Characterization of Alternating Deoxyribonucleic Acid Conformations in Solution by Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy†

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ABSTRACT: Medium length (50–200 bp) alternating purine–pyrimidine DNAs were prepared by sonication of synthetic polymers at low temperature. The products, and the hairpin structures derived from them after melting, were sufficiently small for high-resolution ^{31}P NMR studies. Of the five sequences studied, two DNAs, poly(dG-dC)·poly(dG-dC) and poly(dA-dU)·poly(dA-dU), gave singlet ^{31}P resonances, while three others, poly(dA-dT)·poly(dA-dT), poly(dA-br^{5}U)·poly(dA-br^{5}U), and poly(dI-dC)·poly(dI-dC), exhibited two resolved signals of equal area. This indicates the existence of two distinct alternating phosphodiester backbone conformations for these latter three B-DNAs in solution. Controls of homopolymers, which were also prepared by sonication, showed only singlet ^{31}P resonances. Of the alternating sequence DNAs, only sonicated poly(dG-dC)·poly(dG-dC) exhibited a conformational transition to a high salt (>2.5 M) form which exhibited two well-resolved ^{31}P resonances of equal area. This indicates that the high salt form of poly(dG-dC)·poly(dG-dC) also has an alternating backbone structure, and it is presumed to be a Z-DNA. These results indicate a general response of the DNA backbone conformation to alternating purine–pyrimidine base sequences but with a degree of sequence and environmental specificity which might have functional genetic significance.

NMR methods are valuable in the study of the conformation of DNA and polynucleotides in solution. These methods do have limitations, however, the most notable of which is that the broadness of the resonances increases with molecular size (or correlation time), thus preventing effective studies of very high molecular weight DNA (Hanlon et al., 1976).

To circumvent this problem in our ^{31}P NMR studies of DNA, we initially utilized nucleosome core particles as a source of relatively uniform low molecular weight (145 base pair) material (Shindo et al., 1978, 1980a). In the case of the alternating copolymer poly(dA-dT)·poly(dA-dT) obtained from reconstituted nucleosomes, the narrow ^{31}P NMR signals resulted in the resolution of two components of approximately equal area (Shindo et al., 1979). Calculations showed that these components could not derive directly from chemical shifts due to the effects of the different bases. It was concluded that they derived from two distinct conformational environments of the phosphodiester backbone in ApT and TpA sequences (Shindo et al., 1979), although on the basis of ^{31}P chemical shifts alone these conformations could not be specified.

In order to extend our ^{31}P NMR studies to medium-length deoxynucleotide duplexes of different sequences, it was necessary to have a more facile and general preparative method than the nuclease digestion of reconstituted nucleosomes. Not all DNAs of specific base sequences give rise to such complexes, and those that do provide only limited quantities (Bryan et al., 1979; Simpson & Kunzler, 1979; Rhoades, 1979). We have utilized the continuous sonication procedure described for calf thymus DNA by Davis & Phillips (1978). They used low temperature to achieve efficient DNA breakage and obtained fragments in the desired size range, and we have obtained similar results for commercial synthetic polydeoxynucleotide duplexes. We now report ^{31}P NMR results on products of this sonication process, which confirm and expand our previous findings on the phosphate backbone conformations of DNA.

Experimental Procedures

Polydeoxynucleotides were obtained from P-L Biochemicals, and poly(dA-dT)·poly(dA-dT) was also purchased from Miles Biochemicals, Inc. Usually 25 OD_{260} units were sonicated at the maximum output below cavitation by using a tapered probe with a Heat Systems-Ultrasonics, Model W175 or W225R sonicator. Solutions were 2–4 mL in 0.1 M NaCl contained in a thick-walled glass tube equipped with a nitrogen gas flow and copper–constantan thermocouple encased in a glass capillary. The temperature was monitored continuously with a digital thermometer. The temperature was adjusted as far as possible between 0 and 2 °C, using a CCl_4–MeOH–dry ice bath in a Dewar on a variable height jack or a circulating cooling bath. With the W225R sonicator, the temperature could be adjusted by using the pulse control (>50% duty cycle). After sonication, samples were centrifuged at 20 000 rpm for 30 min at 5 °C or passed through a Millex (Millipore) filter (0.54 μm). When 1 M NaCl was used as solvent, the sample was dialyzed with a Millipore immersible CX filter against 0.1 M NaCl in a test tube and concentrated to ~1 mL.

Polyacrylamide gel electrophoresis was carried out according to the procedure of Maniatis et al. (1975). Materials for gel electrophoresis were obtained from Bio-Rad. Twelve percent 3-mm gels were used, containing either 30% glycerol or 7 M urea. Electrophoresis was performed by using tris(hydroxymethyl)aminomethane (Tris)–borate–ethylenediaminetetraacetic acid (EDTA) buffer at ~300 V and 15 mA with an ISCO Model 492 power supply and a Bethesda Research Laboratory Model V16 vertical slab apparatus. Samples were concentrated to ~5 μL and made ~50% in glycerol containing bromphenol blue (~0.075%) before application to the gel. Standards for size determination were φX174 RF *Hae*III and λ *Hin*dIII fragments (Bethesda Research Laboratory) and nucleosomal DNA (145 bp) from chicken erythrocytes.

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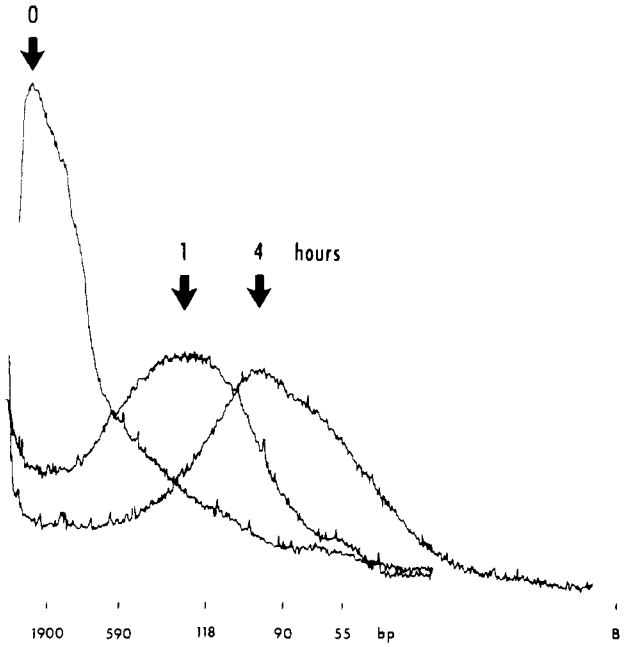

FIGURE 1: Microdensitometer tracing of polyacrylamide (12%) denaturing gel of sonicated poly(dG-dC)·poly(dG-dC). Aliquots taken at 0, 1, and 4 h of sonication appear from left to right; the arrows indicate the maxima. Visualization was achieved by staining with ethidium bromide and photographing under UV light. Sizes of synthetic fragments are standardized relative to ϕX174 RF *Hae*III and λ *Hin*dIII fragments. The intercepts at half-height of the 4-h peak occur at 45 and 195 base pairs (bp). B represents the position of bromphenol blue.

Sample chain lengths were estimated by using the formulation of Kovacic & Van Holde (1977). Bands were visualized by staining with ethidium bromide (0.001%) or "Stains-all" (0.005%). A Joyce-Loebl and Co. double-beam Model IIIB microdensitometer was used to scan the gel negatives.

To test for small fragments (<10 bp) in the products of sonication, aliquots were treated with cold perchloric acid (5%) for 1 h and centrifuged. UV measurements on a Gilford 240 spectrophotometer at 260 nm indicated 3–13% small fragments.

CD spectra were recorded on a Cary 61 spectrophotometer at ambient temperature in a 0.5-cm path-length Opticell quartz cell. Melting curves were obtained at 260 nm with a Beckman Acta III spectrophotometer controlled by a Hewlett-Packard 2100 computer. For concentrated solutions, a 1-mm path-length cell was used.

NMR spectra were obtained at 109.3 MHz on a partially NIH built spectrometer with a Bruker superconducting magnet and Nicolet 1180 computer. Between 5000 and 40 000 scans were acquired for each spectrum in 8K data points with a spectral window of ±4000 Hz and pulse repetition rate of 1.5 s. All spectra were proton decoupled with a power of ~3 W. A line broadening of 3 Hz was applied to the signal prior to Fourier transformation to enhance the signal to noise. Sonicated samples for NMR were usually 10–20 units in 1 mL of 0.1 M NaCl, and EDTA (~0.1 mM) was added. Chemical shifts are positive upfield from trimethyl phosphate as the internal reference signal (to convert to external 85% H_3PO_4, subtract 3.71 ppm).

Results

We have been able to routinely prepare medium-length polydeoxynucleotide duplexes by sonication for 3–4 h at low temperature. The sizes of the products were shown by gel electrophoresis to be in the range 50–200 bp (Figure 1). The size and distribution of the fragments from sonication were approximately the same for the homopolymers, e.g., poly(dA)·poly(dT), as for the alternating copolymers and were the same for sonication in low salt (0.1 M NaCl) as in high salt (1.0 M NaCl). The products of sonication were characterized by UV melting curves (Table I) and by CD spectra, and by both criteria were essentially the same as for the synthetic polymer starting materials, thus indicating the intactness of the materials used for the ^{31}P NMR studies.

Table I: Melting Temperatures of Sonication Products at 260 nm

sample	T_m (°C)
poly(dA-dT)·poly(dA-dT)	57
sonicated poly(dA-dT)·poly(dA-dT)	54 (57)[a]
sonicated polyd(A)·polyd(T)	61[a]
sonicated poly(dG-dC)·poly(dG-dC)	58.5
sonicated poly(dA-dU)·poly(dA-dU)	51.5
sonicated poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U)	66

[a] These samples at concentrations used for NMR experiments in 1-mm UV cell. Other samples diluted to ~0.6 OD_{260} unit in either 5 mM Tris and 0.1 mM EDTA (pH 6.8) or 1 mM cacodylate (pH 7.2).

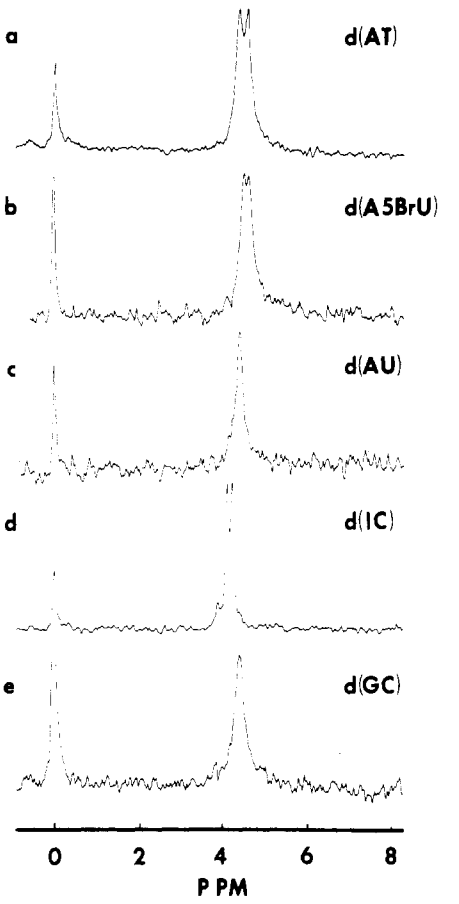

FIGURE 2: ^{31}P NMR spectra at 109.3 MHz of sonicated alternating purine–pyrimidine deoxynucleotide duplexes: (a) poly(dA-dT)·poly(dA-dT) at 24 °C; (b) poly(dA-dbr^{5}U)·poly(dA-dbrU) at 30 °C; (c) poly(dA-dU)·poly(dA-dU) at 31 °C; (d) poly(dI-dC)·poly(dI-dC at 39 °C; (e) poly(dG-dC)·poly(dG-dC) at 23 °C; all in 0.1 M NaCl and 0.05 mM EDTA, pH 6–7, except b in 5 mM Tris and 0.1 mM EDTA.

^{31}P NMR spectra of the synthetic polymer starting materials were very broad (line width $\simeq$ 200 Hz), as found previously (Shindo et al., 1979). The products after sonication gave sharp signals, particularly after being heated above the melting temperature and then being cooled (Figure 2). The formation of smaller hairpin structures (Scheffler et al., 1968) in these samples (ca. 25–100 bp) was shown to occur by electrophoresis on a nondenaturing gel (Figure 3). Of the five alternating

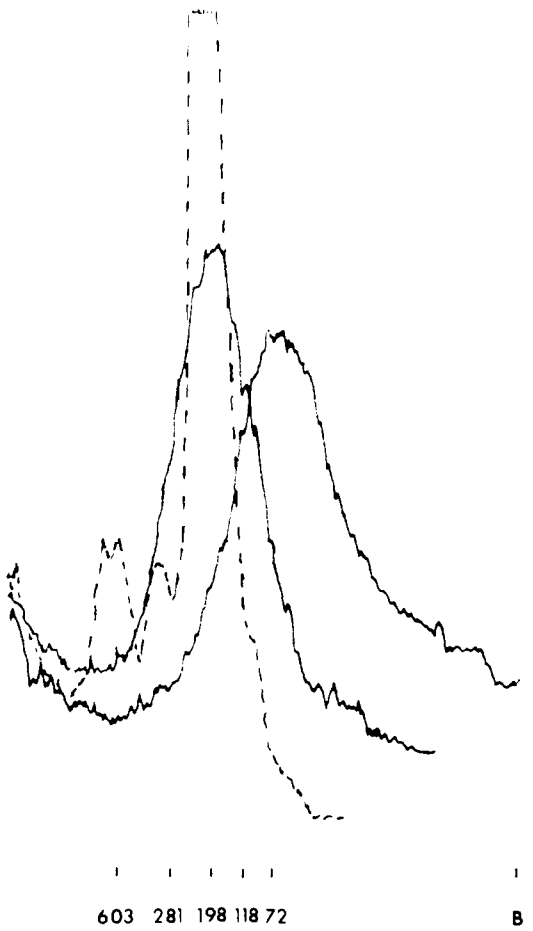

FIGURE 3: Microdensitometer tracing of sonicated poly(dA-dT)·poly(dA-dT) samples on a nondenaturing gel stained with ethidium bromide. The sample containing shorter lengths was derived from that containing longer ones following melting to hairpin structures. The dashed line derives from chicken erythrocyte nucleosome core particle DNA.

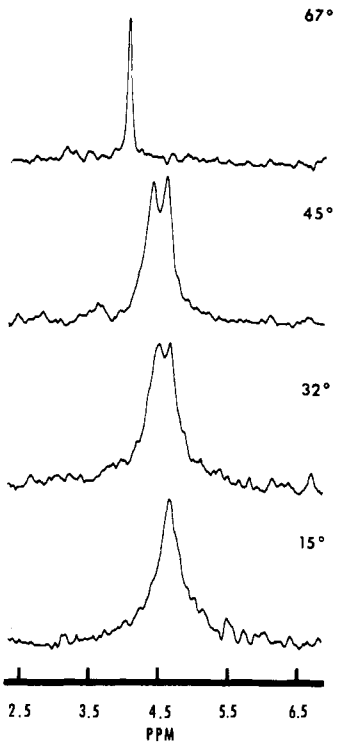

FIGURE 4: Temperature dependence of ^{31}P NMR spectra of sonicated poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U) in 5 mM Tris and 0.1 mM EDTA (pH 6.8) buffer.

purine–pyrimidine sequences studied, two gave singlet ^{31}P spectra and three exhibited "doublets", that is, resonances of equal area arising from a single molecular species (Figure 2).

The separation of the two components (21 Hz, Table II) for sonicated poly(dA-dT)·poly(dA-dT) was essentially the same for both unmelted and melted materials as that observed previously for the 145-bp alternating copolymer (Shindo et al., 1979); when the products were heated above the melting temperature, the doublet collapsed into a singlet but was reformed on cooling. The areas of the two components were equal within the accuracy of the estimation (Table II), as found previously (Simpson & Shindo, 1979). These similarities of the sonicated material prepared in this work to the previous results for 145-bp poly(dA-dT)·poly(dA-dT) indicate that the observation of a doublet resonance is not restricted to a specific-sized material and indeed is apparently not sensitive to the range of sizes. The only limitation is that the material be small enough to give sharp high-resolution spectra as a result of increased mobility and long enough to neglect end effects.

From the separation of the two components ($\times 2\pi$), one can estimate the rate of interconversion of the two conformations to be slower than 125 s^{-1}. It should be emphasized that the NMR result indicates that these are two distinct conformations, which are therefore not rapidly interconverting, although each, no doubt, exhibits significant local mobility (Shindo et al., 1980a).

The ^{31}P chemical shifts and line widths of the samples studied under various conditions are given in Table II. In all cases, when a doublet was observed at room temperature (~22 °C), it was better resolved at higher temperature as a result of the lines sharpening with increased mobility. For sonicated poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U), the line separation also increased slightly (Figure 4). Below room temperature, the singlet resonance for material after sonication occurred at the upfield chemical shift for several samples, indicating that one of the two conformations, which are represented by the doublet at room temperature, may be preferred. Above the melting temperature, a sharp resonance corresponding to the equivalent phosphates of single-stranded poly(dA-dbr^{5}U) was observed, and on cooling, the formation of the doublet down to 26 °C and the singlet down to 20 °C was completely reversible. The fact that the resonance of the melted species was shifted further downfield than the corresponding peak of single-stranded poly(dA-dT) at high temperature made the interpretation clearer for poly(dA-dbr^{5}U) since there was no overlap of the downfield component of the doublet of the room temperature form and the resonance of single-stranded material, as occurs for poly(dA-dT) (Shindo et al., 1979; Simpson & Shindo, 1979). For sonicated poly(dI-dC)·poly(dI-dC), although the doublet was not as well resolved at room temperature, the resonances were generally quite sharp and were well resolved at ~40 °C (Figure 5).

When sonicated poly(dG-dC)·poly(dG-dC) was heated (Figure 6), the singlet resonance sharpened somewhat, and a further component was observed at lower field which increased with increasing temperature and was attributed to single-stranded material. Even after cooling to room temperature, there was no evidence of formation of a doublet for poly(dG-dC)·poly(dG-dC). Similarly, sonicated poly(dA-dU)·poly(dA-dU) gave only a singlet resonance. An attempt was made to see if greater separation of the two components observed for d(AT)·d(AT) or d(IC)·d(IC) could be accomplished by adding aliquots of solutions of Co^{2+} and Tb^{3+} ions. The separation of the components increased only slightly (~7 Hz) before the lines were completely broadened; addition of

Table II: ^{31}P NMR Parameters of Sonicated Polydeoxynucleotides

duplex	T (°C)	salt[a] (M)	chemical shifts[b] (ppm)	separation of doublet[c] (Hz)	line width[d] (Hz)
d(A-T)·d(A-T)	24	0.1	4.324	21	21.5
			4.514		21
	54	0.1	4.133		
	24	4	4.041		
			4.394		
d(A-br^{5}U)·d(A-br^{5}U)	15	5 mM Tris	4.593		
	32	5 mM Tris	4.456	15	20
			4.594		23
	67	5 mM Tris	4.102		
	30	4	lb		
d(A-U)·d(A-U)	30	0.1	4.467		21
	65	0.1	4.191		
	24	4	4.012		
			4.318		
d(I-C)·d(I-C)	38	0.1	4.091	13	10
			4.212		8
	70	0.1	3.776		
			3.959	10	
			4.053		
	30	4	3.836		
			4.057		
d(G-C)·d(G-C)	23	0.1	4.264		29
	68	0.1	4.443		
			4.615		
	23	4	2.817	160	
			4.282		
d(A)·d(T)	24	0.1	4.512		47
d(I)·d(C)	24	0.1	4.525		20

[a] NaCl except where otherside stated; all samples also contain EDTA. [b] In ppm upfield from internal trimethyl phosphate. Actual values from the computer output are quoted. Braces indicate a doublet arising from a single molecular species. lb = line broadened. For comparison, values for single-stranded species are the following: poly(dA), 4.579; poly(dT), 3.991; poly(dC), 4.057; poly(dG), 3.932 ppm. [c] Separation of a clear doublet to the nearest Hz. [d] Line width to the nearest Hz, from curve fitting for doublets using the NTCAP program.

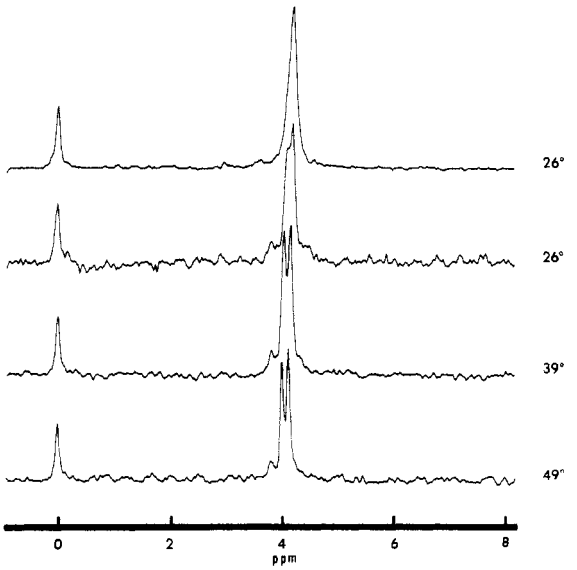

FIGURE 5: Temperature dependence of the ^{31}P NMR spectra of sonicated poly(dI-dC)·poly(dI-dC) in 0.1 M NaCl at pH 5.2. The sample was first observed at 26 °C (top); it was heated to ~70 °C for 10 min and cooled to room temperature. The spectrum was recorded at 26 °C, and then the temperature was increased stepwise.

excess EDTA to these solutions restored the original spectrum.

From a comparison of line widths of hairpin structures (Table II), it seems unlikely that the d(GC)·d(GC) or d-(AU)·d(AU) singlets could contain unresolved doublets (see Discussion), although we found that the line widths do vary somewhat from sample to sample. However, two conformations could be present and interconverting rapidly on the NMR time scale; from the line width for poly(dG-dC)·poly(dG-dC), the rate of interconversion would have to be faster than ~180 s^{-1}.

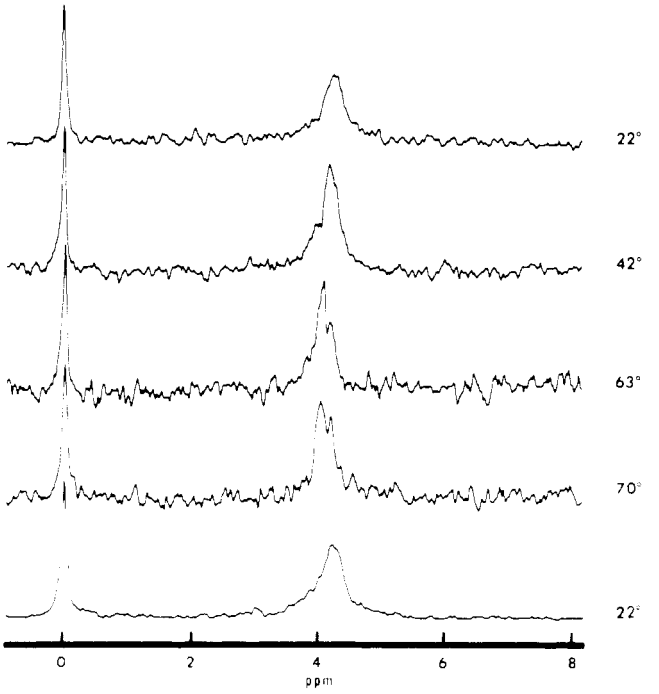

FIGURE 6: Temperature dependence of the ^{31}P NMR spectra of sonicated poly(dG-dC)·poly(dG-dC) in 0.1 M NaCl at pH 6.5. The temperature was increased in steps from 22 to 70 °C, followed by cooling to 22 °C (bottom).

When NaCl concentration was increased to high levels, most of the samples showed little or no change in their CD spectra. With addition of salt, sonicated poly(dA-dT)·poly(dA-dT) exhibited a stepwise change in the ^{31}P NMR spectra such that the downfield component increased in intensity (Figure 7). It is possible that this results from a preference for the confor-

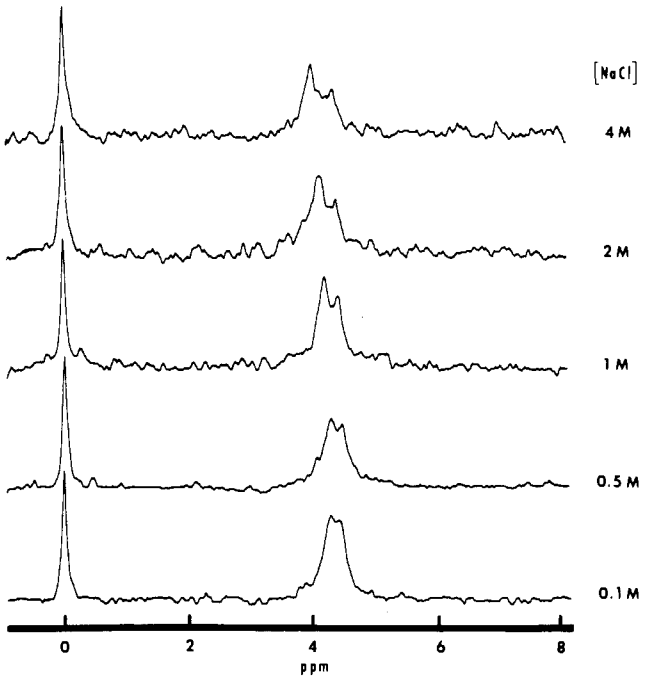

FIGURE 7: Salt dependence of ^{31}P NMR spectra of sonicated poly(dA-dT)·poly(dA-dT) at 22 °C, pH 6.5. Crystalline NaCl was added to give the desired concentration.

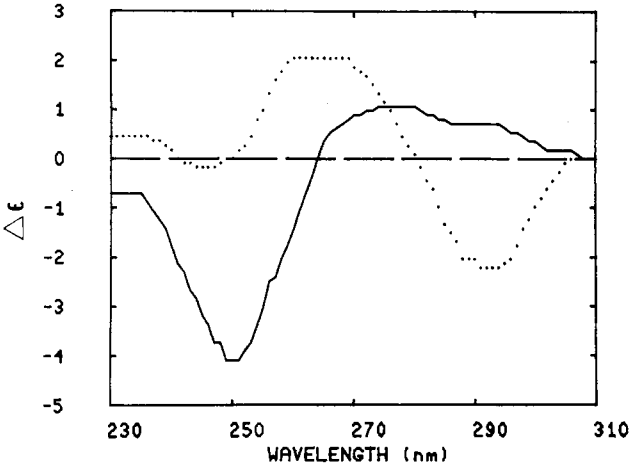

FIGURE 8: CD spectra of sonicated poly(dG-dC)·poly(dG-dC) in 0.1 M NaCl (—) and in ~4 M NaCl (···).

mation represented by this peak or possibly represents the formation of a slightly different structure with equivalent backbone conformations. Sonicated poly(dG-dC)·poly(dG-dC) exhibited the conformational transition described by Pohl & Jovin (1972) in CD spectra at high salt concentrations (Figure 8). ^{31}P NMR spectra also showed this conformational transition (Figure 9) involving first broadening of the signals, followed by the resolution of the two sharp signals of approximately equal area separated by 160 Hz at 4 M NaCl.[1] The absence of a clear transition point is consistent with the conversion from the low to the high salt form being length dependent (Brahms et al., 1976) since there is an approximate 4-fold range of sizes in our samples. Also, loss of intensity and broadening of the residual signal which is observed might

[1] A resonance was observed at ~3 ppm in samples of both sonicated poly(dG-dC)·poly(dG-dC) and poly(dI-dC)·poly(dI-dC) after stepwise heating in low salt and cooling to room temperature. The absence of an alternative explanation for a minor component at this chemical shift value might indicate the formation of a small amount of Z-DNA (5–10%) under these conditions.

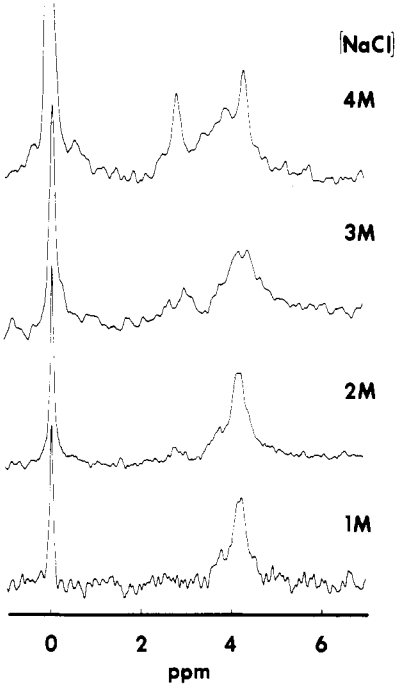

FIGURE 9: Salt dependence of ^{31}P NMR spectra of sonicated poly(dG-dC)·poly(dG-dC) at 22 °C, pH 6.5. Salt concentration was increased by adding crystalline NaCl.

result from collapse of the DNA, which is known to occur in very high salt concentrations (Wilson & Bloomfield, 1979). Addition of excess spermidine (to ~50 mM) to both sonicated poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT) in 50 mM NaCl and 0.2 mM EDTA gave no significant effects on their ^{31}P NMR spectra.

Discussion

Our initial evidence for conformational variance in the DNA backbone came from ^{31}P NMR studies of nucleosomal DNA from chicken erythrocytes (Shindo et al., 1978, 1980a). In order to establish the generality, or otherwise, of a phenomenon which is counter to accepted concepts, a systematic study of the phenomenon is desirable. This is what we have attempted here in relation to the question of the DNA backbone: is it a monotonic repetition as envisaged in the original Watson & Crick (1953) double-helical structure, or can it exhibit distinct conformational variance (Cohen, 1980)?

Such backbone variance was proposed independently by Shindo et al. (1979) from the presence of a doublet of two peaks of equal area in ^{31}P NMR spectra of 145-bp d(A-T)·d(A-T) in solution and by Klug et al. (1979) on the basis of the crystal structure of $d(A\text{-}T)_2$. Patel et al. (1979) also provided ^{31}P NMR evidence for an alternating structure of oligomers of d(G-C)·d(G-C) in high salt concentrations (see below), and they suggested that resolution of the ^{31}P resonances for d(G-G-A-A-T-T-C-C) duplex might indicate sequence dependence of the internucleotide bond angles (Patel & Canuel, 1979). However, results for short olignucleotides may be subject to possible end effects on chemical shifts. Selsing et al. (1978, 1979) have also described a block copolymer which has a conformational A-form–B-form transition, or backbone bend, at an RNA·DNA–DNA·DNA junction.

The results presented here show that medium-length oligodeoxynucleotide duplexes can be conveniently prepared by continuous sonication at low temperature. That this is a general method was shown by the results obtained for several copolymers of different sequences and under different salt conditions.

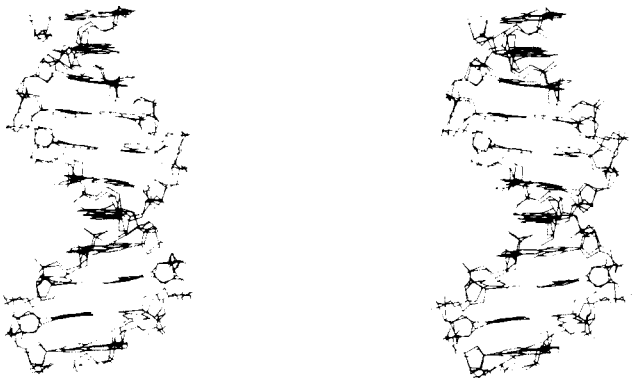

FIGURE 10: Stereoview of structures of B-DNA and alternating B-DNA (coordination of Klug et al., 1979) superimposed as far as possible to illustrate the differences. It should be noted that this latter structure, based on a dinucleotide crystal structure, is not necessarily the same as that present in solution.

The ^{31}P NMR spectra of the medium-length poly(dA-dT)·poly(dA-dT), poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U), and poly(dI-dC)·poly(dI-dC) showed two component resonances of approximately equal area (Figure 2). Some samples were heated above their melting temperatures and then cooled to room temperature, a process which gives rise to hairpin structures (Figure 3). However, the observation of a doublet for unmelted as well as for melted samples and for 145-bp d(A-T)·d(A-T) prepared from nucleosomes (Shindo et al., 1979) shows that hairpin structures are not the origin of such doublets. The present work indicates that the presence of a doublet is a general phenomenon for certain sequences, not dependent on the precise length or method of preparation. The resolution of the resonance of the high-temperature melted component from the doublet for poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U) (Figure 4) helps to clarify this observation in comparison to that for poly(dA-dT)·poly(dA-dT) where the resonances of these two constituents were not resolved, thus giving rise to some ambiguity in interpretation (Shindo et al., 1979; Simpson & Shindo, 1979).

The presence of the doublet in the ^{31}P NMR spectra of sonicated poly(dA-dT)·poly(dA-dT), poly(dA-dbr^{5}U)·poly(dA-dbr^{5}U), and poly(dI-dC)·poly(dI-dC) indicates that these DNAs are characterized by two phosphodiester backbone conformations. In view of the alternating sequences and the equal areas of the resonances over the temperature range 20–40 °C, it is concluded that these are alternating B-DNA conformations which are present in solution (Figure 10).

By contrast, sonicated poly(dG-dC)·poly(dG-dC) and poly(dA-dU)·poly(dA-dU) gave only singlet resonances under the same low salt conditions. In view of the relative values of the line widths (Table II), it seems unlikely that these peaks contain unresolved doublets (Simpson & Shindo, 1979, 1980), although such a possibility cannot yet be completely excluded. A rapid interconversion of various phosphodiester conformations is possible. Recent reports using NMR relaxation data have indicated much greater flexibility in DNA molecules than previously realized (Early & Kearns, 1979; Klevan et al., 1979; Shindo, 1980; Shindo et al., 1980a; Bolton & James, 1980; Hogan & Jardetzky, 1980), although the interpretation of these diverse results in terms of a single structural model has not yet been accomplished. However, such flexibility does not preclude the presence of two discrete backbone conformations, which are demonstrated by the observation of the two resolved ^{31}P resonances described above.

There appears to be no common stereochemical basis in the three DNA sequences for which two backbone conformations have been observed. Substitution at the pyrimidine 5 position may be suspected to play a role in the cases of d(A-T)·d(A-T) and d(A-br^{5}U)·d(A-br^{5}U) compared to d(A-U)·d(A-U). Whether the presence of three hyrogen bonds for a G-C base pair, compared to two for an I-C base pair, is a relevant cause of the difference between d(G-C)·d(G-C) and d(I-C)·d(I-C) is not known.

The effect of increasing salt concentration on the ^{31}P NMR spectra of medium-length sonicated poly(dG-dC)·poly(dG-dC) (Figure 9) was similar to that reported for shorter length synthetic oligomers (8–11 bp) (Patel et al., 1979) and 145-bp material (Simpson & Shindo, 1980). The conversion of this alternating copolymer to a distinct high salt form was originally described by Pohl & Jovin (1972) who termed it the "L form." The appearance of a doublet ^{31}P NMR resonance indicates that this high salt form also has an alternating backbone conformation. Partly on the basis of the conclusions of Klug et al. (1979) on poly(dA-dT)·poly(dA-dT), Patel et al. (1979) termed the high salt form of d(G-C) an "alternating B-DNA." However, Wang et al. (1979) have described a novel left-handed helix for crystalline d(C-G-C-G-C-G), with Watson–Crick base pairing but an unusual zigzag backbone with a dinucleotide repeat unit, and more recently Drew et al. (1980) have described a variant of this structure for d(C-G-C-G). This has been called "Z-DNA," and it appears very likely that this is also the structure of the high salt form of poly(dG-dC)·poly(dG-dC) in solution. Consequently, the ^{31}P NMR results on poly(dG-dC)·poly(dG-dC) in high salt probably correspond to an alternating Z-DNA, and the spectra in Figure 9 would then represent the transition from right-handed B-DNA to left-handed Z-DNA.

^{31}P NMR is here acting as a sensitive probe of the phosphodiester conformations of DNA *in solution*. Although the observation of oligonucleotide crystal structures with alternating backbone variations is suggestive of such structures for polynucleotides (Klug et al., 1979; Wang et al., 1979), only NMR has shown that such structures actually exist for polynucleotides in solution (Shindo et al., 1979; Patel et al., 1979).

The results for the alternating purine–pyrimidine copolymers presented here indicate the generality of the response of the backbone conformation to base sequence, although, as we have pointed out elsewhere (Cohen, 1980), precise backbone conformations corresponding to the ^{31}P chemical shifts exhibited by the alternating copolymers are not known. However structural correlations may now be facilitated; for example, it is most likely that the downfield-shifted component of the doublet in the spectra of the supposed Z-DNA corresponds to the gauche–trans conformation of the phosphodiester backbone of the dG residues rather than the usual gauche–gauche dC conformation (Gorenstein et al., 1976). The difference between the chemical shifts of the two components of the alternating B-DNAs is too small to allow a clear distinction between the two phosphodiester conformations proposed for the tetranucleotide crystal structure (Klug et al., 1979), although the coincidence of the upfield component with that at lower temperature (Figure 4) indicates that this resonance corresponds to the presumed Watson–Crick B-DNA backbone conformation.

Calculations show that the resolution of X-ray fiber diffraction patterns is not sufficient to enable a distinction to be made between B-DNA and alternating B-DNA in the solid state (S. Zimmerman, personal communication). Different conformations of DNA fibers are well-known to occur under different experimental conditions (Arnott, 1970). Arnott et al. (1980) have recently described a left-handed structure with

an alternating backbone conformation for several DNA fibers of alternating purine–pyrimidine sequence. Recent work by Shindo & Zimmerman (1980) and Shindo et al. (1980b), using solid-state ^{31}P NMR methods on DNA fibers including poly(dA-dT)·poly(dA-dT), also support the existence of backbone conformational variance in the solid state.

Apparently neither spermidine nor histones (Simpson & Shindo, 1979, 1980) induce conformational changes in DNA, as evidenced by the ^{31}P NMR spectra. The different DNA conformations described here for different sequences under different environmental conditions could, however, provide a basis for the specificity of different nucleases. These metalloproteins could induce or selectively interact with distinct phosphodiester geometries. Although there has been a great deal of speculation concerning a putative biological role for left-handed DNA, it seems much more likely that alternating B-DNA could have genetic significance in view of the relatively small energy and steric differences between B-DNA and its alternating form than between B-DNA and Z-DNA (Wang et al., 1979). If this is true, the spatial constellation of charged phosphate groups as well as the primary sequence encoded in the bases could provide the essential three-dimensional DNA recognition site for some drugs and selective enzymes, for example, the alternating [TATA...] boxes which are found to be present at the promoter region for RNA polymerase in many genes (Corden et al., 1980). It is noteworthy that such alternating sequences occur very infrequently in DNA (Dykes et al., 1974).

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