Collman, J. P., Brauman, J. I., Collins, T. J., Iverson, B. L., Lang, G., Pettman, R., Sessler, J. L., & Walters, M. A. (1983a) *J. Am. Chem. Soc. 105*, 3038–3052.
Collman, J. P., Brauman, J. I., Iverson, B. L., Sessler, J. L., Morris, R. M., & Gibson, Q. (1983b) *J. Am. Chem. Soc. 105*, 3052–3064.
Eisenberger, P., Shulman, R. G., Kincaid, B. K., Brown, G. S., & Ogawa, S. (1978) *Nature (London) 274*, 30–34.
Figgis, B. N., Gerlock, M., & Mason, R. (1969) *Proc. R. Soc. London, Ser. A 309*, 91–118.
Grady, J. E., Bacskay, G. B., & Hush, N. S. (1978) *J. Chem. Soc., Faraday Trans. 2 74*, 1430–1440.
Heidner, E. J., Ladner, R. C., & Perutz, M. F. (1976) *J. Mol. Biol. 104*, 707–722.
Hoard, J. L. (1975) in *Porphyrins and Metalloporphyrins* (Smith, K. M., Ed.) Chapter 8, Elsevier, New York.
Huber, R., Epp, O., & Formanek, H. (1970) *J. Mol. Biol. 52*, 340–354.
Iball, J., & Morgan, C. H. (1970a) *Acta Crystallogr., Sect. B 23*, 239–244.
Iball, J., & Morgan, C. H. (1970b) *Acta Crystallogr., Sect. B 23*, 349–354.
Lee, P. A., Citrin, P. H., Eisenberger, P. M., & Kincaid, B. M. (1981) *Rev. Mod. Phys. 53*, 769–806.
Norvell, J. C., Nunes, A. C., & Schoenborn, B. P. (1975) *Science (Washington, D.C.) 190*, 568–570.
Padian, E. A., & Love, W. E. (1974) *J. Biol. Chem. 249*, 4067–4078.
Peisach, J., Powers, L., Blumberg, W. E., & Chance, B. (1982) *Biophys. J. 38*, 277–285.
Peng, S.-M., & Ibers, J. A. (1976) *J. Am. Chem. Soc. 98*, 8032–8036.
Phillips, S. E. (1980) *J. Mol. Biol. 147*, 531–554.
Powers, L., Chance, B., Ching, Y., & Angiolillo, P. (1981) *Biophys. J. 34*, 465–498.
Shaanan, B. (1982) *Nature (London) 296*, 683–684.
Spiro, T. G., Woolery, G. L., Brown, J. M., Powers, L., Winkler, M. E., & Solomon, E. I. (1983) in *Cooper Coordination Chemistry: Biochemical and Inorganic Perspectives* (Karlin, D., & Zubieta, J., Eds.) pp 23–41, Adenine Press, New York.
Takano, T. (1977a) *J. Mol. Biol. 110*, 537–568.
Takano, T. (1977b) *J. Mol. Biol. 110*, 569–584.
Teo, B. K. (1981) *J. Am. Chem. Soc. 103*, 3990–4001.
Walek, A., & Loew, G. (1982) *J. Am. Chem. Soc. 104*, 2346–2351.
Woolery, G. L., Powers, L., Winkler, M., Solomon, E. I., & Spiro, T. G. (1984) *J. Am. Chem. Soc. 106*, 86–92.
Wyckoff, R. W. G. (1963a) in *Crystal Structures*, 2nd ed., Vol. 2, pp 226–227, 178–179, Interscience, New York.
Wyckoff, R. W. G. (1963b) in *Crystal Structures*, 2nd ed., Vol. 1, pp 185–186, 29–30, Interscience, New York.

Assignment of Resonances in the ^{31}P NMR Spectrum of d(GGAATTCC) by Regiospecific Labeling with Oxygen-17†

B. A. Connolly and F. Eckstein*

ABSTRACT: The chemical synthesis of the octanucleotide d(GGAATTCC) in which each of the phosphate groups is sequentially replaced by an ^{17}O-containing phosphate group using a polymer-supported phosphoramidite method is described. All seven phosphorus resonances in the ^{31}P spectrum of d(GGAATTCC) can be resolved. Assignment of these resonances to a particular phosphate group in the chain is possible because labeling of a phosphate with ^{17}O causes its particular signal to disappear from the spectrum. Phosphate residues toward the middle of the octamer have ^{31}P NMR shifts similar to those found in polydeoxynucleotides, whereas those toward the ends resemble those of dinucleoside phosphates. These data are interpreted in terms of less flexibility of the phosphate groups in the center of the molecule as compared to those at the ends.

X-ray fiber diffraction patterns of DNA and polydeoxynucleotides have shown that these molecules predominantly exist as right-handed helices in two conformations, designated the A and B forms. With the availibility of single crystals of oligodeoxynucleotides, X-ray structural analysis has provided a more detailed picture of the structure and conformation of such oligonucleotides and in particular has revealed the existence of a left-handed helix, designated the Z conformation. The results obtained with oligonucleotides are generally considered representative of the structure of polynucleotides and DNA (Dickerson et al., 1982; Zimmerman & Pheiffer, 1982; Arnott et al., 1983; Saenger, 1983; Wang et al., 1983; Zimmerman, 1983). Most recently, modern NMR spectroscopic techniques have facilitated the assignment of the protons in such oligodeoxynucleotides (Patel et al., 1982a,b, 1983a,b; Kan et al., 1982; Clore & Gronenborn, 1983; Feigon et al., 1983; Hare et al., 1983; Pardi et al., 1983; Scheek et al., 1983). These studies provide information on the conformation of oligonucleotides in solution and thus complement the data obtained by X-ray structural analysis on the conformation in crystals. In contrast to ^{1}H NMR spectroscopy, ^{31}P NMR spectroscopic data although recorded for a number of oligodeoxynucleotides have been less informative, mainly because the signals observed in the spectra could not be assigned to particular phosphate residues in the oligonucleotide chain (Patel & Canuel, 1979; Gorenstein, 1981; Patel et al., 1982a,b, 1983a). The only exceptions, discussed further below, are the recently published data on the two d(CGCG) and d(TCGA) tetramers (Pardi et al., 1983; Petersheim et al., 1984).

† From the European Molecular Biology Laboratory, D-6900 Heidelberg, FRG (B.A.C.), and Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, D-3400 Göttingen, FRG (F.E.). *Received February 23, 1984.*

0006-2960/84/0423-5523$01.50/0

In the course of our work on the stereochemical course of the reaction catalyzed by the restriction endonuclease *Eco*RI, we have synthesized the octamer d(GGAATTCC), which contains the recognition sequence for this enzyme (Connolly et al., 1984). ^{31}P NMR spectroscopy revealed that, at 10 °C where this oligomer exists as a double helix, all seven phosphorus resonances could be resolved. We now wish to report the assignment of all these resonances to their individual phosphate groups using regiospecific labeling with oxygen-17. Furthermore, we show that increases in the upfield chemical shift of these resonances can be correlated qualitatively to increase in the distance of the phosphate groups from both ends of this oligonucleotide.

Materials and Methods

The octamers were synthesized by a phosphoramidite method on a polymer support as described (Connolly et al., 1984) except that $H_2{}^{17}O$ instead of H_2O was used in the iodine/water oxidation step at those positions where ^{17}O was to be incorporated. The final HPLC purification step was replaced by chromatography on a DEAE-Sephadex A-25 column (1.0 × 20.0 cm) with a linear gradient of 300 mL each of 0.2 M and 1 M triethylammonium bicarbonate. The oligomers so produced were ≥98% pure by the HPLC systems detailed in Connolly et al. (1984).

^{17}O-Enriched water (51.0% ^{17}O, 10.4% ^{16}O, 38.6% ^{18}O) was obtained from the Monsanto Research Corp., U.S. Department of Energy (Miamisburg, OH). Dinucleoside phosphates were obtained from Sigma Chemie, München, or P-L Biochemicals, Milwaukee, WI. Polynucleotides were purchased from Boehringer Mannheim, West Germany. ^{31}P NMR measurements were performed at 10 °C using 5-mm precision tubes on a Bruker WP200SY spectrometer at 81.01 MHz with ^{1}H broad-band decoupling. Trimethyl phosphate was used as the external standard. The polymers were sonicated for 1 h under ice cooling with a Branson sonifier before their spectra were recorded.

Results and Discussion

The interpretation of ^{31}P NMR spectra of oligo- and polydeoxynucleotides clearly depends on the assignment of the resonances observed to individual phosphate groups along the oligo- or polynucleotide chain. Once this is known, the chemical shifts of the resonances could provide useful information on the conformation of these molecules since it is known that the shifts are strongly dependent upon the torsional angles ω and ω' of the phosphate ester group as defined by Sundaralingam (1969), which in turn are influenced by O–P–O bond angles and sugar conformation (Cozzone & Jardetzky, 1976; Gorenstein, 1981).

One approach for assignment of phosphorus resonances in polydeoxynucleotides is the exchange of phosphate for phosphorothioate groups. Assignment is possible because the ^{31}P NMR signals of phosphorothioates occur at considerably lower field than those of phosphates. This method has been used for the assignment of the two resonances in poly[d(A-T)] to the two dinucleoside phosphates d(ApT) and d(TpA) by the enzymatic synthesis of two phosphorothioate-containing polymers, one in which the phosphate group of d(ApT) and the other in which that of d(TpA) were replaced by a phosphorothioate group (Eckstein & Jovin, 1983). A similar study with poly[d(G-C)] allowed us to assign the two resonances in the ^{31}P NMR spectrum of this polymer under conditions where it adopts the Z conformation (Jovin et al., 1983). We had hoped that the availability of d(GGsAATTCC), the phosphorothioate analogue of d(GGAATTCC) where the phosphate group between dG and dA is replaced by a phosphorothioate (Connolly et al., 1984), would in an analogous manner allow us to assign the resonance of the d(GpA) phosphate group. However, inspection of the spectrum of d(GGsAATTCC) and comparison with that of d(GGAATTCC) showed that not only was the d[Gp(S)A] signal shifted downfield, as expected, but also the positions of several of the other phosphate signals were slightly changed. The replacement of this one phosphate with a phosphorothioate group must result in a conformational change in the octamer, which causes small changes in the chemical shifts of some of the unmodified phosphate groups, rendering an unequivocal assignment of the d(GpA) resonance impossible. This contrasts with what was observed with the long-chain polymers mentioned above, where replacement of one class of phosphate group with a phosphorothioate does not result in a change in the chemical shift of the remaining unmodified phosphate groups. Since the assignment of phosphorus resonances was not feasible by phosphorothioate replacement, our attention was drawn to an alternative method based on oxygen isotopic substitution. Petersheim et al. (1984) have recently introduced the ^{17}O-labeling technique to identify the phosphorus resonances in the ^{31}P NMR spectrum of d(CGCG). This method seemed most suitable to solve the problem of assigning the resonances in the octamer. It has been known for several years that when the quadrupolar oxygen-17 nucleus is directly bonded to phosphorus, the phosphorus resonances in the ^{31}P NMR spectrum are broadened to such an extent that they usually disappear [for reviews, see Tsai (1982), Cohn (1982), and Gerlt et al. (1983)]. The preparation of d(GGAATTCC) species in which each of the phosphate groups is sequentially replaced with an ^{17}O-containing phosphate group will thus lead to assignment of all the resonances seen in the ^{31}P NMR spectrum as the ^{17}O-labeled phosphate group signal will disappear from the spectrum. Additionally, this isotope substitution should not cause perturbations in neighboring phosphate groups as was found with chemical substitution with sulfur. Of the two most commonly used methods for the synthesis of oligodeoxynucleotides, we chose the phosphite approach (Matteuci & Caruthers, 1981) for the synthesis of the oxygen-17-containing octamers as it requires the least change in protocol. The only change necessary is the replacement of H_2O by $H_2{}^{17}O$ in the oxidation of the phosphite intermediate to a phosphate by iodine. This has been described before for the introduction of isotopes of oxygen into dinucleoside phosphates (Seela et al., 1983; Potter et al., 1983).

The seven resonances in the ^{31}P NMR spectrum of d(GGAATTCC) can clearly be distinguished under conditions (50 mM NaCl and 10 °C) in which this oligomer exists as a double helix. This spectrum is shown in Figure 1A and is identical with that reported previously (Connolly et al., 1984). Figure 1B–H shows the spectra obtained when one phosphate group at a time is replaced by an ^{17}O-containing phosphate group. As the $H_2{}^{17}O$ used was only 51% enriched in ^{17}O and additionally contained 10.4% ^{16}O and 38.6% ^{18}O, the signals do not disappear completely but are reduced in intensity by about half. Additionally, the residual ^{16}O- and ^{18}O-labeled phosphate groups have slightly different chemical shifts with the ^{18}O species resonating to higher field. This can sometimes be observed, especially in spectrum C of Figure 1, but usually the [^{16}O]- and [^{18}O]phosphate groups resonate too closely to be resolved. In some spectra, noticeably F and G, the resonance of the ^{17}O-labeled phosphate is no longer observable as a single line but merges with the rather broad base of the adjacent signal where it can be detected by the correspondingly

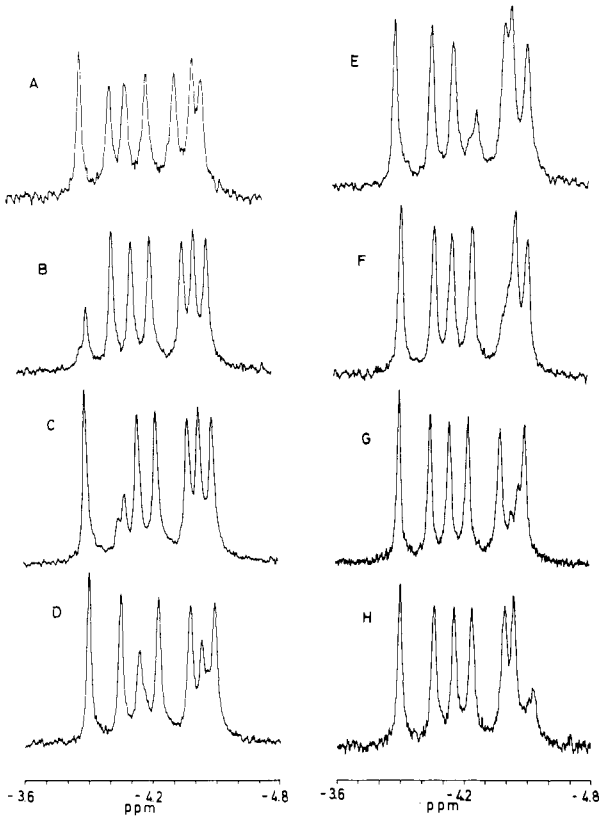

FIGURE 1: ^{31}P NMR spectra of d(GGAATTCC) containing [^{17}O]-phosphate. In spectra A–H the phosphate groups of the following dinucleoside phosphates have been replaced by [^{17}O]phosphate: A, none; B, d(CpC); C, d(GpG); D, d(GpA); E, d(TpC); F, d(ApA); G, d(TpT); H, d(ApT). The samples, approximately 40 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. The spectra were recorded at 10 °C with the following parameters: offset, 1300 Hz; sweep width, 800 Hz; pulse width, 7 μs; 16K; acquisition time, 10.24 s; line broadening, 0.2 Hz; number of transients, between 4000 and 5000.

higher value in the integration. Figure 1B–H shows that in each case one signal is reduced in intensity and this resonance can then be assigned to the phosphate group that was oxidized with $H_2{}^{17}O/I_2$. Only in spectrum D does any ambiguity exist. Here, both the third and sixth signals from the left appear to be reduced in intensity. However, spectrum G shows that the sixth signal must be assigned to the d(TpT) group, and therefore the third resonance in Figure 1 is identified as that of d(GpA).

The values for the chemical shifts of each dinucleoside phosphate in d(GGAATTCC) are tabulated in Table I as are, for comparison, the chemical shifts of the individual dinucleoside phosphates measured under identical conditions and the shifts of phosphate groups in polydeoxynucleotides. The value for d(ApT) in polymers has been previously obtained by the phosphorothioate method using poly[d(A-T)] (Eckstein & Jovin, 1983). However, as these chemical shifts are temperature dependent (Gorenstein, 1981), we have remeasured the spectrum of poly[d(A-T)] again at 10 °C (previously it had been measured at 30 °C) to provide a better comparison with the present data. The chemical shifts of d(CpC) and d(GpG) in polydeoxynucleotides were obtained by recording the ^{31}P NMR spectrum of poly(dG)·poly(dC), whereas those for d(ApA) and d(TpT) resulted from measurements on poly(dA)·poly(dT). Both these polymers gave one peak each in the ^{31}P NMR spectra, showing that both d(CpC)/d(GpG) and d(TpT)/d(ApA) must have similar chemical shifts in these polymers. Due to the lack of appropriate long-chain polymers, we are unable to give a value for the shifts of d(GpA) and d(TpC) in polydeoxynucleotides.

Table I: ^{31}P Chemical Shifts of Dinucleoside Phosphates in d(GGAATTCC)[a]

dinucleoside phosphate	chemical shift[b] in octamer (ppm)	chemical shift in polymer (ppm)	chemical shift of dinucleoside phosphate (ppm)
d(CpC)	−3.89	−4.16[c]	−3.85
d(GpG)	−4.04	−4.16[c]	−4.20
d(GpA)	−4.13		−4.17
d(TpC)	−4.22		−3.90
d(ApA)	−4.37	−4.58[d]	−4.25
d(TpT)	−4.43	−4.58[d]	−4.09
d(ApT)	−4.49	−4.50[e]	−4.13

[a] In 25 mM Hepes, pH 8.0, 25 mM EDTA, and 50 mM NaCl at 10 °C; NMR parameters as given in Figure 1; chemical shift values are ±0.02 ppm. [b] Taken from Figure 1. [c] Values obtained from poly(dG)·poly(dC). [d] Values obtained from poly(dA)·poly(dT). [e] Values obtained from poly[d(A-T)].

A comparison of the chemical shifts of the dinucleoside phosphates and the long-chain polymers shows that the ^{31}P NMR signals of the polymers are shifted to higher field. This effect is well-known and is due to the double-helical nature of the polymers (Gorenstein, 1981). The base stacking and consequent rigidity in a double helix cause both phosphate diester torsion angles ω and ω' (Sundarlingam, 1969) to adopt the gauche, gauche conformation, which is associated with an upfield ^{31}P NMR chemical shift. In the more flexible dinucleoside phosphates the angles ω and ω' tend to be gauche, trans, respectively, and the ^{31}P NMR signals are found at lower field strength. The dimer d(GpG) seems to be anomalous in this respect as it resonates to higher field than the d(GpG) group incorporated into polymeric material. The reason for this is unclear at present but might be caused by self-aggregation of this dimer.

Figure 1 shows that the ^{31}P NMR shifts of the individual phosphate groups in d(GGAATTCC) move toward higher field as the groups approach the center of the octanucleotide. Thus, the chemical shifts of the two terminal dinucleoside phosphates d(CpC) and d(GpG) occur at lowest field whereas that of the central dinucleoside phosphate d(ApT) is found at highest field. This cannot merely be due to a sequence dependence in chemical shift of the phosphate groups in the octanucleotide since, although the isolated dinucleoside phosphates show a shift variation, the linear shift increase is only seen when they are part of the octamer. By comparison of these two sets of values, it becomes clear that those dinucleoside phosphates that are at or close to the center of the octamer experience greater upfield shifts as compared to the isolated dinucleoside phosphates than those toward the end of the helix. Table I further shows that only the central dinucleoside phosphate d(ApT) in the octamer has a chemical shift value that is identical, within experimental error, with that found in a polymer. Thus, as judged by ^{31}P NMR data, this dinucleoside phosphate could have an identical conformation in both the octamer and the polymer. The chemical shifts of the two dinucleoside phosphates adjacent to the center of the octamer, d(TpT) and d(ApA), lie between those observed in the appropriate isolated dimers and polymeric material, indicating an intermediate conformation. A note of caution might be in order here for the justification of taking the ^{31}P NMR shifts in homopolymers as the limiting values for the same dinucleoside phosphates as part of a polydeoxynucleotide of a irregular sequence. At present, not enough material is available to decide whether differences in shifts exist for such polydeoxynucleotides as a function of sequence. We had no access to the polynucleotides that would have

allowed us to determine the limiting values for the chemical shifts of d(GpA) and d(TpC). The latter obviously experiences an upfield shift in the octamer as compared to the dimer, whereas the value for d(GpA) is, rather unexpectedly, similar in both dimer and octamer. At present we are unable to say whether this is due to the extreme flexibility of this phosphate group in the octamer (which occurs toward the end of the helix) or results from d(GpA) having an unusual gauche, gauche conformation. Given the general trends outlined here, it would seem unlikely that the chemical shifts of these two residues recorded in the octamer represent limiting values found in long-chain polymeric material. The terminal dinucleoside phosphate d(CpC) has a very similar chemical shift in the octamer and the dimer that occurs, as expected, to lower field than is found when d(CpC) is incorporated into polymeric material. It is difficult to draw conclusions about the d(GpG) residue in the octamer due to the anomalous chemical shift of the d(GpG) dimer. The interpretation of the ^{31}P NMR spectrum of the octanucleotide d(GGAATTCC) can be summarized by the statement that phosphate residues toward the middle of the molecule have ^{31}P NMR shifts similar to those found in long-chain polymers whereas residues at the ends are more akin to those found for dinucleoside phosphates. Unfortunately, no X-ray structural and no ^{1}H NMR spectroscopic data exist for this octamer yet as they do for the dodecamer d(CGCGAATTCGCG) (Fratini et al., 1982; Patel et al., 1982a,b, 1983a,b; Hare et al., 1983), which would allow a comparison with the data reported here. Hopefully, this situation will change in the future.

Patel & Canuel (1979) have published a ^{31}P NMR spectrum of d(GGAATTCC), recorded at 145.72 MHz and 30.8 °C, in which five resonances could be distinguished. A temperature dependence of these shifts was reported and showed the six resonances could be distinguished at 10 °C, the temperature used in our publication. None of these resonances was assigned to a particular phosphate group. However, a comparison of their and our chemical shift data allows such an assignment, the larger chemical shift values (about 0.06 ppm) reported by Patel & Canuel (1979) at 10 °C in comparison to ours, most likely due to the slight differences in NaCl concentration and pH values used. We suggest that the lowest chemical shift reported in Figure 12 of Patel & Canuel (1979) is due to d(CpC), followed in increasing order by those of d(GpG), d(GpA), and d(TpC), and that the last at −4.5 ppm is due to in this case unresolved d(ApT) and d(TpT) phosphates. The justification for this latter statement is that resolution of these two signals was the most difficult to achieve in our experiments. Accepting this interpretation, it can be seen in Figure 12 of Patel & Canuel (1979) that upon heating and melting the resonances of the central and its two flanking dinucleoside phosphates undergo the most dramatic downfield shift, followed by those of the two dinucleoside phosphates next to the ends and with the two terminal phosphate groups exhibiting the smallest shift. It should be pointed out here that the temperature dependence of the chemical shifts as documented in this figure shows a similar midpoint of thermal transition for all phosphorus resonances, indicating that the observation of six or seven individual resonances at 10 °C is not due to fraying of ends. The downfield shift in the ^{31}P NMR spectrum seen when self-complementary oligonucleotides are melted into random coil structures is also explained by a change in the phosphate diester torsion angles from gauche, gauche in the rigid double helix to gauche, trans in the more flexible single strand (Patel, 1976; Olson, 1975). It is therefore clear that in d(GGAATTCC) the central phosphate groups, which have a high proportion of gauche, gauche structure, should undergo the greatest conformational change and therefore the largest chemical shift change on melting and formation of a gauche, trans single strand. Conversely, the terminal phosphate groups already have a high degree of gauche, trans structure in the double helix and so undergo a lesser conformational and chemical shift change upon melting. Patel (1976), who recorded the ^{31}P NMR spectrum of d(CGCGCG) above and below the melting temperature, without having the assignment of the resonances, also concluded that the phosphate groups in the interior exhibit less conformational flexibility than those closest to the end.

How feasible and general is this method for assigning phosphorus resonances and how does it compare with other approaches? Recently, the phosphorus resonances in the tetramer d(CTAG) have been assigned by using two-dimensional ^{1}H and ^{31}P NMR spectroscopy (Pardi et al., 1983). This method obviously requires less synthesis than that described here. However, this approach is dependent on both the resolution and assignment of the C5′ and C3′ sugar protons. Once these protons have been identified, the phosphate groups can be assigned by selective heteronuclear decoupling. Due to crowding in the C5′ and C3′ region, it has still to be seen whether this method can be applied to longer oligomers.

The method outlined in this paper is clearly applicable to any length of oligonucleotide for which the phosphorus resonances can be resolved and does not require any prior assignment or knowledge of proton resonances. The disadvantage, when compared to approaches based on heteronuclear decoupling, is clearly the larger amount of synthetic work required. However, with the solid-phase phosphite method used here, enough octamer for ^{31}P NMR measurement can be synthesized in about 4 h and the HPLC purification takes about 2–3 h. The two most time-consuming steps, the deblocking with NH_3 and the final DEAE-Sephadex A-25 column, can be carried out overnight with little or no attention from the operator. No modification of the existing methodology is needed to incorporate the ^{17}O regiospecifically into any phosphate group. The synthetic method used by Petersheim et al. (1984) for regiospecific labeling of d(CGCG) is based on phosphotriester chemistry in solution and, at least for longer oligonucleotides, seems less convenient in terms of both the actual synthesis of the oligomer and the introduction of the ^{17}O label than that described here.

In summary, we have assigned the phosphorus resonances in the ^{31}P NMR spectrum of d(GGAATTCC) and shown that only the central region of this octamer approaches a polymeric DNA-like conformation. This octanucleotide and its 5′-phosphorylated derivative are substrates, albeit poor ones, for the restriction endonuclease *Eco*RI. These weak substrate-like properties have been ascribed to additional binding sites present in the normal substrate (long-chain DNA containing the sequence GAATC) but absent in the octanucleotide. The studies of Lu et al. (1981) show that this is indeed part of the reason. However, the slow cleavage could also be due to the difference in overall conformation between d(GGAATTCC) and the same sequence incorporated into polymeric oligomers with a consequent poorer recognition and cleavage by *Eco*RI. Clearly, some caution must be exercised in studies of enzyme–DNA interaction using small easily prepared oligonucleotides and then extrapolating the results obtained to long polynucleotides.

Acknowledgments

We thank B. Seeger for recording the NMR spectra and J. A. Gerlt for communication of results prior to publication.

Registry No. d(GGAATTCC), 70755-49-6.

References

Arnott, S., Chandrasekaran, R., Hall, J. H., Puigjaner, L. C., Walker, J. K., & Wang, M. (1982) *Cold Spring Harbor Symp. Quant. Biol. 47*, 53–65.

Clore, G. M., & Gronenborn, A. M. (1983) *EMBO J. 2*, 2109–2115.

Cohn, M. (1982) *Annu. Rev. Biophys. Bioeng. 11*, 23–42.

Connolly, B. A., Potter, B. V. L., Eckstein, F., Pingoud, A., & Grotjahn, L. (1984) *Biochemistry 23*, 3443–3453.

Cozzone, P. J., & Jardetzky, O. (1976) *Biochemistry 15*, 4853–4859.

Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Fratini, A. V., & Kopka, M. L. (1982) *Science (Washington, D.C.) 216*, 475–485.

Dickerson, R. E., Drew, H. R., Conner, B. N., Kopka, M. L., & Pjura, P. E. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 13–24.

Eckstein, F., & Jovin, T. M. (1983) *Biochemistry 22*, 4546–4550.

Feigon, J., Leupin, W., Dumy, W. A., & Kearns, D. R. (1983) *Biochemistry 22*, 5943–5951.

Fratini, A. V., Kopka, M. L., Drew, H. R., & Dickerson, R. E. (1982) *J. Biol. Chem. 257*, 14686–14707.

Gerlt, J. A., Coderre, J. A., & Mehdi, S. (1983) *Adv. Enzymol. Relat. Areas Mol. Biol. 55*, 291–380.

Gorenstein, D. G. (1981) *Annu. Rev. Biophys. Bioeng. 10*, 355–386.

Hare, D. R., Wemmer, D. E., Chon, S.-H., Drobny, G., & Reid, B. R. (1983) *J. Mol. Biol. 171*, 319–336.

Jovin, T. M., van de Sande, J. H., Zarling, D. A., Arndt-Jovin, D. J., Eckstein, F., Füldner, H. H., Greider, C., Grieger, J., Hamori, E., Kalisch, B., McIntosh, L. P., & Robert-Nicoud, M. (1982) *Cold Spring Harbor Symp. Quant. Biol. 47*, 143–154.

Kan, L.-S., Cheng, D. M., Jayaraman, K., Lentzinger, E. E., Miller, P. S., & Ts'o, P. O. P. (1982) *Biochemistry 21*, 6723–6732.

Lu, A.-L., Jack, W. E., & Modrich, P. (1981) *J. Biol. Chem. 256*, 13200–13206.

Matteucci, M. D., & Caruthers, M. H. (1981) *J. Am. Chem. Soc. 103*, 3185–3191.

Olson, W. K. (1975) *Biopolymers 14*, 1797–1810.

Pardi, A., Walker, R., Rapoport, H., Wider, G., & Wüthrich, K. (1983) *J. Am. Chem. Soc. 105*, 1652–1653.

Patel, D. J. (1976) *Biopolymers 15*, 533–558.

Patel, D. J., & Canuel, L. L. (1979) *Eur. J. Biochem. 96*, 267–276.

Patel, D. J., Kozlowski, S. A., Marky, L. A., Broka, C., Rice, J. A., Itakura, K., & Breslauer, K. J. (1982a) *Biochemistry 21*, 428–436.

Patel, D. J., Pardi, A., & Itakura, K. (1982b) *Science (Washington, D.C.) 216*, 581–590.

Patel, D. J., Kozlowski, S. A., Ikuta, S., Itakura, K., Blatt, R., & Hare, D. R. (1983a) *Cold Spring Harbor Symp. Quant. Biol. 47*, 197–206.

Patel, D. J., Kozlowski, S. A., & Blatt, R. (1983b) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3908–3912.

Petersheim, M., Mehdi, S., & Gerlt, J. A. (1984) *J. Am. Chem. Soc. 106*, 439–440.

Potter, B. V. L., Eckstein, F., & Uzmanski, B. (1983) *Nucleic Acids Res. 11*, 7087–7103.

Saenger, W. (1983) *Principles of Nucleotide and Nucleic Acid Structure* (Cantor, C. R., Ed.) Springer-Verlag, New York, Berlin, Heidelberg, and Tokyo.

Scheek, R. M., Russo, N., Boelens, R., & Kaptein, R. (1983) *Biochemistry 22*, 5943–5951.

Seela, F., Ott, J., & Potter, B. V. L. (1983) *J. Am. Chem. Soc. 105*, 5879–5886.

Sundaralingam, M. (1969) *Biopolymers 7*, 821–860.

Tsai, M. D. (1982) *Methods Enzymol. 87*, 235–279.

Wang, A. H.-J., Fuji, S., van Boom, J. H., & Rich, A. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 197–206.

Zimmerman, S. B. (1982) *Annu. Rev. Biochem. 51*, 395–427.

Zimmerman, S. B., & Pheiffer, B. H. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 67–75.