^{31}P NMR Spectral Analysis of the Dodecamer d(CGCGAATTCGCG)

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ABSTRACT: The resonances in the ^{31}P NMR spectrum of the dodecamer d(CGCGAATTCGCG) have been assigned by use of regiospecific labeling with oxygen-17. At 19 °C the resonances of 9 of the 11 dinucleoside phosphates are resolved. Most noticeably, different chemical shifts are observed for the phosphates of d(GpC) at positions 2 and 10 as well as for d(CpG) at positions 1, 3, 9, and 11, indicating that the position in an oligonucleotide influences the chemical shift. For the central d(GAATTC) portion of this dodecamer, a close relationship between the chemical shift of the phosphate groups and their position in the sequence of the oligonucleotide exists, in that the more central the phosphate residue is the more the signal appears at higher field. This finding parallels that found for the octamer d(GGAATTCC) [Connolly, B. A., & Eckstein, F. (1984) *Biochemistry 23*, 5523–5527]. The signals of the phosphate residues at positions 3 and 9, however, are found at lower field strength than expected from their position in the sequence, indicating a break in conformation at these two locations. A discontinuity of structure is also observed at these positions in the X-ray structure of this dodecamer [Dickerson, R. F., & Drew, H. R. (1981) *J. Mol. Biol. 149*, 761–786] as shown by the anomalous twist angles between the third and fourth as well as the ninth and tenth base pairs. The dependence of the chemical shift on temperature indicates different mobilities for each of the 11 phosphate groups. There seems to be no fraying at the ends but conformational changes particularly at the central A-T base pairs at the center of the molecule, consistent with the data obtained by ^{1}H NMR spectroscopy [Patel, D. J., Kozlowski, S. A., Marky, L. A., Broka, C., Rice, J. A., Itakura, K., & Breslauer, K. J. (1982) *Biochemistry 21*, 428–436].

The past few years have seen a considerable improvement in the methods available for the preparation of oligodeoxynucleotides. Many of these synthetic oligodeoxynucleotides have been crystallized, and their X-ray structural analysis has yielded information on their conformation in the solid state (Wang et al., 1979, 1981, 1983; Dickerson & Drew, 1981; Shakked et al., 1981; Dickerson et al., 1982, 1983; Fratini et al., 1982; Dickerson, 1983). With the recent development of two-dimensional ^{1}H NMR spectroscopy it has been possible to assign all or most of the nonexchangeable ^{1}H resonances for a number of oligodeoxynucleotides, and this has led to information on the conformation of these molecules in solution (Kan et al., 1982; Patel et al., 1982a,b, 1983a,b; Clore & Gronenborn, 1983a,b; Feigon et al., 1983; Pardi et al., 1983; Scheek et al., 1983; Broido et al., 1984; Cheng et al., 1984; Gronenborn et al., 1984; Guittet et al., 1984; Weiss et al., 1984; Wemmer et al., 1984). Obviously, a comparison of the X-ray with the NMR data would be most informative as it provides an insight into similarities or differences of conformations in the crystalline state and in solution. The oligonucleotide for which such a comparative study seems to be most advanced is the dodecamer d(CGCGAATTCGCG). Its X-ray structural analysis has been reported (Dickerson & Drew, 1981; Fratini et al., 1982; Dickerson et al., 1983; Dickerson, 1983) as well as the assignment of most of the ^{1}H NMR resonances (Patel et al., 1982a,b; Hare et al., 1983). It seemed to us that as an additional piece of information the ^{31}P NMR spectrum of this dodecamer should be analyzed as ^{31}P chemical shifts are sensitive indicators of conformations around the internucleotidic phosphate groups. A ^{31}P NMR spectrum of this dodecamer has been published earlier but the assignment of resonances has not been reported (Patel et al., 1982a,b). Such assignments, however, are essential for an interpretation of the spectrum. We have recently suceeded in assigning the ^{31}P NMR resonances for the octamer d(GGAATTCC) by regiospecific labeling of the internucleotidic phosphate groups with oxygen-17 (Connolly & Eckstein, 1984). We report here the assignment of the ^{31}P resonances in the spectrum of the dodecamer d(CGCGAATTCGCG) by the same method and on the basis of this an analysis of the chemical shifts of the resonances of the various internucleotidic linkages as a function of the position in the sequence as well as of temperature.

Materials and Methods

The dodecamers were synthesized by the phosphoramidite method on a polymer support in a syringe essentially as described by Connolly et al. (1984) and Connolly & Eckstein (1984). Oxygen-17 was regiospecifically incorporated into each phosphate group by sequentially oxidizing each phosphite intermediate with iodine in $H_2{}^{17}O$. The following changes were introduced: The 9-phenylxanthen-9-yl (pixyl) group was used for the protection of the 5′-hydroxyl groups of nucleosides (Chattopadhyaya & Reese, 1978; N. Piel, private communication). N^6-Benzoyl-2′-deoxyadenosine, N^4-benzoyl-2′-deoxycytidine, and N^2-(phenylacetyl)-2′-deoxyguanosine were used as the base protected nucleosides, which were prepared by transient protection with trimethylsilyl groups (Ti et al., 1982). After the completion of the synthesis, the removal of the phosphate-protecting methoxy groups and the base-labile groups, and the cleavage of the oligomer from the resin, the oligonucleotides were purified by preparative reverse-phase HPLC[1] on ODS Hypersil (column 250 × 10 mm) with a flow rate of 5 mL/min and the following program of linear gra-

[1] Abbreviations: TEAB, triethylammonium bicarbonate; HPLC, high-pressure liquid chromatography; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid. The numbering of the phosphate groups in the dodecamer is as follows:

1 2 3 4 5 6 7 8 9 10 11

5′-CpGpCpGpApApTpTpCpGpCpG-3′

3′-CpCpGpCpTpTpApApGpCpGpC-5′

11 10 9 8 7 6 5 4 3 2 1

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dients prepared from 100 mM TEAB, pH 7.5 (solvent A), and 100 mM TEAB, pH 7.5, containing 70% CH_3CN (solvent B): t = 0 min, 5% B; t = 3 min, 5% B; t = 10 min, 35% B; t = 16 min, 45% B; t = 25 min, 70% B; t = 28 min, 70% B. The product usually eluted at about 41% B. The higher concentrations of B were used to wash the column. The pixyl group was removed by treatment with 80% acetic acid and extracted with ether. The aqueous phase was evaporated to dryness and the product dissolved in water. No further purification of the oligonucleotides was found to be necessary. The composition of the oligonucleotides was checked by digestion with nuclease P1 and the sequence analyzed by the wandering-spot method (Frank & Blöcker, 1982).

The absorption coefficient of the dodecamer was determined as 193 460 M^{-1} cm^{-1} for the double strand, and a hypochromicity of 22% was obtained by digestion of the dodecamer with snake venom phosphodiesterase. The yields of the final products were approximately 1 μmol, representing 10% overall with respect to the first silica-bound nucleoside.

Oxygen-17-enriched water (^{16}O, 13.8%; ^{17}O, 50.7%; ^{18}O, 35.5%) was obtained from Monsanto Research Corp., U.S. Department of Energy (Miamisburg, OH). The dinucleoside phosphates were purchased from Sigma and P-L Biochemicals. ODS Hypersil (5 μm) for reverse-phase HPLC was from Shandon Southern Products, Astmoor, Runcorn, U.K.

HPLC was performed with a Waters dual-pump 6000 A system in combination with a Waters Model 680 gradient controller and a Model 440 UV detector operating at 280 nm for preparative and at 254 nm for analytical runs. ^{31}P NMR spectra were recorded with a Bruker WP200SY spectrometer operating at 81.01 MHz with ^{1}H broad-band decoupling in 5-mm tubes. The temperature in the NMR probe was determined with a ^{31}P thermometer of the appropriate ionic strength (Gorenstein et al., 1982). Chemical shifts refer to trimethyl phosphate as external standard and are negative when upfield from this reference. A comparison of the shift difference between external and internal trimethyl phosphate showed that it was 0.02 ppm at 30 and 40 °C and 0.1 ppm at 80 °C.

Results and Discussion

There is good evidence that the factor that largely determines the chemical shift of ^{31}P NMR resonances is the phosphate ester torsional angle (Gorenstein et al., 1976; Patel 1976; Gorenstein, 1981, 1984). For internucleotidic linkages, these are the angles O3′–P-O5′–C5′ (α) and C3′–O3′–P-O5′ (ζ) (Saenger, 1983). The resonances of phosphate esters in the gauche,gauche conformation are located at higher field than those in the gauche, trans conformation. In double-stranded polynucleotides the gauche,gauche conformation is energetically favored whereas in single-stranded polymers the gauche,trans conformation is found (Chen & Cohen, 1984). Thus, double-stranded polynucleotides show a downfield shift in the ^{31}P NMR resonances upon melting. The assignment of ^{31}P NMR resonances in oligonucleotides is of interest because the chemical shifts can give an indication of differences in conformation of the individual dinucleoside phosphates resulting from influences by neighboring bases and position in the sequence. In addition, the temperature dependence of the shifts allows conclusions as to the change in torsional angles of the individual internucleotidic linkages as a function of temperature.

We have recently reported that the assignment of resonances in the ^{31}P NMR spectrum of the octamer d(GGAATTCC) can be achieved by succesive replacement of the internucleotidic phosphate diester linkages by oxygen-17-carrying

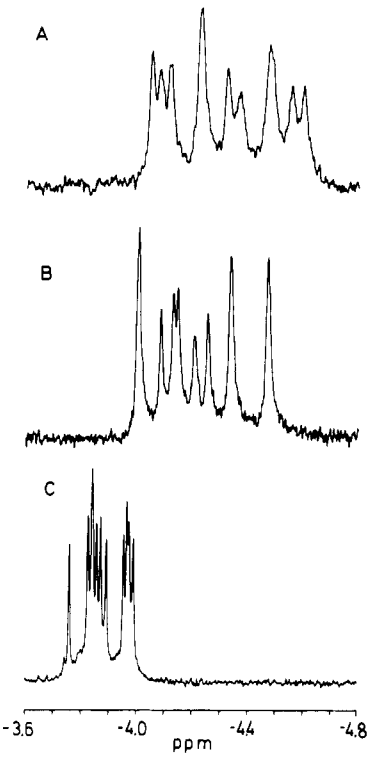

FIGURE 1: ^{31}P NMR spectra of d(CGCGAATTCGCG) at different temperatures: (A) 19 °C; (B) 36 °C; (C) 77 °C. The samples, approximately 70 A_{260} units, were dissolved in 500 μL of D_2O containing 25 mM Hepes, pH 8.0, 25 mM EDTA, and 50 mM NaCl. Parameters were as follows: offset 1300 Hz; sweep width 800 Hz; pulse width 6.5 μs; 16K transients; acquisition time 10.24 s; line broadening between 0.1 and 0.3 Hz; number of transients between 1000 and 2500.

phosphate groups (Connolly & Eckstein, 1984). This is an extension of a method that was first described by Petersheim et al. (1984) and more recently by Shah et al. (1984) for the assignment of resonances in two tetranucleosides. We show here that the same method can be employed for the assignment of resonances in the ^{31}P NMR spectrum of the dodecamer d(CGCGAATTCGCG).

In order to assign each resonance in the ^{31}P NMR spectrum to an individual phosphate group, it is obviously necessary to have a sufficiently resolved spectrum. Figure 1 shows that at 19 °C nine resonances are visible. Of these, seven correspond to one phosphate group each. The positions of the resonances as well as the resolution of the spectra change with temperature. The spectra taken at 36 and 77 °C are also given in Figure 1. The assignment of resonances is based on the decrease of a resonance when the phosphorus carries an oxygen-17. Ideally, such a resonance should disappear from the spectrum completely because of the quadrupolar moment of oxygen-17 [for reviews, see Tsai (1982), Cohn (1982), and Gerlt (1983)]. However, as the $H_2{}^{17}O$ available contains only about 50% oxygen-17, the rest being 13% oxygen-16 and 35% oxygen-18, the resonance is reduced to about half. The residual resonance can often be observed as a single resonance, which is slightly shifed upfield because of the oxygen-18-induced shift. Sometimes, however, it is no longer observable as a single line as it merges with the rather broad base of the adjacent signals. To demonstrate the method two spectra representing the two cases are reproduced in Figure 2 as typical examples for the effect of oxygen-17 labeling. In spectrum A the signal for the dinucleoside phosphate d(CpG) at position 1 in the dodecamer has been labeled with oxygen-17. It can be seen that one of the signals at −4.18 ppm is reduced and

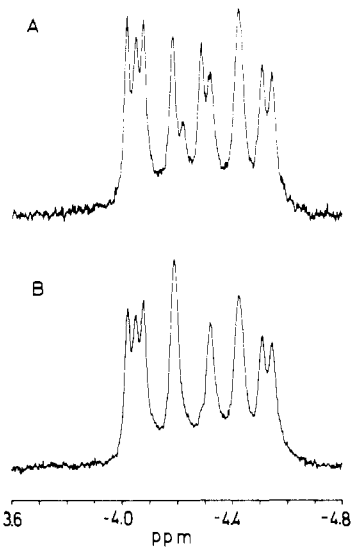

FIGURE 2: ^{31}P NMR spectra of two oxygen-17-labeled d-(CGCGAATTCGCG) samples: (A) dodecamer oxygen-17 labeled at position 1; (B) dodecamer oxygen-17 labeled at position 2. The spectra were recorded at 19 °C with the same parameters as in Figure 1.

Table I: ^{31}P Chemical Shifts of Dinucleoside Phosphates in d(CGCGAATTCGCG) and in d(GGAATTCC) and as Monomeric Units

dinucleoside phosphate	chemical shift in dodecamer (ppm)[a,b]	chemical shift in octamer (ppm)[a-c]	chemical shift as monomeric unit[g] or in polymer[d-f] (ppm)[a]
d(CpG)	−4.02 [11]		−4.08 (−4.26)[d]
	−4.05 [9]		
	−4.07 [3]		
	−4.18 [1]		
d(GpC)	−4.18 [10]		−3.95 (−4.26)[d]
	−4.28 [2]		
d(GpA)	−4.32 [4]	−4.13 [2]	−4.17
d(TpC)	−4.42 [8]	−4.22 [6]	−3.89
d(ApA)	−4.42 [5]	−4.37 [3]	−4.25 (−4.58)[e]
d(TpT)	−4.51 [7]	−4.43 [5]	−3.97 (−4.58)[e]
d(ApT)	−4.54 [6]	−4.49 [4]	−4.13 (−4.50)[f]

[a] In 25 mM Hepes, pH 8.0, 25 mM EDTA, and 50 mM NaCl at 19 °C [b] Numbers in brackets denote the corresponding phosphorus atom. [c] Values taken from Connolly & Eckstein (1984). [d] Values obtained with poly[d(G-C)]. [e] Values obtained with poly[d(A)]-poly [d(T)]. [f] Value obtained with poly[d(A-T)], taken from Connolly & Eckstein (1984). [g] These values are in agreement with those reported by Giessner-Prettre et al. (1984).

slightly shifted upfield. In spectrum B where d(CpG) at position 2 had been labeled, the signal at −4.28 ppm has completely disappeared. In analoguous manner all resonances have been assigned. The assignment of the resonances at 19 °C is given in Table I. At this temperature the signal at highest field is due to d(ApT) of position 6 in the dodecamer. Slightly downfield from it is that of d(TpT) (position 7), followed by the unresolved resonances for d(ApA) and d(TpC) (positions 5 and 8) and further downfield by that for d(GpA) (position 4). At this point the relationship between chemical shift and distance from the center of the oligonucleotide breaks down as neither of the resonances for d(CpG) of positions 3 or 9, which are next in sequence, appear in the spectrum but rather that of d(GpC) (position 2) and then those of d(GpC) and d(CpG) (positions 10 and 1, respectively). It is only then that the resonances for positions 3 and 9 are seen, closely followed by the 3′-terminal residue d(CpG).

We can identify only 11 resonances in our ^{31}P NMR spectra, consistent with a duplex with 2-fold symmetry. This 2-fold symmetry is also evident from the oxygen-17-labeled oligomers because labeling of one phosphate leads to the reduction of only one resonance. If a phosphate group in one strand had a different conformation in the other, ^{17}O-labeling should cause a decrease in two resonances. This observation of 2-fold symmetry is in agreement with what has been observed previously in the ^{1}H NMR spectra (Patel et al., 1982a; Hare et al., 1983) of this oligonucleotide. These observations are in contrast to the lack of such 2-fold symmetry of this duplex in the crystal (Dickerson & Drew, 1981). As has been pointed out by Patel et is higher, approximately 68 °C, mainly due to the higher oligonucleotide concentration but nevertheless in the crystal. It cannot be excluded that it might not be due to dynamic averaging in solution.

The main difficulty in interpretation of the shifts observed in oligonucleotides is the uncertainty to what degree the shifts are influenced by the neighboring bases (sequence effect) and to what extent by their position in an oligonucleotide (positional effect). To dissect these effects one can take the monomeric dinucleoside phosphates as standards for values expected when the conformation is unaffected by sequence as well as position. Inspection of Table I reveals that—as has been reported earlier (Giessner-Prettre et al., 1984)—the shifts for different dinucleoside phosphates are not identical as the bases influence the conformation of the deoxyribose and thus the conformation of the phosphate (Cozzone & Jardetzky, 1976; Gorenstein, 1981). Nevertheless, one might use these values as standards. Synthetic polynucleotides should provide approximate values for shifts to be expected for dinucleoside phosphates when part of a polymer. However, caution should be exercized as the conformation of such synthetic homopolymers or polymers with alternating bases might be different from those of less regular base composition.

Unfortunately, for the dodecamer d(CGCGAATTCGCG) a comparison between shifts of monomeric and polymeric dinucleoside phosphates is limited to five dinucleoside phosphates. The data indicate that the largest conformational change on incorporation into homo- or alternating polynucleotides is observed for d(TpT) ($\Delta\delta$ 0.61 ppm) followed by d(ApT) ($\Delta\delta$ 0.37 ppm) and d(ApA) ($\Delta\delta$ 0.33 ppm), the lowest values being observed for d(CpG) ($\Delta\delta$ 0.18 ppm) and d(GpC) ($\Delta\delta$ 0.31 ppm). This scale indicates that dipurine nucleoside phosphate monomers tend toward a conformation that is more akin to that in polymers than do thymidine-containing ones. This trend is also seen in a comparison of the monomeric dinucleoside phosphates and those incorporated into the dodecamer (Table I).

Quite clearly, the comparison of shift values obtained for the same dinucleoside phosphate in different oligomers should be most valuable in determining to what extend the position determines the shift. At present, the only oligonucleotide with which such a comparison is possible is the octamer d(GGAATTCC) (Connolly & Eckstein, 1984) in which the dinucleoside phosphates at positions 2–6 are identical with those at positions 4–8 in the octamer. This provides an opportunity for assessing the influence of the length of an oligonucleotide on the chemical shifts of the central part of the molecule. The observation is that the corresponding resonances in the dodecamer are at somewhat higher field (Table I). The difference is rather small for the central phosphate group d(ApT) and the two adjacent groups d(TpT) and d(ApT) but becomes more pronounced ($\Delta\delta$ 0.2 ppm) for the groups d(GpA) and d(TpC) at either end of this tetramer. We interpret this to indicate an increase in gauche,gauche conformation of the torsional angles and thus a more polymer-like

conformation. This increase in shift in the dodecamer is presumably due to its greater length and particularly so because it spans slightly more than one turn of a helix whereas the octamer is somewhat short of that.

The dodecamer itself provides an opportunity to determine the influence of position of a dinucleoside phosphate in an oligomer as two dinucleoside phosphates occur more than once. Most surprising is the fact that the values for d(GpC) in position 10 and 2 are different. The value for the latter (−4.28 ppm) is identical with that of poly[d(G-C)] (−4.26 ppm) and about −0.1 ppm further upfield than that of position 10. This is the first example available for the influence of position on the chemical shift. It is particularly valuable as the neighboring nucleotides are the same in both positions. Moreover, both dinucleoside phosphates are the penultimate groups from both ends and one would intuitively expect them both to have the same conformation and thus ^{31}P chemical shift. As this is not so, one can interpret this as a difference in conformation, indicating a more polymer-like conformation at the 5′- than at the 3′-end of the oligomer. The dinucleoside phosphate d(CpG) occurs 4 times in the dodecamer. In positions 3, 9, and 11 it exhibits chemical shifts that are very similar to those of the monomeric dinucleoside phosphate whereas at position 1 this value is higher and approaches that found in poly[d-(G-C)]. This shows again that the same dinucleoside phosphate can exhibit different chemical shifts and, thus, adopt different conformation depending on its position in the oligomer. However, the positional effect cannot be so nicely separated here from the sequence effect as in the case of d(GpC) discussed above as the immediate neighbors are not identical. However, it is remarkable again that the groups in positions 1 and 11, which are both terminal, show such a large difference, paralleling the results obtained for d(GpC).

We have reported earlier (Connolly & Eckstein, 1984) that in the ^{31}P NMR spectrum of the octamer d(GGAATTCC) there is a relationship between the chemical shift of the phosphate group and its position in the sequence, i.e., the closer a phosphate group is positioned toward the center of the octamer, the more the resonance appeared at higher field. This was interpreted as showing that the more central a dinucleoside phosphate is situated in an oligonucleotide the more its conformation resembles that of a polymeric structure. The data reported here for the dodecamer show that this cannot be generalized. It is true that this interpretation is valid for the central hexamer of the dodecamer. However, it has to be emphasized that additional data for the dinucleoside phosphates that are the components of this hexamer are badly needed to check whether they will exhibit shifts at lower field when they are not so centrally located.

It is interesting to note that the close relationship between shifts and position breaks down on either side of the hexamer, at phosphate groups 3 and 9, which appear at lower field than expected from this relationship. Instead, phosphate groups 2 and 10 take their place. That the resonances of d(CpG) at position 3 and 9 appear at unduly low field can probably best be seen by a comparison with the resonance of the phosphate group of d(CpG) in position 1, which appears at higher field ($\delta = -4.18$ ppm) than those of 3 and 9, indicating that δ values of −4.05 and −4.07, respectively, are not the limiting values for d(CpG) when present in a polymer but that higher values are possible.

At this point a comparison of the ^{31}P NMR data with the X-ray structural data of this dodecamer becomes of interest. Inspection of the various representations of the crystal structure of this molecule (Dickerson & Drew, 1981; Dickerson et al., 1982, 1983; Dickerson, 1983) indicates a very regular B-type structure of the central hexamer as judged, e.g., by the local and global twist angles for the base pairs and the position of the local helix rotation vectors along a straight axis. A break occurs in this structure at base steps 3 and 9 in that the twist angles relating base pair 3 to base pair 4 as well as 8 to 9 become unusually small. The local helix rotation vectors for these steps in relation to the central base pairs are shifted away from the central axis into the major groove reminiscent of an A DNA conformation. These vectors are nearly related by a lateral 2-fold axis through the center of the molecule, suggesting to the authors that this deviation from the rather regular B-type structure in the center will also be observable in solution. Indeed, the irregularities we see in the ^{31}P NMR spectrum are at phosphate residues 3 and 9, bridging base pairs 3 and 4, and residues 8 and 9 and so at the positions where the irregularities are seen in the crystal.

If there was a direct correspondence between the structure in the crystal and in solution we should see differences in the ester torsional angles α and/or ζ at positions 3 and 9 (Dickerson & Drew, 1981). However, inspection of these two values obtained from the X-ray structural analysis shows that α is the same for all and ζ for all but two internucleotidic linkages. Only for phosphate residues 10 and its counterpart in the other strand are the δ values shifted to more negative values, indicating a more gauche,trans conformation.

The ^{31}P spectrum does not indicate residue 10 to be particularly downfield; instead, residues 3 and 9 resonate at low field. Thus, the chemical shifts observed in the NMR spectrum cannot be explained by the values for α or ζ found in the X-ray structure. This could indicate either that other factors than α or ζ determine the chemical shift or that the structures in solution and in the crystal are not identical regarding the conformations around these phosphate groups. However, there is agreement by both analyses that a conformational change occurs at either side of the central GAATTC sequence, which is expressed by a change at base pairs 3 and 9 in the X-ray analysis and at the phosphate residues 3 and 9 in the ^{31}P NMR spectrum. It is also of interest to note that phosphate groups 3 and 9 are the positions where distortions are observed in the oligonucleotide in the X-ray structural analysis of the oligonucleotide–*Eco*RI complex (Frederick et al., 1984).

The temperature dependence of the chemical shifts (Figure 3) shows a similar pattern to that observed for d(GAATTCC) (Patel, 1979; Connolly & Eckstein, 1984) in that the resonances at the highest field at low temperature (14 °C) experience the greatest downfield shift with increasing temperature. This is in agreement with the interpretation that the more central a phosphate group is located in an oligonucleotide the more polymer-like the conformation is. A single thermal transition of the midpoint of approximately 58 °C was observed spectrophotometrically (not shown) at an oligonucleotide concentration of approximately 10 μM and 0.12 M Na^+. This agrees quite well with the value reported earlier (Marky et al., 1983). This melting represents the duplex to single-strand transition. The melting point found by ^{31}P NMR spectroscopy is higher, approximately 68 °C, mainly due to the higher oligonucleotide concentration but nevertheless indicating the same transition process. The most cooperative decrease in shift is seen for the central phosphate groups 6–8 and also for group 2. At the other extreme is the complete lack of any sigmoidal character of this temperature dependence of the shift for the resonance of group 4 where the decrease is approximately linear with the increase in temperature. For

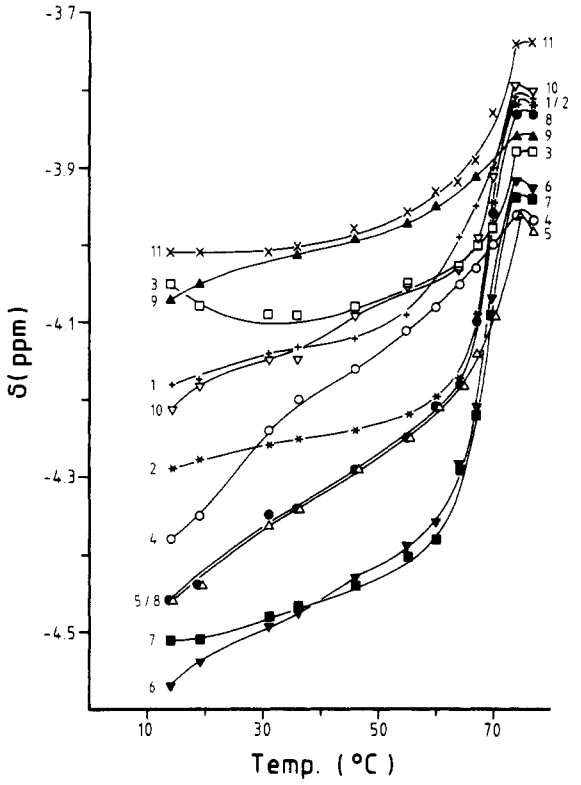

FIGURE 3: Temperature dependence of chemical shifts of the ^{31}P NMR resonances of d(CGCGAATTCGCG). The spectra were recorded as indicated in the legend to Figure 1.

all other groups the curves are intermediate with respect to cooperativity.

It is interesting to analyze the premelting region of these melting curves, bearing in mind that the downfield shift of the ^{31}P resonances is due to conformational changes around the phosphate groups and that these are not necessarily directly related to the melting process, i.e., the breaking of base pairs. The cooperative phase in melting observed by UV is rather broad and is between 40 and 75 °C. This correlates quite well with the ^{31}P shifts for groups 1, 2, and 11, which connect nucleosides involved in G-C base pairs. They exhibit very little changes in shift below the melting. It has already been reported that the ^1H resonances for the dC and dG residues in this dodecamer (Patel, 1982a) show no significant chemical shift changes below the melting region. Thus, the ^{31}P and ^1H NMR studies are in agreement in this respect. Groups 5–8, which connect nucleosides involved in A-T base pairing at the center of the molecule, show definite changes in shifts in the premelting region, i.e., below 40 °C, indicative of a high degree of flexibility of these groups. The ^1H spectra of the dodecamer (Patel, 1982a) indicate that CH_3-5 and H-6 of thymidine at position 8 (but not at position 7) as well as H-2 of deoxyadenosine at position 5 (but not at position 6) undergo temperature-dependent shifts in the premelting region. As discussed, there this does not necessarily have to indicate melting but could be due to changes in propeller twisting and duplex unwinding. This process is facilitated by an increase in temperature as the dielectric constant of water decreases and phosphate–phosphate repulsion increases (Fratini et al., 1982). Whatever the motions are, they are also reflected in the ^{31}P NMR chemical shift.

The shifts of resonances of groups 3, 9, and 4 merit special mention. Group 3 is the only resonance that upon heating shows an upfield shift between 14 and 55 °C, which can be interpreted as transient change of conformation to one of a more polymer character. The shift of group 9 and particularly that of group 4 show very little if any sigmoidal character; thus, there must be a gradual linear change in conformation of these groups. A very similar temperature dependence of the shift to that observed here for group 4, d(GpA), is also seen for d(ApA) in the spectrum of d(GGAATTCC) (Patel & Canuel, 1979; Connolly & Eckstein, 1984). Further work will have to show what determines this unusual melting behavior. It is worth noticing that even at 77 °C when the melting seems to be complete the 11 resonances can still be distinguished. What is most surprising is that even the resonances of the four identical dinucleoside phosphates d(GpC) are clearly separated. If the oligonucleotide had formed a random coil at this temperature, one would expect these resonances to coincide. Thus, we have to conclude that there is still some residual structure even at this high temperature. This is in agreement with the notion that there is still some interaction between the bases of dinucleoside phosphates even in the denaturated state (Michelson, 1963). This can also be demonstrated by the higher hypochromicity observed on enzymatic digestion of a polymer to mononucleosides than upon heat denaturation (Pohl & Jovin, 1972).

In summary it can be stated that the assignment of the ^{31}P NMR resonances and the dependence of their shifts on temperature give an insight into the differences of the various phosphate groups in terms of conformation and mobility. A more detailed analysis of these spectra will probably yield more information on the structure of the dodecamer in solution and thus complement the ^1H NMR spectroscopic studies (Patel et al., 1982a,b; Hare et al., 1983).

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