Eppstein, D. A., Marsh, Y. V., & Schryver, B. B. (1983) *Virology 131*, 341-354.
Graessman, M., & Graessman, R. (1983) *Methods Enzymol. 101*, 482-492.
Haugh, M. C., Cayley, P. J., Serafinowska, H. T., Norman, D. G., Reese, C. B., & Kerr, I. M. (1983) *Eur. J. Biochem. 132*, 77-84.
Hughes, B., & Robins, R. (1983) *Biochemistry 22*, 2127-2135.
Imai, J. & Torrence, P. F. (1984) *Biochemistry 23*, 766-774.
Johnston, M. I., & Torrence, P. F. (1984) in *Interferon* (Friedman, R. M., Ed.) Vol. 3, pp 189-298, Elsevier, Amsterdam.
Kariko, K., & Ludwig, J. (1985) *Biochem. Biophys. Res. Commun. 128*, 695-698.
Knight, M., Cayley, P. J., Silverman, R. H., Wreschner, D. H., Gilbert, C. S., Brown, R. E., & Kerr, I. M. (1980) *Nature (London) 288*, 189-192.
Laemmli, U. K. (1970) *Nature (London) 227*, 680-685.
Laskey, R., & Hills, A. (1975) *Eur. J. Biochem. 56*, 335-341.
Lee, C., & Suhadolnik, R. J. (1985) *Biochemistry 24*, 551-555.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265-275.
Milhaud, P. G., Silhol, M., Faure, T., & Milhaud, X. (1983) *Ann. Virol. 134E*, 405-416.
Minks, M. A., Benvin, S., Maroney, P. A., & Baglioni, C. (1979) *J. Biol. Chem. 254*, 5058-5064.
Rice, A. P., Roberts, W. K. & Kerr, I. M. (1984) *J. Virol. 50*, 220-228.
Schmidt, A., Chernajovsky, Y., Shulman, L., Federman, P., Berissi, H., & Revel, M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4788-4792.
Silverman, R. H., (1984) in *Interferon* (Friedman, R. M., Ed.) Vol. 3, pp 177-188, Elsevier, Amsterdam.
Silverman, R. H., Wreschner, D. H., Gilbert, C. S., & Kerr, I. M. (1981) *Eur. J. Biochem. 115*, 79-85.
Torrence, P. F., Imai, J., & Johnston, M. I. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5993-5997.
Watling, D., Serafinowska, H. T., Reese, C. B., & Kerr, I. M. (1985) *EMBO J. 4*, 431-436.
Williams, B. R. G., & Kerr, I. M. (1978) *Nature (London) 276*, 88-89.
Wreschner, D. H., James, T. C., Silverman, R. H., & Kerr, I. M. (1981) *Nucleic Acids Res. 9*, 1571-1578.

Sequence Dependence of the B to Z Transition in Crystals and Aqueous NaCl Solutions for Deoxyoligonucleotides Containing All Four Canonical DNA Bases†

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Received November 20, 1986; Revised Manuscript Received March 17, 1987

ABSTRACT: A laser Raman study has been made on the conformation of a series of self-complementary octameric deoxynucleotides that contain all four canonical deoxynucleotide bases [guanine (G), cytosine (C), adenine (A), and thymine (T)] in order to determine which sequences will crystallize in the Z form and which sequences will go into the Z form in aqueous solution at high salt concentrations (4–6 M NaCl). All four octadeoxynucleotides, d(TGCGCGCA) (I), d(CACGCGTG) (II), d(CGTGCACG) (III), and d(CGCATGCG) (IV), have been crystallized from low-salt solutions. The Raman spectra of microcrystals show that I, II, and IV crystallize in a rigorous Z form while III crystallizes in the B form. Sequences I and II go into a Z form in 4–6 M NaCl solution at 0 °C while sequences III and IV remain in the B form in 6 M salt. There are substantial differences in the Raman spectra of oligonucleotides in the Z form found in the crystal and in high-salt solutions. The Raman spectra of the Z forms in 6 M NaCl solution at 0 °C are not linear combinations of the Raman spectra of the complete Z form in the crystal and the complete B form in low-salt solutions. The terminal residues of these oligomers do not appear to be in a strict Z form. A detailed analysis of the ring puckers and syn/anti conformation for all of the residues both in solution and in the crystal has been made. From these data together with data found in recent literature, some simple rules are suggested that may prove useful for predicting which DNA sequences containing all four canonical bases will go into the Z form in aqueous solution under high-salt conditions. The tendency of these four sequences to go into the Z form may be ranked I > II > IV > III.

Although the discovery of Z DNA has led to a great deal of discussion concerning its possible biological significance (Rich et al., 1983; Jovin et al., 1983; Rich et al., 1984), it is not clear at the present time which base sequences in DNA will support the left-handed conformation and which environmental conditions are necessary to induce the Z form for a given base sequence. The initial discovery of the left-handed conformation of poly(dG-dC) in 4 M aqueous salt solutions (Pohl & Jovin, 1972) has been followed by several investigations of alternating pyrimidine–purine sequences (Jovin et al., 1983; Rich et al., 1984). There has been an increasing number of reported examples of both polydeoxynucleotides and oligonucleotides that will support this left-handed conformation. With the exception of $d(CG)_n$ where $n \geq 2$, either most of the sequences that may be induced into the Z form

† This work was supported by grants from the National Institutes of Health (5 RO1 GM15547-18, 5 RO1 GM33825-02) and National Science Foundation (DMB-8417199) and by NSF Equipment Grant DMB 8507352.

0006-2960/87/0426-5178$01.50/0

contain one or more modified bases (Behe & Felsenfeld, 1981) or the conditions used to induce the Z form appear to be more severe than that of a 4–6 M aqueous salt solution, the solvent usually tried first. For example, the Z conformation has been induced in d(CGTACGTACG) in the crystalline state with cobalt hexamine (Brennen et al., 1986). Another common practice is to use a combination of high sodium perchlorate concentration and organic solvents. An example is poly(dA-dC)·poly(dG-dT), which will not go into the Z form in 4–6 M salt solution but requires 4 M sodium perchorate and 19% ethanol in order to go into the Z form [Jovin et al. (1983) characterize these conditions as "rather extreme"]. On the other hand, very recently Schorschinsky and Behe (1986) showed that a polymer synthesized by ligation of the non-complementary 10 base pair repeating sequence d-(CGCGCGTGCA) goes into the Z form at 4–6 M salt solution. Because of the method by which the polymer was synthesized it is obvious that it has sticky (i.e., single-stranded) ends, but this will have little or no effect on the conformation for high degrees of polymerization. Indeed an infinitely long polymer of this sequence may be considered to be generated by simply repeating either of the self-complementary base sequences, -CACGCGCGTG- or -GCGTGCACGC-. It will be obvious from the work presented that an oligomer consisting of the former decanucleotide would go into the Z form, while a decanucleotide of the second sequence definitely would not. [The decanucleotide -GCGCGCGCGC- does not even go into the Z form (Quadrifoglio et al., 1983).] Thus, this polymer may be generated by the repetition of a decanucleotide that goes into the Z form or one that will not go into the Z form. We suggest that any polymer that can be shown to be made up a repetition of a self-complementary oligonucleotide that does go into the Z form at high salt will itself go into the Z form at high salt provided that its molecular weight is high enough that end effects are not important. Thus, it does not matter whether the polynucleotide can also be generated by repetition of a sequence that does not go into the Z form if the polymer molecular weight is sufficiently large. This type of analysis shows one reason why it is important to know which oligomeric sequences will go into the Z form.

One of the purposes of this work is to obtain experimental data that will aid any effort to devise some simple rules for predicting the tendency of any given sequence to go into the Z form. In addition, it would be useful to predict the occurrence of more than one conformation in a double-stranded sequence, i.e., which parts of the oligomer will be in the Z form and which in the B form. As was shown in the initial work of Thamann et al. (1983) there is a band at 625–630 cm^{-1} characteristic of a guanine residue in the Z form while this band is at 680–685 cm^{-1} in the B form. As we will discuss below, there are Raman bands of adenine and thymine characteristic of the Z form. We will show that in the crystals of these octameric nucleotides the Z form is complete—there are no bands characteristic of the B form in the Raman spectra of the crystals. In solution this is not the case. Here the degree of Z form is a function of the salt concentration, and the temperature. Furthermore, the degree of Z conformation is strongly sequence dependent even among those oligomeric sequences that go into the Z form. The occurrence of the A and T residues in the Z form depends largely on their relative position in the sequence, with the tendency to go out of the Z form increasing when the A and T residues approach the ends of the oligomer thereby exposing a longer d(CG) sequence. (These considerations do not necessarily hold in either a crystal or a polymer where all of the residues may be held into a rigorous Z form by the requirements of three-dimensional order in the case of a crystal or one-dimensional order in the case of a polymer.) Recently in this laboratory it was shown that of the four self-complementary sequences of tetranucleotides, d(CGCG), d(GCGC), d(CCGG), and d-(GGCC), only the first would support the Z conformation at high salt (Thomas & Peticolas, 1984). It seemed worthwhile to extend these investigations to include oligomers that contain A and T as well as C and G. Since it seemed unlikely to us that any self-complementary hexamers other than $d(CG)_3$ would go into the Z form in aqueous salt solution, we began our studies with the four self-complementary octamers containing one A and one T residue. One of these octamers (IV) had already been shown to exist in the Z form in the crystal by Benevides et al. (1984), and we use their Raman results as our starting point. It will be shown that two of these octamers go into the Z form both in the crystal and in 4–6 M salt solution (I, II), one does not go into the Z form either in the crystal or in 6 M salt solution (III), and one goes into the Z form in the crystal but not in the high-salt solution (IV). However, we have found that IV will go into a partial Z form at 25% EtOH and saturated salt solution. Thus, we may rank these four oligonucleotides in order of their tendency to go into the Z form as I > II > IV > III.

Materials and Methods

The five oligodeoxynucleotides, $d(CGCGCGCG)_2$, $d(CGCATGCG)_2$, $d(CGTGCACG)_2$, $d(CACGCGTG)_2$, and $d(TGCGCGCA)_2$, and the dinucleotide, d(CG), were synthesized by the University of Oregon Biotechnology Laboratory utilizing an Applied Biosystem Model 380 DNA synthesizer that uses phosphoramidite chemistry. The oligodeoxynucleotides following trityl group removal were purified by reversed phase HPLC on a Vydac C4 10 mm × 250 mm column. The octamers were eluted with an acetonitrile and 0.1 M triethylamine acetate (pH 6.5) gradient. Purified fractions were concentrated to dryness by rotary evaporation. The samples were dissolved in double-distilled water and lyophilized. The dissolving and lyophilization procedure was repeated three times to eliminate residual triethylamine acetate. Oligodeoxynucleotide purity was estimated to be greater than 99% based upon synthetic coupling yields and chromatographic analysis. It should be noted that none of the oligonucleotides studied have a terminal phosphate.

The solution samples of the octadeoxynuclotides were prepared in the following manner. Portions of the octadeoxynucleotides were dissolved in 0.5 or 6.0 M NaCl solutions at an approximate DNA concentration of 0.4 OD/μL, which corresponds to about 25 μg/μL. After the samples were allowed to reach equilibrium, 4 μL of each sample containing about 100 μg of oligomer was transferred into a capillary mounted on the Raman sample holder made of a thermostated copper block. The Raman spectra of all the oligodeoxynucleotides were measured at a temperature of 2.0–10.0 °C. The concentration of oligomer, the salt content, and the experimental temperature were chosen to ensure that the DNAs form double-stranded duplexes. For the dinucleotide, d(CG), spectra at low salt and high salt were measured in a similar manner.

Crystallization of these oligomers was obtained by the vapor diffusion technique. The initial solvent contained 30 mM sodium cacodylate, 15 mM $MgCl_2·6H_2O$, and 5 mM spermine. For several weeks this solution was equilibrated with a solution of 10–45% 2-methyl-2,4-pentanediol. For large crystals we used a classical Raman apparatus, while for very small crystals we used a newly developed intracavity laser Raman micro-

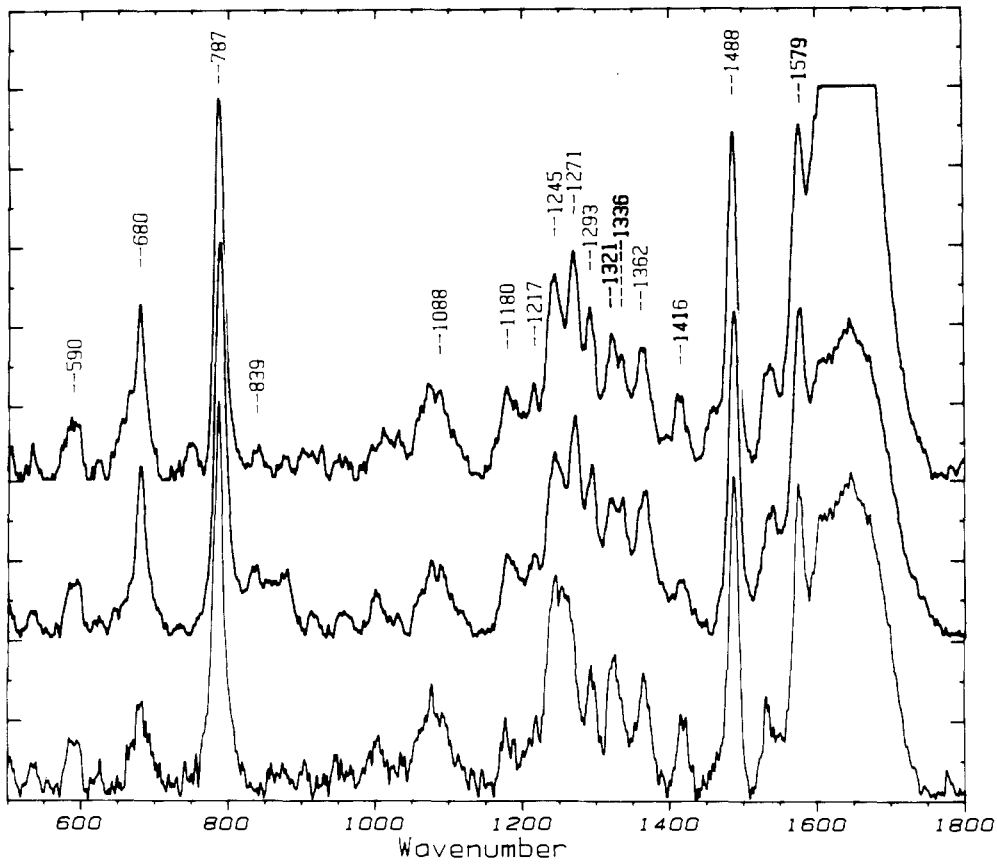

FIGURE 1: Raman spectra of 4.0% d(CG) in 0.1 M NaCl at +55 °C (bottom) and at −15 °C (middle) and in saturated NaCl solution at −15 °C (top) for the region of 500–1800 cm^{-1}.

scope. The crystals of $d(TGCGCGCA)_2$ appeared cubic with a dimension of about 200 μm on a side, and the crystals of $d(CACGCGTG)_2$ appeared hexagonal in shape and were 30–60 μm on a side, while d(CGTGCACG) were very small cubic crystals about 50–100 μm to a side. For study in the classical Raman system, the crystal along with a small amount of mother liquor was drawn up into a 15-μL disposable capillary pipet. The pipet was then mounted in a 100-μL disposable capillary pipet sealed on one end. The two pipets were then sealed together at the open end. Crystals sealed in this manner were quite stable, and Raman spectra could be taken at leisure. The crystal samples were grown in a drop suspended from the underside of a microscope cover slide suspended over the pentanediol solution. The whole crystal growing assembly was mounted under the microscope objective, which was focused into the interior of a crystal attached to the underside of the microscopic cover glass. X-ray diffractions studies on some of the larger crystals of the oligomer (III) have been begun in collaboration with Professor Brian Matthews, Institute of Molecular Biology.

The classical Raman apparatus includes a Spectra Physics Model 165 argon ion laser and a Spex 1301 double monochromator interfaced to a HP 200 series computer capable of computer-controlled data acquisition. The 514.5-nm laser line with 200-mW output power was used for obtaining the Raman spectra. Fifteen scans were collected for each spectrum with a spectral resolution of 4 cm^{-1}. The Raman microscope is described elsewhere (Patapoff et al., unpublished results). It consists of a Zeiss industrial model microscope with a 100× objective. The crystal was illuminated by a 1-μm^3 spot from a focused laser beam using an epiilluminator, and the Raman spectrum was obtained in back-scattering geometry with a Spex triplemate and an EMI image intensifier with a Tracor Northern vidicon optical multichannel analyzer. Raman spectra taken on the microscope had a satisfactory signal to noise after about 500 scans, which took about 5 min.

Results and Discussion

In order to develop a working hypothesis for the sequence dependence of the Z conformation in 4–6 M salt solution, it is useful to know the smallest dC-dG sequence that can exist in the Z form in aqueous salt solution. We have previously shown in this laboratory that the d(CGCG) sequence goes into the Z form at high salt and low temperature (Thomas & Peticolas, 1984), but the question of whether the simple self-complementary deoxy dimer, d(CG), would form a duplex structure that would go into the Z form arises. Figure 1 shows the Raman spectrum of an aqueous solution of d(CG) taken at 0.1 M salt at −15 and +55 °C and in saturated salt solution at −15 °C. It is apparent that both low-temperature spectra show the characteristic Raman frequencies and intensities of the normal B-type DNA. When the 0.1 M salt solution is heated, the duplex dimer dissociates into two single d(CG) stands and the 835-cm^{-1} band disappears. Indeed, all of our attempts using alcohol and sodium perchlorate to force the d(CpG) duplex to go into the "Z form", i.e., the guanine in the syn conformation, failed. In every case only the B-form Raman spectrum was observed. Thus, although Wang et al. (1979) have shown from X-ray diffraction measurements that the repeat unit of Z DNA is d(CG), this dimer itself will not support the left-handed form in concentrated NaCl solutions. It seems reasonable to suppose that at least a string of two dimers, d(CGCG), are required to initiate a Z conformation in aqueous salt solutions. Furthermore, it appears that the longer the sequence $(CG)_n$, the more stable the Z form will be. These observations will be useful in our interpretation of

Table I: Dominated Changes of the Raman Bands upon Transition from B Form to Z Form Conformations (cm^{-1})

B form	assignment	change	Z form	assignment
		increasing	628	C3′-endo/syn G
682	C2′-endo/anti G	decreasing		
786	C2′-endo/anti C + P-R[a]	increasing	786	
832	C2′-endo/anti G	decreasing		
		increasing	867	P-R[a]
1180	C–N str[b] for C, G	splitting	1180	C–N str for C
			1188	C–N str for G
1242	G, C	shift and increase	1247	G, C
1267	C, G	increasing	1267	C, G
1318	G	increasing	1319	C3′-endo/syn G
1336	C2′-endo/anti G	decreasing		
1366	C, G	shift	1362	C, G
1420	C2′-endo/anti G	shift	1428	C3′-endo/syn G

[a] Phosphate and ribose mode. [b] Base external carbon–nitrogen stretching vibration.

experimental results discussed below since we will emphasize the apparent importance of the sequency of pyrimidine–purine dimers.

The oligonucleotide d(CGCATGCG) has been found to adopt a Z form when crystallized from alcohol solutions (Fujii et al., 1985). Benevides et al. (1984) have examined by Raman spectroscopy both the crystalline and solution forms of this oligomer. The Z form was readily identifiable in the crystalline state, and a B form was observed under the solution conditions employed. Their observation implies that the oligomer may adopt a Z form in concentrated sodium chloride solutions although this is not specifically stated in their paper. Starting out with such experimental evidence, we examined the four self-complementary deoxyoctanucleotides that have alternating pyrimidine–purine sequences and contain one thymine and one adenine. Our goal has been twofold: first, to determine which sequences will go from the B to the Z form as the concentration of salt is increased to 4–6 M and, second, to compare the Raman spectra of Z form in solution with the Z form in the crystal where the constraints are much greater. In this way we determine any change in the Z form for a given oligonucleotide upon going from the crystal to the solution. These experimental observations should be most useful for the development of semiempirical theoretical methods for predicting the relative ability of oligonucleotides of moderate length, <20 base pairs that contain unmodified bases, to adopt a left-handed Z helix in concentrated salt solutions.

In discussion of our experimental results, it will be useful to present a simple analysis based upon the order of the pyrimidine-purine dimer sequences that make up the oligomer. The dimeric unit is chosen as the basic unit of analysis because the repeat unit of the Z structure is a dimeric pyrimidime-purine unit. The relative stability of pyrimidine–purine pairs in the Z form is taken as (CG) > (TG) = (CA) ≥ (TA) (Wang et al., 1984). On the basis of the observation that d(CGCG) is the minimal sequence that will support the Z form in aqueous salt solutions (Thomas & Peticolas, 1984), we propose that sequential (CG) pairs tend to stabilize the Z form and sequential (TG) and (CA) pairs tend to offer at best neutral contributions to the stability of the Z form, while sequential (AT) sequences will disrupt the Z helix leading to some change from a strict Z conformation. The relative stability of the Z form for a series of oligonucleotides can then be estimated by examination of the contributions of individual dimer units and by estimating the contributions of sequential dimer units.

Consider, for example, a simple analysis of the four self-complementary octadeoxynucleotides that contain one A and one T. If these are considered to be made of pyrimidine–purine dinucleotide units, they may be written as (TG)(CG)(CG)-(CA), (CA)(CG)(CG)(TG), (CG)(TG)(CA)(CG), and (CG)(CA)(TG)(CG). Note that each of these octamers contains two (CG) pairs and one (CA) and one (TG) pair. They differ only in the order of permutation of these pairs. The contributions of the individual dimer units is the same for the four octamers. On the other hand, the first two oligomers contain two sequential (CG) pairs and the non-(CG) pairs are separated. For the third and fourth oligomers sequential (TG)(CA) and (CA)(TG) pairs are present and the (CG) units are separated. In view of the apparent importance of sequential (CG) units in stabilizing the Z form, the first two members of the set of octamers may reasonably be expected to be more stable in the Z form than the latter two members, which may be expected to remain in the B form in high-salt solutions. The Raman spectrographic analysis of this set of self-complementary octamers indicates that these predictions are valid. Thus, although at the present time these considerations must be considered as rationalizations of the Raman results, they may be the first step toward a more general theory of the sequence dependence of the Z form in concentrated salt solutions.

The conformational analysis of these octamers in aqueous salt solutions is assisted by comparison to the reference Raman spectra of the octadeoxynucleotide d(CGCGCGCG) taken in 0.5 and 6.0 M NaCl at 10 °C, shown in Figure 2. The high-salt spectrum is a typical Raman spectrum of Z DNA (Thamann et al., 1983) in solution. In Figure 2, the bands showing no change upon going from the B to Z forms are shadowed in black. All of the nonshadowed bands change in

Table II: Observed Changes in the Raman Spectra of Z DNA from Crystal Form to Solution (cm^{-1})[a]

$d(TGCGCGCA)_2$			$d(CACGCGTG)_2$		
frequency in soln at 0.0 °C	change	frequency in crystal	frequency in soln at 0.0 °C	change	frequency in crystal
682 (G)	decrease		678 (G)	shift	675 (G)
			716 (G)	decrease	
750 (T, Z(b))	increase	750 (T, Z(b))	732 (A)	decrease	
866 (Z(b))	shift	852 (Z(bb)	746 (T, Z(b))	shift	749 (T, Z(b))
1031	increase	1031	864 (Z(b))	shift	856 (Z(b))
1094 (P(st))	decrease	1094 (P(st))	1030	increase	1030
	increase	1302 (A)	1094 (P(st))	decrease	1094 (P(st))
1319 (G)	increase	1319 (G)	1267 (C)	increase	1267 (C)
1336 (G, A)	decrease			increase	1302 (A)
1374 (G, A)	decrease		1374 (G, A)	decrease	
			1388 (G)	decrease	

[a] Key: A, C, G, and T, vibrational modes associated with adenosine, cytidine, guanosine, and thymidine; P(st), phosphate symmetric stretching vibration; Z(b), vibrational mode associated with backbone of Z conformation.

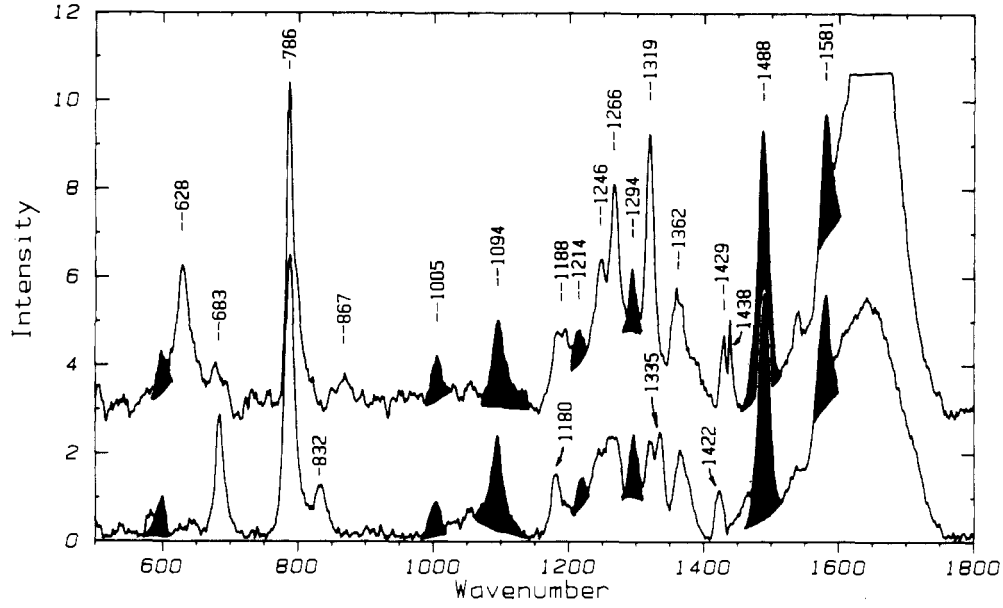

FIGURE 2: Raman spectra of model octamer DNA, $d(CGCGCGCG)_2$, in the B form in 0.5 M NaCl (bottom) and in the Z form in 6.0 M NaCl solution (top) at 10.0 °C. Two spectra are normalized according to the height of 1094-cm^{-1} band, and the Raman bands that show negligible changes for the B to Z transition are shadowed with black.

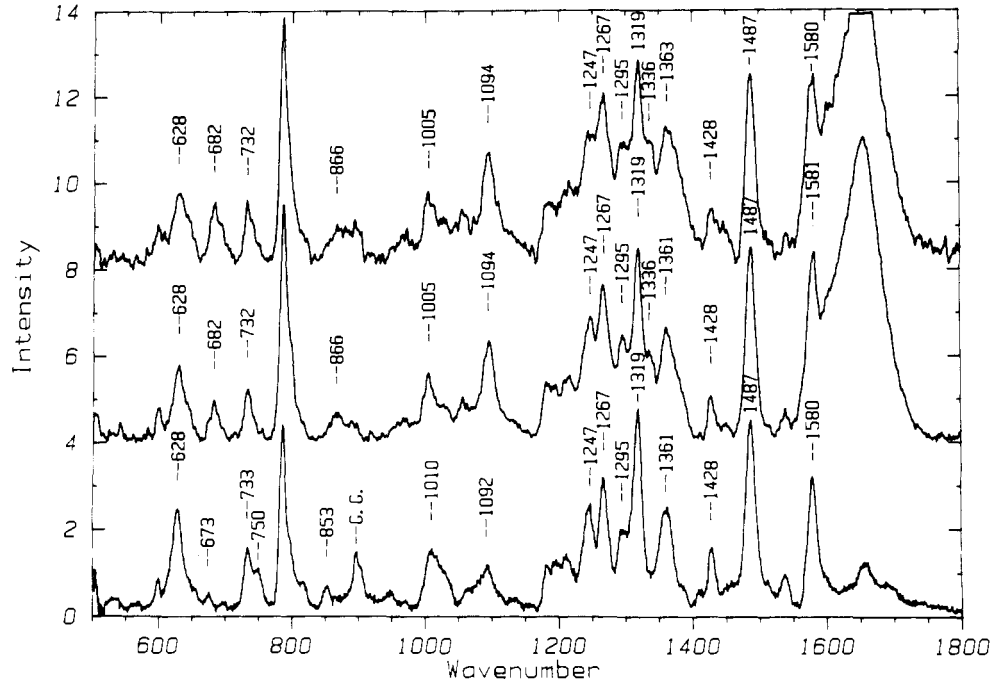

FIGURE 3: Raman spectra of $d(TGCGCGCA)_2$ in 6.0 M NaCl solution at 10.0 °C (top), at 0.0 °C (middle), and in its crystal state (bottom). The three spectra were normalized according to the 1488-cm^{-1} Raman band.

either intensity or frequency on going from the B to Z forms. Table I lists characteristic frequency changes in the Raman spectrum for the B to Z transition observed in solution. Figure 2 permits the analysis of the vibrations attributed to C, G, and the sugar–phosphate backbone. However, in addition the other Raman spectra will contain bands due to A and T. In this case we will compare our spectra in solution with the Raman spectrum reported by Benevides et al. (1984) for the oligomer $d(CGCATGCG)_2$ in the crystal.

The Raman spectra of the four octamers, $d(TGCGCGCA)_2$, $d(CACGCGTG)_2$, $d(CGTGCACG)_2$, and $d(CGCATGCG)_2$, in 0.5 M aqueous salt solution are very similar and are typical for the B genus DNA—so much so that we will not present them here. Instead, it is more worthwhile to compare the Raman spectra of these oligomers in the crystalline form and in concentrated salt solutions at low temperature and room temperature. The last two oligomers are in the B form in concentrated salt solutions and yield Raman spectra typical of all of these oligomers when they are in the B form.

Detailed Comparison of the Raman Spectra of the Octamers in Crystal and Concentrated Salt Solutions. (*A*) *$d(TGCGCGCA)_2$.* The bottom Raman spectrum in Figure 3 was obtained from a single crystal of $d(TGCGCGCA)_2$. From this high-quality spectrum, it is easy to show that this oligo DNA is in a Z conformation. The bands at 628, 751, 853, 1247, 1266, 1319, 1360, and 1428 cm^{-1} are all marker bands for the Z form. It is also observed that the bands at 682, 833, 1336, and 1422 cm^{-1}, found in the B-form conformation, are absent in this spectrum. There are other Raman bands associated with adenine and thymine bases. The band at 672 cm^{-1} is due to C2′-endo/anti T, which can only be seen in this Z form, since it is obscured often by the 682-cm^{-1} band in the

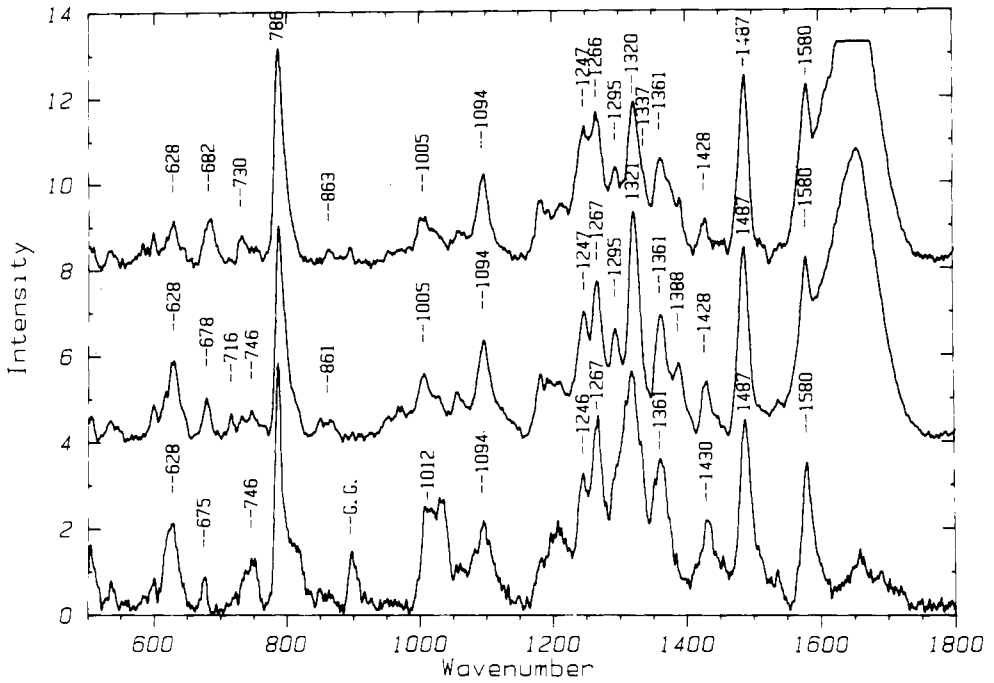

FIGURE 4: Raman spectra of d(CACGCGTG)$_2$ in 6.0 M NaCl solution at 10.0 °C (top), at 0.0 °C (middle), and in its crystal state (bottom), normalized according to the height of the 1488-cm^{-1} Raman band.

B form and 675 cm^{-1} for C2′-endo/syn of the terminal guanosine in the Z form. It is interesting to note that the 733-cm^{-1} band of adenine is present in this spectrum because it is absent in all of the other crystalline spectra when the oligomers are in the Z form. We tend to assign it to the C2′-endo/syn adenosine furanose mode. It belongs to neither C2′-endo/anti nor C3′-endo/anti, because of the absence of both 1342- and 1336-cm^{-1} bands. The evidence of its not being due to a C3′-endo/syn mode will be discussed later with d(CACGCGTG)$_2$.

The top two spectra in Figure 3 are for the 6 M salt solution of d(TGCGCGCA)$_2$, both of which present the same general features with some variations in the relative intensities of some Raman bands. The two big differences from the crystal spectrum are the presence of bands at 682 and 1337 cm^{-1} and the changes at 751, 1319, and 1374 cm^{-1}. It is clear that in the solution state at low temperature one of the guanine residues is in the C2′-endo/anti conformation, and it is probably the second base to the terminal. Higher temperatures cause more G to flip into the C2′-endo/anti conformation, as indicated by the top spectrum in Figure 3 where bands at 628 and 682 cm^{-1} have nearly the same intensity and the ratio of 1319/1337 bands is reduced. It is not so easy to distinguish whether terminal A is in C2′-endo/anti or C3′-endo/anti, but it is reasonable to assume that the former conformation is more likely due to the favorable nearest-neighbor interactions.

(*B*) *d*(*CACGCGTG*)$_2$. The bottom spectrum in Figure 4 shows the Z-form crystal Raman spectrum of this oligomer. Different Z-form conformations of guanosine residues in the crystal and solution are also observed for d(CACGCGTG)$_2$. This may be seen from the 675-cm^{-1} band in the crystal, which changes to 678 cm^{-1} for solution at low temperature and 682 cm^{-1} at higher temperatures. The Z-marker band at 628 cm^{-1} is more intense for the crystal, and it decreases in solution with an increase of temperature. This would be explained if the terminal guanosine residue is in a C2′-endo/syn conformation in the crystal and a conformation close to C2′-endo/anti at the elevated temperature in the solution. The 677-cm^{-1} band is assigned to the conformation close to the C3′-endo/anti G. Although it seems nearly 10 wavenumbers higher than the normal C3′-endo/anti form found in the Raman spectrum of the A-form DNA, there might be different interactions involved here for this particular guanosine. Sequence dependence and the terminal effect could be the major two factors among them. Other evidence come from 716 and 1388 cm^{-1}, both of which have been shown to be associated with A-form DNA (Nishimura et al., 1986; Benevides et al., 1986). Information concerning the adenosine conformation may be obtained from the band at 732 cm^{-1} in solution at 12 °C that decreases at 2.0 °C and nearly disappears in the crystal. It is assigned to adenosine in the following conformations: C3′-endo/anti at 12 °C, C3′-endo/syn at 2 °C and in the crystal. Our result is in disagreement with the statement that 728 cm^{-1} does not change when adenosine is in C3′-endo/syn conformation as it is in the Z form (Benevides et al., 1986). However, a comparison of the Raman spectrum of the crystas of d(TGCGCGCA) and d(CACGCGTG) shows that the 732 cm^{-1} does change when the adenosine changes, probably due to a difference in the conformation of the terminal A in the former octamer from the A in the latter. The height of 1320 cm^{-1} may also contain a partial contribution from the C3′-endo/anti G conformation, which gives a strong band at 1321 cm^{-1}.

(*C*) *d*(*CGTGCACG*)$_2$. Figure 5 indicates clearly that the crystal of d(CGTGCACG)$_2$ has a conformation similar to that observed in solution. Because this crystal was very small, the Raman spectrum was taken with the microscope. The presence of bands at 683, 833, 1338, and 1422 cm^{-1} shows that both crystal and solution DNA are in a B-form conformation. There are certain differences in band intensities shown between the two spectra in Figure 5, and we think that they are due to the orientation of the crystal in the microscope in a specific relation to the polarization direction of the excitation laser. Orientation effects have been found to be very important in the Raman spectra of crystals taken by the laser Raman microscope (unpublished work of this laboratory).

(*D*) *d*(*CGCATGCG*)$_2$. The Raman spectrum of this oligo DNA (Figure 6) in the crystalline state has been published (Benevides et al., 1984), and it is assigned to the Z form. The present work shows that this oligomer will not adopt Z con-

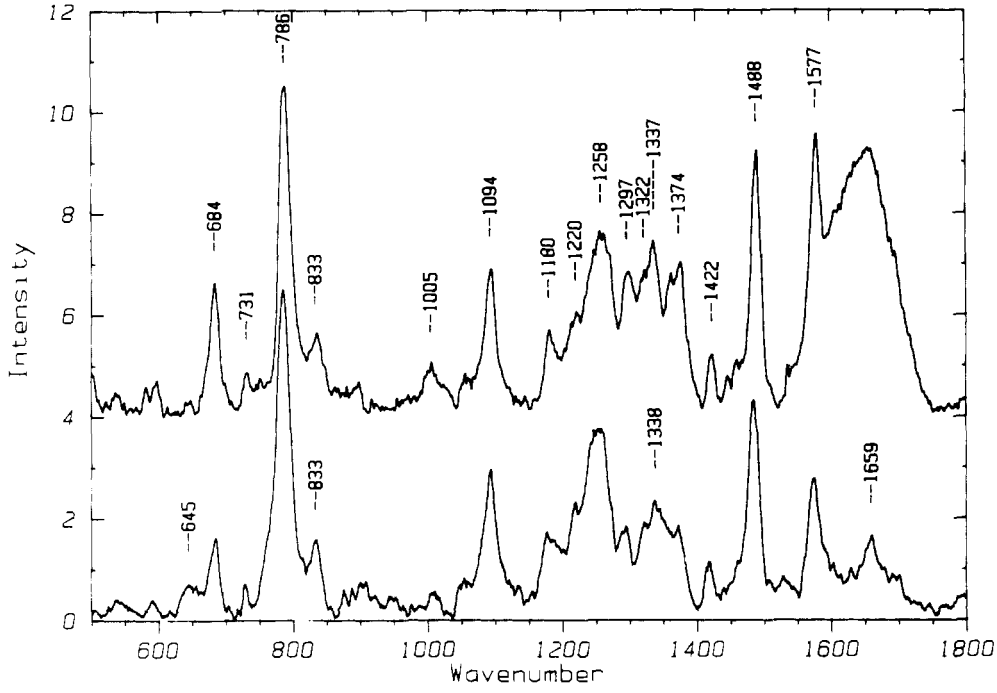

FIGURE 5: Raman spectra of d(CGTGCACG)$_2$ in 6.0 M NaCl solution at 0.0 °C (top) and in its crystal state (bottom). These spectra demonstrate that this oligomer exists in the B form under each of these conditions.

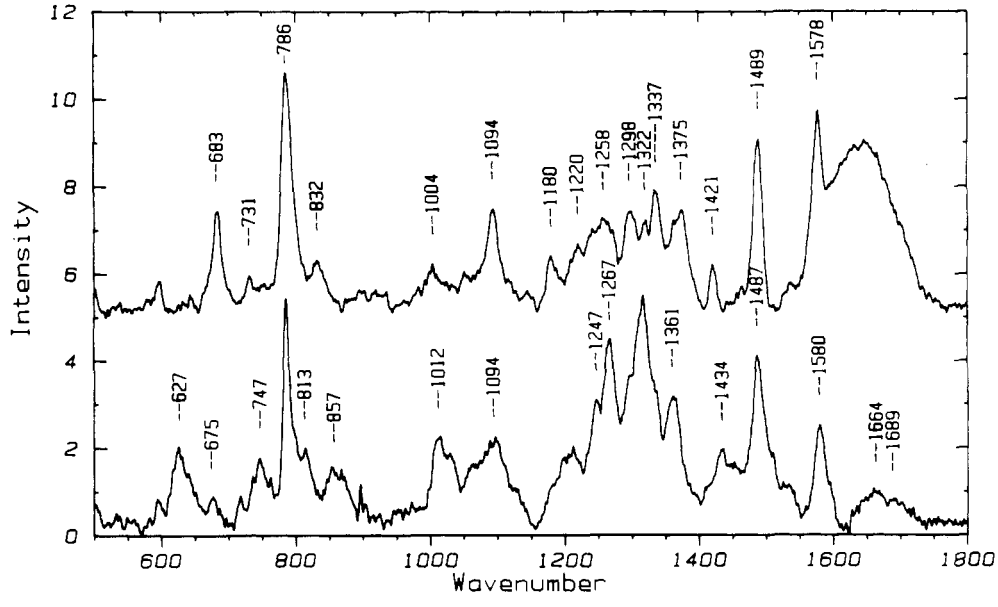

FIGURE 6: Raman spectra of d(CGCATGCG)$_2$ in 6.0 M NaCl solution at 0.0 °C (top) and in its crystal state (bottom). The spectra were normalized according to the 1488-cm^{-1} Raman band. It is apparent that this oligomer is in the Z form in the crystal but in the B form in solution.

formation in 6.0 M NaCl solution. However, we have observed that in saturated NaCl/25% ethanol solution d(CGCATGCG)$_2$ has a Z-type conformation very similar to that of d(CACGCGTG)$_2$ in salt solution at 2.0 °C for the region of 550–860 cm^{-1}. This shows that in the solution state d(CGCATGCG)$_2$ has the ability to adopt a modified Z form. From this result we conclude that 6.0 M NaCl solution is a milder condition for inducing the Z conformation in an oligomer than a 25% ethanol solution saturated with salt.

From the Raman band assignments discussed in detail above, it is now possible to suggest with some certainty that the two oligonucleotides, d(TGCGCGCA)$_2$ and d(CACGCGTG)$_2$ which adopt Z form in both crystal and high-salt solution, have the conformations in the crystalline state and in aqueous solution at 2.0 °C given in Chart I.

It appears that the tetramer d(CGCG)$_2$, which occurs at the center of this octamer, has the same conformation in the high-salt solution state at 2.0 °C and in the crystalline state. According to the above model, sequences that possess a sort of "B–Z or A–Z junction" in aqueous solution are actually observed. These "junctions" appear to consist of the coexistence of the interior bases in a Z-type conformation and the terminal bases in a conformation that resembles an A-type or B-type environment but is much more disordered as evidenced by the weakness of the A and B backbone marker bands at 807 and 835 cm^{-1}. This indicates that it is possible to have a discontinuity that only involves two base pairs. It is true that the terminal effects play a role in the formation of the junction in these oligomers. It is possible that such B–Z and A–Z junctions may exist in the conformation of native DNAs

Chart I

For $d(TGCGCGCA)_2$:

SOLUTION				CRYSTAL			
	3′	5′			3′	5′	
C2′-endo/anti	A	T	C2′-endo/anti	C2′-endo/syn	A	T	C2′-endo/anti
C2′-endo/anti	C	G	C2′-endo/anti	C2′-endo/anti	C	G	C3′-endo/syn
C3′-endo/syn	G	C	C2′-endo/anti	C3′-endo/syn	G	C	C2′-endo/anti
C2′-endo/anti	C	G	C3′-endo/syn	C2′-endo/anti	C	G	C3′-endo/syn
C3′-endo/syn	G	C	C2′-enod/anti	C3′-endo/syn	G	C	C2′-endo/anti
C2′-endo/anti	C	G	C3′-endo/syn	C2′-endo/anti	C	G	C3′-endo/syn
C2′-endo/anti	G	C	C2′-endo/anti	C3′-endo/syn	G	C	C2′-endo/anti
C2′-endo/anti	T	A	C2′-endo/anti	C2′-endo/anti	T	A	C2′-endo/syn
	5′	3′			5′	3′	

For $d(CACGCGTG)_2$:

SOLUTION				CRYSTAL			
	3′	5′			3′	5′	
C3′-endo/anti	G	C	C3′-endo/anti	C2′-endo/syn	G	C	C2′-endo/anti
C2′-endo/anti	T	A	C3′-endo/syn	C2′-endo/anti	T	A	C3′-endo/syn
C3′-endo/syn	G	C	C2′-endo/anti	C3′-endo/syn	G	C	C2′-endo/anti
C2′-endo/anti	C	G	C3′-endo/syn	C2′-endo/anti	C	G	C3′-endo/syn
C3′-endo/syn	G	C	C2′-endo/anti	C3′-endo/syn	G	C	C2′-endo/anti
C2′-endo/anti	C	G	C3′-endo/syn	C2′-endo/anti	C	G	C3′-endo/syn
C3′-endo/syn	A	T	C2′-endo/anti	C3′-endo/syn	A	T	C2′-endo/anti
C3′-endo/anti	C	G	C3′-endo/anti	C2′-endo/anti	C	G	C2′-endo/syn
	5′	3′			5′	3′	

when suitable base sequences and a suitable environment are present. Finally, it should be noted that although the simple analysis given above correctly predicts that two of the octomers will go into the Z form in high salt and that two will remain in the B form, it does not distinguish between these two octamers. Plainly, the d(CGTGCACG) goes into the Z form much less readily than d(CGCATGCG) since the latter crystallizes in the Z form and goes into the Z form in 25% ethanol saturated with salt while the former remains in the B form under all of these conditions. It may be that the CGC and GCG sequences that occur in the latter octamer tend to induce the Z form since the former does not have these sequences. Thus, to develop a more quantitative theory it may be that triplets as well as dimers must be considered.

An alternative possibility to the analysis given above has been put forward by the reviewers. They have suggested that, in addition to the existence of a variation in the torsional angles at the ends of the Z-form duplexes in high-salt solutions, one must consider the possibility of an equilibrium between fully left-handed and fully right-handed forms. Such an equilibrium appears to be rulled out by the following argument. If we assume that we have an equilibrium mixture of B and Z forms in aqueous solution at high salt and low temperatures, then the Raman spectrum of this mixture should be linear combination of the Ramn spectra of the pure Z form observed in the crystal and the pure B form observed in solution at low ionic strength. From this linear combination one could calculate the fraction of each form present at high salt. However, computer analysis of the Raman spectra of these Z-form duplexes in high-salt solution and low temperature shows that these spectra cannot be generated by any linear combination of the right-handed and left-handed forms. This is because it is the base vibrations and not the backbone vibrations that indicate the existance of a mixture of conformations. The observed Raman spectra of the Z-form oligomers can only be explained from the model spectra by assuming that the residues on the ends of the duplexes (which are different in base composition from those in the interior) are not in the Z form while those in the interior of the duplex are in the Z form. These arguments only apply to measurements made in aqueous solutions that are 6 M in NaCl and at or near 0 °C. We have begun to study the temperature dependence of the B to Z transition, and we find that at higher temperatures that an equilibrium between the B and Z forms does exist.

It should be noted that self-complementary structures have been mostly used for the study of Z DNA, but this is probably due to convenience in synthesis. It seems highly likely that a duplex octamer, which contains a single base replacement such as d(TGCGCGCG), would go into the Z conformation. However to test this assumption, it would be necessary to synthesize both this octamer and its complement, d-(CGCGCGCA).

In conclusion it appears that it is possible to interpret the data on the sequence dependence of the salt-induced B to Z transition from the known Z-form stability of dimeric units with the incorporation of the cooperative stabilization and destabilization of sequential dimer pairs using the rules developed here. These rules also allow the prediction of the sequence dependence of the salt-induced B to Z transition of longer oligonucleotides containing only the four canonical DNA bases. The method at this stage is rudimentary, but it is hoped that it will prove useful for the examination of the B to Z transition in longer sequences. Perhaps it may be a step in the development of a more quantitative approach that will ultimately allow for the inclusion of modified bases. Another conclusion from this work is that although the overall Z conformation of the oligo DNAs in concentrated salt solutions is similar to that in the crystal, more disorder in the local conformation can exist in the residues at the ends of the oligomer in solution. This effect becomes even more profound with increasing temperature. Although we have not discussed the biological implications of the sequence dependence of the B to Z transition in this paper, it is apparent that understanding the B to Z transition in these sequences can play a role in helping to predict the sequence dependence of Z DNA formation in naturally occurring DNAs such as superhelical DNA (Johnston & Rich, 1986; Hagen et al., 1986; Ellison et al., 1986).

ACKNOWLEDGMENTS

We gratefully acknowledge the work of Dr. M. A. Lindorfer, who carried out the syntheses of the oligonucleotides.

Registry No. I, 108817-44-3; II, 108817-45-4; III, 108817-46-5; IV, 108817-47-6.

REFERENCES

Behe, M., & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1619–1623.

Benevides, J. M., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H., Rich, A., & Thomas, Jr., G. J. (1984) *Nucleic Acids Res. 12*, 5913–5925.

Benevides, J. M., Wang, A. H.-J., Kyogoku, Y., van der Marel, G. A., van Boom, J. H., & Thomas, G. J., Jr. (1986) *Biochemistry 25*, 41–50.

Brennan, R. G., Westhof, E., & Sundaralingam, M. (1986) *J. Biomol. Struct. Dyn. 3*, 649–665.

Ellison, M. J., Quigley, G. J., Wang, A. H.-J., & Rich, A. (1986) in *Biomolecular Stereodynamics* (Sarma, R. H., Ed.) Vol. 4, Adenine, Guilderland, NY.

Fujii, S., Wang, A. H.-J., Quigley, G. J., Westerink, H., van der Marel, G., van Boom, J. H., & Rich, A. (1985) *Biopolymers 24*, 243–250.

Hagen, F., Miller F., Jovin, T., van der Sande, J., & Rattner, J. B. (1986) in *Biomolecular Stereodynamics* (Sarma, R. H., Ed.) Vol. 4, Adenine, Guilderland, NY.

Johnston, B. H., & Rich, A. (1986) in *Biomolecular Stereodynamics* (Sarma, R. H., Ed.) Vol. 4, Adenine, Guilderland, NY.

Jovin, T. M., McIntosh, L., Arndt-Jovin, D. J., Zarling, D. A., Robert-Nicoud, M., van de Sande, J. H., Jorgenson, K. F., & Eckstein, F. (1983) *J. Biomol. Struct. Dyn. 1*, 21–58.

Nishimura, Y., Torigoe, C., & Tsuboi, M. (1986) *Nucleic Acids Res. 14*, 2737–2748.

Pohl, F. M., & Jovin, T. M. (1972) *J. Mol. Biol. 67*, 375–396.

Quadrifoglio, F., Marzini, G., Yathindra, N., & Cred, R. (1983) *Jerusalem Symp. Quantum Chem. Biochem. 16*, 61–74.

Rich, A., Nordheim, A., & Azorin, F. (1983) *J. Biomol. Struct. Dyn. 1*, 1–20.

Rich, A., Nordheim, A., & Wang, A. H.-J. (1984) *Annu. Rev. Biochem. 53*, 791–846.

Schorschinsky, N., & Behe, M. J. (1986) *J. Biol. Chem. 261*, 8093–8095.

Thamann, T., Lord, R. C., Wang, A. H.-J., & Rich, A. (1983) *Nucleic Acids Res. 9*, 5443–5457.

Thomas, G. A., & Peticolas, W. L. (1984) *Biochemistry, 23*, 3202–3207.

Wang, A. H.-J., Quigley, G. S. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680–682.

Wang, A. H.-J., Hakoshima, T., van der Marel, G., van Boom, J. H., & Rich, A. (1984) *Cell (Cambridge, Mass.) 37*, 321–331.

Formation of a Left-Handed RNA Double Helix: Energetics of the A–Z Transition of Poly[r(G-C)] in Concentrated $NaClO_4$ Solutions†

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Received December 30, 1986; Revised Manuscript Received March 13, 1987

ABSTRACT: Ultraviolet spectroscopic and nuclear magnetic resonance (NMR) studies have shown that poly[r(G-C)] in a solution of 4 M $NaClO_4$ undergoes a transition to a left-handed Z-RNA helix upon raising the temperature to 60 °C [Hall, K., Cruz, P., Tinoco, I., Jr., Jovin, T. M., & van de Sande, J. H. (1984) *Nature (London) 311*, 584–586]. In the present report, the transition temperature of this particular order/order transition is shown to increase with decreasing $NaClO_4$ concentration to about 110 °C, above which only the helix-to-random coil transition is detectable. The reversibility and cooperativity of the helix/helix conversion has facilitated the quantitative evaluation of the transition enthalpy by means of differential scanning microcalorimetry. In 5 M $NaClO_4$, the transition temperature is 43 °C, the conversion enthalpy 4.2 kJ (1.0 kcal) per mole of base pair, and the corresponding entropy change 13 J (3.1 cal) deg^{-1}. The van't Hoff enthalpy for the same process, determined from the temperature dependence of the optical transition, is 0.26 MJ (62 kcal) per mole of cooperative unit. The ratio of the two enthalpy values yields an apparent cooperative length for the A–Z transition of poly[r(G-C)] of ~60 base pairs, indicative of a concerted all-or-none process.

Some of the early attempts to solve the three-dimensional structure of DNA single crystals involved oligonucleotides composed exclusively of alternating guanine and cytosine residues (Wang et al., 1981; Dickerson et al., 1981). These sequences provide very stable secondary structures in small oligomers as well as in polymers and lack the difficulties associated with the corresponding homopolymer pair. Unexpectedly, the first crystal structure of the duplex of the hexanucleotide $d(C\text{-}G)_3$ did not reveal the familiar right-handed helix of B-DNA but rather a left-handed conformation designated Z-DNA (Wang et al., 1981). It provided a structural basis for the previously reported cooperative optical transitions and anomalous circular dichroism spectra of poly[d(G-C)] at high salt concentrations (Pohl & Jovin, 1972). Numerous

† This investigation was supported by the Max Planck Society and the Deutsche Forschungsgemeinschaft (DFG, Schwerpunktprogramm Biophysik der Organisation der Zelle) and by the Fonds der Chemischen Industrie.

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0006-2960/87/0426-5186$01.50/0