DNA Length and Concentration Dependencies of Anisotropic Phase Transitions of DNA Solutions

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ABSTRACT Critical concentrations for the isotropic to cholesteric phase transitions of double-stranded DNA fragments in simple buffered saline (0.1 M NaCl) solutions were determined as a function of DNA contour length ranging from ~50 nm to 2700 nm, by solid-state ^{31}P NMR spectroscopy and polarized light microscopy. As expected for semirigid chains, the critical concentrations decrease sharply with increasing DNA length near the persistence length in the range from 50 to 110 nm, and approach a plateau when the contour length exceeds 190 nm. The biphasic region is substantially wider than observed for xanthan, another semirigid polyelectrolyte approximately twice as stiff as DNA, primarily because of low critical concentrations for first appearance of the anisotropic phase, C_i^*, in DNA samples ≥110 nm (320 base pairs) long. The limiting C_i^* for DNA ≥490 nm long is exceptionally low (only 13 mg/ml) and is substantially lower than the C_i^* of ~40 mg/ml reported for the stiffer xanthan polyelectrolyte. The much higher values of the critical concentrations, C_a^*, for the disappearance of the isotropic DNA phase (≥67 mg/ml) are modestly higher than those observed for xanthan and are predicted reasonably well by a theory that has been applied to other semirigid polymers, if a DNA persistence length in the consensus range of 50–100 nm is assumed. By contrast, the broad biphasic region and low C_i^* values of DNA fragments ≥190 nm long could only be reconciled with theory by assuming persistence lengths of 200–400 nm. The latter discrepancies are presumed to reflect some combination of deficiencies in current theory as applied to chiral, strong polyelectrolytes such as DNA, and sequence-dependent variations in DNA properties such as flexibility, curvature, or interaction potential. The propensity of DNA to spontaneously self-order at low concentrations well in the physiological range may have biological significance.

INTRODUCTION

Cellular DNA contains all of the information required for an organism to function; hence these chromosome-spanning molecules achieve formidable sizes. DNA must be packaged to be secluded from ordinary cell activities while remaining accessible for transcription or replication. Efficient packing requires DNA ordering, yet accessibility requires a fluid structure. These are the defining characteristics of liquid crystals, and liquid crystal-like organization has been proposed for DNA in several biological contexts, including certain bacterial nucleoids, dinoflagellate chromosomes, sperm heads, and virus capsids (Livolant and Bouligand, 1978; Livolant, 1984a,b; Lepault et al., 1986; Rill et al., 1989; Booy et al., 1991; Kellenberger and Arnold, 1992; Reich et al., 1994; Bloomfield, 1996). The liquid crystalline phases of concentrated solutions of DNA formed in vitro have remained of interest because of this biological relevance, and as models for understanding phase transitions of semirigid polyelectrolytes (see Bloomfield, 1996, for a recent review). The ease with which DNA can be biochemically manipulated makes it possible, in principle, to experimentally test advances in theories of phase transitions of lyotropic polymer liquid crystals. DNA structure is not monotonous, however, so it remains uncertain how well the behavior of DNA mimics that of simpler polymers. Here we show that there are significant differences between the critical behaviors of solutions of moderately long DNA molecules and solutions of xanthan, a double-helical polysaccharide polyelectrolyte with a hard core diameter, charge density, and persistence length similar to those of double-stranded DNA (Sato et al., 1990; Inatomi et al., 1992).

Isotropic to nematic phase transitions in solutions of monodisperse rodlike particles were first described theoretically by Onsager (1949) and Flory (1956a,b). Formation of an anisotropic phase in equilibrium with an isotropic phase is predicted above a critical concentration C_i^*, and a fully liquid crystalline phase above another critical concentration C_a^*. The critical concentrations are inversely related to the rod axial ratio, approaching an asymptotic limit at large axial ratios. Recent theoretical advances provide a more realistic description of semirigid polymers by including the effects of charge and chain flexibility (Khokhlov and Semenov, 1981; Odijk, 1986; Stroobants et al., 1986; Sato et al., 1990; Inatomi et al., 1992; Chen, 1994; Tkachenko and Rabin, 1995; Sheng et al., 1996). For a given axial ratio, the critical concentrations increase significantly, relative to the rod limit, with increasing chain flexibility (decreasing persistence length) (Khokhlov and Semenov, 1981; Odijk, 1986). The increased critical concentrations reflect the fact that the configurations of semiflexible polymers are much less constrained in crowded solutions than the configurations of rigid rods of equal length. Theory can now explain nearly quantitatively the phase behaviors of certain un-

Received for publication 8 April 1997 and in final form 8 September 1997.

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0006-3495/97/12/3154/10 $2.00

charged polymers in organic solvents (Conio et al., 1984; Itou and Teramoto, 1988), as well as the behavior of xanthan, a natural polysaccharide and strong polyelectrolyte (Sato et al., 1990; Inatomi et al., 1992).

DNA is a modestly flexible, chiral, strong polyelectrolyte. The anisotropic phases, primarily a twisted nematic or "cholesteric" phase, formed by concentrated DNA solutions have been characterized in several contexts (Luzzati et al., 1961; Maniatis et al., 1974; Maret et al., 1975; Iizuka, 1977; Brian et al., 1981; Rill et al., 1983; Brandes and Kearns, 1986; Livolant and Bouligand, 1986; Rill, 1986; Podgornik et al., 1995; Bloomfield, 1996). Critical concentrations were determined for the isotropic to anisotropic phase transitions of short (~500 Å) DNA fragments isolated from nucleosome core particles by Strzelecka and Rill (1987, 1990, 1991). Recently we examined the solution behavior of very long DNA (mean length ≈ 8 kbp) and showed that the critical concentration, C_i^*, for the first appearance of the anisotropic phase (13 mg/ml) was much lower than observed for nucleosome DNA, and much less than expected for the consensus static persistence length of DNA (~50 nm) (Merchant and Rill, 1994). By contrast, the critical concentration for disappearance of the anisotropic phase, C_a^*, was consistent with this persistence length.

The present investigation addresses in detail the dependence of phase transitions of DNA in simple saline solutions on contour DNA length. Critical concentrations for anisotropic phase formation were determined by solid-state ^{31}P NMR spectroscopy and polarized light microscopy. A wide biphasic region was observed regardless of DNA length, and the critical concentrations C_i^* for DNA longer than nucleosome size were much lower than expected for a persistence length near 50 nm. These deviations from theory and the behaviors of simple semiflexible polymers suggest that sequence-dependent variations in DNA flexibility or other properties may influence DNA packing.

MATERIALS AND METHODS

DNA isolation

DNA fragments with most probable lengths of 170 bp and 336 bp were isolated from mono- and dinucleosomes obtained by digesting calf thymus chromatin with micrococcal nuclease (Strzelecka and Rill, 1987). Intact nuclei isolated from thymus glands were washed twice in homogenization medium and resuspended in 0.25 M sucrose in 50 mM 3-(*N*-morpholino)propanesulfonic acid, 25 mM KCl, 5 mM $MgCl_2$, and 10 mM ϵ-aminocaproic acid (to inhibit endogenous proteases), plus 2 mM $CaCl_2$, adjusted to a final pH of 8 with solid Tris. Nuclei were preincubated at 37°C for 10 min, then incubated for 40 min after the addition of 6200 units of micrococcal nuclease (Sigma). The digestion of the chromatin provided a "ladder" of nucleosomal DNA fragments that were slightly longer than nucleosome core length and multiples thereof, as indicated by electrophoresis on a 1% agarose gel. Digestion was stopped by the addition of EGTA (pH 6.5) to a final solution concentration of 5 mM. Nuclei were recovered by centrifugation, and the supernatant was saved. The nuclei were further lysed by suspending the pellet in saline EDTA (75 mM NaCl, 24 mM disodium EDTA, at pH 6.5) and blending in a homogenizer. The supernatant was again saved after centrifugation. This procedure was repeated until the concentration of the DNA in the supernatant was less than 0.5 mg/ml (determined by absorbance measurements at 260 nm). The supernatants were pooled and the DNA was treated to remove protein and RNA as described previously (Strzelecka and Rill, 1987). The DNA was then precipitated with 1.2 volumes of isopropanol and washed twice with 70% ethanol, and alcohol was removed by brief vacuum drying.

High-molecular-weight DNA was isolated from partially purified calf thymus nuclei, using procedures analogous to those above, excluding nuclease digestion. The longer DNA fragments in this study (570 bp and 1.4 kbp) were obtained by sonication of the high-molecular-weight DNA, followed by size fractionation. Sonication was performed in a cold room with a Brinkmann homogenizer (model TT 10/35) fitted with a PTA 10 s generator, for 40 min at a power setting of 4.

DNA size fractionation

DNA samples from both sources were fractionated according to length, using saline solutions of polyethylene glycol (PEG) (Lis, 1980). DNA was dissolved in PEG fractionation buffer (50 mM Tris, 10 mM EDTA, 1.1 mM NaCl, pH 7.4) and precipitated with various concentrations of PEG 6000. The higher molecular weight components obtained from chromatin digests (three nucleosome lengths, ~500 bp and longer) were precipitated with 8% PEG 6000, leaving mono- and dinucleosome-length DNA in the solution. The dinucleosome DNA fragments (~340 bp) were removed from the mononucleosome DNA (170 bp) by precipitation with 11% PEG. Mononucleosome DNA fragments were further precipitated with 14% PEG to remove any shorter DNA fragments that might be present. Several rounds of dissolution/precipitation with slight adjustments in PEG concentration were required to obtain pure mono- and dinucleosome-length DNA fractions. PEG was eliminated by repeated precipitation from 70% ethanol, and removal of PEG was confirmed by proton NMR as before (Merchant and Rill, 1994).

Sonicated high-molecular-weight DNA was also fractionated with PEG. The ~1.4-kbp DNA fragments were precipitated by 7% PEG 6000, and the ~570-bp fragments were precipitated by 8% PEG. DNA fragments significantly longer or shorter than these lengths were removed by 6% or 11% PEG precipitations, respectively. The PEG was removed as above.

The size distributions of mono- and dinucleosome DNA samples were determined by electrophoresis on a 6% polyacrylamide gel with a 123-bp ladder (BRL Biochemicals) as length markers. The size distributions of longer DNA were determined by electrophoresis on a 1% agarose gel with a 1-kb ladder (BRL Biochemicals) as length markers. Gels were stained with ethidium bromide and photographed under 365-nm illumination, using a Polaroid MP3 camera with type 665 positive/negative film. Negatives were scanned on a Desktop scanner model DNA 35 and analyzed by Quantity One one-dimensional analysis (Version 2.2, software from PDI, Inc.). Marker mobilities were used to compute length distribution curves for the sample DNA as before (Merchant and Rill, 1994). A plot of Δ area/area versus DNA size (i.e., the weight distribution) was used to obtain the most probable DNA length, as well as the range of fragment sizes present in the sample as determined from the distribution width at half-maximum (Fig. 1). Sample polydispersities, reflected by the ratios of the weight average to number average molecular weights, M_w/M_n, were determined by numerical integration. Characteristics of the samples are summarized in Table 1.

For phase transition studies, the DNA fragments were finally dissolved in buffered sodium chloride (0.1 M NaCl, 10 mM sodium cacodylate, 5 mM EDTA, pH 6.5). A few concentrated solutions were prepared by centrifugal ultrafiltration with a Centriprep filtration device (Amicon) and sometimes were further concentrated in Ultrafree-PF 30,000 NMWL (nominal molecular weight limit) units (Millipore Corp.). Other samples were prepared by dilution of these samples after NMR measurements were completed. Most samples were equilibrated overnight. Samples with exceptionally high viscosities were equilibrated more thoroughly, as described previously for samples of the very long DNA (Merchant and Rill, 1994). Three or more concentration measurements for each sample were made spectrophotometrically from the absorbancies at 260 nm, using

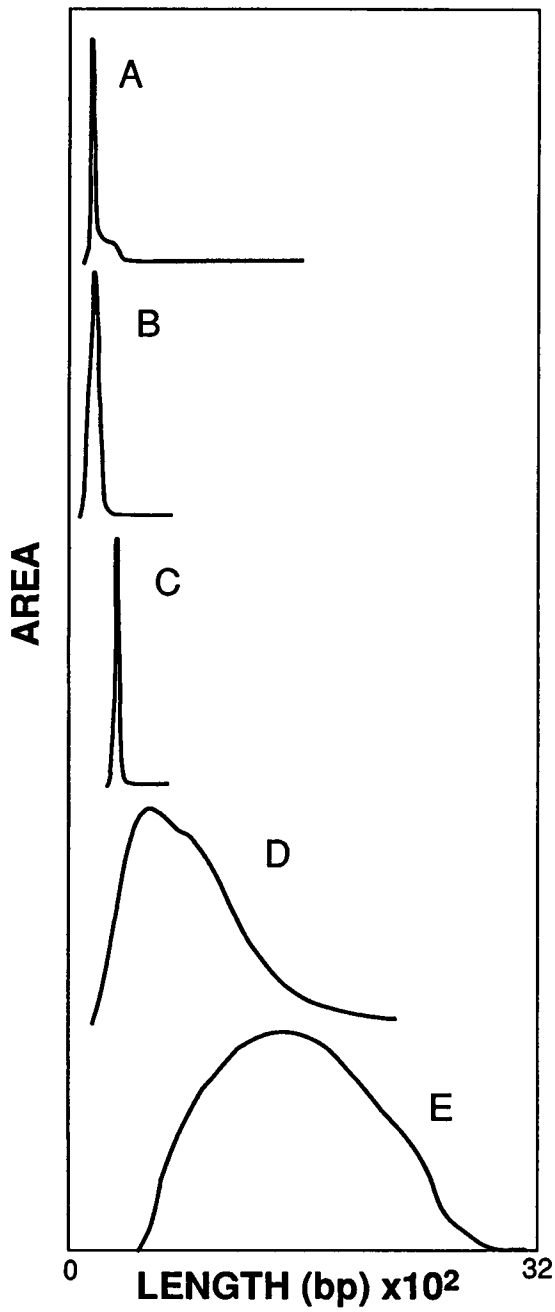

FIGURE 1 Weight distributions of DNA lengths obtained by numerical integration of the densitometer scan and calibrated using the marker DNA fragments indicated in the text. (*A*) Nucleosome core DNA ($L \approx 50$ nm). (*B*) Nucleosome DNA ($L \approx 58$ nm). (*C*) Dinucleosome DNA ($L \approx 114$ nm). (*D*) 8% PEG fraction of sheared calf-thymus DNA ($L \approx 193$ nm). (*E*) 7% PEG fraction of sheared calf thymus DNA ($L \approx 490$ nm).

dilutions of aliquots withdrawn by a positive displacement micropipette, assuming an extinction coefficient of E_{260nm} of 20 for a 1 mg/ml solution.

MR spectroscopy

^{31}P NMR spectra were taken on a nonspinning sample at a frequency of 109 MHz. The sweepwidth was ±8 kHz, and a pulse repetition time of 20 s was used with gated proton decoupling during acquisition. Typically 300-1000 scans were acquired for each data set (depending on the concentration of the sample). Data were transferred to the Peak-Fit program (Jandel Scientific), which was used to deconvolute the two superimposed resonances characteristic of the isotropic and anisotropic phases, as formerly described (Merchant and Rill, 1994). Integrated areas of the narrow and broad resonances were used to calculate the fractions of molecules (F_i and F_a) in the isotropic and anisotropic phases, respectively (see also Results).

Microscopy

DNA solutions were observed between cross-polarizers with a polarized light microscope (Nikon Microphot Pol FX/A). Typically 20–30 μl were placed on a microscope slide, and a glass coverslip was sealed in place with Cytoseal Liquid Coverslip (Stephens Scientific) on the edge. Slides were left to equilibrate overnight, then viewed. The samples with longer DNA fragments (1.4 kb) at higher concentrations appeared similar to the very high-molecular-weight DNA and were examined accordingly (Merchant and Rill, 1994).

RESULTS

Critical concentrations determined by ^{31}P NMR spectroscopy

DNA solutions between the critical concentrations C_i^* and C_a^* are biphasic, containing both isotropic and anisotropic (cholesteric) phases. ^{31}P NMR spectra of biphasic solutions are characterized by two superimposed resonances: a sharp Lorentzian due to rapidly moving molecules in the isotropic phase, and a broad Gaussian due to DNA molecules with slow, coupled motions in the anisotropic phase. A close correspondence between the chemical shifts of isotropic and anisotropic resonances, and poor phase separation in the solution itself, were noted for DNA fragments of greater than dinucleosome length. By contrast, samples of the shorter nucleosome core DNA (146 bp) and mononucleosome length DNA (170 bp) readily phase separate, and discernibly different chemical shifts are observed for the isotropic and anisotropic resonances (Strzelecka and Rill, 1987, 1991; Strzelecka et al., 1988; Rill et al., 1991).

The area under each resonance, after deconvolution, indicates the fractions of molecules in the isotropic (F_i) and anisotropic phase (F_a). These fractions can be converted to concentrations by noting that

$$\alpha C_a^* = CF_a \quad \text{and} \quad (1-\alpha)C_i^* = CF_i \qquad (1)$$

where C is the overall concentration, C_i^* and C_a^* are the concentrations in the two phases, and α is the fraction of the total volume in the anisotropic phase. The fractional volume of the anisotropic phase is not known at a given concentration, but for monodisperse samples the composition of each phase should remain constant at constant temperature throughout the biphasic region, regardless of the overall concentration C. This condition allows estimation of C_i^* and C_a^* from the variations in F_i and F_a, with C based on the relationships derived earlier (Strzelecka and Rill, 1990; Merchant and Rill, 1994), which predict linear dependen-

TABLE 1 Lengths, length distributions, and critical concentrations of DNA samples

DNA (bp)	Length* (nm)	Range# (bp)	Range# (nm)	SD§ (bp)	M_W/M_n¶	C_i^*‖ (mg/ml)	C_a^*‖ (mg/ml)
147	50	135–162	46–55	±12	1.07	135	271
170	58	131–210	44–71	±32	1.07	122	200
336	114	311–355	105–120	±19	1.01	48	138
570	190	257–1140	87–386	NA	1.23	23	110
1450	490	766–2400	262–804	±690	1.14	13	89
8000	2700	4k–>23k	1352–7774	NA	ND	13	67

* Most probable DNA lengths were determined by electrophoresis on polyacrylamide or agarose gels along with DNA restriction fragments of known lengths.

Reported ranges correspond to the lengths at half-heights on the mass distribution curves.

§ Standard deviations were calculated from densitometer scans of bands if they were approximately Gaussian. NA, Not applicable because of irregular band shape.

¶ The ratios of the weight average and number average lengths determined by numerical integration of the densitometer scans. ND, Not determined.

‖ Critical concentrations determined from extrapolations of NMR data (see Materials and Methods). Data for the shortest DNA (50 nm) were taken from Strzelecka and Rill (1991). Data for the longest DNA (2700 nm) were taken from Merchant and Rill (1994).

cies of $C \cdot F_i$ and $C \cdot F_a$ on C over the biphasic region:

$$C \cdot F_i = -\frac{C \cdot C_i^*}{(C_a^* - C_i^*)} + \frac{C_i^* \cdot C_a^*}{(C_a^* - C_i^*)}$$

and (2)

$$C \cdot F_a = \frac{C \cdot C_a^*}{(C_a^* - C_i^*)} - \frac{C_i^* \cdot C_a^*}{(C_a^* - C_i^*)}$$

Data were plotted as $C \cdot F_a$ versus C, and $C \cdot F_i$ versus C, for each fragment length. These plots were not linear over the whole range observed (Fig. 2), perhaps because of some preferential fractionation of the longer DNA fragments in the polydisperse samples into the anisotropic phase, or the onset of another anisotropic phase at high concentrations. The critical concentrations for appearance of the anisotropic phase, C_i^*, and disappearance of the isotropic phase, C_a^*, were estimated by linear extrapolation from approximately the midpoints of the biphasic concentration range to the extremes where F_a or F_i, respectively, were zero (see Fig. 2). Values of the critical concentrations, along with other sample characteristics, are given in Table 1. We consider these estimates to be reasonable because the extrapolations are short, and the data on which they are based extend over a significant fraction of the total data range. Extrapolation errors are unlikely to exceed the aggregate uncertainties of the measurements, estimated to be 5–10%, and errors of this magnitude do not affect the major conclusions drawn below. Possible effects of sample polydispersity are small, as discussed in a later section.

Plots of critical concentration dependencies on DNA contour length are shown in Fig. 3. The critical concentrations C_i^* for the first appearance of the anisotropic phase were low (<25 mg/ml for DNA ≥ 190 nm), with a weak dependence on length from 190 nm to over 2500 nm. A strong length dependence was observed for the shorter DNA fragments. An approximately parallel length dependence was noted for C_a^*. The biphasic region was wide over the whole length range.

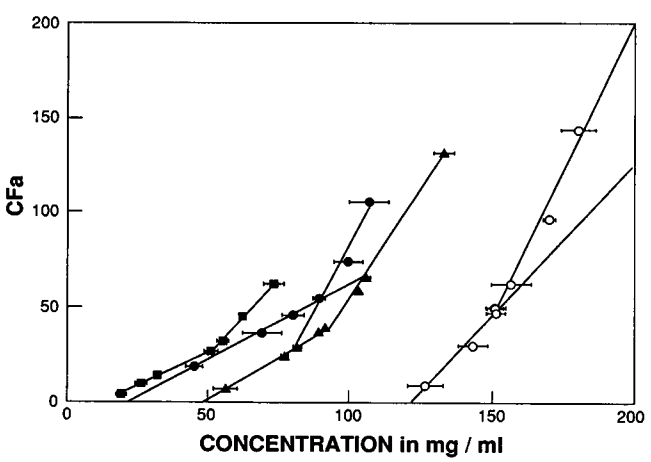

FIGURE 2 The total DNA concentration multiplied by the fraction of molecules in the anisotropic phase ($C \cdot F_a$) versus the total DNA concentration in mg/ml. DNA lengths are 58 nm (○), 114 nm (▲), 193 nm (●), and 490 nm (■). The critical concentrations C_i^* were obtained by extrapolation of these curves to 0% anisotropy ($F_a = 0$). Analogous plots of ($C \cdot F_i$) were used to determine C_a^*.

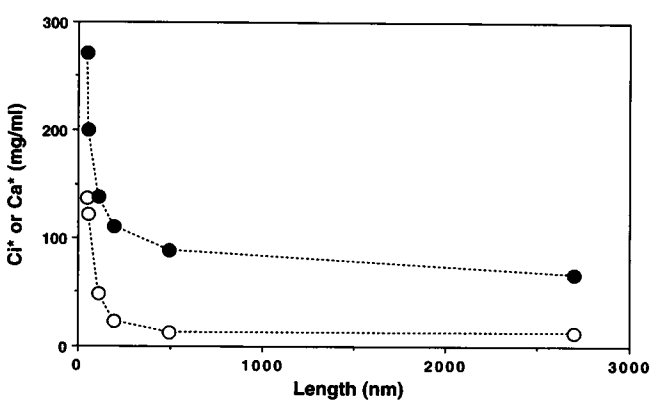

FIGURE 3 Dependencies of the observed DNA critical concentrations, C_i^* (○) and C_a^* (●), on most probable contour length (see also Table 1).

Polarized light microscopy

Although the ^{31}P NMR spectra are expected to be highly sensitive to DNA ordering, independent verification of the existence of the anisotropic phase was desired. The existence of birefringence was confirmed by polarized light microscopy for all samples where deconvolution of the NMR spectrum indicated the presence of a small amount of anisotropic phase. The anisotropic phases of the shorter DNA fragments (50–114 nm) displayed well-developed features, as described for nucleosome core-length DNA (Strzelecka et al., 1988; Rill et al., 1991). Discrete spherulites and globules were observed in samples with low anisotropy, and distinct fingerprint textures typical of the cholesteric phase were displayed by samples with higher concentrations (not shown). Similar textures were noted for samples of 190-nm DNA. This is an important observation, because C_i^* was very low (23 mg/ml) in comparison to shorter DNA. The 490-nm fragments in the lowest concentration solutions showed a weak, unstructured birefringence, whereas samples higher in concentration showed highly birefringent but uncharacterized textures similar to that of very long DNA (Merchant and Rill, 1994).

Potential influence of length heterogeneity on critical concentrations

Polydispersity is expected to broaden the biphasic region observed for real polymer samples, as some fractionation will occur during phase separation, with the longer polymer chains preferentially partitioning into the anisotropic phase. It is important to estimate whether polydispersity could broaden the biphasic region of DNA solutions to the extent observed. The influence of polydispersity on phase separations of solutions of semiflexible polyelectrolytes has been analyzed theoretically for certain limiting cases. Inclusion of bidispersity in the theories of phase transitions for rigid, rodlike molecules by Odijk and Lekkerkerker (1985) provides some insight into the potential effects of polydispersity. For the case when the ratio of lengths of the two components is small (akin to the DNA samples studied here), numerical analysis indicated that the presence of some longer rods in a solution of shorter ones decreases both C_i^* and C_a^* relative to a pure solution of short rods. Decreases in critical concentrations are proportional to the ratio of rod lengths. This implies that C_i^* of a bidisperse or polydisperse solution will be lowered relative to that of the shortest rods, but will never be lower than C_i^* of a "pure" solution of longest rods within each sample. Similarly, C_a^* of a bidisperse or polydisperse solution will not be greater than that of the "pure" solution of the shortest molecules.

Keeping this in mind, C_i^* and C_a^* values were calculated for values of the contour lengths in each sample corresponding to the half-height positions (designated L_{min} and L_{max}) of the mass distributions of lengths, as well as the most probable contour length, assuming a persistence length of 50 nm and D_{eff} = 5.6 nm (Table 2). This simplistic comparison provides estimates of widths of the biphasic region that are much narrower than observed, even for the more polydisperse samples of the longer DNA.

TABLE 2 Estimated effect of length heterogeneity on the predicted critical concentrations

Length (nm)*	C_i^* (mg/ml)#	C_a^* (mg/ml)#
L_{max} = 71	110	121
L_{med} = 58	115	125
L_{min} = 44	122	132
L_{max} = 120	102	113
L_{med} = 114	103	114
L_{min} = 105	104	115
L_{max} = 386	94	106
L_{med} = 193	98	109
L_{min} = 87	107	118
L_{max} = 804	92	104
L_{med} = 491	93	105
L_{min} = 262	96	108

* L_{max} and L_{min} refer to the lengths at half-height positions of the mass distribution of lengths, respectively. L_{med} refers to the most probable length.

Critical concentrations calculated according to Odijk (1986).

Chen and Cui (1994) used a perturbation method to analyze the influence of modest polydispersity on the partitioning of semiflexible polymers according to length between the isotropic and nematic phases. To first order, they found that

$$\frac{L_N}{L_I} = 1 - A(\alpha)\left(\frac{M_w}{M_n} - 1\right) \tag{3}$$

where L_N and L_I are the number average chain lengths in the nematic and isotropic phases, and M_w/M_n is the ratio of weight average to number average molecular weights. $A(\alpha)$ is a function of the ratio, α, of the chain contour length to the Kuhn statistical segment length. $A(\alpha)$ increases sharply and monotonically from the rigid rod limit of -1.84 at α = 0 to about -0.4 when $\alpha \cong 1$, then slowly approaches a limit of -0.333 as α exceeds 1; thus partitioning of unequal length chains decreases significantly with increasing chain flexibility. Values of L_N/L_I calculated for the DNA samples studied here were ≤ 1.08, excepting the sample with a median length of 190 nm, for which L_N/L_I = 1.16. Partitioning of DNA fragments between isotropic and nematic phases in these samples is therefore expected to be modest, and to have modest effects on the critical concentrations.

DISCUSSION

This study describes the variations in critical concentrations for phase transitions of simple saline solutions of DNAs over a wide range of contour lengths. The range includes short DNAs, near the persistence length, for which the contour length is expected to strongly influence the critical concentrations, and extends to DNAs from a few to many persistence lengths, easily long enough for critical concentrations to be dominated by chain flexibility. Although

DNA behaves qualitatively as expected, the biphasic region is very broad, especially for DNA longer than a few persistence lengths. This breadth can be attributed to the critical concentrations for the onset of the anisotropic phase (<25 mg/ml for DNAs ≥ 190 nm), which are much lower than observed for other semirigid polymers (Itou and Teramoto, 1988; Sato et al., 1990; Inatomi et al., 1992), including xanthan, another strong polyelectrolyte. Solutions of high-molecular-weight xanthan exhibit a narrow biphasic region and a critical concentration, C_i^*, of ~40 mg/ml, roughly twice that of DNA; yet this polyelectrolyte double helix has a persistence length (100 nm) approximately twice the commonly cited persistence length of DNA (Sato et al., 1990; Inatomi et al., 1992).

It is of interest to consider the anomalous behavior of DNA within the framework of theories of semirigid chain behavior. As noted by Khoklov and Semenov (1981) and Odijk (1986), the ratio of the contour length to the persistence length is the major factor determining critical concentrations of anisotropic phases for wormlike, semiflexible chains. Other, length-independent factors affect the critical concentrations of chains if they are polyelectrolytes, because charge screening must be taken into account, and the solutions are three-component systems. These factors have been approximated by treating the solutions as two-component systems in which the hard core particle diameter is replaced by an effective diameter, D_{eff}, which reflects charge screening by counterions; and a twist parameter h is defined to account for the twisting effect of repulsive interactions between two parallel rods. Possible influences of the intrinsic chirality of polymers such as DNA, xanthan, and polybenzyl-L-glutamate are not considered. Both D_{eff} and h depend on the ion double layer and hence are functions of the polyelectrolyte charge density and effective ionic strength. Decreasing the ionic strength increases D_{eff} and significantly reduces $C^*_{i\text{ or }a}$. Critical concentrations are much more sensitive to ionic strength-dependent changes in D_{eff} than h, as explained previously (Merchant and Rill, 1994); hence variations in h are not included in the calculations that follow.

Values of D_{eff} for various NaCl concentrations were calculated from Poisson-Boltzmann theory by Stigter (1977). These calculations are strictly appropriate only for infinitely dilute solutions, far from the present case. Aside from nonideal effects, the contribution of counterions from the polyelectrolyte itself must be included in the effective ionic strength. For the present case, the effective ionic strength was equated to the concentration of M^+ added as NaCl and buffer salts, plus a contribution from DNA assumed to be 24% of the total DNA concentration in nucleotides. In all cases except the highest concentrations of nucleosome core DNA, the ionic strength calculated in this manner ranged from ~0.11 M to 0.2 M. Because of uncertainties in the appropriateness of both the theory and the ionic strength, calculations were performed for a range of effective DNA diameters, rather than a single value. The range chosen was D_{eff} = 5.6–4.4 nm, corresponding to ionic strengths of 0.1–0.2 M, as calculated by Stigter (1977). The lower limit is most appropriate for critical concentrations of ≥100 mg DNA/ml, whereas the upper limit is most appropriate for the lowest critical concentrations. Calculations were also performed with D_{eff} = 4 nm at the very high DNA concentrations corresponding to C_a^* for the shortest DNA samples, because of the increased counterion contribution to the effective ionic strength.

The degree to which chain flexibility dominates the critical behavior of semirigid chains depends on the ratio $N_p = L/P$ of chain contour length, L, to persistence length, P. According to Odijk (1986), the critical concentrations c_i and c_a in dimensionless units in the limit of long chains with $N_p = L/p \gg 1$ are given by

$$c_i = 5.409 + \frac{1.910}{N_p} + \delta c_i$$

and (4)

$$c_a = 0.6197 + \frac{1.781}{N_p} + \delta c_a$$

where the electrostatic twist contribution is included as $\delta c_i = 4.72h$ and $\delta c_a = 4.72h$. In the present case, h was assumed to be 0.15 for DNA (Stroobants et al., 1986).

Flexibility plays a modest role for chains significantly shorter than the persistence length, and the critical concentrations are close to those expected for rigid rods. Odijk's expressions for critical concentrations when $N_p \ll 1$, modified from Khokhlov and Semenov, are given by

$$c_i = 4.99 + \frac{3.340}{N_P} + \delta c_i \quad \text{and} \quad c_a = \frac{4.486}{N_P} - 1.458 + \delta c_a \tag{5}$$

Finally, Odijk proposed a combination of the above equations for the case where $N_P \approx 1$:

$$c_i = \frac{3.34 + 5.97N_P + 1.585N_P^2}{N_P(1 + 0.293N_P)} + \delta c_i$$

and (6)

$$c_a = \frac{4.486 + 11.24N_P + 17.54N_P^2}{N_P(1 + 2.83N_P)} + \delta c_a$$

The dimensionless concentrations are related to the usual units of concentrations in mg/ml by

$$C^*_{i\text{ or }a} = c_{i\text{ or }a}\left[\frac{4}{\pi}\left(\frac{1}{D_{eff} \cdot P \cdot L}\right)\left(\frac{M}{N_{Av}}\right)\right] \tag{7}$$

Equation 6 is most appropriate for all but the longest DNA samples examined, and nearly converges with Eq. 4 when $N_p > 5$. Table 3 compares the determined critical concentrations to those calculated for different assumed effective diameters and persistence lengths, using Eq. 6, except when $N_p > 5$ ($L > 250$ nm).

TABLE 3 Predicted dependencies of DNA critical concentrations on contour length and persistence length

D_{eff} (nm)	Length (nm)	Predicted C_i^* (mg/ml) for persistence length (P, nm)*						$C_{i\,exp}$* (mg/ml)
		P = 400	P = 300	P = 200	P = 100	P = 50	P = 30	
4.4	50			89	**116**	**170**	244	137
5.6				70	91	**134**	191	
4.4	58		72	80	**107**	162	236	122
5.6			56	63	84	**127**	185	
4.4	114	41	**45**	**54**	81	137	211	48
5.6		32	36	**43**	64	107	166	
4.4	193	**30**	34	43	71	127		23
5.6		**23**	**27**	34	56	100		
4.4	491	**20**	24	34	61			13
5.6		**16**	**18**	27	48			
4.4	2700	**15**	20	29				13
5.6		**12**	**15**	23				

D_{eff} (nm)	Length (nm)	Predicted C_a^* (mg/ml) for persistence length (P, nm)*						$C_{a\,exp}$* (mg/ml)
		P = 400	P = 300	P = 200	P = 100	P = 50	P = 30	
4.0	50					193	**283**	271
4.4				95	119	175	**257**	
5.6				75	93	138	202	
4.0	58					**184**	275	200
4.4				85	110	168	250	
5.6				67	86	132	**197**	
4.4	114			55	84	**146**	231	138
5.6				43	66	**115**	182	
4.4	193		35	44	75	**139**	224	110
5.6			27	35	59	**109**	176	
4.4	490	20	26	36	**68**	132		89
5.6		16	20	28	54	**104**		
4.4	2700	17	22	33	**65**	129		67
5.6		13	17	26	**51**	102		

* Predicted critical concentrations were calculated according to the method of Odijk (1986), using effective diameters (D_{eff}) calculated according to the method of Stigter (1977) for 1:1 salt concentrations of 0.1–0.2 M. Values best matching those observed are shown in bold.

The length dependencies of phase transitions observed for DNA (Fig. 3) are qualitatively in accord with expectations in that the critical concentrations decrease sharply with increasing length near the persistence length, whereas the critical concentrations for longer fragments are relatively insensitive to changes in contour length because of the dominance of chain flexibility. The upper critical concentrations, C_a^*, of the longer DNAs agreed well with calculations according to Odijk (Table 3 and Fig. 4) when the persistence length was assumed to be in the consensus range of 50–100 nm. Theoretical C_a^* values for core and mononucleosome-length DNA, ~50 nm and 58 nm, were somewhat below the actual values. In contrast, the low values of the critical concentrations C_i^* (Table 3 and Fig. 5), which

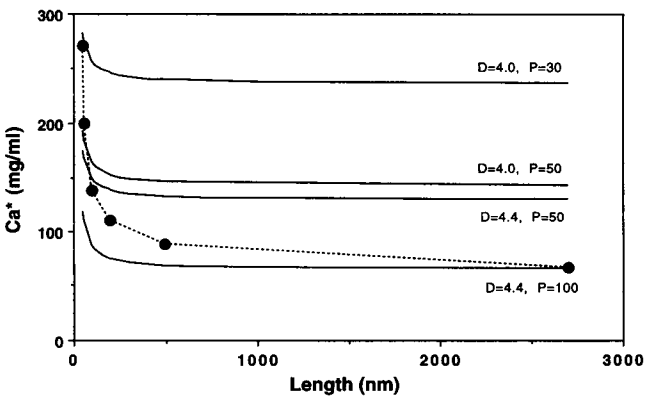

FIGURE 4 Comparison of actual (- -●- -) and theoretical values (——) of C_a^* as a function of length for the indicated persistence lengths (P) and effective diameters (D).

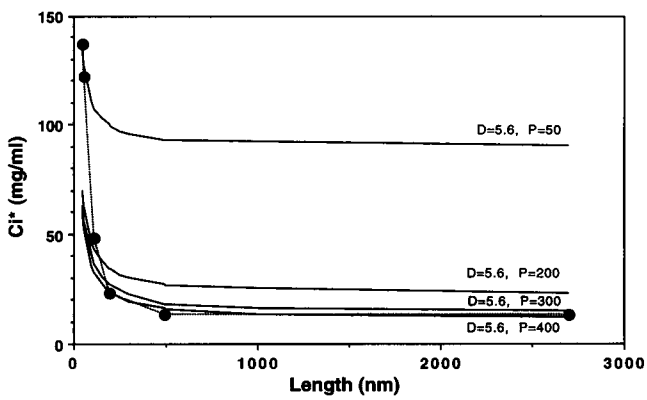

FIGURE 5 Comparison of actual (- -●- -) and theoretical values (——) of C_i^* as a function of length for the indicated persistence lengths (P) and effective diameters (D).

produce an extremely broad biphasic region for the longer DNAs, cannot possibly be reconciled with a 50–100-nm persistence length. An assumption of a much larger persistence length of 300–400 nm was required to accurately predict observed C_i^* values for the longest DNA samples.

Variations in DNA persistence length

A major difference between the basic properties of DNA and other, simpler polymers such as xanthan is the variation in chemical structure along the chain. Although to a first approximation DNA possesses a uniform double-helical structure, there are well-documented disparities in local DNA curvature dependent on the base sequence (Trifonov, 1985; Koo et al., 1986; Crothers et al., 1990, 1992; Hagerman, 1990; Brukner et al., 1995; Harvey et al., 1995; Olson et al., 1995; Robinson and Drobny, 1995; Olson, 1996). The flexibility of DNA may also be sequence dependent (Hagerman, 1988; Sarai et al., 1989; Fujimoto and Schurr, 1990; Lyubchenko et al., 1993; Heath et al., 1996). Such local variations in DNA properties are expected to influence its critical solution behavior.

The finding that the statistically averaged conformations of certain DNA sequences are curved necessitates reexamination of the persistence length as a measure of chain stiffness. Curvature alone reduces the DNA persistence length, independent of chain flexibility. The relative contributions of statistically averaged or "static" curvature and dynamic chain bending to the measured persistence length have been approximated by the relation (Schellman and Harvey, 1995)

$$\frac{1}{P} = \frac{1}{P_{\text{static}}} + \frac{1}{P_{\text{dynamic}}} \tag{8}$$

Dynamic bending rigidities measured for DNA indicate dynamic persistence lengths that are much larger than the consensus equilibrium value near 50 nm. Schurr and coworkers (Fujimoto and Schurr, 1990; Schurr et al., 1992) have proposed 150 nm as the current best estimate of the dynamic persistence length, but dynamic persistence lengths as high as 250 ± 34 nm have been reported (Hustedt et al., 1993). Nonetheless, these estimates fall short of the persistence length assumptions required to reconcile theory with the observed C_i^* of long DNA.

Furthermore, although current theories do not take into account the influence of chain curvature, it seems reasonable that curved chain segments should be more difficult to pack in orderly fashion than straight segments, thereby increasing both critical concentrations. This expectation may be reflected in the higher than predicted C_a^* values of the 50- and 58-nm DNA samples, which were isolated from nucleosome cores and mononucleosomes. It has been proposed that sequence-dependent DNA curvature facilitates wrapping of DNA around histone octamers in nucleosomes (Trifonov and Sussman, 1980; Satchwell et al., 1986). Nucleosome reconstitution experiments with DNA polymers of defined sequence support this concept (Rhodes, 1979; Simpson and Künzler, 1979; Kunkel and Martinson, 1981; Shrader and Crothers, 1989).

Weakly attractive DNA interactions

A number of investigators have detected aggregation or clustering of short (near nucleosome length) DNA fragments beginning at concentrations well below those required for appearance of the true anisotropic phase detected by polarized light microscopy or extreme broadening of the ^{31}P NMR signal (Fulmer et al., 1981; Mandelkern et al., 1981; Fried and Bloomfield, 1984; Härd and Kearns, 1986; Nicolai and Mandel, 1989; Wissenberg et al., 1994, 1995). Clustering does not appear to be dependent on inorganic cations or protein contamination (Wissenberg et al., 1995). This phenomenon is not well understood and cannot be explained in the context of conventional theories that consider primarily repulsive electrostatic interactions and excluded volume contributions to phase separation. Odijk and co-workers (Odijk, 1994; Wissenberg et al., 1995) have suggested that clustering is caused by a weakly attractive component of DNA-DNA interactions arising from the third virial coefficient (see also Ray and Manning, 1994). Such attractive components may also vary with DNA sequence. Weak interactions acting cooperatively over long distances might contribute to lowering the critical concentration for anisotropic phase formation by selected segments of long DNA fragments.

In summary, theories that predict with reasonable accuracy the phase behaviors of several semiflexible polymers with uniform structures do not describe the behavior of DNA well. Solutions of long, heterogeneous sequence DNA begin to form an anisotropic phase at much lower concentrations than is predicted based on the consensus persistence length 50–100 nm, or has been observed for xanthan, a polyelectrolyte with a longer persistence length than DNA. Furthermore, DNA solutions are biphasic over a much larger concentration range than has been observed for xanthan or is predicted if a uniform persistence length is assumed. Segmental variations in persistence length or curvature due to variations in DNA sequence are established. These sequence-dependent variations could account for significant broadening of the biphasic region. If stiffness is the critical factor, then DNA persistence lengths as large as 300–400 Å appear to be required to explain why the anisotropic phase appears at extraordinarily low concentrations. Whereas some DNA segments may be unusually stiff, these values seem unrealistically large. Postulated weakly attractive interactions between DNA segments could assist in lowering this critical concentration. Further developments in theory are required to treat more thoroughly the polyelectrolyte character and local structure variability of DNA.

Regardless of the origins, it is clear that solutions of long DNA begin to order at modest concentrations (<15 mg/ml), well within the physiological range in eukaryotic nuclei and bacterial nucleoids. Long DNA is in a fully liquid crystal-

line phase at concentrations less than 70 mg/ml. The ready ability of long DNA to self-order is consistent with models that describe as liquid crystalline the packing of DNA in several natural settings. The biological significance of sequence-dependent variations in DNA flexibility or curvature is thought to be expressed, in part, by facilitating binding of proteins at specific sites: histones (Trifonov and Sussman, 1980; Rhodes, 1979; Simpson and Künzler, 1979; Kunkel and Martinson, 1981; Satchwell et al., 1986; Shrader and Crothers, 1989) or specific gene regulatory proteins (Liu-Johnson et al., 1986; Kahn and Crothers, 1992; Lyubchenko et al., 1993). That is, both the physical properties (flexibility, curvature) and chemical properties (gene coding, protein recognition) of DNA play important physiological roles. The correlations suggested here between DNA base sequence, flexibility and interactions, and bulk phase transitions remain to be established. Such correlations may also have physiological significance for the large-scale organization of DNA, e.g., in chromatin, sperm heads, or virus capsids. For example, atypically flexible or curved DNA segments can be viewed as defects in a stiff polymer. Advantage might be taken of these defects to change the DNA chain direction in response to a variety of packing requirements.

We thank Drs. Dick Rosanske and Tom Gedris of the Florida State University Magnetic Resonance Laboratory for technical assistance, and Dr. Teresa Strzelecka for advice.

This work was supported in part by National Science Foundation grant BCS-9311901 and the Florida State University Center for Materials Research and Technology.

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