Ultrapolymorphic DNA: B, A, Z, and Z* Conformations of Poly(dA-dC)·Poly(dG-dT)†

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ABSTRACT: The synthetic polynucleotide poly(dA-dC)·poly(dG-dT) at low ionic strength is shown to undergo conformational changes in the presence of [tris(2-aminoethyl)amine]zinc(II) chloride (ZnN4). At 100 μM ZnN4, circular dichroism and ^{31}P NMR spectra show the formation of Z DNA. With an increase of the concentration up to 600 μM, an A-like form is obtained, and at still higher concentration, the polynucleotide reverts to the original B form. Experiments on polynucleotide samples in which some sequence errors were observed showed that spermine was necessary as well as ZnN4 to induce the Z form. At higher concentrations of spermine and ZnN4, a second Z form (Z*) is observed. Raising the ionic strength inhibits the formation of the Z form, whereas the presence of ethylene glycol favors it.

The left-handed helical or Z conformation of DNA has been well-defined by X-ray crystallographic studies on $d(CpG)_3$ (Wang et al., 1979, 1981), and these results explained the previously reported anomalous CD[1] spectra of poly(dC-dG) under high-salt conditions (Pohl & Jovin, 1972). A wide variety of factors have been shown to influence this B to Z transition. The transition is facilitated in ethanolic solution (Pohl, 1976), by certain divalent metal ions (van de Sande et al., 1976; Zacharias et al., 1982; Russell et al., 1983) or by methylation (Behe & Felsenfeld, 1981).

However, long sequences of $d(CG)_n$ are extremely rare in biological systems, and since an alternating purine–pyrimidine repeat would seem to be the most likely candidate to adopt a Z form, interest has recently focused on other sequences. X-ray studies have shown (Hakoshima et al., 1983; Wang et al., 1984; Brennan & Sundaralingam, 1985) that when A-T base pairs are inserted into a G-C sequence the DNA can still adopt a Z form. Recently, this has also been reported from solution studies (Patel et al., 1985).

The sequence $d(AC)_n{\cdot}d(GT)_n$ is commonly found in eukaryotic genomes and is an interesting candidate for the formation of a Z helix. (Acetylamino)fluorene modification (AAF) (Wells et al., 1982), high-salt conditions with $NiCl_2$ (Taillandier et al., 1984; Taboury & Taillandier, 1985), high salt/ethanol (Jovin et al., 1983), or the topological stress of supercoiling (Haniford & Pulleyblank, 1983) favor the transition to the Z form. Recently, a $(TG)_{25}$ sequence in the rat somatostatin gene has been shown to form Z DNA (Hayes & Dixon, 1985). At high temperature and high salt, poly(dA-dm^5C)·poly(dG-dT) adopts the Z form (MacIntosh et al., 1983).

As we have recently shown (Fazakerley, 1984; Woisard et al., 1985) that micro- or nanomolar concentrations of certain zinc complexes induce the Z form in poly(dC-dG) and poly(dm^5C-dG), we have investigated whether one of these complexes could induce the Z form in poly(dA-dC)·poly(dG-dT) under conditions much milder than hitherto found.

MATERIALS AND METHODS

Poly(dA-dC)·poly(dG-dT) was purchased from P-L Biochemicals or from Boehringer. The polynucleotide was exhaustively dialyzed with 0.5 M NaCl, 1 mM EDTA, and 2 mM sodium cacodylate, pH 7, followed by dialysis with 0.1 M NaCl–2 mM buffer, then 0.01 M NaCl–2 mM buffer, and finally against buffer alone. Most of the results reported below were of batch 719–97 from P-L Biochemicals. We noted that results on subsequent batches were not reproducible (see Results) and that with one batch from Boehringer we were unable to obtain any results at all. We attempted to control these polynucleotides by running the proton NMR spectra at high temperature under conditions where the polynucleotide is melted. In many cases, two thymidine H^6 or methyl resonances of varying relative intensity were observed, and integration of base proton resonances did not give a base ratio of 1:1:1:1. The discrepancy was as high as 25% in one lot from Boehringer. We believe that different results on different batches are entirely due to the proportion of sequence errors.

Tris(2-aminoethyl)amine was purchased from Fluka A.G. The zinc complex was prepared by mixing equimolar quantities of the amine ligand and $ZnCl_2$. Titrations are carried out by adding microliter quantities of stock solutions of the zinc complex or spermine. Circular dichroism spectra were recorded on a Jobin Yvon Mark V dichrograph. ^{31}P nuclear magnetic resonance spectra at 20 °C were recorded at 121 MHz on a large-bore Bruker MSL-300 spectrometer. Spectra correspond to 20000–80000 acquisitions.

RESULTS

Titration of Poly(dA-dC)·Poly(dG-dT) with ZnN4. The B-form circular dichroism (CD) spectrum of poly(dA-dC)·poly(dG-dT), Figure 1A, remains unaltered by titration with [tris(2-aminoethyl)amine]zinc(II) chloride (ZnN4) up to a concentration of 50 μM. At 55 μM ZnN4, the positive band at 280 nm disappears, the positive band at 267 nm has increased in intensity and shifted to 262 nm, and the negative band at 250 nm has decreased in intensity, Figure 1A. The initial transition is very slow. Typically, the CD spectrum evolved over a period of ca. 2.5 h before reaching equilibrium. On further addition of ZnN4, the negative band at 295 nm increases in intensity, and the negative band at 250 nm disappears. The time to reach the equilibrium spectrum becomes

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[1] Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; ZnN4, [tris(2-aminoethyl)amine]zinc(II) chloride.

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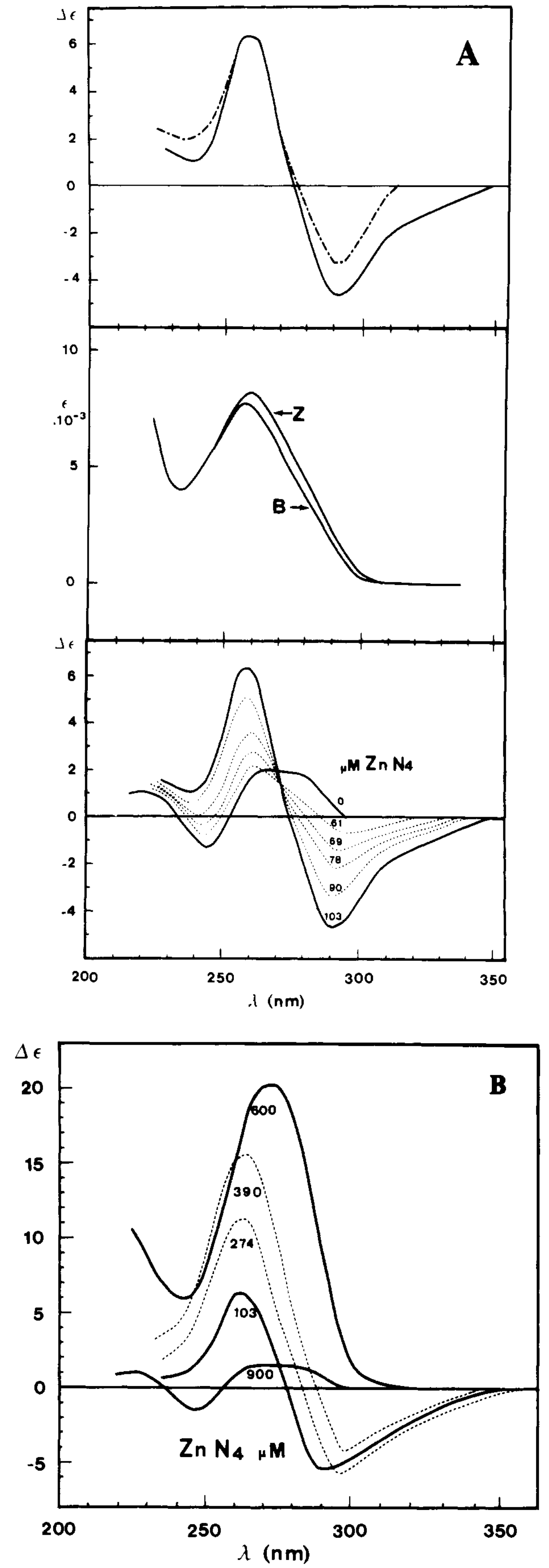

FIGURE 1: Changes in CD spectra of poly(dA-dC)·poly(dG-dT) induced by ZnN4. Conditions: 0.05 mM nucleotide, 2 mM sodium cacodylate, pH 7.2 at 20 °C. (A) (Bottom) B → Z transition; (center) UV spectra of the B and inverted CD forms; (top) spectra before and after sonication. (B) Z → A* → B transitions.

very much shorter as the total concentration of ZnN4 is increased. In the latter stages of the transition, equilibrium was reached 5 min after addition of an aliquot of ZnN4. The transition is cooperative and displays an isosbestic point at 275 nm, and a limiting spectrum is reached at 100 μM ZnN4, Figure 1A. This limiting spectrum is reminiscent of the CD spectrum of the Z form of poly(dC-dG) (Pohl & Jovin, 1972) except that the negative band tails to 350 nm. This same tailing has been observed with the Z form of AAF-modified poly(dA-dC)·poly(dG-dT) (Wells et al., 1982).

Attempts to carry out this titration at much higher polynucleotide concentrations failed as in each case the DNA visibly aggregated. A solution that showed the limiting inverted spectrum at a concentration of 40 μM in nucleotide was centrifuged for 5 min in an Eppendorf centrifuge, and this resulted in a loss of ca. 5% of the optical density. However, at much higher velocity, 15 000 rpm, the loss in optical density was greater than 95%, whereas for the polynucleotide in the absence of ZnN4 the loss was less than 5%; ZnN4 must induce some aggregation of poly(dA-dC)·poly(dG-dT), and the observed dichroism above 300 nm is consistent with this.

DNA exhibits a pronounced tendency to form aggregates in the presence of bi- or trivalent metal ions especially at low ionic strength. As a result, differential light scattering will make a contribution to the CD spectrum, and this contribution can be large (Bustamante et al., 1983).

We prepared a more dilute solution (0.015 mM nucleotide) giving the same limiting inverted spectrum as shown in Figure 1A. When the temperature was raised to 60 °C, the CD spectrum remained unchanged over a period of several hours. Heating alone was not sufficient to breakdown the aggregation as shown by the tail toward 350 nm. We therefore gently sonicated the solution in the cuvette in a Bransonic 220 sonicator for 1 h at 60 °C. The spectrum was recorded immediately at the same temperature (Figure 1A). The negative band at 295 nm is about 30% less intense, and the tailing toward 350 nm has disappeared. We observe no differential light scattering above 315 nm. The intensity of the positive band at 267 nm has not changed. The observed spectrum has the same form as that for the Z form of poly(dA-dC)·poly(dG-dT) observed by others (Jovin et al., 1983; Taboury & Taillandier, 1985) although the relative intensities of the positive and negative bands are not exactly the same. After 30 min we observed that the tailing begins to reappear in the CD spectrum.

The B → Z transition also induces changes in the UV spectrum of the DNA. The UV spectra corresponding to the B form and to the inverted CD spectral form are also shown in Figure 1A. The small red shift is typical of the B → Z transition, and the observed spectrum is very similar to that observed for the polynucleotide in high-salt/ethanol solution (Jovin et al., 1983).

Upon further addition of ZnN4, no change was observed in the CD spectrum until the concentration reached 250 μM. Above this concentration the positive band increased considerably in intensity and shifted to 270 nm, and in the later stages the negative band at 295 nm disappears, Figure 1B. This transition is very much less, if at all, cooperative, and the limiting spectrum is reached at 600 μM ZnN4. Equilibrium was reached rapidly, 2–5 min after addition of each aliquot of ZnN4. The final spectrum showed only a positive band. At low polynucleotide concentration, little or no tailing of the circular dichroism towards 350 nm was observed although at a concentration 100 μM in nucleotide this was seen, indicating aggregation. The CD spectrum resembles that of an A-form

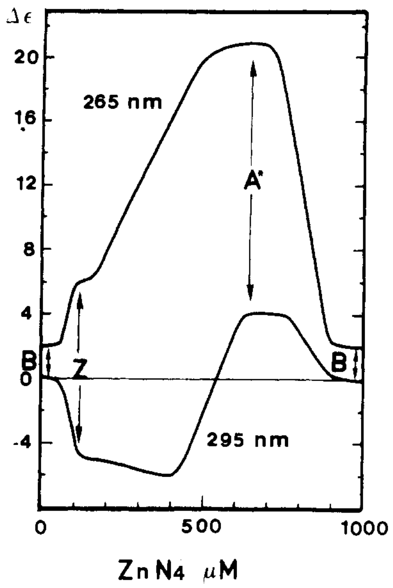

FIGURE 2: Change of the CD amplitude of poly(dA-dC)·poly(dG-dT) at two wavelengths, 265 and 295 nm, as a function of ZnN4 concentration.

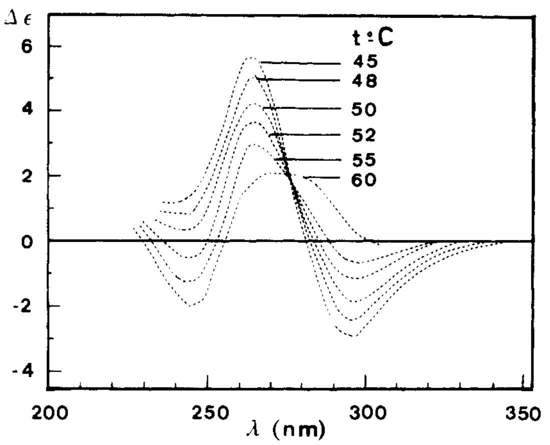

FIGURE 3: CD spectra of the thermal transition of the poly(dA-dC)·poly(dG-dT)–ZnN4 complex.

helix. The spectrum, however, does not show the small negative band at about 305 nm previously observed for an A form (Zimmer et al., 1982) and could rather correspond to the sum of spectra of an A form and a modified aggregated form.

Further addition of ZnN4 up to a concentration of 900 μM gave no change in the CD spectrum, but above this concentration a rapid and cooperative transition occurred, resulting in the original B-form CD spectrum, Figure 1B. The three transitions observed are more clearly visualized by the changes in molar ellipticity at constant wavelength as a function of ZnN4 concentration as shown in Figure 2.

The above titrations were carried out at 2 mM ionic strength, and when this was increased up to 10 mM with NaCl, the required concentration of ZnN4 for the midpoint of the first transition to the inverted spectrum increased by a factor of ca. 4. By contrast, solutions containing 20% ethylene glycol, also at 2 mM ionic strength, gave a midpoint transition of 18 μM ZnN4.

The CD spectra of poly(dA-dC)·poly(dG-dT), titrated to 90% of the first transition, as a function of temperature are shown in Figure 3. When the temperature was raised from 40 to 60 °C, the reverse transition toward the B form was observed. The intensity of the band at 295 nm was used to calculate the concentration of each species, and a van't Hoff plot of the transition is shown in Figure 4 from which a ΔH of 160 kJ/mol was found.

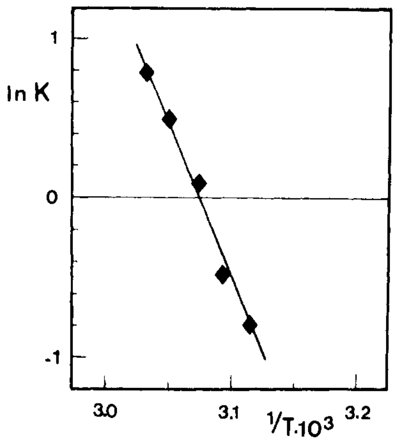

FIGURE 4: van't Hoff plot of the thermal transition in Figure 3.

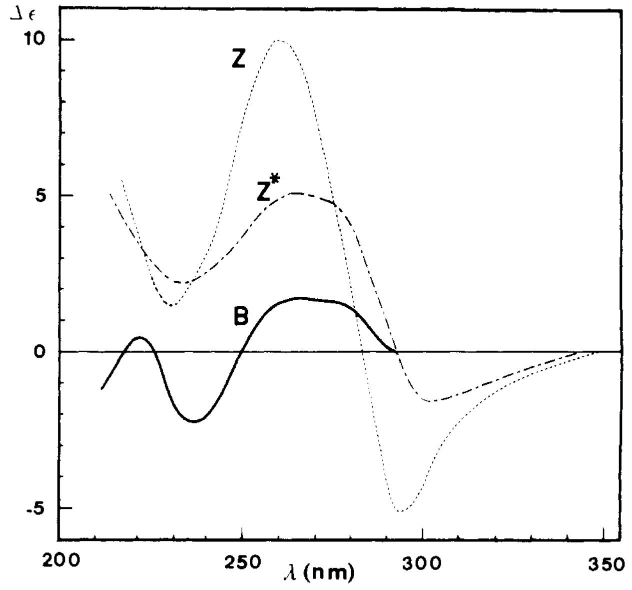

FIGURE 5: Limiting CD spectra of the transitions induced by ZnN4 and spermine in poly(dA-dC)·poly(dG-dT). Conditions: 0.05 mM nucleotide, 2 mM sodium cacodylate, pH 7.2 at 20 °C.

The results presented above obtained with poly(dA-dC)·poly(dG-dT) batch 719-97 from P-L Biochemicals were highly reproducible. However, as noted under Materials and Methods, results obtained with different batches were not. For comparison and also because yet another transition was observed, we also report results on batch 517-94 from P-L Biochemicals. For this second batch, the first transition to the inverted form required the presence of spermine. A titration with ZnN4 of poly(dA-dC)·poly(dG-dT) at 2 mM ionic strength and 4 μM spermine showed a transition midpoint to the inverted spectrum at 150 μM ZnN4. The limiting spectrum is identical with that observed in Figure 1A. Upon further addition of spermine and ZnN4, the positive band at 262 nm increases in intensity while the negative band remains unchanged. At 10 μM spermine and 325 μM ZnN4, the spectrum, Figure 5, has evolved toward the spectrum shown in Figure 1B of the form that displays only a positive band. However, this transition is only ca. 10% complete. Further addition of spermine and ZnN4 results in a highly cooperative transition to another form in which both the positive and negative bands have decreased in intensity and shifted to higher wavelengths, Figure 5. The limiting spectrum shown in Figure 5 was obtained at 12 μM spermine and 375 μM ZnN4. No

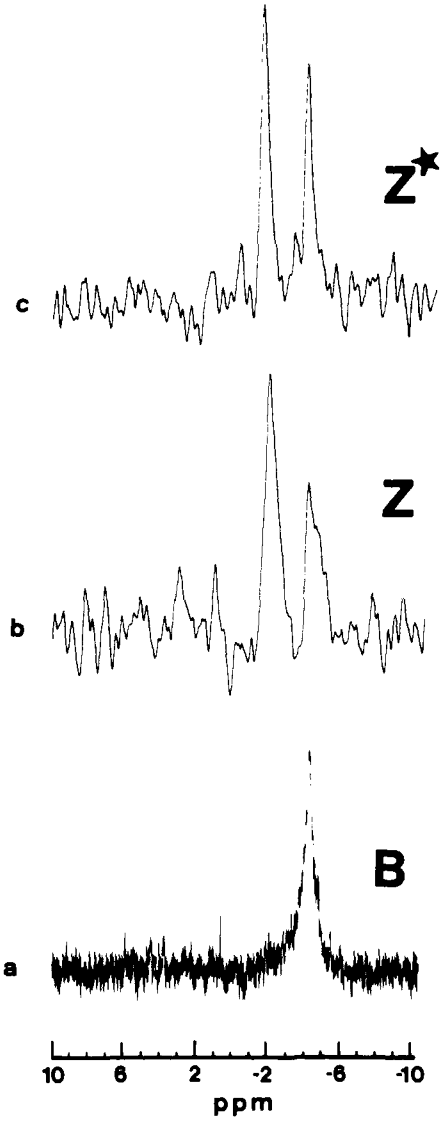

FIGURE 6: ^{31}P spectra recorded at 121 MHz of poly(dA-dC)·poly(dG-dT), batch 719-97. Conditions: 0.025 mM nucleotide, pH 7.2 at 20 °C. (a) Without ZnN4; (b) 70 μM ZnN4; (c) Polynucleotide batch 517-94, 120 μM ZnN4, and 4 μM spermine.

further change was observed in the CD spectrum upon further addition of spermine and ZnN4, although when these concentrations reached 50 μM and 1 mM, respectively, the DNA aggregated and precipitated from solution. This type of behavior was observed with different batches of poly(dA-dC)·poly(dG-dT) although the concentrations of ZnN4 and spermine required to reach the limiting spectra varied by a factor of 2.

The two inverted CD spectra shown in Figure 5 both display a tail toward 350 nm similar to that shown in Figure 1A before sonication. When the same procedure of sonication (as described above) at 60 °C was repeated with these samples, the tailing disappeared and no dichroism was observed above 315 nm. In both cases, the negative band was reduced in intensity by ca. 30%, but the positive band was unchanged. The UV spectra of these two forms are identical with that shown in Figure 1A.

Solutions of poly(dA-dC)·poly(dG-dT) in the presence of spermine and ZnN4 displaying the first inverted CD spectrum showed the reversed thermal stability relative to that shown in Figures 3 and 4. The transition back to the B form was observed upon cooling these solutions, and a van't Hoff plot (not shown) gave a ΔH of −71 kJ/mol.

Table I: Phosphorus Chemical Shifts of Poly(dA-dC)·Poly(dG-dT)[a]

concn of ZnN4 (μM)	shift, δ	$\Delta\delta$
0	−4.18	0^b
70	−2.00 −4.44	2.44^b
450	−3.8	0^b
120 + 4 μM spermine	−1.70 −4.14	2.44^c

[a] Solutions were 25 μM nucleotide and 2 mM sodium cacodylate, pH 7.2. Chemical shifts are reported relative to external trimethyl phosphate. [b] Batch 719-97 from P-L Biochemicals. [c] Batch 517-94 from P-L Biochemicals.

^{31}P NMR. While most of the limiting CD spectra reported above correspond well to known DNA conformations, alone they are not sufficient to unambiguously characterize the different conformations. We have therefore carried out ^{31}P NMR measurements on solutions displaying the different limiting spectra. As all of these forms aggregate in the presence of ZnN4, these spectra were recorded at very low DNA concentration, 25 μM in nucleotide.

In the absence of ZnN4, poly(dA-dC)·poly(dG-dT) shows a single resonance at −4.18 ppm, Figure 6a. We observed no splitting of the resonance such as has been observed for poly(dA-dT) (Cohen et al., 1981). This solution was titrated with ZnN4 to give the inverted CD spectrum shown in Figure 1A. The ^{31}P spectrum of the solution, Figure 6b, shows two resonances at −4.44 and −2.00 ppm. Both resonances are broad with the resonance at −4.44 ppm showing a line width ca. 50% greater than that of the resonance at −2.00 ppm. While accurate integration is obviously not possible, the two resonances are of similar area. Upon addition of ZnN4 to give a solution displaying the second limiting CD spectrum with only a positive band, Figure 1B, the ^{31}P spectrum (not shown) gave only a single resonance at −3.8 ppm.

We have also measured ^{31}P spectra of the batch 517-94 where the first inverted spectrum was obtained in the presence of 4 μM spermine. A solution giving the same inverted CD spectrum gave the same ^{31}P spectrum as in Figure 6b. The second limiting spectrum obtained in the presence of spermine, Figure 5, also gave two ^{31}P resonances as shown in Figure 6c. The resonances at −1.7 and −4.14 ppm are somewhat narrower than those of the first inverted form and are both shifted 0.3 ppm downfield relative to this form. The ^{31}P NMR results are summarized in Table I.

DISCUSSION

The CD spectra reported above show the existence of three forms of poly(dA-dC)·poly(dG-dT) other than that of the initial B form. The first limiting spectrum obtained, Figure 1A, is similar to that seen for the Z form of poly(dC-dG) (Pohl & Jovin, 1972) and poly(dA-dm^{5}C)·poly(dG-dT) (MacIntosh et al., 1983) at high salt and high temperature and reported for poly(dA-dC)·poly(dG-dT) (Jovin et al., 1983; Taboury & Taillandier, 1985) at high salt in the presence of ethanol of $NiCl_2$. In the latter case, the solution studies were limited to CD spectra.

The first limiting form gives a ^{31}P spectrum typical of DNA with a dinucleotide repeat unit in which the two phosphates are in quite different environments. The separation between the two resonances of ca. 2.4. ppm is somewhat greater than the 1.5 ppm observed for poly(dA-dm^{5}C)·poly(dG-dT) (MacIntosh et al., 1983), poly(dC-dG), or poly(dm^{5}C-dG) (Patel et al., 1982). The question arises as to whether this spectrum corresponds to a single species in solution or to an equilibrium between two species where exchange is slow on the ^{31}P NMR time scale. First, the CD spectrum is a limiting one. Figure

2 shows clearly a plateau region on further addition of ZnN4, and we have never observed a species giving greater negative ellipticity at 295 nm. Second, the transition to the species showing only a positive CD band accompanied by a collapse of two resonances gives a resonance not coincident with either of them. Thus, from the CD and NMR spectra we conclude that the first transition we observe is to a Z form of poly(dA-dC)·poly(dG-dT). As noted above, the inital rate of conversion is slow as would be expected for such a transition. The rate becomes much faster after the midpoint of transition is reached. This would indicate that the process is not "all or nothing" but that initially sections of polynucleotide adopt a Z form and that filling in the gaps is facilitated by regions already in the Z form.

The second limiting CD spectrum, shown in Figure 1B, is most probably that of an A-type helix, and the ^{31}P spectrum is in accord with this in that only a single resonance is observed. The ^{31}P spectrum of poly(rG-dC) (Wu & Behe, 1984) in the A form shows a single resonance at −4.1 ppm. Thus, as a function of ZnN4 concentration the CD and NMR spectra show the series of transitions B → Z → A* → B.

CD titrations reported above in the presence of spermine show first the same initial transition to a Z form. Following this, we observe the beginning to the A*-form spectrum. A pure A form is never reached, and the third transition yields a somewhat less convincing Z-type CD spectrum. However, the ^{31}P spectrum is unambiguously that of a Z form. The separation between the resonances remains constant, but both are shifted 0.3 ppm to low field. X-ray crystallographic studies on the left-handed form of d(CpG)$_3$ (Wang et al., 1979, 1981) showed two types of Z form, which were denoted Z^{I} and Z^{II}. The two conformations differed principally in the phosphate backbone conformation rather than in the base–base interactions. It is difficult to predict if a small change in the base–base interactions could produce the observed CD spectra. As we cannot correlate our results with those obtained in the crystal state, we denote the second left-handed form as Z*. The series of transitions observed as a function of the concentration of spermine and ZnN4 can be summarized as B → Z → (Z + A*) → Z*.

CONCLUSIONS

We have observed that under mild conditions poly(dA-dC)·poly(dG-dT) is able to adopt all the known principal DNA conformations, B, A, and two Z forms. We have previously suggested (Woisard et al., 1985) that ZnN4 induces the Z form in poly(dC-dG) by binding directly to the guanosine N^7 and that the crucial role of the amine ligand is to form hydrogen bonds to the same and to the adjacent residues. With poly(dA-dC)·poly(dG-dT), coordination to the N^7 position of both guanosine and adenosine may occur. While ZnN4 could only be described as a very simple model, it is interesting to note that the environment of the zinc, a nitrogen cage that retains the 2+ charge of the metal ion, is similar to that found in many zinc enzymes.

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Registry No. ZnN4, 45979-84-8; poly(dA-dC)·poly(dG-dT), 55684-99-6; spermine, 71-44-3.

REFERENCES

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

Brennan, R. G., & Sundaralingam, M. (1985) *J. Mol. Biol. 181*, 561–563.

Bustamante, C., Tinoco, I., & Maestre, M. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3568–3572.

Cohen, J. S., Wooten, J. B., & Chatterjee, C. L. (1981) *Biochemistry 20*, 3049–3055.

Fazakerley, G. V. (1984) *Nucleic Acids Res. 12*, 3643–3648.

Hakoshima, T., Wang, A. H., van Boom, J. H., & Rich, A. (1983) *Nucleic Acids Res. 12*, 213–219.

Haniford, D. B., & Pulleyblank, D. E. (1983) *Nature (London) 302*, 593–609.

Hayes, T. E., & Dixon, J. E. (1985) *J. Biol. Chem. 260*, 8145–8156.

Jovin, T. M., MacIntosh, L. P., 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*, 21–57.

MacIntosh, L. P., Greiger, I., Eckstein, F., Zarling, D. A., van de Sande, J. H., & Jovin, T. M. (1983) *Nature (London) 294*, 83–86.

Nordheim, A., & Rich, A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1821–1825.

Patel, D. J., Kozlowski, S. A., Nordheim, A., & Rich, A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1413–1417.

Patel, D. J., Kozlowski, S. A., Hare, D. R., Reid, B., Ikuta, S., Lander, N., & Itakura, K. (1985) *Biochemistry 24*, 926–935.

Pohl, F. M. (1976) *Nature (London) 260*, 365–366.

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

Russell, W. C., Precious, B., Martin, S. R., & Bayley, P. M. (1983) *EMBO J. 2*, 1647–1653.

Taboury, J. A., & Taillandier, E. (1985) *Nucleic Acids Res. 13*, 4469–4483.

Taillandier, E., Taboury, J. A., Adam, S., & Liquier, J. (1984) *Biochemistry 23*, 5703–5706.

van de Sande, J. H., MacIntosh, L. P., & Jovin, T. M. (1982) *EMBO J. 1*, 777–782.

Wang, A. H., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680–686.

Wang, A. H., Quigley, G. J., Kolpak, F. J., van der Marel, G., van Boom, J. H., & Rich, A. (1981) *Science (Washington, D.C.) 211*, 171–176.

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

Wells, R. D., Miglietta, J. J., Klysik, J., Larson, J. E., Stirdivant, S. M., & Zacharias, W. (1982) *J. Biol. Chem. 257*, 10166–10170.

Woisard, A., Fazakerley, G. V., & Guschlbauer, W. (1985) *J. Biomol. Struct. Dyn. 2*, 1205–1220.

Wu, H. Y., & Behe, M. J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7284–7287.

Zacharias, W., Larson, J. E., Klysik, J., Stirdivant, S. M., & Wells, R. D. (1982) *J. Biol. Chem. 257*, 2775–2782.

Zimmer, C., Tymen, S., Marck, C., & Guschlbauer, W. (1982) *Nucleic Acids Res. 10*, 1081–1091.