Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 2508–2511, June 1979
Chemistry

"Alternating B-DNA" conformation for the oligo(dG-dC) duplex in high-salt solution

(nucleic acid structure/Pohl–Jovin transition/base pair overlaps/glycosidic torsion angles/phosphodiester linkages)

DINSHAW J. PATEL*, LITA L. CANUEL*, AND FRITZ M. POHL†

*Bell Laboratories, Murray Hill, New Jersey 07974; and †University of Konstanz, Konstanz, West Germany

Communicated by Frank Bovey, March 23, 1979

ABSTRACT The high resolution ^{1}H and ^{31}P NMR spectra of the $(dG-dC)_8$ duplex have been recorded in low- and high-salt solutions in order to evaluate the structural aspects of the salt-induced transition of oligo(dG-dC) in solution [Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* 67, 375–396]. The NMR data require that the $(dG-dC)_8$ duplex in 4 M NaCl adopt an "alternating B-DNA" conformation for which the symmetry unit repeats every two base pairs. By contrast, the oligomer duplex in low-salt solution is of the regular B-DNA type in solution. The chemical shift parameters for oligo(dG-dC) in high-salt solution demonstrate that every other glycosidic torsion angle and phosphodiester linkage adopts a different conformation from that observed in regular B-DNA. We demonstrate further that the generation of the "alternating B-DNA" structure is facilitated by introduction of halogen atoms at the 5 position of pyrimidine and that this probably reflects the greater overlap of this position with adjacent base pairs in high salt solution. An "alternating B-DNA" model has recently been proposed for alternating deoxy purine-deoxy pyrimidine polynucleotides based on the x-ray structure of pdA-dT-dA-dT [Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z. & Steitz, T. A. (1979) *J. Mol. Biol.*, in press].

Conformational variations in the structure of nucleic acid duplexes may play a fundamental role in the recognition of specific DNA sequences by proteins (1). Pohl and Jovin (2) demonstrated that the circular dichroism of oligo(dG-dC) and poly(dG-dC) inverts in the presence of a high salt concentration. This reversible transition exhibits an activation energy of 22 kcals in both directions (2). The conformational transition was also observed when 60% (final concentration) ethanol (3) or the antibiotic mitomycin was added to poly(dG-dC) in aqueous solution (4). The trypanocidal agent ethidium bromide binds to the low-salt form of poly(dG-dC) more strongly than to the high-salt form (5).

The salt-induced transition was not observed for the synthetic RNA poly(G-C) or the synthetic DNAs poly(dA-dT) and poly(dG)·poly(dC) (2). These results suggest that conformational features related to helical type, base composition, and base sequence play a crucial role in governing the presence or absence of the structural transition.

Drew *et al.* (6) have observed that crystals of dC-dG-dC-dG grown in low- and high-salt solutions exhibit different space groups, and they demonstrated a reversible transition between the two crystalline states.

We have monitored the ^{1}H and ^{31}P NMR parameters for $(dG-dC)_n$ as a function of salt concentration in an attempt to elucidate the structural aspects of the salt-induced conformational transition. Because the NMR line widths are narrower at the oligomer level, this paper discusses the NMR chemical shifts for oligo(dG-dC) of chain lengths 16 and 20–30.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

EXPERIMENTAL

The isolation and characterization of $(dG-dC)_n$ oligomers of chain lengths 16 ($n = 8$) and 20–30 ($n = 10–15$) have been reported (7). They were passed through Sephadex G10 columns prior to the recording of NMR spectra.

High-resolution 360-MHz proton NMR spectra were recorded in the continuous wave (CW) mode for the exchangeable protons and the continuous wave and Fourier transform (FT) modes for the nonexchangeable protons on a Bruker HX-360 NMR spectrometer interfaced to a Nicolet BNC-12 computer. The chemical shifts are referenced relative to internal standard 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). High-resolution 145.7-MHz phosphorus NMR spectra were recorded in the Fourier transform mode, and the chemical shifts are referenced relative to internal standard trimethylphosphate $[(CH_3O)_3PO]$.

RESULTS AND DISCUSSION

Oligo(dG-dC) Melting Transition. The helix–coil transition of $(dG-dC)_{10-15}$ has been monitored at the nonexchangeable proton resonances in low-salt (10 mM NaCl/1 mM phosphate) solution. The resonances are well resolved and the individual base and sugar protons shift as average peaks through the melting transition (Fig. 1). The base protons can be readily assigned, whereas it is not yet possible to definitively differentiate among protons on the sugar ring linked to the guanosine and cytidine residues. The cytidine H-5 and H-6 protons, which shift upfield on duplex formation, are sensitive indicators of base pair overlap geometries in the duplex state. By contrast, the sugar H-1′ chemical shifts predominantly monitor changes in the glycosidic torsion angles associated with the duplex-to-strand transition.

The magnitude and direction of the chemical shift changes associated with the duplex-to-strand transition of $(dG-dC)_8$ (Fig. 1) and poly(dG-dC) are similar to values reported for the melting transition of poly(dA-dU) (8), poly(dA-dT) (8), and poly(dI-dC) (9). These alternating purine-pyrimidine polynucleotide duplexes adopt B-DNA type overlap geometries in solutions (8, 10).

Salt-Induced $(dG-dC)_8$ Transition. Circular dichroism spectra have been recorded for oligo(dG-dC) duplexes as a function of salt concentration. The extent of the structural transition for $(dG-dC)_8$ as a function of NaCl is plotted in Fig. 2. The curve exhibits a transition midpoint at $\approx$2.5 M NaCl with the low-salt form predominating below 1 M NaCl and the high salt form, above 4 M NaCl.

Hydrogen Bonding. The 360-MHz exchangeable proton NMR spectra (12.5–14.0 ppm) of the $(dG-dC)_8$ duplex in 0.2 M NaCl and 4.0 M NaCl have been recorded in H_2O solution. The temperature dependence of the chemical shifts of the ex-

Abbreviation: DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

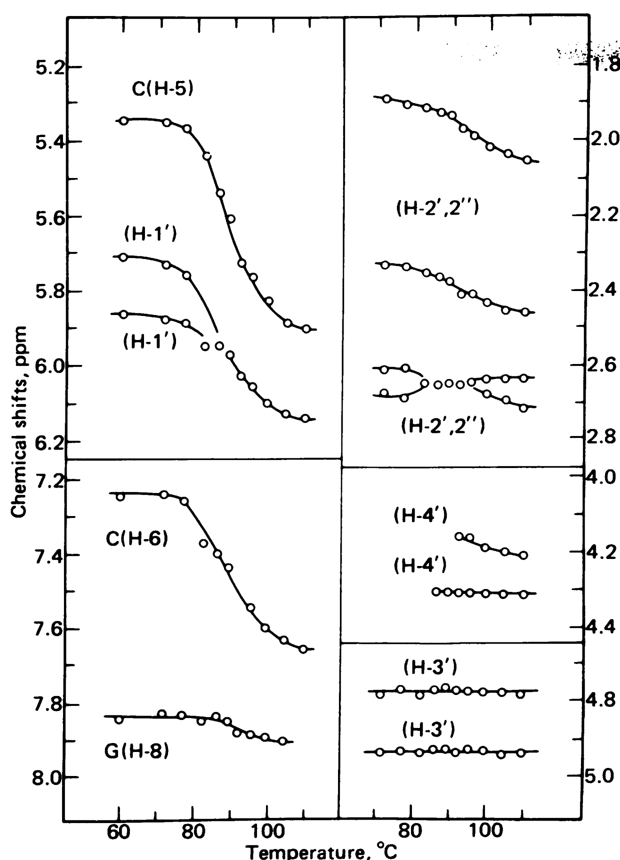

FIG. 1. Temperature dependence of base and sugar proton chemical shifts (relative to DSS) of (dG-dC)$_{10-15}$ in 10 mM NaCl/1 mM phosphate/1 mM EDTA/^2H_2O.

changeable proton in low- and high-salt solutions are plotted in Fig. 3.

Previous NMR studies have demonstrated that the guanosine H-1 proton in an intact dG.dC base pair resonates between 12 and 13.5 ppm in nucleic acid duplexes (11, 12). This resonance is observed for (dG-dC)$_8$ in 0.2 M NaCl and in 4.0 M NaCl (Fig. 3), indicating that both the low- and high-salt forms of oligo(dG-dC) form duplex structures. The hydrogen bonding

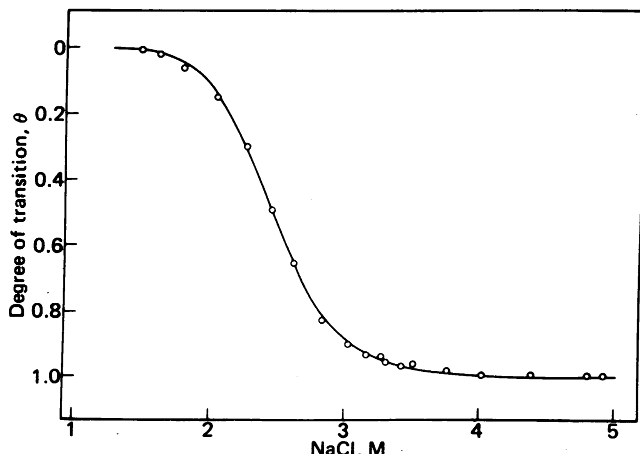

FIG. 2. Degree of salt-induced conformational transition for (dG-dC)$_8$ as a function of NaCl concentration deduced from circular dichroism experiments.

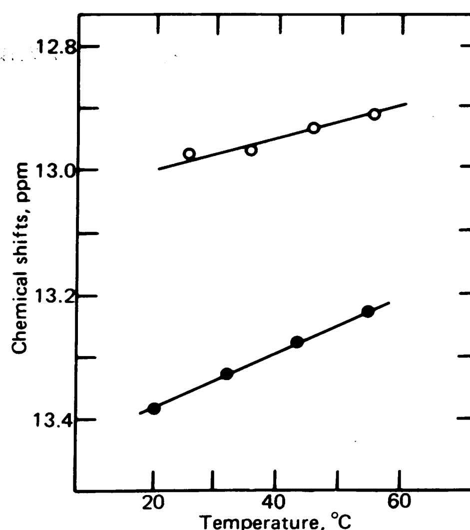

FIG. 3. Temperature dependence of the guanosine H-1 proton of (dG-dC)$_8$ in 1 mM cacodylate/0.1 mM EDTA/H_2O in the presence of 0.2 M NaCl (○) or 4.0 M NaCl (●).

arrangement is most likely to be of the Watson–Crick type in both states.

The observed downfield shift (0.3–0.4 ppm) of the guanosine H-1 proton of (dG-dC)$_8$ when the NaCl concentration was increased from 0.2 M to 4.0 M NaCl suggests a decrease in the ring current contributions (13, 14) at this proton from stacked adjacent base pairs for the conformation in high-salt solution.

Base Pair Overlaps. The temperature dependence of the chemical shifts of the base and sugar resonances of the (dG-dC)$_8$ duplex in low-salt solution (no NaCl, 0.2 M NaCl, and 1.0 M NaCl) and high-salt solution (4.0 M NaCl) are compared in Fig. 4 *A* and *B*, respectively. Selective chemical shift changes are observed in the spectral region 5.0–6.0 ppm between the two forms. The proton NMR spectra of (dG-dC)$_8$ in 2.5 M NaCl are superpositions of the spectra of the low- and high-salt forms, which is indicative of slow exchange between the two conformations on the NMR time scale. The data do not permit a correlation of known assignments of (dG-dC)$_8$ in low-salt solution (Fig. 4*A*) to those in high-salt solution (Fig. 4*B*).

The (dG-dC)$_8$ guanosine H-8 proton can be assigned in both states, because it can be selectively deuterated after heating in ^2H_2O at high temperature. This resonance exhibits the same chemical shift in low- and high-salt solution (Fig. 4). The resonance at 7.25 ppm is well separated from the spectral region at 5.0–6.2 ppm; therefore, this resonance at the same chemical shift in low- and high-salt solutions must correspond to the cytidine H-6 proton (Fig. 4). The position of the (dG-dC)$_8$ cytidine H-5 proton in high-salt solution has not been definitively assigned at this time. The similar chemical shifts for the guanosine H-8 and cytidine H-6 protons in low- and high-salt solutions (Fig. 4) suggest that the cytidine H-5 proton at 5.30 ppm in the low-salt structure (Fig. 4*A*) is unlikely to undergo a large shift on conversion to the high-salt structure. This permits the resonance at 5.16 ppm to be tentatively assigned to the cytidine H-5 resonance in high-salt solution (Fig. 4*B*).

A comparison of the chemical shifts of the nonexchangeable base protons of (dG-dC)$_8$ in the duplex state demonstrates that the guanosine H-8 and cytidine H-6 resonances are unperturbed in the presence of high-salt solution (Fig. 4). By contrast, the cytidine H-5 proton appears to shift upfield by 0.15 ppm in 4.0 M NaCl solution (Fig. 4), which is indicative of an increased ring current contribution from adjacent base pairs (13, 14) at

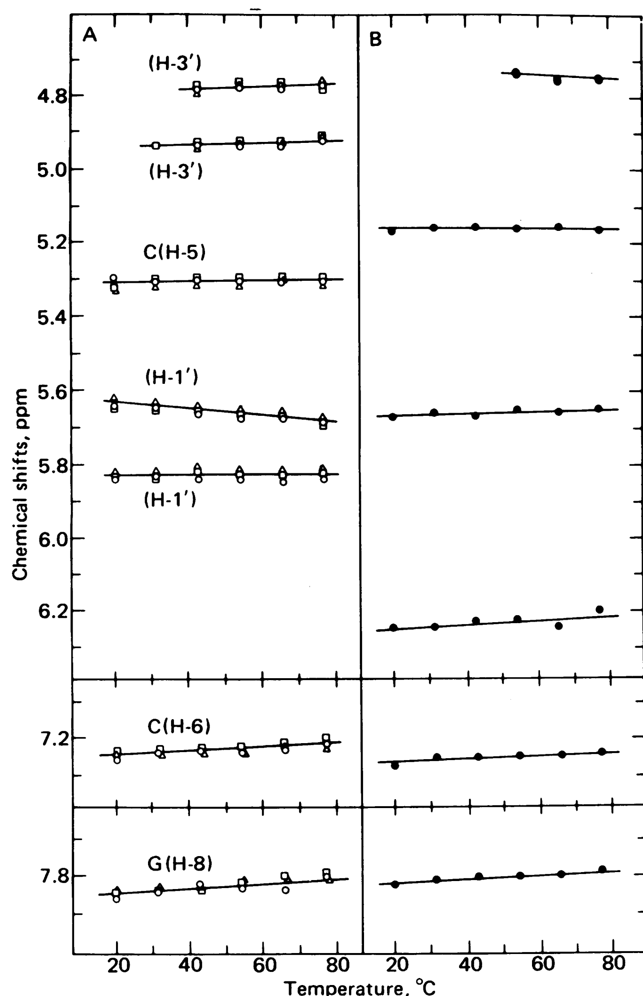

FIG. 4. Temperature dependence of the base and sugar proton chemical shifts (relative to DSS) of $(dG\text{-}dC)_8$ in 1 mM cacodylate/0.1 mM EDTA/^2H_2O for no added NaCl (□), 0.2 M NaCl (○), 1.0 M NaCl (△), and 4.0 M NaCl (●). (*A*) Low-salt form. (*B*) High-salt form.

this position. The above data suggest that the base pair overlap geometries for the oligo(dG-dC) duplex change on the addition of salt such that there is somewhat less overlap at the guanosine H-1 position and greater overlap at the cytidine H-5 position with adjacent base pairs.

Glycosidic Torsion Angles. The $(dG\text{-}dC)_8$ sugar H-1′ resonances are observed at 5.66 ppm and 5.84 ppm in low-salt solution (Fig. 4*A*) and at 5.66 ppm and 6.23 ppm in high-salt solution (Fig. 4*B*). The H-1′ resonances cannot be assigned to the sugars linked to the guanosine and cytidine residues of the oligo(dG-dC) duplex in either state at this time. However, it is readily apparent that one of the two sugar H-1′ protons undergoes a large downfield shift of 0.5 ± 0.1 ppm in high-salt solution (Fig. 4). The sugar H-1′ chemical shift is a sensitive indicator of the glycosidic torsion angle (15), and this suggests that either the guanosine or the cytidine glycosidic torsion angle in the oligo(dG-dC) duplex changes dramatically during the salt-induced conformational transition.

Sugar Pucker Geometries. The chemical shifts of the sugar H-3′ protons remain unchanged during the duplex-to-strand transition of $(dG\text{-}dC)_{10\text{-}15}$ in 10 mM buffer solution (Fig. 1). By contrast, the sugar H-3′ chemical shifts of $(dG\text{-}dC)_8$ in low-salt solution (4.77 ppm, 4.93 ppm) and high-salt solution (superimposable at 4.74 ppm) demonstrate a selective upfield shift of ≈0.2 ppm for one of the two sugar H-3′ resonances on addition of 4 M NaCl (Fig. 4). The sugar H-3′ proton chemical shifts may be sensitive to torsion angle changes about the C3′—C4′ backbone bond, which in turn is a measure of the pucker of the sugar ring.

Backbone Phosphate Torsion Angles. Previous ^{31}P NMR investigations have suggested that the internucleotide phosphate chemical shifts are sensitive to changes in the ω′(O3′-P) and ω(P-O5′) polynucleotide backbone torsion angles (16–18). We observe a single ^{31}P resonance at 4.20 ppm for $(dG\text{-}dC)_8$ in low-salt solution (Fig. 5, spectrum A), which suggests that the internucleotide phosphates linking dC(3′-5′)dG and dG(3′-5′)dC exhibit similar ω′,ω rotation angles. The ^{31}P resonances of oligo(dG-dC) are quite broad in low-salt solution, which requires that the chemical shifts be dispersed about 4.2 ppm.

By contrast, two well-resolved ^{31}P resonances of approximately equal area are observed for $(dG\text{-}dC)_8$ in 4 M NaCl solution with chemical shifts of 2.85 ppm and 4.34 ppm (Fig. 5, spectrum B). These two resonances correspond to the internucleotide phosphates linking dC(3′-5′)dG and those linking dG(3′-5′)dC, though we are currently unable to deduce which of these oligo(dG-dC) resonances shifts 1.5 ppm downfield in high-salt solution. The ^{31}P NMR results demonstrate that one of the two types of internucleotide phosphates of $(dG\text{-}dC)_8$ changes its ω′,ω rotation angles upon transfer from low- to high-salt solution.

Salt-Induced Poly(dI-br^{5}dC) Transition. Pohl and Jovin (2) noted that the circular dichroism spectrum of poly(dI-dC) does not invert in high-salt solution. We have now recorded the circular dichroism spectrum of poly(dI-br^{5}dC) and have ob-

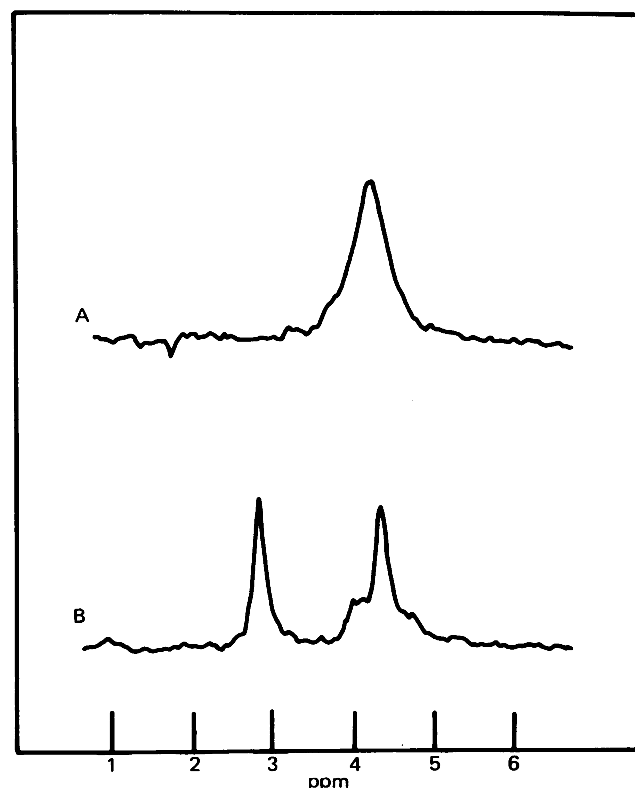

FIG. 5. The 145.7-MHz Fourier transform NMR spectra (1–6 ppm upfield from standard trimethylphosphate) for $(dG\text{-}dC)_8$ in 1 mM cacodylate/0.1 mM EDTA/H_2O in the presence of 0.2 M NaCl, pH 6.7, 27°C (spectrum A) and 4.0 M NaCl, pH 6.0, 27°C (spectrum B).

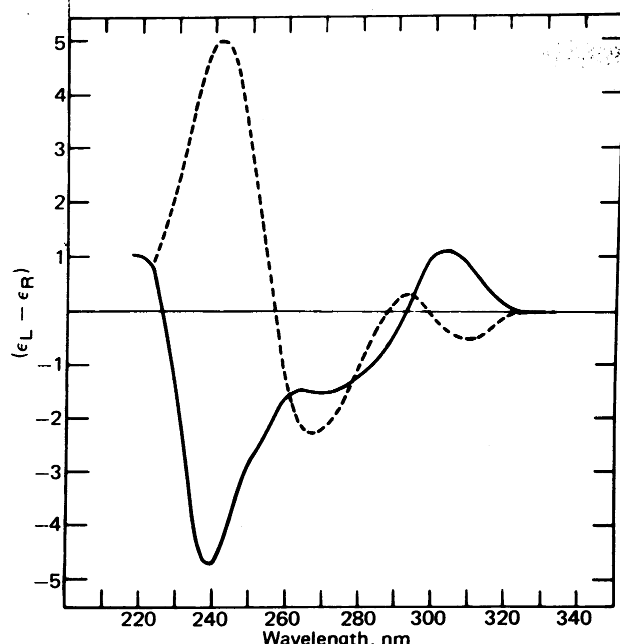

FIG. 6. Circular dichroism spectra of poly(dI-br^{5}dC) in 10 mM cacodylate/1 mM EDTA/H_2O, pH 6.8, in the presence of 0.1 M NaCl (—) or 4.0 M NaCl (- - -).

served an inversion of the spectrum with a transition midpoint at 3.3 M NaCl (Fig. 6). The results demonstate that the introduction of halogen atoms at the 5 position of cytidine stabilizes the high-salt conformation of alternating deoxypurine-deoxycytosine polynucleotide duplexes in solution. This may be related to the greater overlap of the cytidine 5 position with adjacent base pairs in the high-salt structure of such alternating sequence duplexes, as suggested earlier from the cytidine H-5 chemical shift data. By contrast, we did not observe an inversion of the circular dichroism spectra of either poly(dA-br^{5}dU) or poly(dA-i^{5}dU) in high-salt solution.

Possible Role of Metal Ion Binding in Stabilizing the High-Salt Conformation. The NMR chemical shift parameters demonstrate that every other glycosidic torsion angle and phosphodiester linkage for the oligo(dG-dC) duplex in high-salt solution adopts a different conformation from that observed in B-DNA. Computer model building studies have demonstrated that oxygens on pairs of adjacent phosphates can be 4 Å apart for direct coordination to metal ions when the glycosidic torsion angles alternate between RNA and DNA values and the backbone phosphate torsion angles alternate between *gauche, gauche* and *gauche,trans* values (19). It is therefore conceivable that pairs of adjacent phosphates generate metal ion binding sites in the oligo(dG-dC) conformation in high-salt (4 M Na^+ and 1.5 M Mg^{++}) solutions.

"Alternating B-DNA" Conformation. The concept of an "alternating B-DNA" conformation was first proposed by Klug and coworkers (20) for the structure of poly(dA-dT) in solution based on some structural features observed in the x-ray analysis of pdA-dT-dA-dT (21) and the effect of the enzyme DNAse I on the polymer (22). In summary, the "alternating B-DNA" conformation exhibits A-DNA type sugar pucker and glycosidic torsion angle at the purine residues but changes to B-DNA type sugar pucker and glycosidic torsion angle at the pyrimidine residues. The internucleotide phosphates linking purine(3′-5′)pyrimidine adopt ω',ω backbone O-P rotation angles similar to B-DNA but ω' changes for those linking pyrimidine(3′-5′)-purine. The pyrimidine 5 position is located more directly over the preceding purine ring in the "alternating B-DNA" models, which led Klug and coworkers to propose that halogen atoms at position 5 of the pyrimidine ring would stabilize this conformation (20, 21).

The NMR data to date suggests that the oligo(dG-dC) duplex in high-salt solution adopts an "alternating B-DNA" conformation for which the symmetry unit repeats every two base pairs. These conclusions have been confirmed by parallel studies on poly(dG-dC) as a function of salt concentration. A choice among possible models for the "alternating B-DNA" conformation of $(dG\text{-}dC)_n$ in high-salt solution must await the results of selective labeling studies to determine which glycosidic torsion angle (pyrimidine or purine) and which phosphodiester linkage [dG(3′-5′)dC or dC(3′-5′)dG] adopts a conformation different from that observed in B-DNA.

We thank Professor A. Klug for sending us a preprint of his "alternating B-DNA" model for poly(dA-dT). We also thank Professors H. Sobell and H. Berman for helpful discussions and preprints.

1. Arnott, S., Chandrasekaran, R. & Selsing, E. (1975) in *Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions*, eds. Sundaralingam, M. & Rao, S. T. (Univ. Park, Baltimore, MD), pp. 577–596.
2. Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375–396.
3. Pohl, F. M. (1976) *Nature (London)* **260**, 365–366.
4. Mercado, C. M. & Tomasz, M. (1977) *Biochemistry* **16**, 2039–2046.
5. Pohl, F. M., Jovin, T. M., Baehr, W. & Holbrook, J. J. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3805–3809.
6. Drew, H. R., Dickerson, R. E. & Itakura, K. (1978) *J. Mol. Biol* **25**, 535–543.
7. Pohl, F. M. (1974) *Eur. J. Biochem.* **42**, 495–504.
8. Patel, D. J. (1978) *J. Polym. Sci., Polym. Symp.* **62**, 117–141.
9. Patel, D. J. (1978) *Eur. J. Biochem.* **83**, 453–464.
10. Early, T. A., Olmstead, J., Kearns, D. R. & Lezins, A. G. (1978) *Nucleic Acids Res.* **5**, 1955–1970.
11. Kearns, D. R., Patel, D. J. & Shulman, R. G. (1971) *Nature (London)* **229**, 338–339.
12. Patel, D. J. & Tonelli, A. E. (1974) *Biopolymers* **13**, 1943–1964.
13. Giessner-Prettre, C., Pullman, B., Borer, P. N., Kan, L. S. & T'so, P. O. P. (1976) *Biopolymers* **15**, 2277–2286.
14. Arter, D. B. & Schmidt, P. G. (1976) *Nucleic Acids Res.* **3**, 1437–1447.
15. Giessner-Prettre, C. & Pullman, B. (1977) *J. Theor. Biol.* **65**, 171–188.
16. Gueron, M. & Shulman, R. G. (1975) *Proc. Natl. Acad. Sci. USA.* **72**, 3482–3485.
17. Patel, D. J. (1974) *Biochemistry* **13**, 2396–2402.
18. Gorenstein, D. G., Findlay, J. B., Momii, R. K, Luxon, B. A. & Kar, D. (1976) *Biochemistry* **15**, 3796–3803.
19. Berman, H. M., Broyde, S. & Neidle, S. (1979) in *Stereodynamics of Molecular Systems*, ed. Sarma, R. (Pergamon, Elmsford, NY), in press.
20. Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z. & Steitz, T. A. (1979) *J. Mol. Biol.*, in press.
21. Viswamitra, M. A., Kennard, O., Shakked, Z., Jones, P. G., Sheldrick, G. M., Salisbury, S. & Falvello, L. (1978) *Nature (London)* **173**, 687–688.
22. Scheffler, I. E., Elson, E. L. & Baldwin, R. L. (1968) *J. Mol. Biol.* **36**, 291–304.