously observed shifts of about 1.2 to 1.5 ppm. The integrated intensity for the upfield group of ^{31}P resonances in the high salt and methanol solution is greater than can be accounted for by the five expected phosphates. We attribute this extra intensity to some residual non-Z form of DNA (probably aggregated B-DNA).

Proton-NMR spectra of the brominated decamer in a 0.2*M* NaCl solution in D_2O and in a 2.9*M* NaCl, 33 percent methanol-d_4, and 67 percent D_2O solution are shown in Fig. 3. The spectral differences are much greater than would be expected from the change in solvent alone. Large chemical-shift differences (greater than 0.4 ppm) are observed for the AH8, AH2, and TMe resonances in the two spectra. In contrast, we also found that the spectrum of the decamer in a low salt, methanol (33 percent), and D_2O solution is nearly identical to that in just low salt and D_2O. In the absence of methanol, addition of salt results in a broadening of the resonances with little change in the chemical shifts from the low salt solution (aggregated B-DNA).

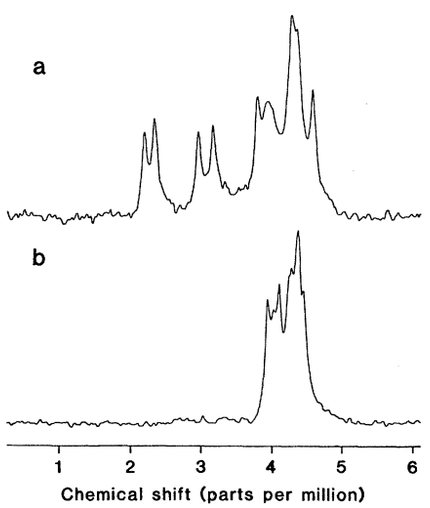

Fig. 2. Phosphorus-NMR spectra of d(br^{5}C-Gbr^{5}CGATbr^{5}CGbr^{5}CG) at 109 MHz and 40°C in aqueous solution (D_2O) containing 3*M* NaCl and 29 percent methanol-d_4 (a) and 1*M* NaCl (b). Chemical shifts are relative to external trimethylphosphate in 1*M* NaCl. Spectra were acquired with a sweep width of ±4000 Hz and a recycle delay of 1 second with broadband proton decoupling. Samples (280 μl) were contained in 5-mm, 528-pp NMR tubes (Wilmad). The sodium form of the decamer was prepared by cation exchange (Dowex AG-50), and the DNA was lyophilized, redissolved in D_2O, and stored frozen until use. The NMR samples (1 to 2 m*M* in duplex) were dried in the NMR tube under a stream of nitrogen and redissolved in 99.996 percent D_2O (Stohler Isotopes) or D_2O and 99.5 percent methanol-d_4 (Aldrich). Solid NaCl was added to reach the desired concentration. Samples were redried for the proton NMR experiments as necessary.

These spectra demonstrate that d(br^{5}CGbr^{5}CGATbr^{5}CGbr^{5}CG) is in a different conformation in the high salt and methanol solution from that in the low salt solution. To obtain information about the spatial relationship between various protons in the DNA molecule, and therefore information about the structure, the 2D-nOe on the decamer was measured (*11, 12*). Such measurements give spectra in two frequency domains; the resonance intensity from protons that do not cross-relax with other protons during the mixing time appear on the diagonal, and dipole-dipole (or chemical exchange) interactions between spatially proximate protons appear as off-diagonal cross-peaks. In a 2D-nOe experiment, all major dipolar interproton interactions are detected, and under appropriate conditions, the intensity of the cross-peak can be related to the interproton distances (*17*).

In the low salt solution, the decamer gives a pattern of nOe cross-peaks consistent with a B-DNA conformation (*18, 19*). In contrast, in the high salt and methanol solution, it has an nOe spectrum consistent with those observed for Z-DNA (*6, 18*). In particular, a large nOe is observed between the guanine H8 proton resonances, as well as the thymine H6 resonance, and their sugar H1′ resonances. Cross sections of a 2D-nOe spectrum of the decamer in the high salt and methanol solution show the nOe cross-peaks between the aromatic resonances and the rest of the spectrum (Fig. 4). Assignments of the GH8 and AH8 resonances were confirmed by chemical exchange with deuterium at high temperature. The TH6 resonance was identified by its strong nOe cross-peak to the T-methyl resonance. The complete assignments to specific bases for the molecule in both the B- and Z-DNA conformations were determined by analysis of 1D- and 2D-nOe spectra (*18*). The observation of large nOe cross-peaks between the GH8 and TH6 resonances and their H1′ sugar resonances indicate that these bases are all in the *syn* conformation. The relatively small nOe's from the other CH6 and AH8 resonances to the H1′ sugars and their large nOe's to the H2′ and H2″ sugar resonances indicate that the br^{5}dC and the dA bases are in the *anti* conformation.

The AT base pairs in this decamer disrupt the purine-pyrimidine alternation in the rest of the molecule; therefore, formation of Z-DNA for this sequence requires that the pyrimidine T adopt the *syn* conformation. A *syn* conformation for thymine has also been observed in the recent Z-DNA crystal structure of

d(br^{5}CGATbr^{5}CG) (*10*). The determination of the *syn* conformation for the T-base is important because it provides evidence that the central AT base pairs, in addition to the flanking CG regions of the decamer, are in the Z-DNA conformation. Furthermore, we have found that spectra of the imino protons from the AT base pairs in the Z-DNA conformation indicate that out-of-alternation AT base pairs in Z-DNA are less stable than in B-DNA.

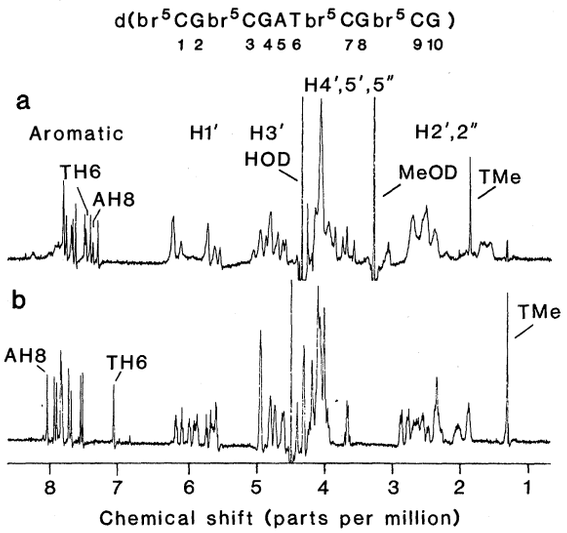

Fig. 3. Proton-NMR spectra of d(br^{5}CGbr^{5}CGATbr^{5}CGbr^{5}CG) at 500 MHz and 45°C in aqueous D_2O solution containing 2.9*M* NaCl and 33 percent methanol-d_4 (a) and 0.2*M* NaCl (b). Samples were prepared as described in Fig. 2. Chemical shifts were determined relative to an HDO peak that had been calibrated to 2,2-dimethyl-2-silapentane-5-sulfonate as a function of temperature and solvent conditions. Assignments of proton type and specific assignments of some of the base proton resonances that show the largest chemical shift differences in the two solvents are indicated. Spectra were collected with a pulse of about 60° and a 2-second recycle delay, and they were processed, before Fourier transformation, by convolution difference. A residual broad component, which was processed out by the convolution difference, appeared in all spectra of the high salt and methanol solution and is tentatively attributed to a small amount of aggregated B-DNA that remained in the sample.

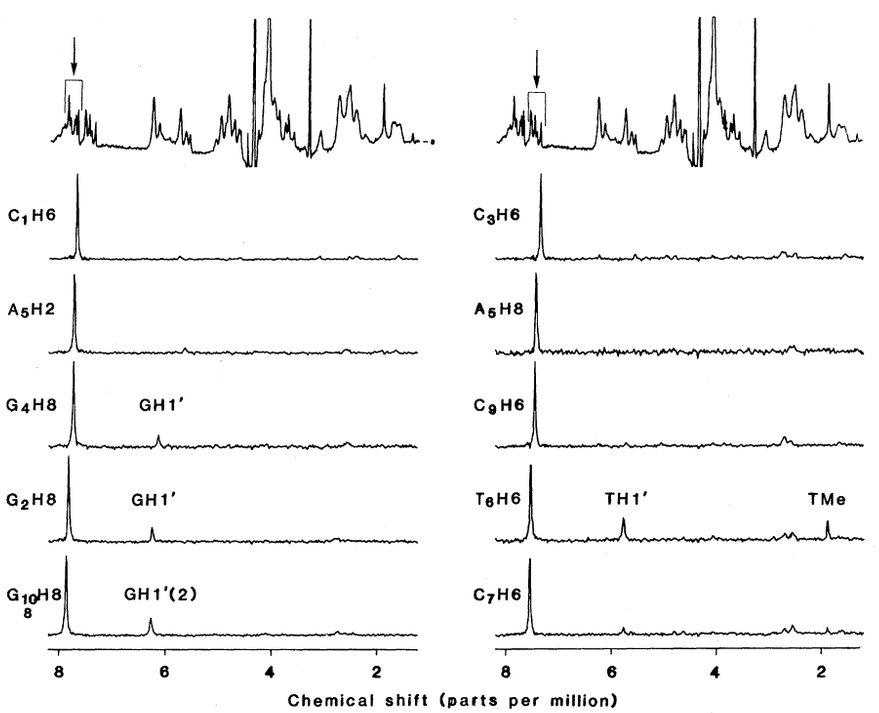

Fig. 4. Portion of a 2D-nOe spectrum of d(br^{5}CGbr^{5}CGATbr^{5}CGbr^{5}CG) at 45°C in an aqueous solution containing 2.9*M* NaCl and 33 percent methanol-d_4. Cross sections through the aromatic proton resonances are shown. Assignments to specific bases are indicated. The large nOe cross-peaks from the GH8 and TH6 proton resonances to their H1′ sugar resonances indicate that these bases are in the *syn* conformation. The 2D-nOe experiment was the usual three-pulse $[P_2\text{-}t_1\text{-}P_2\text{-}\tau_m\text{-}P_2\text{-}t_2\text{-}D_5]_n$ experiment (*12*). For the experiment shown, the mixing time τ_m was 270 milliseconds, and D_5 was 2 seconds. Spectra were collected by 32-step phase cycling (*23*) to allow processing in the pure absorption mode. Spectra were collected with 1024 complex points in t_2 and approximately 256 transients in t_1, which was zero-filled to 512 transients, resulting in a data matrix of 1024 points by 512 points. Spectra were line-broadened by 4 Hz in both dimensions.

The data presented here provide evidence for Z-DNA formation in solution in a DNA oligomer with a nonalternating purine-pyrimidine sequence. They show that under appropriate conditions thymine can adopt a *syn* conformation in a DNA duplex. This suggests that the barrier to the *syn* conformation for pyrimidine nucleotides in DNA is not as great as previously suggested (*20, 21*). This is a significant result in light of recent experiments that indicate that Z-DNA may exist in regions of negatively supercoiled plasmids that are not strictly alternating purine-pyrimidine in sequence as judged by anti–Z-DNA antibody binding (*1, 8*). In addition, the data obtained here, in conjunction with the Z-DNA crystal structure of d(br^{5}CGATbr^{5}CG), show that DNA that has only partial alternating purine-pyrimidine sequences can form a regular Z-DNA helix under appropriate conditions.

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