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Toroidal Condensation of Z DNA and Identification of an Intermediate in the B to Z Transition of Poly(dG-m^{5}dC)·Poly(dG-m^{5}dC)†

T. J. Thomas‡ and Victor A. Bloomfield*

Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108
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ABSTRACT: Using a combination of spectroscopic techniques, quasi-elastic laser light scattering (QLS), and electron microscopy (EM), we have been able to show that the B to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is accompanied by extensive condensation of the DNA in both low and high ionic strength buffers. At low concentrations of NaCl (2 mM Na^+), an intermediate rodlike form, which exhibits a circular dichroism (CD) spectrum characteristic of an equimolar mixture of B and Z forms, is observed. This is produced by the orderly self-association of about four molecules of the polymer after prolonged incubation of a concentrated solution at 4 °C. On addition of 5 μM $Co(NH_3)_6^{3+}$, the CD spectrum of the intermediate changes to that of the Z form, which is visualized as a dense population of discrete toroids on an EM grid stained with uranyl acetate. On the other hand, addition of NaCl to a solution of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in the absence of any multivalent ion condenses the polymer to toroidal structures at the midpoint (0.75 M NaCl) of the B to Z transition. Further addition of NaCl unfolds these toroids to rodlike structures, which show characteristic Z-form CD spectra. These results show that Z DNA can take up a variety of tertiary structural forms and indicate that its inverted CD spectrum is due to its left-handed helical sense rather than to differential scattering artifacts.

Alternating purine–pyrimidine sequences of the type poly(dG-dC)·poly(dG-dC) and its substituted derivatives are known to undergo a transition from a right-handed B to a left-handed Z conformation under a variety of conditions (Pohl & Jovin, 1972; Wang et al., 1981; Zacharias et al., 1982; Dickerson et al., 1982; Behe & Felsenfeld, 1981; Revet et al., 1983; Moller et al., 1984). Various spectral studies have confirmed the essential identity of the structures observed in poly(dG-dC)·poly(dG-dC) at high salt (Thamann et al., 1981; Patel et al., 1979) and in fibers (Arnott et al., 1980) as well as in single crystals of alternating deoxyguanosine–deoxycytidine oligonucleotides (Wang et al., 1979; Crawford et al., 1980; Drew et al., 1980). The inversion of the circular dichroism (CD) spectrum accompanying the B to Z transition is similar for poly(dG-dC)·poly(dG-dC), poly(dG-m^{5}dC)·poly(dG-m^{5}dC), and other substituted derivatives. Recently, there has been considerable interest in the B to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (Zimmerman, 1982; Rich et al., 1984) since the methylation of DNA is known to play an important role in gene regulation (Doerfler, 1983) and since the B to Z transition of this polymer occurs at physiological or lower salt conditions, especially in the presence of the ubiquitous cellular components, the polyamines (Behe & Felsenfeld, 1981). Additionally, C-5-methylation of cytosine has been shown to stabilize the Z form, perhaps by increased hydrophobic interactions (Fujii et al., 1982).

A variety of trivalent cations that effect the B to Z transition in poly(dG-m^{5}dC)·poly(dG-m^{5}dC) are known to induce the collapse of native DNA's into compact structural forms that are visualized by EM as toroids, folded fiber rods, and spheroids (Gosule & Schellman, 1976, 1978; Chattoraj et al., 1978; Eikbush & Moudrianakis, 1978; Widom & Baldwin, 1980; Allison et al., 1981). Even monovalent cations can cause extensive association or condensation of Z DNA (Castleman & Erlanger, 1983; Revet et al., 1983). The toroidal condensation of DNA by polyamines and other cations is an in vitro model system to study the energetic and kinetic factors involved in the organization of DNA in virus capsids, since toruslike

† This work was supported by NIH Research Grant GM 28093.
‡ Present address: Section of Rheumatology, Department of Medicine, University of Minnesota, Minneapolis, MN 55455.

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structures have been observed in electron micrographs of disrupted λ (Chattoraj et al., 1978) and T4 (Earnshaw et al., 1978) bacteriophages and herpes simplex virus (Furlong et al., 1972). Considerable structural detail in spermidine-condensed toroids of calf thymus DNA has been elucidated by Marx & Ruben (1981). Wilson & Bloomfield (1979) showed that DNA collapsed into compact structural forms when 89–90% of the phosphate charge was neutralized, as calculated by Manning's (1978) counterion condensation theory, regardless of the ionic conditions. This theory treats ions as point particles; hence, any trivalent cation should have the same effect. However, extensive studies on DNA condensation using spermidine^{3+}, $Co(NH_3)_6^{3+}$ (Widom & Baldwin, 1979, 1983a; Thomas & Bloomfield, 1983a), and a series of spermidine homologues (Allison et al., 1981; Schellman & Parthasarathy, 1984) demonstrated definite structural specificity in the condensation of DNA. Similar cation-specific effects were observed in the B to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) by a series of divalent cations, spermidine, and $Co(NH_3)_6^{3+}$ (Behe & Felsenfeld, 1981) and by N^1- and N^8-acetylspermidines (Thomas et al., 1984). Both the B to Z transition and the condensation of DNA are prevented by intercalating drugs like ethidium bromide (Pohl et al., 1972; Widom & Baldwin, 1983b). Although similar effects may have different causes (e.g., preferential interactions, structural perturbations), these analogies in the two cation-induced structural transitions prompted us to search for a unifying mechanism in the B to Z transition and condensation of DNA.

The B to Z transition has been studied mainly by spectroscopic techniques that are insensitive to tertiary structural modifications of DNA. Native DNA's give typical B-form CD spectra in both extended wormlike and condensed toroidal forms (Gosule & Schellman, 1978). The possibility has been raised (Tinoco, 1980; Bustamante et al., 1983; Castleman & Erlanger, 1983) that tertiary structural modifications, particularly if accompanied by aggregation, may contribute to substantial changes in the CD spectrum through differential scattering of polarized light. While significant differential scattering effects have not been observed in most of the CD spectral changes associated with the B–Z transition, this possibility must be borne in mind. Perhaps more fundamentally, the inability of current theory to predict or rationalize the CD spectra of the various forms of double-helical DNA suggests that CD not be relied on as the sole tool for investigating the B—Z transition. It should be viewed as a technique complementary to hydrodynamics and electron microscopy (EM) in studying condensed structures and conformational transitions.

Experimental Procedures

Materials. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was purchased from P-L Biochemicals. It was dissolved in a buffer containing 100 mM NaCl and 1 mM sodium cacodylate, pH 7.0, to a concentration of about 1 mg/mL. This stock solution was diluted in appropriate buffers for the CD, QLS, and EM experiments. All solutions were filtered through 0.45-μm type HA Millipore filters before use. $Co(NH_3)_6^{3+}$ was a gift from Prof. Robert Bryant of the Chemistry Department, University of Minnesota. All other chemicals were reagent grade.

Methods. CD spectra were recorded on a Jasco J-41 spectropolarimeter. Concentrations of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) were determined by absorbance measurements (A_{260}) in a Beckman DU-8 spectrophotometer.

Quasi-elastic laser light scattering (QLS) and static light scattering experiments were done in an instrument described earlier (Thomas & Bloomfield, 1983b) with an Ar^+ laser operated at a wavelength, λ, of 4880 Å. In the static light scattering mode, the weight-average molecular weight, M_w, was determined by measuring the excess intensity of light scattered by the polymer solution over that of the solvent and by constructing Zimm plots (Pletcher et al., 1980). In the QLS mode, the apparent diffusion coefficients, D_{app}, were determined at various scattering angles, θ, in the range 30–135° from Koppel's (1972) cumulant analysis of the autocorrelation function. The heterogeneity of the sample was assessed by the polydispersity parameter μ_2/Γ^2, where Γ and μ_2 are the first and second cumulants. Γ is equal to $D_{app}q^2$, where the scattering vector $q = (4\pi n/\lambda) \sin(\theta/2)$; n is the solution refractive index. For relatively small and symmetrical particles, it may be shown that $M_w/M_n \approx 1 + \mu_2/\Gamma^2$ (Kubota & Chu, 1983). For such particles, e.g., condensed DNA that is spheroidal or toroidal in shape, D_{app} is independent of scattering angle and equals the translational diffusion coefficient D_T. However, for larger particles like uncondensed DNA, the autocorrelation function also contains information on the segmental or rotational motions of the chain. These additional motions contribute to an angular dependence of D_{app}, which then extrapolates to D_T at $q = 0$. For random coils, this has been interpreted (Schurr, 1977) as corresponding to translation at low q and segmental diffusion at high q. For rodlike or elongated particles, an angular dependence of D_{app} reflects the contribution of rotational or flexural modes at high angle. In this work, we monitored the collapse of DNA from extended wormlike coils to toroidal condensates; hence, all D_{app} values were extrapolated to $q = 0$ to obtain D_T. The hydrodynamic radius R_h was calculated from the Stokes–Einstein equation $R_h = kT/(6\pi\eta D_T)$, where η is the solvent viscosity. All QLS experiments were repeated 4–6 times; the standard deviation in D_{app} was ±2%.

Electron microscopy was done on a Hitachi H 6010 instrument. Carbon-coated copper grids were glow discharged for 1–2 min, dipped in absolute alcohol for 1 s, and dried in air. A drop of the poly(dG-m^{5}dC)·poly(dG-m^{5}dC) solution with the appropriate concentration of NaCl or $Co(NH_3)_6^{3+}$ was placed on the grid and allowed to dry in air for 30 min. A second drop of the solution was applied after blotting the first drop with filter paper. This process was repeated 4–6 times, after which a drop of 1% uranyl acetate (pH 5.1) was placed on the grid and blotted with a filter paper after 10 s. The grid was then dried in air for EM examination.

Sedimentation coefficients were measured in a Beckman Model E analytical ultracentrifuge equipped with photoelectric scanner and electronic speed control. The solutions used for these measurements had $A_{260} \approx 0.6$.

Results

Figure 1 shows the CD spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under different ionic and storage conditions. At 100 mM NaCl, the spectrum is characteristic of the B form. However, on incubation of a concentrated solution of this polymer at low ionic strength at 4 °C, the B form is converted to an intermediate form with a CD spectrum characteristic of an equilibrium mixture of B and Z forms, as typically seen at the midpoint of the B to Z transition (Pohl & Jovin, 1972). From this intermediate, either the B or the Z form could be generated by the addition of NaCl or $Co(NH_3)_6^{3+}$, respectively. We first observed this behavior with a solution of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) that had been stored for 4 months at 4 °C in low salt, as detailed in the legend to Figure 1. In later experiments we found that the intermediate form could be produced by dialyzing a concentrated solution ($A_{260} \approx 2$) for a few days against a buffer containing 1 mM NaCl

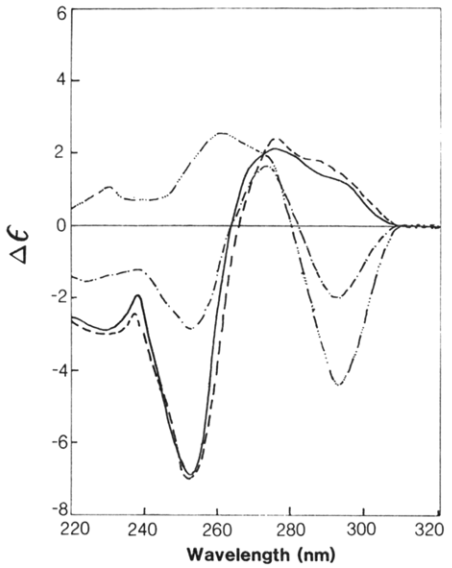

FIGURE 1: CD spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under different ionic and storage conditions: (A) (—) 100 mM NaCl, recorded 24 h after preparing solution; (B) (–·–) 1 mM NaCl + 1 mM sodium cacodylate kept at 4 °C at a polymer concentration of 125 μg/mL, CD spectrum recorded 4 months after preparing the solution; (C) (–···–) 5 μM $Co(NH_3)_6^{3+}$ added to (B) at a concentration of 35 μg/mL; (D) (– –) (B) diluted to 35 μg/mL and made to 100 mM NaCl by adding a small volume of concentrated NaCl solution. All solutions were filtered through 0.45-μm type HA Millipore filters. All CD spectra except (A) were recorded at a DNA concentration of 35 μg/mL at pH 7.0, 30 min after dilution with buffer. These solutions were subsequently used in QLS, sedimentation, and EM studies.

and 1 mM sodium cacodylate at pH 7. However, in dilute solutions ($A_{260} \approx 0.2$), the change in CD spectrum proceeded completely to the Z form. In the B to Z transition, A_{260}/A_{295} decreased from 4.2 to 2.6, with about 3.5 for the intermediate.

We monitored the storage stability of poly(dG-Gm^{5}dC)·poly(dG-m^{5}dC) by measuring the absorption spectra and diffusion coefficients of the B-form solutions A and D described in the legend to Figure 1. Solution D, which had been stored for 4 months, had a slightly lower D_T than did solution A (5.2×10^{-8} cm^0/s compared with 5.8×10^{-8} cm^2/s), indicating the presence of some residual aggregation. There was no indication of degradation of the polymer after extended storage. Both of these solutions showed some angular dependence of D_{app}, as expected for large wormlike coils.

We repeated these low-salt experiments in the presence of 0.15 mM ethylenediaminetetraacetic acid (EDTA) and found that the CD spectrum remained unchanged in the B form after prolonged incubation at 4 °C. Thus, the B to intermediate and B to Z transitions reported here are apparently caused by traces of multivalent ions associated with the polymer. This accords with the recent observation by Chen et al. (1984) that as little as one $Co(NH_3)_6^{3+}$ or spermine^{4+} bound per 40–50 nucleotides can cause the B to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) at low ionic strength. At higher polymer concentrations, the transition equilibrated at an intermediate stage, presumably due to the additional Na^+ associated with the polymer, which makes the total effective Na^+ concentration significantly higher than that in the buffer alone or in a solution with a low concentration of polymer. Na^+ is an effective competitor of multivalent cations both in the B to Z transition (Behe & Felsenfeld, 1981) and in condensation (Wilson & Bloomfield, 1979) of DNA.

Under the low-salt conditions (and in the absence of EDTA) in which we observed the intermediate form, Mandelkern et al. (1981) reported the association of mononucleosomal DNA fragments into bundles. We used light scattering and electron microscopy to search for the presence of such an ordered self-association of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) during the B to Z transition. Figure 2 shows electron micrographs of the B, intermediate, and Z forms. Since we used a high concentration (35 μg/mL) for these EM studies, the B form in panel A shows entangled chains of the polymer. The intermediate form in panel B shows no such entanglements, but instead, a number of rods, toroids, and spheres are observed. The Z form in panel C, produced by the additional of 5 μM $Co(NH_3)_6^{3+}$, shows a dense population of toroids only.

It is of interest to compare these EM results with those determined in solution by QLS, given in Table I. For B-form

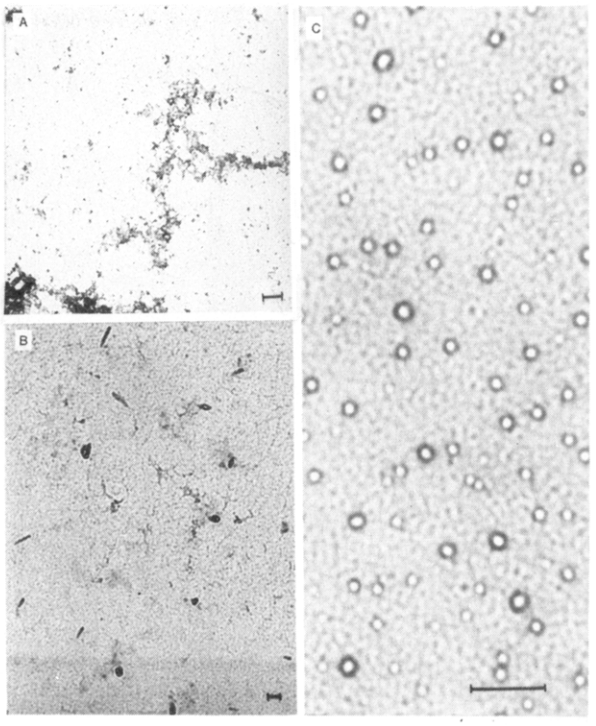

FIGURE 2: Electron micrographs of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in B-form (A), intermediate-form (B), and Z-form (C) conformations prepared from solutions whose CD spectra are shown in Figure 1. Grids for EM were prepared by staining the solutions with 1% uranyl acetate. Micrographs were taken with a Hitachi H 6010 instrument. Bar represents 100 nm.

Table I: Hydrodynamic Parameters and Molecular Weights of B, Intermediate, and Z Forms[a] of Poly(dG-m^{5}dC)·Poly(dG-m^{5}dC)

	B form	intermediate form	Z form
ionic conditions	100 mM NaCl	1 mM NaCl + 1 mM sodium cacodylate	1 mM NaCl + 1 mM sodium cacodylate
M_r	9×10^{5b}	(3.8 ± 0.5) $\times 10^{6c}$	2×10^{6d}
solution structure[d]	entangled chains	rods, toroids, etc.	toroids
D_T (10^{-8} cm^2/s)	5.8[e]	1.8	5.2
hydrodynamic radius (Å)	430	1200	410
μ_2/Γ^2	0.2	0.15	0.1

[a] All conformational assignments are made on the basis of CD spectra (Figure 1). [b] From sedimentation coefficient. [c] From sedimentation coefficient and Zimm plot of total light scattering intensity. [d] From electron micrographs. [e] D_{app} extrapolated to $q = 0$; see text for details.

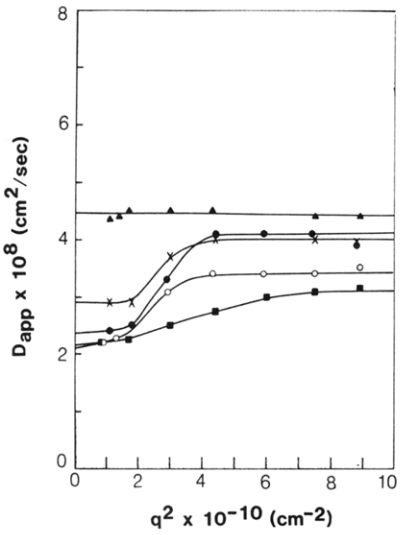

FIGURE 3: Angular dependence of the apparent diffusion coefficients of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) at 0.1 (●), 0.5 (×), 0.75 (▲), 1.0 (■), and 2.5 (○) M NaCl. The polymer, obtained from P-L Biochemicals ($s_{20,w}$ = 8.6 S), was extensively dialyzed from a buffer of 10 mM NaCl and 1 mM sodium cacodylate, pH 7.0, and filtered through 0.45-μm type HA Millipore filters. Small amounts of 5 M NaCl were added to 1-mL volumes of the polymer (concentration 7.5×10^{-5} M phosphate) to give the desired NaCl concentrations.

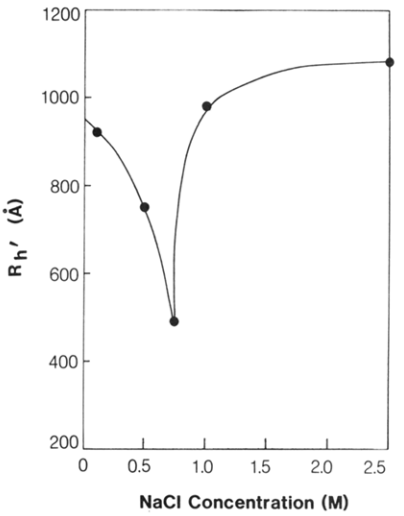

FIGURE 4: Effect of NaCl on the hydrodynamic radius of poly(dG-m^{5}dC)·poly(dG-m^{5}dC). The midpoint of the B to Z transition, determined from CD spectra, is 0.75 M NaCl.

polymer, the value of D_T is in the range expected for wormlike coil DNA of this molecular weight. The intermediate form has a lower D_T value and a higher molecular weight, consistent with limited association into particles containing about four monomer chains on the average. The Z form has a higher D_T value, consistent with formation of compact toroids. However, the hydrodynamic radius, 410 Å, is considerably larger than the 100–150 Å measured from EM in panel C. As described under Methods, cumulant analysis of QLS data allows assessment of polydispersity. The values of μ_2/Γ^2 are all fairly low, indicating only moderate heterogeneity of these preparations and decrease in passing from the B through the intermediate to the Z form. This is consistent with the electron micrographs in Figure 2. Although we attempted to decrease the polydispersity of the B form by passing it over a Sepharose 4B column, no decrease in μ_2/Γ^2 was observed for the peak fractions.

Since the inversion of the CD spectrum of poly(dG-dC)·poly(dG-dC), the first indication of a change in handedness of the double helix, was first observed at high NaCl concentrations (Pohl & Jovin, 1972) and since the B to Z transition is still studied by many laboratories under high-salt conditions, we conducted EM and QLS experiments on poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in a region of high ionic strength that can provoke the transition in the absence of multivalent cations. The angular dependence of D_{app} over a range of [NaCl] from 0.1 to 2.5 M is shown in Figure 3. These solutions had a QLS polydispersity index μ_2/Γ^2 of 0.15–0.25. The q independence at 0.75 M NaCl (the midpoint of the B to Z transition according to CD measurements), compared with the variation with q at higher and lower salt, suggests that the polymer is spherical and/or small compared to $1/q$ at the midpoint of the transition, while it has an extended conformation on either side of the transition. This is borne out by the hydrodynamic radius of the polymer, calculated from D_T, as a function of [NaCl] shown in Figure 4. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) clearly undergoes a collapse at the midpoint of the B to Z transition. D_T at this point is $(4.4 \pm 0.2) \times 10^{-8}$ cm^2/s, a value typically found for spermidine-collapsed DNA (Thomas & Bloomfield, 1983a).

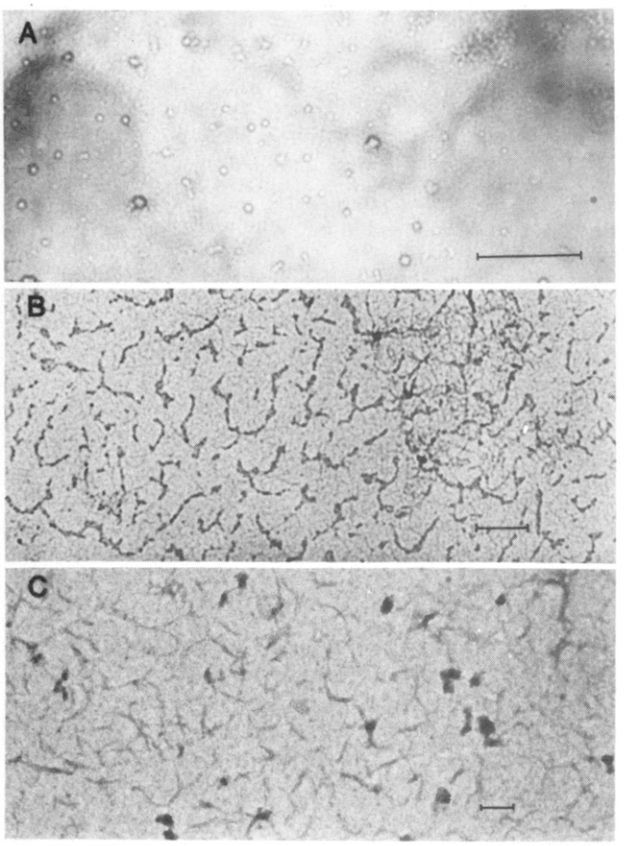

FIGURE 5: Electron micrographs of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) at 0.75 (A), 1.0 (B), and 2.5 (C) M NaCl, taken with a Hitachi H 6010 microscope after being stained with 1% uranyl acetate. The bar is 100 nm.

The structure of these solutions were examined by EM, with results shown in Figure 5. At 0.1 M NaCl, the micrograph of the B form is essentially identical with that in Figure 2A and is not repeated here. However, at 0.75 M NaCl (the midpoint of the transition) the structures (Figure 5A) are small, discrete toroids consistent with the QLS data. The CD spectrum at the salt concentration is similar to that of the intermediate form shown in Figure 1. At 1 M NaCl, the CD spectrum changes to that of a typical Z form, while EM (Figure 5B) shows a population of flexible rods, such as might be produced by unfolding and subsequent limited associaton

of the toroids. These rods become thicker at higher salt concentration (Figure 5C).

Discussion

We have characterized the solution of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in three conformational states, B, Z, and an intermediate form, identified by CD spectra. The existence of an intermediate with extensively reduced rms dimensions was recently suggested theoretically (Olson et al., 1983). Zacharias et al. (1983) have shown that a condensed intermediate form with a CD spectrum resembling that of ψ DNA (Jordan et al., 1972) is an intermediate in the B to Z transition of poly(dG-dC)·poly(dG-dC) induced by sodium acetate. However, the ψ-form structure was found after the midpoint of the B to Z transition. The results found in this study identify an intermediate at the midpoint of the transition under two quite different ionic conditions.

In the first case, incubation of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in low salt produces an intermediate structure that appears in EM mainly as a rodlike form with other less regular structures, perhaps including toroids, as minor species. Molecular weight and hydrodynamic properties of this intermediate (Table I) indicate that it is formed by association of about three to four chains of poly(dG-m^{5}dC)·poly(dG-m^{5}dC). The rods have a length of 2400 ± 250 Å and a width of 240 ± 50 Å. These dimensions are comparable to those reported by Eikbush & Moudrianakis (1978) for compacted native DNA in the B form. They are consistent with an orderly assembly of three to four chains, folded back every 2500 Å. On addition of 5 μM $Co(NH_3)_6^{3+}$ to this rodlike phase, a remarkably dense population of small toroids is generated. The hydrodynamic radii of these toroids determined by QLS, about 400 Å, is quite close to those reported for $Co(NH_3)_6^{3+}$–condensed λ (Widom & Baldwin, 1983a) and T4 (Thomas & Bloomfield, 1983a) phage DNA's. However, the toroidal structures observed on the EM grids are significantly smaller, ranging from 100- to 150-Å radius. This discrepancy could be due to contraction of the toroids during grid preparation involving dehydration protocols. Under similar conditions, biological structures are known to shrink by as much as 20–50% (Boyde et al., 1977). Fulmer & Bloomfield (1982) also found that the lengths and dimensions of chromatin fragments were reduced by approximately 30% when EM data were compared with solution dimensions. A typical toroid shown in Figure 2 has a circumference of about 570 Å and radius of 34 Å, corresponding to a M_r of about 2×10^6 if a density of 1.7 g/cm^3 is assumed, and is sufficient to accomodate two chains of poly(dG-m^{5}dC)·poly(dG-m^{5}dC). This is in agreement with the suggestion by Schellman & Parthasarathy (1984) that two or more molecules of DNA participate in toroid formation when the M_r is less than a critical value somewhere between 7×10^6 and 14×10^6.

The EM's of Z-DNA toroids presented in Figure 2 differ in two important respects from those of B-DNA condensates reported earlier. First, previous studies on the compaction of native B-form DNA's (Chattoraj et al., 1978; Widom & Baldwin, 1980; Eikbush & Moudrianakis, 1978) reported toroids and a variety of other structures, including toroidal aggregates, in contrast to the large and pure population of discrete toroids reported here. Second, in most published condensation experiments, the DNA concentration was kept below 1 μg/mL. At higher DNA concentrations, extensive aggregation was generally seen (Chattoraj et al., 1978; Wilson & Bloomfield, 1979). In this study, a poly(dG-m^{5}dC)·poly(dG-m^{5}dC) concentration of 40 μg/mL was used. The difference in the experimental procedure is that we used a stock solution, produced after prolonged low-temperature incubation, that had a CD spectrum of the intermediate form. This intermediate form appears to act as a nucleation site for the simultaneous transition to Z form and the collapse of rods into toroids under the influence of $Co(NH_3)_6^{3+}$.

Events are somewhat different in high salt. In contrast to the rods and other irregular structures observed with the intermediate form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in low salt, we find toroidal structures at the midpoint of the B to Z transition in 0.75 M NaCl. The hydrodynamic radius is a minimum at this salt concentration, and D_{app} is essentially independent of q, indicating a highly condensed structure. The solution dimensions and EM appearance of these toroids are similar to those formed with 5 μM $Co(NH_3)_6^{3+}$ in low salt, and there is the same discrepancy between the radii determined by QLS and EM, suggesting shrinkage of the toroids in uranyl acetate stained preparations. Again in contrast to the low-salt behavior, when [NaCl] is increased above 0.75 M, the toroidal intermediates appear to unfold into rods, which have typical Z-form CD spectra. The lengths of these rods are typically 2400 Å, which, when compared with the anticipated DNA duplex length of 5040 Å for a M_r of 9×10^5, suggests that the chain is folded back once or twice. These dimensions are similar to those of the intermediate-form rods observed in low salt and are consistent with the Z-form rods observed by Castleman & Erlanger (1983) with poly(dG-dC)·poly(dG-dC) at 4 M NaCl.

A significant result of this study is the finding of toroidal structures in poly(dG-m^{5}dC)·poly(dG-m^{5}dC) solutions containing only NaCl, when the polymer is at the midpoint of the B to Z transition (Figure 5). This is another of an increasing number of findings that association of Z DNA into organized structures can take place in the absence of multivalent cations. High salt has been shown to induce parallel association of the Z form of poly(dG-dC)·poly(dG-dC) into a structure with a few filaments (Revet et al, 1983), and heating of poly(dG-dC)·poly(dG-dC) in 2.5 M NaCl to 60 °C for 15 min, followed by slow cooling to room temperature, led to the appearance of toroidal structures that were apparently in the Z conformation (Castleman & Erlanger, 1983). From previous work, the conditions under which toroidal condensates were identified as a stable form of B DNA (Chattoraj et al., 1978; Eikbush & Moudrianakis, 1978; Wilson & Bloomfield, 1979; Widom & Baldwin, 1980; Marx & Ruben, 1983, 1984) all seemed to involve trivalent cations in aqueous solution. Only in aqueous methanol solutions were divalent cations sufficient. The requirement for trivalent cations in aqueous solution was empirically correlated (Wilson & Bloomfield, 1979) with partial neutralization of the linear charge density of DNA as calculated by counterion condensation theory (Manning, 1978). It appeared that 89–90% of the charge had to be neutralized before condensation could occur, though later experiments with $Co(NH_3)_6^{3+}$ showed that ca. 85% could suffice (Widom & Baldwin, 1983a; Thomas & Bloomfield, 1983a). With monovalent cations and B DNA, charge neutralization is calculated to be only 76%; with Z DNA it is only 74%.

Condensation presumably occurs when a balance among electrostatic, solvation, chain stiffness, van der Waals, and ligand bridging forces is achieved (Riemer & Bloomfield, 1978; Rau et al., 1984; Schellman & Parthsarathy, 1984). Since polyelectrolyte repulsion is greater with lower charge neutralization and chain stiffness increases as B DNA converts to Z DNA, at least for poly(dG-dC)·poly(dG-dC) (Thomas & Bloomfield, 1983b), the formation of toroidal condensates

at 0.75 M NaCl must imply considerably enhanced attractive forces between double-helical segments in Z-form or intermediate-form DNA relative to B DNA. This is consistent with the observations in several laboratories that Z DNA has a strong tendency to associate or precipitate (Jovin et al., 1983; Revet et al., 1983; Shin & Eichhorn, 1984). As the NaCl concentration is raised above 0.75 M, the balance of forces is changed, and stiff rods containing several Z DNA molecules become the dominant structures. It may be that trivalent cations are required to stabilize the toroidal form outside the transition region.

Our results and those of others show that DNA, under conditions where it exhibits Z-form spectra, can display substantial structural polymorphism, being found as toroids, bundled rods, and wormlike chains. It thus behaves like B-form DNA, which preserves its characteristic spectral properties upon condensation from a wormlike chain to a toroid. This independence of spectral measures of secondary structure from the state of folding or association of the polymer argues strongly that the inversion of the CD spectrum accompanying the B to Z transition reflects a change in handedness of the double helix, rather than differential scattering artifacts associated with extensive orderly packing, as suggested by Castleman & Erlanger (1983).

We have also shown that poly(dG-m^{5}dC)·poly(dG-m^{5}dC) can undergo a conformational transition from B form to Z form that involves a rodlike or toroidal intermediate, depending on the salt concentration. These intermediates have structures very similar to those observed by Eikbush & Moudrainakis (1978) with native DNA. The toroids, whether they exhibit B-form or Z-form CD spectra, are hydrodynamically virtually identical. But, the compaction of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is accompanied by a reversal in handedness of the chain.

Registry No. Poly(dG-m^{5}dC), 51853-63-5.

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Nucleotide Sequence of a cDNA Clone Encoding Mouse Protamine 1†

Kenneth C. Kleene, Robert J. Distel, and Norman B. Hecht*

Department of Biology, Tufts University, Medford, Massachusetts 02155
Received July 5, 1984

ABSTRACT: The nucleotide sequence of a 404-base cDNA encoding the cysteine-rich, tyrosine-containing mouse protamine has been determined. This insert, isolated from a mouse testis cDNA library, encodes a polypeptide of 50 amino acids of which 28 are arginine, 9 are cysteine, and 3 are tyrosine. The insert contains the complete 3′ noncoding region of 151 bases and most of the 5′ noncoding region. The predicted amino acid sequence of mouse protamine 1 is about 80% homologous to boar protamine and 67% homologous to bull protamine and contains the central, highly basic domain of four arginine clusters found in the trout protamines. The identification of a cDNA clone for a mouse protamine will facilitate studies of the evolution, regulation, and protein–DNA interaction of this nuclear protein unique to haploid spermatogenic cells.

During spermatogenesis in vertebrates, the histones on chromatin are replaced by protamines, basic proteins 27–65 amino acids long containing 40–70% arginine and relatively little lysine (Coelingh et al., 1972; Bellvé, 1979; Bellvé & Carraway, 1978; Kistler et al., 1976; Tobita et al., 1983; Gaastra et al., 1978; Nakano et al., 1976; Sautière et al., 1981). This change in chromatin, occurring late in the haploid phase of sperm differentiation, produces a DNA–protamine complex that is extremely compact, devoid of nucleosomal structure, and inactive in RNA synthesis (Kierszenbaum & Tres, 1975). The protamines of placental mammals (Coelingh et al., 1972; Bellvé, 1979; Bellvé & Carraway, 1978; Kistler et al., 1976; Tobita et al., 1983; Gaastra et al., 1978) are distinct from those of fish and birds (Nakano et al., 1976; Sautière et al., 1981) because they contain 8–18% cysteine, which serves to further condense and stabilize the mammalian sperm nucleus by forming disulfide bridges. Although the expression and evolution of the protamine gene family in the trout have been extensively studied by Dixon and his colleagues (Iatrou & Dixon, 1977; Aiken et al., 1983), studies of the protamines in mammals have been restricted to the synthesis, structure, and function of these unique proteins (Coelingh et al., 1972; Bellvé, 1979; Bellvé & Carraway, 1978; Kistler et al., 1976; Tobita et al., 1983; Gaastra et al., 1978; Rodman et al., 1984; Bellvé et al., 1975; Goldberg et al., 1977; Mayer et al., 1981; Balhorn et al., 1984).

We have previously isolated a cDNA clone from a mouse testis cDNA library and shown by RNA blots that it is complementary to a small, highly abundant, testis-specific mRNA (Kleene et al., 1983). This mRNA is translationally regulated: it is first present at high levels in round spermatids but is first translated in elongating spermatids (Kleene et al., 1983, 1984), the stage when protamines are synthesized in mice (Bellvé et al., 1975; Goldberg et al., 1977; Mayer et al., 1977; Balhorn et al., 1984). The properties of this mRNA led us to suspect that it encoded a protamine. This suspicion has been confirmed in the present study by DNA sequencing, which demonstrates that this cDNA clone (pMP1-1) encodes mouse protamine 1, the cysteine-rich, tyrosine-containing protamine.

Materials and Methods

Clone pMP1-1 was isolated from a mouse testis cDNA library in pUC8 by colony hybridization using a nick-translated insert from the plasmid pIC3 as a probe (Kleene et al., 1983, 1984). Plasmid pIC3 was 1 of 17 of 750 clones from a testis cDNA library that hybridized much more strongly to ^{32}P-labeled cDNA copied from cytoplasmic poly(A+) RNA of round spermatids than pachytene spermatocytes (Kleene et al., 1983). The nucleotide sequence of the cDNA inserts was determined on both strands by the method of Maxam & Gilbert (1978). The 3′ and 5′ termini of restriction fragments were labeled by using the Klenow fragment of *Escherichia coli* DNA polymerase I and T4 polynucleotide kinase, respectively (Maxam & Gilbert, 1978). In some cases, the complementary strands were separated by denaturation with methylmercury and electrophoresis on 5–8% acrylamide gels (Schwarzbauer et al., 1983).

Results and Discussion

The nucleotide sequence of both strands of the 404-base insert of pMP1-1 was determined by using the strategy depicted in Figure 1. There was complete agreement in the sequences determined from each strand. The first initiation codon (AUG) is located 78 bases from the 5′ end of pMP1-1 and appears in the context of a sequence (GCACCAUGG) that closely matches the consensus sequence (CC$^{A}_{G}$CCAUGG) for initiation of protein synthesis in eukaryotic cells (Kozak, 1984). The amino acid sequence deduced from the following nucleotide sequence encodes a polypeptide of 50 amino acids

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