Volume 10 Number 11 1982 Nucleic Acids Research

Length changes in solution accompanying the B-Z transition of poly (dG-m^{5}dC) induced by $Co(NH_3)_6^{3+}$

Holly Ho Chen, Elliot Charney* and Donald C.Rau*

Department of Chemistry, George Mason University, 4400 University Drive, Fairfax, VA 22030, and *Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Building 2, Room B1-03, Bethesda, MD 20205, USA

Received 2 March 1982; Revised and Accepted 21 April 1982

ABSTRACT

Transient electric dichroism measurements have been used to observe the rotational relaxation times of 145 base pair fragments of poly (dGm^{5}dC) and random sequence DNA in solution. From these the lengths of the fragments are calculated and the interbase pair separation or rise per base pair (RPB) calculated. The observations show that even in low salt, the addition of very low concentrations of trivalent $Co(NH_3)_6^{3+}$ results in a transition of the dGm^{5}dC polymer from B-form to Z-form with a change in the RPB from 3.4 ± .06Å to 3.7 ± .06Å, the latter form defined by the criterion of an inverted circular dichroism spectra similar to that observed at high salt in the absence of $Co(NH_3)_6^{3+}$. The 145 base pair DNA and poly (dGm^{5}dC) are found to be essentially fully extended rods in low salt (0.2 - 2 mM Na^+) solutions.

INTRODUCTION

The view that base sequence dependent conformations of DNA are of biological significance in the cell is becoming increasingly plausible as more and more structures are studied (e.g. 1-4). The most drastic change observed thus far is the transition of poly (dG-dC) from a right handed B form helix to a left-handed structure designated the Z-form (5). In order to bridge the gap between solid state dimensions determined by X-ray diffraction and solution conformation, in this paper we examine the

0305-1048/82/1011-3561$2.00/0

change in length that accompanies the transition from B to Z forms. These lengths can be very accurately determined by measuring the rotational diffusion coefficients (which for rods are approximately dependent on L^3) obtained from the field free relaxation kinetics in a transient electric dichroism experiment.

There appear to be several basic methods for inducing the B-Z transition (3, 6, 7). The addition of Na^+ in molar concentration or of 60% ethanol is sufficient to affect the transition. Both of these procedures have drawbacks. It is not feasible to do transient electric dichroism at high salt concentrations and there is always the possibility the DNA conformations in nonaqueous solutions (i.e., with added ethanol) do not reflect aqueous solution structures. Alternatively, the observations of Behe and Felsenfeld (7) are particularly appealing for application to electric dichroism. They report that the methylated poly (dG-m^5dc) alternating copolymer is able to undergo the B-Z transition at low salt concentrations with very small amounts of added cobalt hexamine ($Co(NH_3)_6^{3+}$). Using better than 95% monodisperse 145 base pair poly (dG-m^5dC) derived from nucleosome trimming, we are able to obtain accurate diffusion coefficients in aqueous solution for Na^+ concentrations in the range 0.2 - 2 mM with up to 10 μM $Co(NH_3)_6^{3+}$ added to induce the Z-form. We find that values of the rise per base pair (RPB) observed in solution from the relaxation kinetics of the electric dichroism are in excellent agreement with the values obtained from X-ray diffraction. We find an RPB at 3.4 ± .06 A for the B-form structure; while the Z-form length is characterized by an RPB at 3.7 ± .06 A and is independent of the amount of added $Co(NH_3)_6^{3+}$.

EXPERIMENTAL

DNA: The 145 base pair sample of poly (dG-m^5dC) was a gift of Drs. J. Nickol and M. Behe. The sample was obtained by nuclease trimming of nucleosomes reconstituted with polydisperse synthetic poly(dG-m^5dC) as

described by Simpson and Kunzler for poly (dA-dT) (8). As judged by gel electrophoresis the sample is about 95% pure, with the remaining 5% contributed by 125 and 135 base pair fragments. The 145 base pair nucleosomal DNA was a gift of Dr. J. McGhee, obtained by nuclease trimming of bulk chicken erythrocyte chromatin. The samples were exhaustively dialyzed against pH 7.0 phosphate buffer ($[Na^+]$ = 1.5 mM) and stored in the cold. Samples for electric dichroism were prepared by diluting the poly (dG-m^5dC) or random sequence DNA stocks into appropriate phosphate buffer Na_2EDTA solution to arrive at the desired Na^+ concentration and 50 μM EDTA. Since such small amounts of +3 ions are needed to induce the transition at very low salt concentrations, it is necessary to keep EDTA in solution. In order to induce the Z form, $Co(NH_3)_6\ Cl_3$ (a gift of Jon Widom) is added and the sample heated to 50-60°C to ensure complete conversion.

Electric Dichroism:

The technique has been well described elsewhere (9). Basically, it involves using a square pulse electric field to orient the sample in a specially constructed cell. Changes in the absorbance of light polarized either parallel or perpendicular to the applied field are measured and can be related to the orientation of the chromophores, with respect to the electric axis of the molecule, and to the overall degree of orientation of the molecules. For the purposes of this paper, it is the decay of the induced dichroism signal that occurs after the field pulse that is the important observation. For a rigid elongated molecule, the dichroism relaxation curves is described by a single exponential kinetics, or $\rho(t) = \rho_0 e^{-t/\tau}$, where ρ is the reduced dichroism ($\Delta A_{\parallel}/A$, for light polarized parallel with the applied field) and τ is the relaxation time of the molecule which can be related to the rotational diffusion coefficent D_0, by $\tau = 1/6D_0$.

For data processing, the output signal from the photomultiplier which measures the dichroism decay is digitized by a Nicolet 1090 AR oscillo-

scope, with a 0.5 μsec/channel resolution interfaced with a Hewlett Packard 9825 minicomputer. In order to improve our signal to noise ratios, the data is signal averaged. Typically, 200 field pulses are summed to give a final curve. During the course of an experiment the magnitude of the dichroism is routinely monitored to ensure sample integrity.

For all experiments, the cell is thermostated at 2°C and DNA concentrations are between 15-45 μM in nucleotides.

RESULTS

Although a relation between high salt poly(dG-dC) and Z-DNA has been found by laser Ramamn studies (10), a firm correspondence between the appearance of an inverted CD spectrum (relative to the classical spectrum) and the left handed Z structure has not been established. It is an entirely reasonable inference and common practice, however, to use CD spectra to monitor the B-Z transition. It should be kept in mind that the rotational relaxation times we observe are technically only for two forms defined by the CD spectra and we cannot unequivocally determine that the Co^{3+} induced form is the left-handed Z form as defined by X-ray crystallography.

Typical CD spectra of poly (dG-m^5dC), at a low salt concentration (2 mM Na^+) with and without added $Co(NH_3)_6^{3+}$ are shown in figure 1. Both curves are in good agreement with the changes in CD spectra observed by others for the B to Z transition at higher salt concentrations and induced by other means (3, 6, 7). Using the change in the magnitude of the signal at 293 nm to monitor the extent of the transition, figure 2 shows the B-Z titration curve as a function of the cobalt hexamine concentration. At these low salt concentrations, the midpoint of the transition occurs at approximately 1 $Co(NH_3)_6^{3+}$/20-30 DNA-phosphates.

All relaxation measurements were taken within the Kerr region, e.g. at low enough field strengths (E) such that the observed dichroism, ρ, is linearly proportional to E^2. Within the Kerr region, we observe no dependence

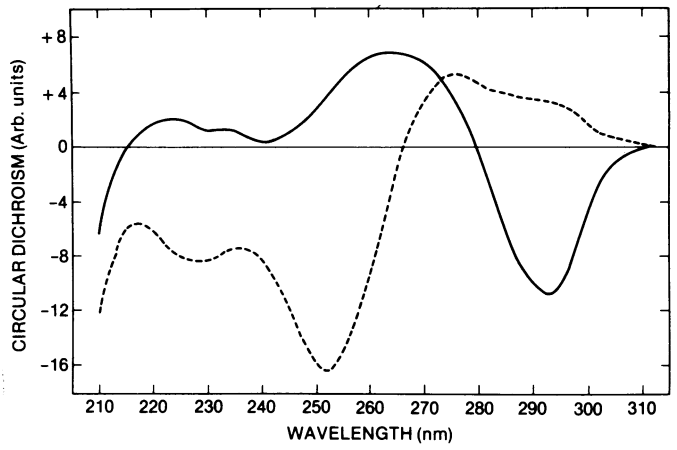

Figure 1 The circular dichroism spectra of the double helical complex of poly (dG-M^5dC) pH 7.0 phosphate buffer ([Na^+] = 1.5 mM in the presence (——) and absence (----) of $Co(NH_3)_6^{3+}$

of the dichroism decay kinetics on the applied field strength. Within the limits of our detection, the relaxation kientics for the B and Z forms of (145 bp poly (dG-m^5dC) and random sequence (145 bp DNA) can be well described by a

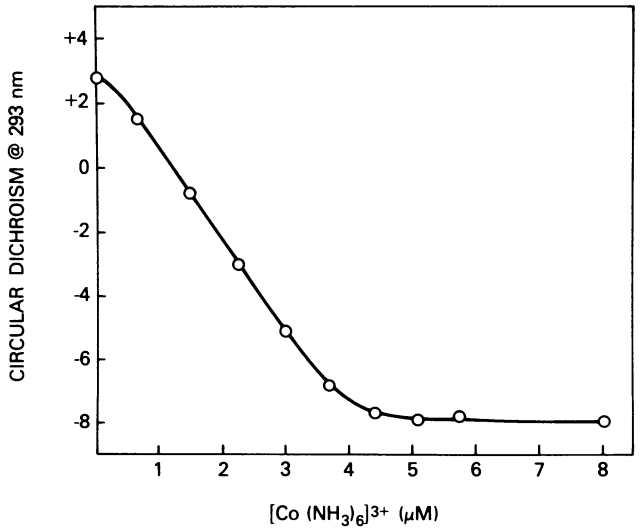

Figure 2 The titration of the B-Z transition of poly (dG-M^5dC) in varying concentrations of $Co(NH_3)_6^{3+}$

single exponential for at least the initial 95% of the decay. Figure 3 shows typical semilog decay plots for the three samples (not all points obtained in an experiment are shown). As can be observed, the relaxation of the 145 bp, B-form (-$Co(NH_3)_6^{3+}$) poly (dG-m^5dC) is within experimental error of the decay kinetics for the 145 bp, "random sequence" DNA. From the standpoint of overall length or, alternatively, the average rise per base pair, therefore, we find no sequence specific difference in the conformation of B form alternating poly (dG-m^5dC) and "random sequence" DNA. When cobalt hexamine is added, however, the relaxation time significantly increases, indicating a lengthening of the Gm^5C polymer. The relaxation time of the CD defined Z conformation is independent of the amount of $Co(NH_3)_6^{3+}$ added once the circular dichroism changes have begun plateauing and extending to $Co(NH_3)_6^{3+}$ concentrations just under those at which random sequence DNA collapses (about one $Co(NH_3)_6^{3+}$/3 DNA phosphates; cf. 11). The cobalt hexamine induced Z-conformation we observed is, therefore, a well defined structure

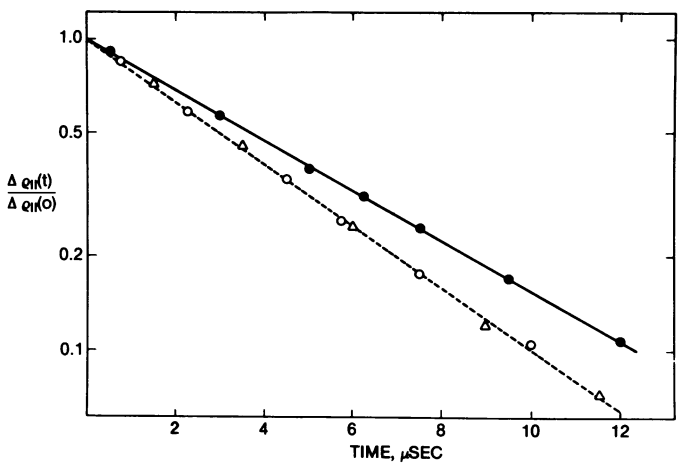

Figure 3 The orientation relaxation of 145 base pair fragments as measured by the decay of the parallel dichroism $\rho_{11}(\tau)$ plotted on a logarithmic scale. ● - poly (dG-M^5dC) with $Co(NH_3)_6^{3+}$; o - poly (dG-M^5dC) without $Co(NH_3)_6^{3+}$; Δ - random sequence DNA without $Co(NH_3)_6^{3+}$. All in pH = 7.0 phosphate buffer.

both hyprodynamically and by CD.

To within our experimental 5% error, the rotational diffusion coefficient of each sample is independent of polynucleotide concentration in the range 15-45 μM in base pairs. More importantly for calculating hydrodynamic lengths, relaxation times varied by less than 5% in decreasing the ionic strength from 2 mM to 0.2 mM. We infer from this that the B and Z forms of 145 base pair fragments of poly (dG-m^5dC) are, for our practical purposes, rodlike and fully extended.

DISCUSSION

Since, to within the limits of error of our apparatus, the decay kinetics of both the B and Z forms of the 145 bp poly (dG-m^5dC) are well described by a single exponential and since the relaxation times of each form are, to within 5%, independent of ionic strength in the range 0.2-2 mM, we will assume that the structures are rigid and maximally extended. The relaxation time of the exponential, τ, is related to the rotational diffusion coefficent, D_0, of the particle by, $\tau = 1/6D_0$. For rigid cylinders, D_0 is related to the structure's physical dimensions by (12)

$$1/D_0 = \frac{\pi \eta_0 L^3}{3kT} (\ln(L/2b) + \gamma_r)^{-1}$$

where

η_0 = solvent viscosity

L = length of the cylinder

b = hydrodynamic radius of the cylinder.

In terms of the dependence of D_0 on the change in the size of the structure, the L^3 factor dominates, making relaxation times obtained through dichroism a very sensitive function of the overall length. The term γ_r in parentheses is an end effect correction. There now exist three functional forms for γ_r that are extensively used. A theoretical treatment by Broersma (13)

has the form, $\gamma_r = 0.757-7(0.27-(\ln(L/b))^{-1})^2$. Tirado and Garcia de la Torre (14) have fit D_0 numerical results for cylinders built up with small spheres to give the approximation, $\gamma_r = -0.662 + 0.917(L/2b) - 0.050(L/2b)^2$. Finally, Mandelkern and Crothers (as quoted in reference 12) have fit empirical data obtained with macroscopic models to the form, $\gamma_r = .577 + 6.51(0.49 - (\ln(L/2b)^{-1})^2$. In the calculations that follow, these three forms lead to a range of lengths for each τ that differ by about 1-2%. Given an approximate error of 5% in relaxation times, however, this is all the accuracy we could expect anyway. Without additional experimental data for the translational diffusion coefficient, a value for the hydrodynamic diameter must be assumed in order to calculate the lengths. Fortunately, D_0 is rather insensitive to the choice of b and, furthermore we are interested mainly in a comparative length study of B and Z forms, not primarily in an absolute number for the rise per base pair. For the B-form DNA we assume the commonly used by hydrodynamic diameter of 26A (13), while, for the Z-form, since X-ray diffraction (4) indicates this structure is about 2A thinner, we assume a diameter of only 24A.

Table 1 gives the numerical results for both the average length and

TABLE 1

	B-form	Z-form
τ	4.3 ± 0.2 μsec.	5.4 ± 0.2 μsec.
RPB (this study)	3.4 ± 0.05 A	3.7 ± 0.05 A
RPB[a] (X-ray)	3.4	3.6 - 3.8

a) Values taken from the review by S. B. Zimmerman (1981), Annual Review of Biochemistry, in press.

average rise per base pair (RPB) calculated from our rotational diffusion coefficients of the B and Z forms, as well as recent values derived from X-ray diffraction on poly (dG-m^5dC) (15). Quite surprisingly, the RPB values calculated from our relaxation times are in good agreement with the reported X-ray diffraction numbers. Although it is generally expected that a DNA fragment 145 base pairs long would be at least, somewhat flexible, the absolute correspondence between X-ray and relaxation time RPBs could be interpreted as showing that both the 145 bp B and Z forms are maximally extended at an ionic strength of 0.2 mM. The absence of a significant ionic strength dependence of the relaxation time up to I = 2 mM is also consistent with a maximally extended rod conformation. A similar behavior has been observed by Elias and Eden (13) working with restriction fragments up to 124 bp. Whether this apparent breakdown of traditional wormlike chain statistics is due to increased electrostatic repulsions caused by end effects or not is not known. At an ionic strength of 1 mM the Debye shielding length is about 100A ($1/\kappa$), a value large enough to cause a significant decrease in the number of Manning condensed ions at the ends resulting in increased end-to-end repulsions.

Alternatively, it has not been conclusively shown that the additional drag due to the concurrent rotation of the ion atmosphere surrounding a polyelectrolyte will be negligible in comparison with the hydrodynamics of the rod itself. Although the data of Hagerman (16) would suggest this effect is unimportant, there is evidence that the contribution of ion atmosphere drag does make a measurable contribution to translational diffusion coefficients (17, 18). Regardless of the reason, both the 145 bp B and Z forms of poly (dG-m^5dC) appear to be fully extended. At the very least, we can conclude that the rise per base pair increases by 9% in going from B to Z form DNA. The strongest conclusion we can make is that the RPB increases from 3.4 ± .06A in the B form to 3.7 ± .06A in the Z form, in absolute accord with the X-ray data.

Recently, Wu et al. (19) reported very similar results for column fractionated poly (dG-dC) fragments with added alcohol. The data represented here augments the conclusions of their study. First, there is no appreciable difference in the apparent rise per base pair between B-form poly (dG-m^5dC) and random sequence DNA. Secondly, to the level of the apparent RPB, there is neither an appreciable difference between the Z-form structures of poly (dG-dC) and poly (dG-m^5dC) nor does the structure depend on whether the Z-form is induced by a high volume fraction of ethanol or by adding very small amounts of $Co(NH_3)_6^{3+}$ to aqueous solutions. In some respects, this last conclusion is somewhat surprising. In B-form DNA, the distance across the minor groove between the helical paths of phosphates on opposite strands is only about 15-20% shorter than the distance across the major groove, the phosphate to phosphate distances from one strand to another is much more unequal in Z-form DNA (cf. 4), with a closest P-P approach distance of only about 7A across the minor groove. In view of the very small amounts of added +2 and +3 counterions that are necessary to induce a Z-form conformation (20), one would suspect that counterion-phosphate bridging between these close phosphates is important in stabilizing the Z-form structure. It would appear, however, from the very similar results obtained with $Co(NH_3)_6^{3+}$ in these experiments and in the alcohol study (19) that if there is specific $Co(NH_3)_6^{3+}$-phosphate bridging, the equilibrium Z-form RPB is not influenced by this bridging.

REFERENCES

1. Leslie, A. G. W., Arnott, S., Chandrasekaran, R., Ratliff, R. L. 1980. J. Mol. Biol. 143, 49-72.
2. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K., Dickerson, R. E. 1980. Nature 287, 755-758.
3. Pohl, F. M., Jovin, T. M. 1972. J. Mol. Biol. 67, 375-396.
4. Wang, A. H.-J., Quigley, G. J., Klopak, F. J., Crawford, J. L, van Boom, J. H., van der Marel, G., Rich, A. 1979. Nature 282, 680-686.
5. Arnott, S., Chandrasekaran, R., Birdsall, D. L., Leslie, A. G. W., Ratliff, R. L. 1980. Nature 283, 743-745.

6. Pohl, F. M. 1976. Nature 260, 365-366.
7. Behe, M., Felsenfeld, G. 1981. Proc. Natl. Acad. Sci. U.S.A. 78, 1619-1623.
8. Simpson, R. T., Künzler, P. 1979. Nucleic Acids Res. 6, 1387-1415.
9. Fredericq, E., Houssier, C. 1973. "Electric Dichroism and Electric Birefringence", Clarendon Press, Oxford, England.
10. Thamann, T. J., Lord, R. C., Wang, A. H., Rich, A. 1981. Nucleic Acids Research 9, 5443-5457.
11. Widom, J., Baldwin, R. L. 1980. J. Mol. Biol. 144, 431-453.
12. De la Torre, J. G., Bloomfield, V. A. 1981. Quart. Reviews Biophys. 14, 81-140.
13. Elias, J. G., Eden, D. 1981. Biopolymers 20, 2369-2380.
14. Tirado, M. M., Garcia de la Torre, J. 1980. J. Chem. Phys. 73, 1986-1993.
15. Behe, M., Zimmerman, S., Felsenfeld, G. 1981. Nature 293, 233-235.
16. Hagerman, P. J. 1981. Biopolymers 20, 1503-1535.
17. Lin S. C., Lee, W. I. Schurr, J. M. 1978. Biopolymers 17, 1041-1064.
18. Varoqui, R., Schmitt, H. A. 1972. Biopolymers 11, 1119-1136.
19. Wu, H. M., Dattagupta, N., Crothers, D. M. 1981. Proc. Natl. Acad. Sci. U.S.A. 78, 6808-6811.
20. Chen, H. H., Szu, S. C., Behe, M., in preparation.