B to Z Transition of Double-Stranded Poly[deoxyguanylyl(3′–5′)-5-methyldeoxycytidine] in Solution by Phosphorus-31 and Carbon-13 Nuclear Magnetic Resonance Spectroscopy†

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ABSTRACT: The B to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) has been monitored by ^{31}P nuclear magnetic resonance (NMR) spectroscopy as a function of concentration of several salts. In 5 mM Tris- [tris(hydroxymethyl)aminomethane] HCl buffer two peaks of equal area (0.27 ppm separation) are observed at −4.2 ppm (upfield from trimethyl phosphate), indicating an alternating (right-handed) B conformation. Upon addition of NaCl, CsF, $MgCl_2$, or $Co(NH_3)_6Cl_3$, a signal arises at −3 ppm that is characteristic of the left-handed Z form. This signal is half of a doublet (1.3–2.1 ppm separation) and is tentatively assigned to the tg^+ phosphodiester conformation in the Z form. The midpoints of the cooperative B to Z transitions with these salts are in good agreement with the values reported from circular dichroism (CD) studies for this copolymer. The ^{13}C NMR spectra at natural abundance of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in the alternating B conformation in low salt show a broad resonance for the C2′ and no clear resonance for the C3′. This is in contrast to the alternating B form of poly(dA-dT)·poly(dA-dT) under similar conditions, which exhibits a well-resolved doublet for both C2′ and C3′ that is presumed to derive from alternating C2′-endo and C3′-endo deoxyribose conformations. In the ^{13}C NMR spectrum of the Z form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC), two major peaks are observed for the C2′, which may also indicate an alternating sugar conformation.

Variation in the secondary structure of DNA due to different base sequences has recently received a great deal of attention. Of particular note are studies relating to the putative significance of left-handed Z DNA (Nordheim et al., 1981), which is found in crystals of oligonucleotides with alternating d(G-C) sequences (Wang et al., 1979, 1981; Crawford et al., 1980; Drew et al., 1980). Conformational states of double-stranded polydeoxynucleotides in solution have been characterized by ^{31}P NMR[1] spectroscopy (Shindo et al., 1979; Cohen et al., 1981; Chen & Cohen, 1983). Specifically, a doublet was observed for poly(dG-dC)·poly(dG-dC) in high NaCl concentrations (Cohen et al., 1981), corresponding to the presumed Z DNA in solution, with essentially the same spectral parameters as those observed for oligomers (Patel et al., 1979). However, these d(G-C)-containing copolymers precipitate in the very high salt concentrations required to bring about the B–Z transition. Additionally, the high salt content adversely affects the ease of obtaining NMR spectra, not only due to reduced solubility and, hence, reduced sensitivity but also due to line broadening as a result of aggregation in solution.

Behe & Felsenfeld (1981) showed by CD and UV spectroscopy that poly(dG-m^{5}dC)·poly(dG-m^{5}dC) undergoes the same transition to the Z form as the unmethylated copolymer but at much lower salt concentrations. Not only does this bring the B–Z transition within the realm of physiological significance, but it also allows extensive studies of the Z form to be carried out by NMR spectroscopy in solution. We report here our results of such studies using ^{31}P and ^{13}C NMR spectroscopy. While this work was being prepared for publication, Patel et al. (1982) published a preliminary report of ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in low and high salt.

Experimental Procedures

Materials. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was synthesized from dGTP and 5-methyl-dCTP with poly(dI-dC)·poly(dI-dC) as the template according to the procedure described previously (Behe & Felsenfeld, 1981). After treatment with *Hha*I restriction enzyme, and in some cases followed by treatment with DNase I (Patel et al., 1982), the purified polymer gave a single transition during thermal-melting analysis, indicating the absence of residual template. The average size of this copolymer ranged between 150 and 700 base pairs (bp) as determined by gel electrophoresis. A sample of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was also purchased from P-L Biochemicals and was sonicated for 3 h (Chen et al., 1981) to reduce its size (final size ranged from 50 to 230 bp). For some of the ^{13}C NMR studies, the copolymer prepared in this laboratory was sonicated for 1 h. Poly(dA-dT)·poly(dA-dT) was obtained from P-L Biochemicals and was sonicated for 3 h before use. CsF was purchased from Aldrich and $Co(NH_3)_6Cl_3$ from Eastman Kodak.

^{31}P NMR Studies. The NMR samples usually contained 1–1.5 mg of poly(dG-m^{5}dC)·poly(dG-m^{5}dC), 5 mM Tris-HCl pH 8.0 buffer, and 0.1 mM EDTA in 1 mL of 25% D_2O/H_2O. Aliquots of NaCl, CsF, $MgCl_2$, or $Co(NH_3)_6Cl_3$ solutions were added to obtain the desired salt concentrations. The NMR spectra were obtained at 109.299 MHz on a Nicolet spectrometer with a Bruker superconducting magnet and a Nicolet 1180 computer and were recorded at 50–53 °C in the deuterium-lock mode with 4K data points and a spectral window

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[1] Abbreviations: NMR, nuclear magnetic resonance; CD, circular dichroism; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

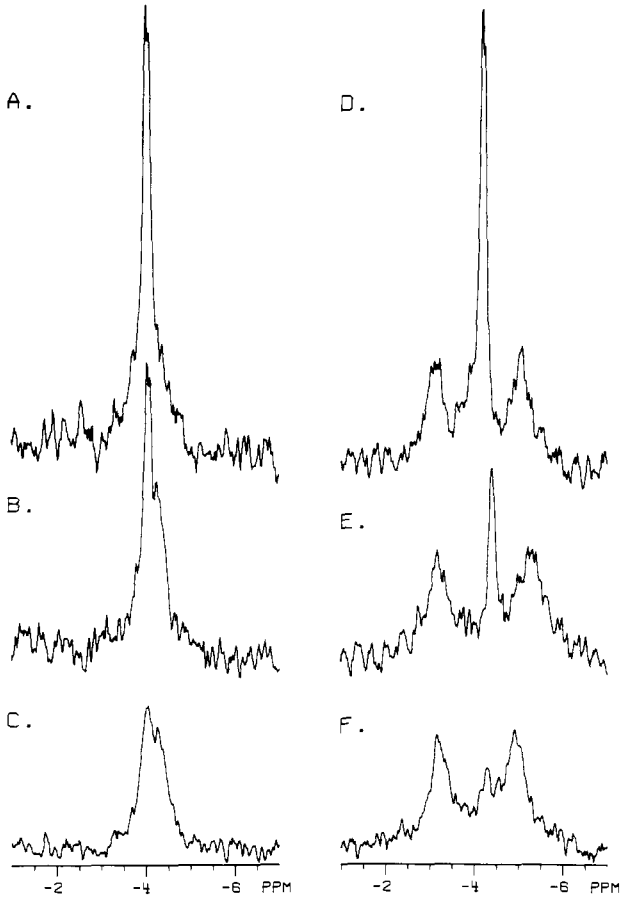

FIGURE 1: Purification of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) as followed by its ^{31}P NMR spectra: (A and D) before purification; (B and E) after *Hha*I digestion; (C and F) after further DNase I digestion. Spectra A, B, and C were in 5 mM Tris-HCl pH 8.0 buffer and 0.1 mM EDTA. Spectra D and E were in 1.5 mM $MgCl_2$, and spectrum F was in 2 mM $MgCl_2$ in addition to the buffer. All were taken at 50 °C except (D), which was at 65 °C.

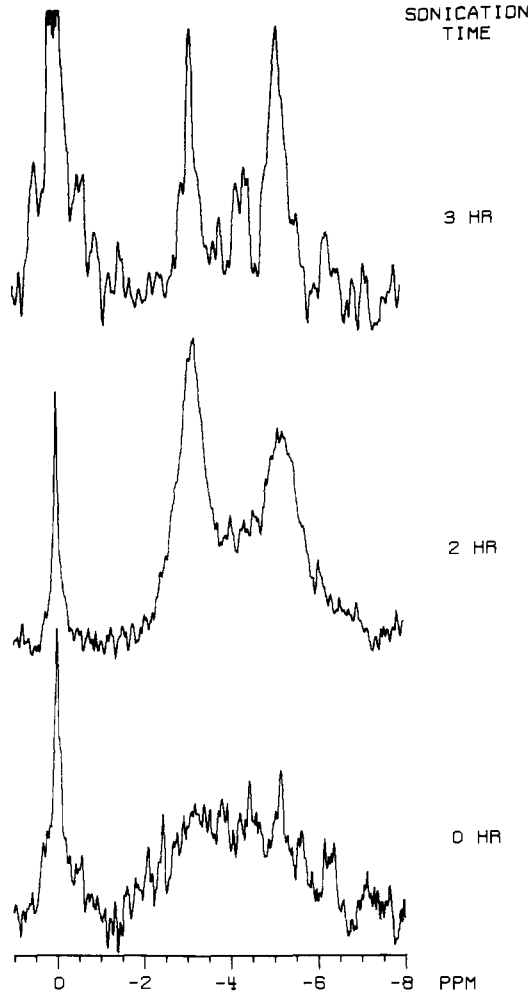

FIGURE 2: Effect of sonication on the ^{31}P NMR line width of poly(dG-m^{5}dC)·poly(dG-m^{5}dC). The copolymer was purchased from P-L Biochemicals. The molecular-size distributions as determined by gel electrophoresis were 900–3000 bp, 70–270 bp, and 50–200 bp for samples sonicated for 0, 2, and 3 h, respectively. All samples contained 5 mM Tris-HCl pH 8.0 buffer, 0.1 mM EDTA, and 1.5 mM $MgCl_2$, and the spectra were recorded at 50 °C.

of ±2000 Hz. Typically 5000–20000 scans were acquired, and a line broadening of 5 Hz was applied prior to Fourier transformation. All chemical shifts are quoted negative upfield from internal trimethyl phosphate (tmp). The relative peak areas in the spectra were obtained by fitting with the NTCCAP program available in the Nicolet 1180 computer and were estimated to have a standard error of ca. ±5%.

^{13}C NMR Studies. The ^{13}C NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) were obtained at 37 °C with 5 mg of the copolymer in 1 mL of D_2O containing the same buffer as above. Spectra of poly(dA-dT)·poly(dA-dT) were obtained under similar conditions except that the sample concentration was 5 mg in 0.3 mL of 0.1 M NaCl/33 mM phosphate buffer/1 mM EDTA. The spectra were observed at 125.757 MHz on a Nicolet NT500 spectrometer with an Oxford superconducting magnet and a Nicolet 1280E computer and were recorded with 32K data points, a spectral window of ±15000 Hz, a pulse width of 16 μs (75°), and a 2.5-s repetition time. A bilevel decoupling sequence was used to avoid overheating. In general, between 60000 and 90000 scans were accumulated, and a 20-Hz line broadening was applied. Chemical shifts are relative to internal $^{13}CH_3CN$.

Results

^{31}P NMR Studies and the B to Z Transition. ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) above and below the B to Z transition are shown in Figure 1. A sharp resonance at −4.0 ppm observed in material untreated with either *Hha*I or DNase I (Figure 1A,D) was attributed to the presence of the poly(dI-dC)·poly(dI-dC) template. This was supported by the presence of two transitions during thermal-melting analysis. In material treated with *Hha*I, this sharp component was removed, but a second impurity (ca. 33%) with slightly broader line width became visible (Figure 1B,E). The single melting transition of this *Hha*I-treated material indicated the absence of poly(dI-dC)·poly(dI-dC). Material that was further treated with DNase I showed only a residual (ca. 10%) peak due to this second impurity (Figure 1C,F). Because of the relative broadness of this residual peak (Figure 1B,C,E,F) compared to that of the unpurified preparation (Figure 1A,D) and to poly(dI-dC)·poly(dI-dC) itself under comparable conditions (not shown) and its apparently higher melting temperature than that of poly(dI-dC)·poly(dI-dC), this second impurity was attributed to poly(dG-m^{5}dC)·poly(dI-dC) hybrid. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) purchased from P-L Biochemicals was considerably larger in size than the material synthesized by us (Figure 2). Consequently, we sonicated it (Chen et al. 1981); and it was found to contain only small quantities (ca. 10%) of the residual impurity (Figures 2 and 3). Material that gave rise to the spectra in Figures 4–6 was prepared in this laboratory and was purified via *Hha*I treatment. The presence of the residual component, usually ca. 30–33%, was easily distinguished and corrected for.

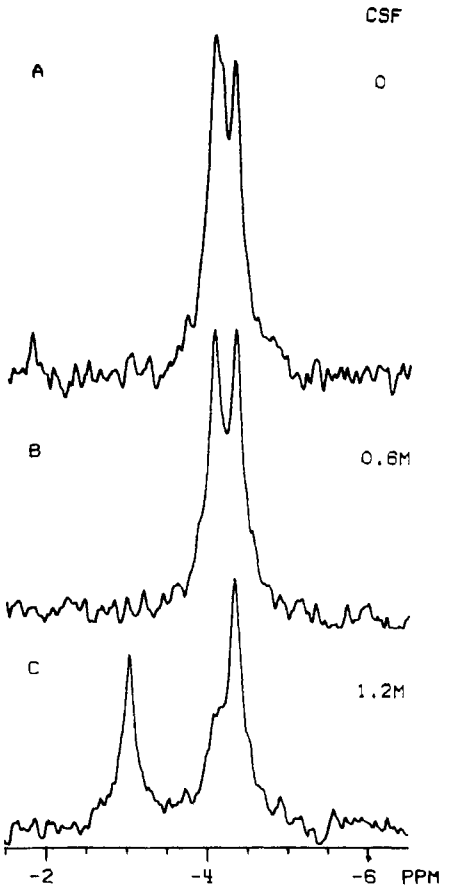

FIGURE 3: Effect of CsF concentration (M) on ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (obtained from P-L Biochemicals) at 50 °C. The copolymer had been sonicated for 3 h.

Table I: Salt Concentrations[a] at the Midpoint of the B-Z Transition from ^{31}P NMR Studies

salt	poly(dG-dC)· poly(dG-dC)	poly(dG-m^{5}dC)· poly(dG-m^{5}dC)
NaCl	3000 (3000)[b]	700
CsF	≫3590 (~4800)[b]	800
$MgCl_2$	900 (600)[b]	0.5
$Co(NH_3)_6Cl_3$		0.1 (~1)[b]

[a] All concentrations are in millimoles. [b] Salt concentration at which aggregation occurred.

Our ^{31}P NMR results indicate that poly(dG-m^{5}dC)·poly(dG-m^{5}dC) undergoes the B to Z transition, as evidenced by the appearance of the signal at ca. −3 ppm (Figures 3–6), at much lower salt concentrations than that for poly(dG-dC)·poly(dG-dC). This is in excellent agreement with the studies of Behe & Felsenfeld (1981). As shown in Table I, the Mg^{2+} concentration at the midpoint of the transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was 3 orders of magnitude lower than that of the unmethylated polymer. Likewise, the transition of the methylated d(G-C) could be induced at a NaCl (or CsF) concentration 4 (or >5) times lower than that required for unmethylated d(G-C). Because of the lower salt requirement, little or no aggregation of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) occurred during the course of the transition. By contrast, extensive line broadening and loss of signal intensity as a result of precipitation and severe aggregation were observed for poly(dG-dC)·poly(dG-dC) in NaCl and $MgCl_2$; and in CsF, no transition could be achieved before 90% of the polymer precipitated (Table I).

At low salt concentrations, a splitting in the resonance at ca. −4.1 ppm was observed for poly(dG-m^{5}dC)·poly(dG-m^{5}dC)

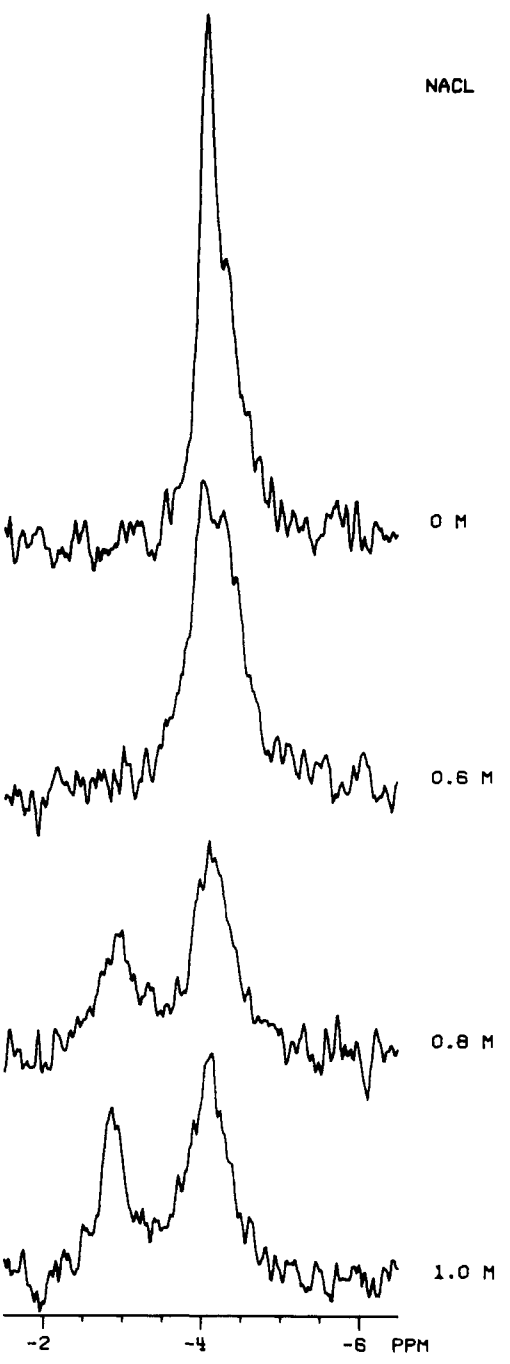

FIGURE 4: ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) as a function of NaCl concentration (M) at 50 °C. The copolymer was prepared in this laboratory and was purified via *Hha*I digestion. The hybrid impurity was estimated to constitute ca. 30% of the entire material.

(Figures 1C and 3A,B). This was similar to the doublet previously reported for poly(dA-dT)·poly(dA-dT) (Shindo et al., 1979; Simpson & Shindo, 1979; Cohen et al., 1981; Chen & Cohen, 1983) but not observed for poly(dG-dC)·poly(dG-dC) (Patel et al., 1979; Simpson & Shindo, 1980; Cohen et al., 1981; Chen & Cohen, 1983). The presence of the doublet may be attributed to an alternating phosphodiester-backbone conformation in the right-handed B form for poly(dG-m^{5}dC)·poly(dG-m^{5}dC). The resolution of this doublet was quite distinct from the presence of the impurity resonance described above, which could be subtracted as a constant contribution for a given sample and which coincided with the downfield component of the doublet (Figures 1 and 4–6). The downfield and upfield components ($\Delta\delta$ = 29 Hz, 0.27 ppm)

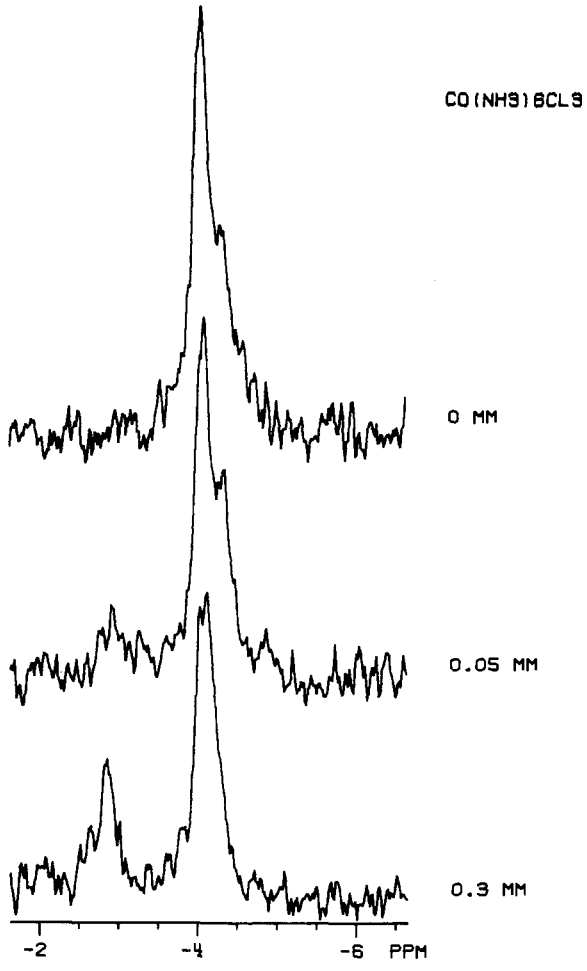

FIGURE 5: ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) as a function of $Co(NH_3)_6Cl_3$ concentration (mM) at 50 °C. See Figure 4 for details.

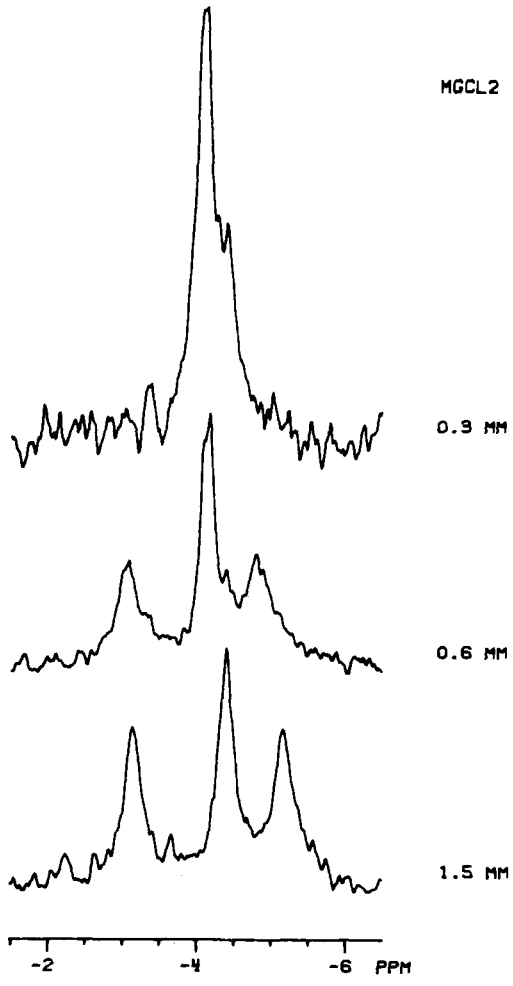

FIGURE 6: ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) as a function of $MgCl_2$ concentration (mM) at 50 °C. See Figure 4 for details.

presumably correspond to predominantly tg^- and g^-g^- conformations, respectively (Chen & Cohen, 1983). It appears in the case of CsF that the more downfield component of the two was preferentially transformed into the characteristic downfield-shifted Z-form resonance at −3 ppm (Figure 3), which represents mainly the tg^+ conformation, although this relationship was not clear for the other salts.

Except for the appearance of the new signal at ca. −3 ppm, there were only minor changes in chemical shifts below the B to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (Figures 3–6). The only significant change observed for this copolymer was the upfield shift of the upfield component after the transition as a result of Mg^{2+} addition (Figure 6). This shift was similar to the upfield shifts observed for other polydeoxynucleotide duplexes on addition of Mg^{2+} (Chen & Cohen, 1983). But the selective nature of the effect in this case enabled us to separate the resonance of the residual hybrid impurity from that of the g^+g^+ phosphodiester component of the Z form, which was presumably superimposed in the presence of CsF, NaCl, and $Co(NH_3)_6Cl_3$ (Figures 3–5).

^{13}C NMR Studies. Poly(dA-dT)·poly(dA-dT) exhibited a well-resolved doublet for the C2′ and C3′ carbon resonances with a separation of ca. 1.68 ppm (Figure 7). This indicates that the alternating conformation of this sequence not only exists in the phosphate backbone (Shindo et al., 1979; Cohen et al., 1981) but also extends to the deoxyribose sugar moieties. Our result agrees well with that of Shindo (1981) on 145-bp poly(dA-dT)·poly(dA-dT) observed at 68 MHz, although our resolution and sensitivity at 125 MHz are considerably better.

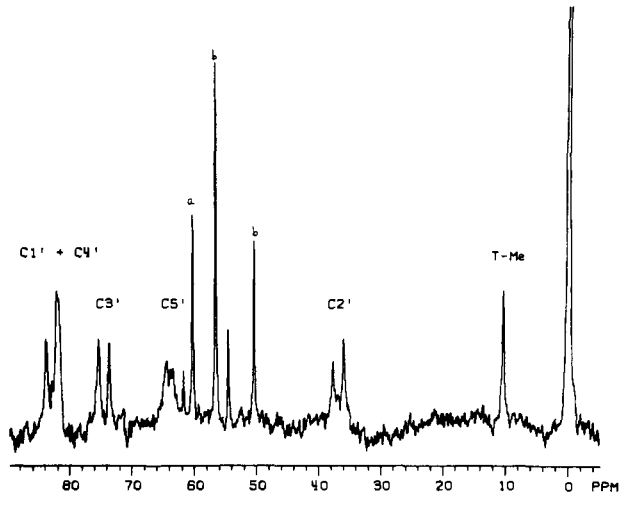

FIGURE 7: ^{13}C NMR spectrum at 125 MHz of sonicated poly(dA-dT)·poly(dA-dT) (50–200 bp long) at 37 °C. The sample contained 0.1 M NaCl, 33 mM sodium phosphate buffer (pH 7.0), and 1 mM EDTA. Peaks marked a and b are traces of Tris and EDTA, respectively.

By contrast, the right-handed alternating B form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC), characterized by its ^{31}P NMR spectrum before ^{13}C NMR analysis, did not show similar doublets for C2′ and C3′. Instead, a broad resonance for C2′ and an apparent multiplet for C3′ (overall line width ca. 500 Hz) were observed (Figure 8A). For the left-handed Z form of this

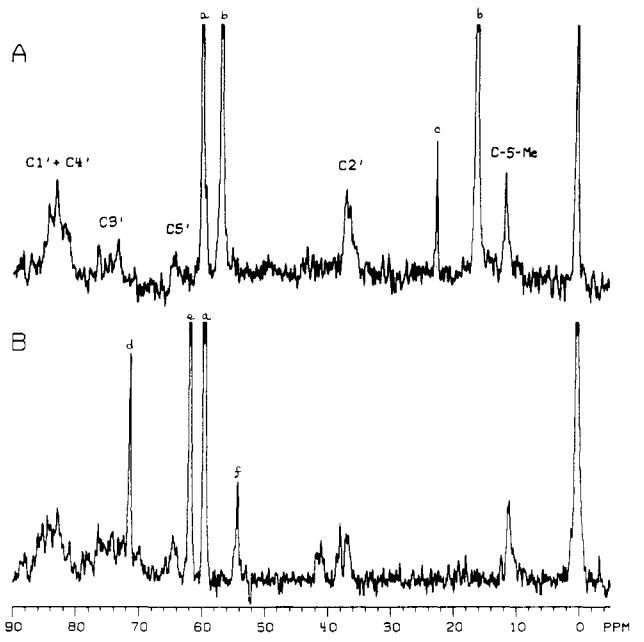

FIGURE 8: ^{13}C NMR spectra at 125 MHz of the (A) right-handed B form and (B) left-handed Z form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) at 37 °C. The sample in (A) was purified via *Hha*I treatment and had a size distribution of 150–700 bp. The sample in (B) had been further purified with DNase I and sonicated for 1 h to reduce its size to 50–200 bp. It contained 4 mM $MgCl_2$ in addition to the Tris buffer. The presence of the Z form in (B) was confirmed by its ^{31}P NMR spectrum under identical conditions. The sharp singlet at 37.9 ppm was attributed to the hybrid impurity (ca. 10%). Peaks marked a, b, and d are Tris, ethanol, and trimethyl phosphate, respectively. Peaks marked c, e, and f are unidentified impurities.

copolymer (150–700 bp), also checked by ^{31}P NMR under identical conditions, the C2′ resonance appeared to be a broad doublet ($\Delta\nu_{1/2}$ = 480 Hz) with a separation of ca. 3.82 ppm while the C3′ resonance remained unclear (spectrum not shown). When the size of this copolymer was reduced to 50–200 bp by means of sonication for 1 h (Chen et al., 1981), the doublet of C2′ was sharpened ($\Delta\nu_{1/2}$ = 140 Hz) with a separation of 4.61 ppm and possible other fine structure perhaps arising from the residual 10% impurity (cf. Figure 1F); but the C3′ resonance still remained unclear (Figure 8B). While no solid conclusion can be drawn about poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in either the B or Z form from these spectra, several attempts were made to obtain improved spectra under various instrumental conditions without notable success. Since the same samples gave perfectly well-resolved ^{31}P spectra, it was concluded that these results are characteristic of this polymer under those conditions.

Discussion

Several left-handed structures have been described in crystals of oligomers of d(G-C) (Wang et al., 1979, 1981; Crawford et al., 1980; Drew et al., 1980). Two variants reported by Rich and his co-workers (Wang et al., 1981), termed Z_I and Z_{II}, differed in the absence of binding of hydrated Mg^{2+} ion between the P–O of pdC and the N7 of the adjacent dG in the 3′ direction. The structure determined by Dickerson and his co-workers (Drew et al., 1980), although very similar to Z_I, was different in detail and was termed Z′. Arnott and his co-workers observed similar left-handed conformations in fibers of three alternating purine/pyrimidine copolymers (Arnott et al., 1980). It remains to be established what the detailed structure of a Z-form DNA is in solution.

Circular dichroism (CD) is an excellent tool for monitoring the transition from B to Z DNA (Pohl & Jovin, 1972) and has been used extensively for this purpose (Behe & Felsenfeld, 1981; Patel et al., 1979; Vorlíčková et al., 1980; Quadrifoglio et al., 1981). However, CD is inadequate to provide structural details of either form in solution. NMR spectroscopy can, in principle, provide detailed structural correlations, and in fact, some ^1H NMR studies of the chemical shifts of base protons in poly(dG-dC)·poly(dG-dC) have been favorably compared with calculated values for Z DNA (Mitra et al., 1981).

We wished to characterize the phosphodiester backbone in the Z form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) by observing its ^{31}P NMR spectra. Previous reports of ^{31}P NMR on (dG-dC)$_8$ (Patel et al., 1979), 145-bp d(G-C) derived from semisynthetic nucleosomes (Simpson & Shindo, 1980), and sonicated poly(dG-dC)·poly(dG-dC) (Cohen et al., 1981) all showed a well-resolved doublet of equal intensity ($\Delta\delta$ = 1.46 ppm) for the Z form in high NaCl concentrations. But it should be noted that a broad component was always present underneath the upfield signal due to either aggregation or other unknown factors. In the presence of $MgCl_2$ or CsF, poly(dG-dC)·poly(dG-dC) (ca. 1 mg/mL) precipitated during or even below the B to Z transition (Table I). Behe & Felsenfeld (1981) have shown that poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is more tractable to work with than its unmethylated analogue, since it undergoes the B to Z transition at much lower ionic strengths, in a physiological range. We have successfully observed by means of ^{31}P NMR spectroscopy the expected transition for the methylated copolymer in CsF, NaCl, Co$(NH_3)_6Cl_3$, and $MgCl_2$ (Figures 3–6). The cooperative transition was accompanied by the appearance of a signal at −3 ppm, which finally corresponded to half of the total phosphate intensity of the copolymer. The transition midpoints (Table I) are in good agreement with those reported by Behe & Felsenfeld (1981) from CD studies. The downfield and upfield components presumably correspond predominantly to the tg^+ conformation of dG-pm^{5}dC and the g^+g^+ conformation of m^{5}dC-pdG (Wang et al., 1981; Gorenstein, 1981), respectively. The separation between the doublet was 1.27–1.35 ppm in CsF, NaCl, and Co$(NH_3)_6Cl_3$ and 1.99–2.07 ppm in $MgCl_2$. The larger separation observed in $MgCl_2$ (Figure 6) is considered to arise from preferential binding of Mg^{2+} ions to the g^+g^+ phosphodiester conformation, resulting in an upfield shift of its resonance. As a result of this selective binding of Mg^{2+} ions, a third constant component for a given preparation was clearly resolved at −4.0 to −4.2 ppm. The relative proportion of this impurity depended on the details of the purification procedure used (Figure 1). The same component was apparently present but was not resolved in Tris buffer alone (Figure 1B,C) or in CsF, NaCl, and Co$(NH_3)_6Cl_3$ (Figures 3–5). It is unlikely that the impurity was the poly(dI-dC) template because of the different line widths (see Figure 1B,C vs. Figure 1A).

Patel et al. (1982) recently presented ^{31}P NMR spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) purified via DNase I digestion, in 0.1 M Tris/10 mM EDTA and in 1.5 M NaCl. Only a slight asymmetry was observed in the low-salt spectrum, and only a small shoulder was present in their high-salt spectrum. These authors offered no explanation for the doublet observed in low-salt solution and did not make specific conformational assignments of the phosphate signals.

With poly(dG-m^{5}dC)·poly(dG-m^{5}dC) at least 90% pure, a resolved doublet of approximately equal intensity with a separation of 0.27 ppm (29 Hz) was clearly observed in low-salt solutions (Figures 1C and 3A,B). This phenomenon corresponds quite closely to the doublet observed in ^{31}P spectra of poly(dA-dT)·poly(dA-dT) and several other alternating

purine/pyrimidine copolymers in low salt concentrations (Cohen et al., 1981; Chen & Cohen, 1983). It strongly suggests that in solutions of low ionic strength poly(dG-m^{5}dC)·poly(dG-m^{5}dC) exists in a right-handed alternating B form with a dinucleotide repeat unit. By analogy to our previous analysis (Chen & Cohen, 1983), the upfield and downfield components of the doublet can be assigned to dG-pm^{5}dC in a predominantly g^-g^- conformation and to m^{5}dC-pdG in a predominantly tg^- conformation (Gorenstein, 1981), respectively, although this is speculative. The two phosphodiester conformations apparently exchange at a rate slower than $2\pi \times 29$ Hz or 180 s^{-1} or are completely stable and noninterchanging. However, an alternating structure was not found in the unmethylated analogue poly(dG-dC)·poly(dG-dC). Previous studies (Cohen et al., 1981; Chen & Cohen, 1983) have shown that even sonicated poly(dG-dC)·poly(dG-dC) of only 100–200 bp gave rise to a single phosphate signal in low- or intermediate-salt solutions, indicating a regular B form with a uniform phosphate backbone and a mononucleotide repeat unit or possibly rapidly interchanging conformations.

Shindo (1981) observed two signals for both C2′ and C3′ in the ^{13}C NMR spectra at 68 MHz of 145-bp poly(dA-dT)·poly(dA-dT) derived from semisynthetic nucleosome core particles. He concluded that the alternating conformation of this copolymer extends into the interior of the double-helical structure. We demonstrate here that sonicated poly(dA-dT)·poly(dA-dT) (50–200 bp long) showed an even better resolved doublet for both C2′ and C3′ carbon resonances at 125 MHz (Figure 7), thus confirming that the alternating conformation of this sequence exists not only in the phosphodiester backbone but also in the deoxyribose rings. It should be added that since these carbon atoms are closer to the bases than are the phosphorus atoms, aromatic ring effects could be partly responsible for the large values of $\Delta\delta$ observed. According to Shindo's peak assignments, the narrower upfield component ($\Delta\nu_{1/2}$ = 70 Hz) in both C2′ and C3′ doublets can be assigned to dA and the broader downfield component ($\Delta\nu_{1/2}$ = 95 Hz) to dT. On the basis of the findings of Viswamitra et al. (1978) in (dA-dT)$_2$ crystals that dT deoxyribose is in the C2′-endo conformation and dA deoxyribose is in the C3′-endo conformation, we can further assign the two dA resonances at 35.9 and 73.6 ppm to C2′ and C3′ of the C3′-endo conformation, respectively, and the two dT resonances at 37.6 and 75.5 ppm to C2′ and C3′ of the C2′-endo conformation, respectively. The separation in each doublet (ca. 1.7 ppm) suggests that the two sugar conformations undergo a very slow exchange process at a rate of <1.3 ms^{-1} or do not exchange at all. However, it should be pointed out that each signal could derive from several conformations in fast exchange and may only be predominantly in one form.

Levy et al. (1981) have compared the line widths of the sugar carbons of nucleosome-length DNA (120 and 160 bp) at different magnetic field strengths, i.e., 22.6–100.6 MHz, and found significant chemical shift dispersions of these carbon resonances at 67.9 and 100.6 MHz. Our study at 125.7 MHz indicates that the frequency range expected from the C2′ and C3′ resonances in sonicated poly(dA-dT)·poly(dA-dT) would be about 290 Hz [i.e., doublet separation, 210 Hz, + $^1/_2$ (70 + 95 Hz) line widths of individual components], which is consistent with Levy's et al. (1981) results. However, the fact that C2′ and C3′ atoms of this copolymer presumably exist in only two distinct conformations allows the C2′ and C3′ resonances to be resolved into two separate components, and the true spectroscopic line widths to be determined.

Although the phosphodiester backbone of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in low-salt solutions exhibits a right-handed alternating conformation (see above), the sugar moieties of this copolymer do not appear to exist in two distinct, slowly exchanging conformations as those observed in poly(dA-dT)·poly(dA-dT). The broad signal of the C2′ resonance and the apparent multiplicity of the C3′ resonance of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (Figure 8A) do not allow clear-cut conclusions to be made. Although the absence of a doublet for C2′ may indicate a uniform conformation throughout the copolymer chain, this is not conclusive. The C3′ atoms may exist in several conformations with possible exchange between them. On the other hand, the left-handed Z form of this copolymer (sonicated, 50–200 bp) showed a widely separated ($\Delta\delta$ = 4.61 ppm) doublet (Figure 8B), suggesting that the C2′ atom of dG and the C2′ atom of m^{5}dC are in at least two distinctly different conformations, with a slower rate limit for interconversion (<0.3 ms^{-1}) than that in poly(dA-dT)·poly(dA-dT). However, it is not clear what changes, if any, occurred for the C3′ conformations during the B to Z transition. Further studies to obtain a higher ^{13}C S/N ratio for these and other copolymers are currently under way.

It is known that a large proportion of d(C-G) sequences that exist in eucaryotic DNA are methylated at the C5 position (Razin & Riggs, 1980), and this methylation may be correlated with the turning off of gene activity (McGhee & Ginder, 1979). Thus, it is possible that the conformational forms and selective effects we have observed for poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in solution may indeed have functional genetic significance.

Registry No. Poly(dG-m^{5}dC), 51853-63-5.

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Solubilization and Partial Purification of RNA Polymerase from Pea Chloroplasts†

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ABSTRACT: DNA-dependent RNA polymerase of pea chloroplasts is tightly bound to a complex that contains chloroplast DNA, DNA polymerase, RNA polymerase, and many other proteins. This complex transcribes all the sequences of chloroplast DNA in vitro. The RNA polymerase has been solubilized and purified from this complex by fractionation in glycerol gradients and in DEAE-cellulose and Sepharose 6B columns. The purified enzyme was completely dependent on the exogenous DNA. The native molecular size of the enzyme was found to be more than 500 000 by native gel electrophoresis and glycerol gradients. Chloroplast RNA polymerase was obtained in 1500-fold purification, starting from the Triton-disrupted chloroplasts. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis has shown that chloroplast RNA polymerase may contain polypeptides of 180, 140, 110, 95, 65, 47, and 27 kdaltons. The enzyme has been found to be extremely sensitive to salt concentrations and had a temperature optimum of 30 °C. The purified enzyme is not inhibited by either α-amanitin or rifampicin.

The structural organization of the pea chloroplast (ct) genome has been extensively studied (Tewari, 1979). Pea ctDNA was found to contain one rRNA gene and 30–40 tRNA genes (Meeker & Tewari, 1980). The hybridization of pea ctDNA with ct polysomal RNA showed that RNA molecules equivalent to a complete single-stranded chain of ctDNA were transcribed in vivo (Oishi et al., 1981). The ribosomal RNA gene was localized in the restriction-endonuclease map of pea ctDNA (Chu et al., 1981; Palmer & Thompson, 1981). The gene for the large subunit of ribulose-bisphosphate carboxylase and two photodependent genes also were identified and mapped in pea ctDNA (Oishi & Tewari, 1983). So that one can understand the mechanism of transcription of the stable and messenger RNA genes of pea ctDNA, purified DNA-dependent RNA polymerase must be obtained from chloroplasts to develop an in vitro transcription system that faithfully mimics the in vivo transcription of ctDNA.

DNA-dependent RNA polymerase from chloroplasts of higher plants was found to be tightly bound with ctDNA and the thylakoid membranes (Tewari & Wildman, 1969). Early attempts to solubilize the enzyme from the membrane were unsuccessful despite hypotonic treatment, homogenization, freezing and thawing, salt extraction, or detergent treatment. Similar attempts with other higher plants were not successful. Hallick et al. (1976) purified a complex from *Euglena* that contained RNA polymerase bound to ctDNA. This complex, named a transcriptionally active chromosome, was found to carry out chain elongation and chain initiation. The RNA polymerase could not be dissociated from the bound ctDNA of this transcription complex. However, Bottomley et al. (1971) were successful in solubilizing and purifying the RNA polymerase from corn leaves. The critical step in obtaining the soluble RNA polymerase from corn was extraction of the membranes in the presence of 4 mM EDTA[1] at 37 °C. The purified ctRNA polymerase was found to contain a number of polypeptides at different stages of purification, but at least two polypeptides of 180 and 140 kdaltons always were found to be associated with the chloroplast RNA polymerase (Smith & Bogorad, 1974). Kidd & Bogorad (1979) compared 180-kdalton subunits from maize chloroplast and type II nuclear DNA-dependent RNA polymerase; but despite similar molecular mass, these 180-kdalton subunits were not the same as shown by one- and two-dimensional peptide mapping techniques. Data from these investigators also showed that the 160-, 43-, and 28-kdalton polypeptides from maize type II nuclear RNA polymerase, and 140-, 42-, and 27-kdalton polypeptides from maize chloroplast RNA polymerase, are unique. The purified RNA polymerase of maize was found to preferentially transcribe maize ctDNA sequences incorporated in cloned chimeric bacterial plasmids (Jolly & Bogorad, 1980). Preferential transcription was found to be dependent on the presence of a 27.5-kdalton polypeptide and the template being in the supercoiled form.

In this paper, we report on the solubilization and purification of RNA polymerase from pea chloroplasts. The enzyme was obtained in 1500-fold purification from Triton-disrupted

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; PMSF, phenylmethanesulfonyl fluoride; BSA, bovine serum albumin; SSC, standard saline citrate; DEAE, diethylaminoethyl.

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