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Chain flexibility and hydrodynamics of the B and Z forms of poly(dG-dC).poly(dG-dC)

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ABSTRACT

The solution properties of the B and Z forms of poly(dG-dC).poly(dG-dC) have been measured by static and dynamic laser light scattering. The radius of gyration, persistence length, translational and segmental diffusion coefficients, and the Rouse-Zimm parameters have been evaluated. The persistence length of the Z form determined at 3 M NaCl is about 200 nm compared to 84 and 61 nm respectively for the B forms of poly(dG-dC).poly-(dG-dC), and calf thymus DNA, both determined at 0.1 M NaCl. The data on persistence length, diffusion coefficients and the Rouse-Zimm parameters indicate a large increase in the chain stiffness of Z DNA compared to the B form. These results are opposite to the ionic strength effects on random sequence native DNAs, for which the flexibility increases with ionic strength and levels off at about 1 M NaCl.

INTRODUCTION

The conformational transition in certain synthetic DNAs such as poly (dG-dC).poly(dG-dC) (1), poly(dG-me^{5}dC).poly(dG-me^{5}dC) (2) and poly(dA-dC).poly(dG-dT) (3) from the right-handed B to the left handed Z helical form in the presence of salts (4), alcohols (5,6), complexes (2,7), and polyamines (2) has invoked much interest in recent years (8-11). Single crystal (12-15) and fiber diffraction (16) patterns of oligomers and polymers containing the dG-dC sequences have shown the existence of left handed helices in the solid state. NMR (17) and Raman (18) spectra of poly(dG-dC).poly-(dG-dC) in high salt solutions reveal the same in solutions. The B to Z transition in poly(dG-dC).poly(dG-dC) and its methylated analog is accompanied by dramatic changes in physicochemical and biological properties. X-ray crystallographic (12-15) and NMR (19,20) studies show that the helical repeating unit in the Z form is two instead of one as in the B form. The Z form is thinner and more extended with a rise per base residue of 3.7 A compared to 3.4 A for the B form (8,21,22). The Z form DNA does not intercalate ethidium bromide (23) and it does not form chromatin (24) in the presence of histones. Recent theoretical studies on the molecular-mechanical

properties (25,26) of B and Z DNAs indicate that the B form is much more flexible than the Z form. The solution properties (23,24) of the Z form are consistent with this. However, no quantitative measurment of the chain flexibility of Z form DNA has been reported so far.

Laser light scattering provides an excellent tool to study the static and dynamic properties of biological macromolecules in solution (27). Ionic effects on the chain flexibility and hydrodynamic properties of calf thymus and Col E_1 DNAs have been studied by total intensity and quasielastic laser light scattering (28-32). At high ionic strengths in NaCl or LiCl, these DNAs contract in size with an increase in the translational and segmental diffusion coefficients (28-29) and a decrease in the effective Stokes' radius, radius of gyration and persistence length (27-32). These data as well as the internal motion parameters determined by the Rouse-Zimm theory (33-35) indicate an increase in the flexibility of natural DNAs at high ionic strengths (30,32). Calf thymus DNA has been shown to undergo the B to C transition under these conditions (36,37) whereas poly(dG-dC).poly(dG-dC) undergoes the B to Z transition. In contrast, theoretical calculations and observed reactivities of Z DNA show it to be less flexible than B DNA. Therefore, in this work, we evaluate the flexibilities of B and Z forms of poly(dG-dC).poly(dG-dC) and compare them with B form calf thymus DNA.

MATERIALS AND METHODS

Calf thymus DNA was obtained from Miles Laboratories as a salt free white powder, certified to contain 0.3% protein and 0.36% RNA. It was dissolved in a buffer containing 0.1 M NaCl, 10 mM sodium phosphate, pH 7.0 (0.1 M NaCl buffer) and sonicated at 0-5°C for 30 min. with a Branson sonifier. The sonicated DNA was fractionated through a column of Sepharose 2B under the same buffer conditions. The fraction used for light scattering studies was found to have absorbance ratios of $A_{260/280}$ = 1.82 and $A_{260/230}$ =2.1, melting temperature of 85°C (in 0.1 M NaCl) and about 40% increase in 260 nm absorbance on melting, showing that the DNA has very low protein contamination (38) and exists in the double stranded form (39). Poly(dG-dC).poly(dG-dC) was from P.L. Biochemicals. It was dissolved in 0.1 M NaCl buffer and fractionated through a Sepharose 2B column. Two peak fractions of poly(dG-dC).poly(dG-dC) were combined and used for light scattering experiments.

Solutions for light scattering experiments were made by diluting stock solutions (~ 0.25 mg/mL) to concentrations of 0.01 to 0.1mg/mL with 0.1 M NaCl buffer or 3M NaCl solution, as needed for the B and Z forms. The NaCl

solution for the Z form was prepared by diluting a 5 M solution filtered through 0.45 μ m type HA Millipore filters. The concentration of the filtered solution was determined by measuring the refractive index using an American Optical Corp. digital Abbe Refractometer. All diluted solutions were kept for about 16 hours at 4°C before using for light scattering measurements. Samples were clarified by filtering through 0.45μm Millipore type HA filters, followed by centrifuging at 2800 g for 2-3 hours. Concentrations of the filtered solutions were determined by measuring absorbance at 260 nm in a Beckman DU-8 spectrophotometer. The B and Z conformations of poly(dG-dC).poly(dG-dC) at 0.1 and 3 M NaCl solutions were ascertained by measuring CD spectra in a Jasco J-41C spectropolarimeter.

Light Scattering Aparatus

Detailed descriptions of the light scattering apparatus have been given previously (40-42). Basically, the set-up consists of a Lexel Model 95 Argon ion laser operating at 4880 A, a light scattering cell (10x75mm borosilicate culture tube) contained in a thermostatted bath of refractive index matching fluid (Dow Corning 550 Fluid), an ITT FW-130 photon counting photomultiplier mounted on a goniometer arm, a Products for Research FW 130/159 preamplifier-amplifier-discriminator unit and a Langley-Ford Model 1096 Correlator. Data were acquired and analysed with a Digital Minc-23 minicomputer.

Molecular Weight and Radius of Gyration

Measurements of the excess intensity of light scattered by macromolecular solutions provide information about the weight average molecular weight M and the radius of gyration R_g through the relations (43):

$$\frac{Kc}{R} = \frac{B(dn/dc)^2}{I-I_B} = \frac{1}{M_{app}}$$

$$\frac{Kc}{R} = \frac{1}{M}\left(1+\frac{16\pi^2}{3\lambda^2} R_g^2 \sin^2(\theta/2)\right)$$

where I and I_B are the scattering intensities of the DNA solution and buffer, K is an optical constant, R is the Rayleigh ratio, c is the weight concentration, dn/dc is the refractive index increment, B is an instrument constant, M_{app} is the apparent molecular weight, λ is the wavelength of light and θ is the scattering angle. The instrument constant B has been determined by using a standard solution, 3 ml of Sequanal grade benzene (Pierce), filtered with a 50 nm Millipore filter and sealed in a cell (40). A value for dn/dc of 0.171 was taken for the 0.1 M NaCl solutions of calf thymus DNA and poly

(dG-dC).poly(dG-dC) and 0.152 for the poly(dG-dC).poly(dG-dC) solution in 3 M NaCl (32). Intensities of the scattered light were determined from solutions of DNAs and buffers at angles varying from 30-120°. The M_{app}^{-1}(=Kc/R) values were computed at different angles and extrapolated to $\theta = 0$ and $c = 0$ by Zimm plots. The molecular weight M and radius of gyration R_g were determined from the intercept and slope of the angular dependence of Kc/R.

Diffusion Coefficients and Effective Hydrodynamics Radii

The apparent diffusion coefficients D_{app} and the effective Stokes' radii R_h of the DNAs were determined by quasielastic laser light scattering (QLS) (44,45). The autocorrelation functions were analyzed according to Koppel's comulant method (46) and the effective hydrodynamic radius was calculated from the Stokes-Einstein equation:

$$R_h = kT/6\pi\eta D$$

where η is the viscosity of the solution.

RESULTS

Molecular Weight, Radius of Gyration and Persistence Length

Figure 1 shows the Zimm plots for sonicated calf thymus DNA and poly(dG-dC).poly(dG-dC) in the B and Z forms. The molecular weights and radii of gyration determined from these plots are presented in Table 1. The Kratky-Porod persistence length is related to the radius of gyration by the following equation (39):

$$\langle R_g^2 \rangle = 2a\mathcal{L}\left[\frac{1}{6} - \frac{a}{2\mathcal{L}} + \frac{a^2}{\mathcal{L}^2} - \frac{a^3}{\mathcal{L}^3}(1-e^{-\mathcal{L}/a})\right]$$

where $\mathcal{L}$ is the contour length and a is the persistence length. In the case of high molecular weight DNA, where $\mathcal{L} \gg a$, the radius of gyration is related to the mean square end to end distance by $\langle R_g^2 \rangle = \langle L^2 \rangle/6$ and $\langle L^2 \rangle = 2a\mathcal{L}$. Thus, from a knowledge of the molecular weight and radius of gyration, the persistence length can be calculated. Values are given in Table I.

The persistence length calculated from the radius of gyration and the contour length (39) does not take into consideration the intra-molecular excluded volume of the polymer chains. A more realistic value of the persistence length (a_ϵ) is calculated by applying the excluded volume (ϵ) correction (47):

$$\langle R_g^2 \rangle = \frac{N_o^{\epsilon-1}}{(\epsilon+2)(\epsilon+3)}\left[1 - \frac{\epsilon+3}{2N(\epsilon+1)}\right]$$

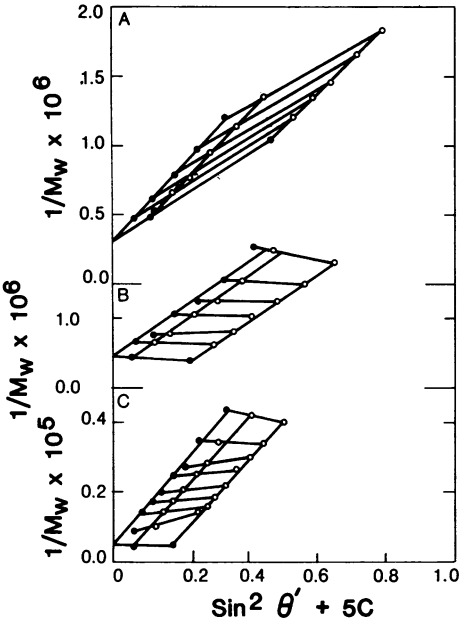

Fig. 1. Zimm plots for calf thymus DNA (A), Poly(dG-dC).Poly(dG-dC) B form (B) and Poly(dG-dC).Poly (dG-dC) Z Form (C).

where $N = \mathcal{L}/2a$. From ζ, a is calculated using the relation, $b_{app} = b_o \times N^{\varepsilon/2}$, where b(=2a) is the statistical segment length (39). For making the excluded volume correction, values of ε were taken as 0.112 and 0.062 at 0.1 and 3 M NaCl. These values of ε calculated from viscosity (48) and light scattering measurements (31) are in satisfactory agreement with other values in the literature (49,50). The values of a_o calculated with the excluded volume correction are also given in Table I.

Diffusion coefficients, Stokes' Radius and Rouse-Zimm Parameters

Figure 2 shows the angular dependence of D_{app} for the three DNA

TABLE I. Molecular Weight, Radius of Gyration and Persistent Length

Solution Properties	Calf Thumus	Poly(dG-dC).Poly(dG-dC)	
Conformation (CD)	B	B	Z
NaCl Conc., M	0.1	0.1	3.0
Molecular Weight (M)	3.5×10^6	2.2×10^6	2.35×10^6
Radius of Gyration (R_g), nm	207	188	305
Persistence Length (a), nm	71.2	93.8	208
Corrected value of a (a_o), nm	61.3	83.7	201

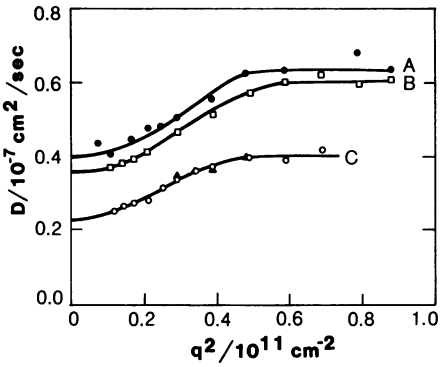

Fig. 2. Angular dependence of the apparent diffusion coefficient for calf thymus DNA (A), Poly(dG-dC). Poly(dG-dC) B form (B) and Poly (dG-dC).Poly(dG-dC) Z Form (C). Concentration of DNA is 0.025 mg/mL. Additional data in C (▲) is concentration of 0.075 mg/mL for Z form. Diffusion coefficients are not corrected for solvent viscosity. $\theta'=\theta/2$.

samples. For most of the QLS experiments, the DNA concentration is about 0.025 mg/mL. An increase in DNA concentration to 0.075 mg/mL does not change the diffusion coefficients as seen in Fig. 2. The apparent diffusion coefficient plotted against q^2 ($=4\pi n/\lambda \sin^2(\theta/2)$) shows two plateau regions in agreement with the work of Parthasarathy et al. (29). The extrapolated value of D_{app} at $q^2= 0$ yields the translational diffusion coefficient D_t and the plateau value of D_{app} at higher values of q^2 is related to the segmental diffusion coefficient, D_s (29,30). The midpoint of the transition between D_t and D_s, q_m^2 is related to the distance b between the Rouse-Zimm beads (33-35):

$$b^2 = 8/q_m^2$$

The Einstein relaxation time τ_E for the free draining Rouse-Zimm model is:

$$\tau_E = b^2/2D_s$$

The number of beads 'n' comprising the random-walk molecule is related to the radius of gyration R_g and b by the relation:

$$R_g^2 = n\, b^2/6$$

The values of D_t, D_s, q_m^2, b, τ_E, and n are presented in Table II.

DISCUSSION

The data presented in Tables I and II, obtained from static and dynamic light scattering experiments, clearly show that the chain stiffness or rigidity of calf thymus DNA is markedly less than that of the Z form of poly(dG-dC).poly(dG-dC). The stiffness of the B form of poly(dG-dC).poly(dG-dC) falls between these extremes. Detailed studies of the solution properties of random sequence, native DNAs have been reported in the literature (28-32,51-53). However, we are not aware of any report on the persistence length, diffusion coefficient or Rouse-Zimm parameters of

TABLE II. Hydrodynamic Properties and Rouse-Zimm Parameters

	Calf Thymus DNA	Poly(dG-dC).Poly(dG-dC)	
		B Form	Z Form
Translational diffusion coefficient*(D_{t-20-w}), $cm^2/sec.$	4.0×10^{-8}	3.5×10^{-8}	3.1×10^{-8}
Effective Stokes' radius, nm	54.0	61.0	70.0
Segmental diffusion coefficient (D_s), $cm^2/sec.$	6.3×10^{-8}	6.1×10^{-8}	5.8×10^{-8}
q_m^2, cm^{-2}	3.2×10^{10}	3.5×10^{10}	2.5×10^{10}
$b(=8/q_m^2)$, nm	158	151	178
$\tau_E(=b^2/2D_s)$, msec.	1.98	1.84	2.71
$n(=6\langle R_g^2\rangle/b^2)$	10.3	9.3	17.6

*The diffusion coefficients have been corrected to standard conditions (20 C water) using a value of 1.46 cp for the viscosity of 3 M NaCl solution.

poly(dG-dC).poly(dG-dC) in the B or Z form.

It has been demonstrated that the persistence length of DNA is independent of molecular weight (51); but the values reported in the literature vary widely from 27 to 350 nm for B form DNA (39,52). Recent determinations show that the persistence length of Col E_1 DNA in 0.2 NaCl is about 50 nm without the excluded volume correction (31,32). The excluded volume correction may contribute to a decrease of about 13 nm from this value. Borochov et al. (31) have also shown that the persistence length of Col E_1 DNA ($M=4.35 \times 10^6$) decreases from 68 nm at 0.005 M NaCl to 40 nm in 0.2M NaCl and levels off at about 27 nm in high salt (1-4 M NaCl) solutions (all these values are corrected for excluded volume). The value of a_o determined in this work for calf thymus DNA at 0.1 M NaCl is 61 nm compared to the most recent value from Eisenberg's group of 54 $\pm$ 6 nm for calf thymus DNA (51) and 40 nm for Col E_1 DNA (31,32). Most of the earlier values determined from light scattering measurements fall at 50 $\pm$ 10 nm (51-53) and those determined by other methods fall at 60 $\pm$ 10 nm (51,54). Our value of a for calf thymus DNA is on the higher side and this may be due to the polydispersity of the sample. However, this value should suffice for a comparative evaluation of a for calf thymus DNA and poly(dG-dC).poly(dG-dC).

The decrease in the chain stiffness with increasing ionic strength is

caused by electrostatic repulsions between the phosphate negative charges. As the ionic strength is increased, the phosphate charges are effectively screened and the DNA becomes more flexible. We have found that the ionic strength effect on the chain flexibility of poly(dG-dC).poly(dG-dC), as measured by the persistence length, is opposite to that on Col E_1 DNA, because of the formation of the Z form at high salt.

The persistence length of the Z form (3 M NaCl) is more than double that of the B form. Some difference is expected, though the magnitude is surprising, since the poly(dG-dC).poly(dG-dC) in the Z form is a left handed helix (8-17) with physico-chemical and biological properties different from the right handed B form. The Z form DNA has been described as a thinner, grooveless, rigid structure when compared to the B form (8,10). The Z form bases are sheared together with a mean propeller twist of 0-7° (15,21,25). On the other hand, the B form is characterized by an average base twist of about 17° from the perpendicular plane (15,21,25). The Z form does not have the flexibility to wrap around histones to form chromatin (24) and it fails to react with DNAse I and II (55). NMR studies reveal the Z form to be more stable than the B form at high temperatures (20). Nuclease digestion surface adsorbed Z form shows cuts every 13.6 base pairs whereas the B form is cut at 10.6 base pairs (55). It reacts with micrococcal nuclease (55), N-acetoxy -2-aminofluorene (56) and certain antibodies (57) more slowly than does the B form. Antibodies specific to the Z form have been isolated and characterized (58,59). There is also a dramatic decrease in the hydrogen exchange rate in the Z form (60). All these properties indicate a structurally distinct, more rigid structure in Z DNA compared to the B term. This is quantitatively confirmed by our measurements of the persistence length of these two forms.

Data presented in Table I show that poly(dG-dC).poly(dG-dC), even in the B form (0.1 M NaCl), is more rigid than calf thymus DNA under the same conditions. It has long been recognized that poly(dG-dC).poly(dG-dC) differs in a number of properties from other synthetic and native DNAs (61-64). Theoretical calculations on the potential energy and steric accessibility show that the amino group of guanine has a decisive role in the reactivities of poly(dG-dC) (65). The propeller twisting of the low salt form of poly(dG-dC)$^-$.poly(dG-dC) lies between those for calf thymus DNA and the Z form (25). NMR (20) and CD (66) measurements at high temperatures show that poly(dG-dC).poly (dG-dC) in the B form (low ionic strength) has a tendency to change to the Z form at about 70°C. The decrease in the propeller twisting is at the expense of stacking interactions for hydrogen bonding, thus stabilizing the base

pairing but at the same time decreasing the flexibility of the chain. These considerations suggest that poly(dG-dC).poly(dG-dC) in the low salt form is more rigid than calf thymus DNA under the same conditions. Our measurement of persistence length confirms this quantitatively.

Calf thymus DNA undergoes a conformational transition to a C form at high salt concentrations (36,37). Parthasarathy et al. (29) have shown that this transition is accompanied by an increase in the translational diffusion coefficient and a decrease in the Stokes' radius. For the B to Z transition of poly(dG-dC).poly(dG-dC), we find the opposite result. The translational diffusion coefficient of a wormlike chain is given by the equation (67):

$$D_t = \frac{M_L RT}{3\pi\eta_o NM}\left(C_1 \ln\left(\frac{L}{d}\right) + C_2 + C_3 L + D_4 L^2 + C_5 L^3\right)$$

where M_L is the mass per unit length and N is Avogadro's number. L and d are the length and hydrated diameter of DNA expressed in terms of the persistence length. The values of the constants C_1-C_5 are slightly dependent upon d. Theoretical values of D_t for the B and Z forms of poly (dG-dC).poly(dG-dC) with the molecular weights used in this study are 3.0×10^{-8} and 2.4×10^{-8} cm^2/sec, compared to the experimental values of 3.5×10^{-8} and 3.1×10^{-8} cm^2/sec. The experimentally determined diffusion coefficients are slightly lower than the theoretical values perhaps due to z-averaging in a polydisperse solution, but the relative magnitude of the decrease in D_t with increase in a is consistent with the theoretical prediction. The translational diffusion coefficient of calf thymus DNA determined in this work is about 4.0×10^{-8} cm^2/sec, compared to $3.8 \times 10^{-8} cm^2/sec$ extrapolated from the molecular weight dependence of D_t reported by Chen et al. (68)

The Rouse-Zimm parameters b, D_s and τ_E provide information about segmental motions in the polymer chain. Recently, Schmitz (28) and Parthasarathy et al. (29,30) have shown that for calf thymus DNA the values of D_s and q_m^2 increase with ionic strength whereas b and τ_E decrease. The data in Table II show the opposite trend. Wu et al. (21) studied the rotational relaxation times (τ_r) of poly(dG-dC).poly(dG-dC) in the A, B and Z forms and showed that τ_r increases in the B to Z transition but τ_r decreases in the B to C transition. We find that the Einstein relaxation time (τ_E) increases in B to Z transition. An increase in the values of b and τ_E again shows that the flexibility of DNA decreases. The conformational perturbations in the low salt form of poly(dG-dC).poly(dG-dC)

are not sufficient to cause drastic changes in the Rouse-Zimm parameters; hence, the values for B form calf thymus DNA and B form poly(dG-dC).-poly(dG-dC) are similar.

In conclusion, this study clearly shows that the flexibility of poly-(dG-dC).poly(dG-dC) decreases at high ionic strength, a trend opposite to that reported for native DNAs, because of the formation of Z DNA. In this study, we have compared the B and Z forms under different ionic conditions. In work now under way, we are studying other polynucleotides in which the B-Z transition can by provoked by addition of multivalent cations while maintaining essentially constant ionic strength.

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