Spontaneous Ordering of DNA

EFFECTS OF INTERMOLECULAR INTERACTIONS ON DNA MOTIONAL DYNAMICS MONITORED BY $^{13}$C AND $^{31}$P NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Effects of intermolecular DNA interactions on the motional dynamics of defined length (147-, 234-, and 437-nucleotide pair (np)) double-stranded DNA were examined by $^{13}$C and $^{31}$P nuclear magnetic resonance spectroscopy. At a critical high concentration varying inversely with length, DNA undergoes a spontaneous transition to an ordered, liquid crystalline-like state. Ordering is accompanied by the appearance of distinct opalescence, and an increase in solution viscosity. An apparent standard heat of fusion of $-38$ kcal/mol of helix ($-0.13$ kcal/mol of DNA phosphate) and an entropy change of $-0.13$ entropy units (per mol of helix) were determined from the temperature dependence of the phase transition of 147-np DNA. The average phosphodiester configuration, monitored by Raman spectroscopy, was typical of B-form DNA above and below the phase transition. NMR spectra and relaxation data show that intermolecular interactions are strong at concentrations well below the phase transition and cause stepwise uncoupling of internal motions at specific sites. Motions of the exocyclic C5' carbon, but not other sugar carbons, are frozen in $-50\%$ of concentrations above 46 mg/ml. Rapid C2' motions are unaffected below the critical concentration (193 mg/ml at 32 °C), and still occur in the ordered phase. We conclude that rigid internal motions of DNA monitored by NMR consist mostly of coupled, periodic bending deformations and partially uncoupled local motions within the sugar ring. Rapid, extensive C2' motions can occur without strong coupling to other sugar carbon motions. From a comparison of $^{13}$C and $^{31}$P NMR data, we conclude that $^{31}$P NMR at best yields an incomplete representation of DNA dynamics.

Multinuclear ($^{1}$H, $^{31}$P, $^{13}$C) NMR studies have shown that relatively short (100-300 np), double-stranded DNA molecules undergo rapid local (internal) motions with correlation times of a few nanoseconds (1-7). This conclusion is based on observations of relatively narrow spectral lines, short T1's, and significant $^{13}$C [($^{1}$H) Nuclear Overhauser Enhancement Factor]

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1 The abbreviations used are: np, nucleotide pairs; NOE, Nuclear Overhauser effect (NOEF = NOE - 1); T1, spin-lattice relaxation times.

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**Materials and Methods**

**DNA Preparation**—Several hundred milligrams of short, double-stranded DNA fragments, relatively homogeneous in length, were isolated from calf thymus chromatin digested with micrococcal nuclease (Worthington) as described previously (6). DNA lengths were determined by electrophoresis on 8% polyacrylamide gels under non-denaturing conditions (16) using λX174 replicative form DNA digested with HaeIII endonuclease (Bethesda Research Laboratories) as size markers. Length distributions were determined using a Gelman DCD-16 scanning densitometer after staining with toluidine blue.

Median lengths of DNA samples were 147, 234, and 437 np. Length distributions of 147- and 234-np DNA samples were approximately symmetric, with about 90% of the DNA within ±20% of the median length. The length distribution of the 437-np sample was bimodal; ~30% of the sample migrated as a broad band with a median length of about 600 np. Assays using S1 nuclease (17) and thermal denaturation curves indicated that ~6% of the samples were single-stranded.

Atomic absorption measurements indicated levels of all heavy metals were <1 ppm. Prior to NMR experiments, samples were ethanol precipitated, lyophilized for 4 h, and then dissolved in phosphate buffer (7 mM Na2HPO4, 19 mM NaH2PO4, 2 mM Na2EDTA, 3 mM NaH2, in 10% D2O, final pH 7.25) containing 50 mM sodium trimethylsilylpropane sulfonate (final Na+ = 0.12M). Inorganic phosphate and the silicic acid compound were used as internal intensity standards for 31P and 13C NMR measurements, respectively. DNA concentrations were determined from the absorbance at 254 nm assuming an extinction coefficient of 6.8 x 10^4 mol of DNA phosphate.

**NMR Measurements**—Carbon-13 spectra were obtained at 67.89 MHz on a Bruker HX-270 modified for quadrature detection with a wide bore solenoid and 20-mm probe (Florida State University) and at 90.56 MHz on a Bruker WM-360WB spectrometer with 20-mm probe (Syracuse University). Temperatures of measurements were 32 ± 2 °C at 67.9 MHz and 28 ± 2 °C at 90.56 MHz. Gated decoupling was used to eliminate nuclear Overhauser effects in most cases; two-level decoupling was used to allow NOE, but prevent radiofrequency heating of samples (18). Phosphorus-31 spectra were obtained at 60.7 MHz on the in-house design multinuclei, Fourier transform spectrometer (SEMINOLE) (19) at Florida State University. Peak areas were determined from baseline-flattened spectra; in all cases, pulse conditions were strictly comparable (for 13C, 2-s pulse intervals were used with 90 °C pulses). Pulse intervals in all cases were at least 3 times the measured τr values.

Experiments to examine effects of DNA concentration were conducted using a 20-mm tube containing inner, concentric tubes of different diameters that contained buffer. As the sample concentration (initially ~300 mg/ml) was gradually decreased, the size of the inner tube containing buffer was also decreased, and the heights of solutions in the inner and outer tubes were kept constant and equal. In this manner the amount of DNA, the concentration of internal standard, and the total volume observed by the detection coil were kept constant at all concentrations.

**Absorption Measurements**—Changes in turbidity of DNA solutions were monitored using a 20-mm tube containing inner, concentric tubes of different diameters that contained buffer. As the sample concentration (initially ~300 mg/ml) was gradually decreased, the size of the inner tube containing buffer was also decreased, and the heights of solutions in the inner and outer tubes were kept constant and equal. In this manner the amount of DNA, the concentration of internal standard, and the total volume observed by the detection coil were kept constant at all concentrations.

**Results**

**Cooperative Association of DNA at High Concentrations**—During preparation of concentrated (>200 mg/ml) solutions of nucleosome core length 147-np DNA, we noted that solutions suddenly became cloudy after addition of an increment of solid. This phenomenon was followed quantitatively by measuring the apparent absorbance at 700 nm versus DNA concentration (Fig. 1). The A700 nm of solutions of 147-np DNA increased abruptly at an apparent critical concentration of 175 mg/ml (at 25 °C) and continued to increase linearly with concentration up to 240 mg/ml, the highest concentration examined. A marked increase in viscosity accompanied onset of turbidity, and turbid solutions exhibited distinct opalescence (birefringence). Both of these properties suggest formation of an ordered, anisotropic phase. No precipitation was observed after prolonged standing.

**The Dependence of Critical Concentration on DNA Length**—Similar experiments with 234- and 437-np length DNA fragments showed that the critical concentration decreased approximately linearly with increasing DNA length (Fig. 1, inset). The shape of the A700 nm versus concentration curve of the 437-np sample differed from that of the smaller DNA’s, having a greater slope below the critical concentration and a smaller slope after the transition. The latter behavior was probably due to greater polydispersity of the 437-np sample, which should decrease the apparent cooperativity of the transition (20). Turbidity changes before the major transition, observed by eye at concentrations as low as 40 mg/ml, are not readily explained in terms of sample polydispersity and may reflect formation of microscopic clusters or nucleation centers (see “Discussion”).

**Temperature Dependence of the Phase Transition**—Cooling or heating 147-np DNA solutions at concentrations near the critical concentration caused rapid changes in turbidity, hence ordering must be driven by a favorable enthalpy change. Transition from disordered to ordered states occurred over a narrow temperature range (10–30 °C at a concentration of 180 mg/ml) in a highly cooperative manner consistent with a true phase transition (Fig. 2). A plot of ln Kc vs 1/T was linear (Fig. 2, inset). An apparent standard heat of fusion (ΔHf) of -38 kcal/mol of DNA helix (0.13 kcal/mol of phosphate) and an entropy change (ΔSf) of -0.13 enzyme units/mol of helix were calculated by standard methods assuming that the sample was fully dissociated and associated at 35.8 °C and 8.7 °C, respectively.

**Effects of DNA Concentration on the 13C NMR Spectrum**

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**Fig. 1.** The DNA phase transition monitored by changes in apparent absorbance (turbidity) at 700 nm for 147- (○), 234- (□), and 437-np (●) length DNA. Straight line segments were determined by linear least squares regression analyses. Inset, dependence of the critical concentration for the phase transition on the inverse of DNA length.
of 147-np DNA-\(^{13}\)C NMR spectra with gated proton decoupling (NOE-suppressed) were obtained at 67.9 MHz for 147-np DNA over a range of concentrations (79 to 305 mg/ml) above and below the phase transition (Fig. 3). Additional spectra at lower concentrations (6.5 to 46 mg/ml) were obtained at 90.6 MHz. Sugar and base \(^{13}\)C integrated resonance intensities generally (excepting C2') decreased significantly with increasing concentrations, both above and below the phase transition, but linewidths did not simultaneously broaden. Distinctly different changes in intensities were noted for different DNA \(^{13}\)C sites (Figs. 3, and 4). The C5' integrated intensity was only 55% that of other sugar carbons at the lowest concentration examined (6.5 mg/ml) and decreased sharply with increasing concentration. Above the critical concentration the C5' resonance essentially disappeared. In contrast, the C2' sugar carbon intensity was almost independent of DNA concentration from 6.5 to 193 mg/ml, and dropped significantly only above the critical concentration. Concentration dependencies of intensities of other carbon resonances were intermediate between those of C2' and C5'. The C1',4' and C3' sugar carbon intensities were constant from 6.5 to 46 mg/ml, then dropped progressively. The loss of C3' intensity at all concentrations was considerably greater than that of C1',4'. Changes in base carbon intensities roughly paralleled those of C1',4' (Fig. 3). With the exception of C2', intensity changes were not accompanied by significant line broadening (Fig. 3). The C2' linewidth increased approximately linearly with concentration from 79 to 305 mg/ml, doubling in this range. Spectra were also obtained for 147-np DNA at concentra-
trations of 79 and 193 mg/ml with continuous two-level broadband decoupling, giving $^{13}$C [$^1$H] NOEs. Within error, there were no changes in NOEs of any sugar carbons with concentration.

**Effects of Concentration on the $^{31}$P NMR of DNA—Phosphorus-31 spectra were obtained at 60.7 MHz on samples described above under conditions both yielding and suppressing nuclear Overhauser effects (Fig. 5). The $^{31}$P integrated signal intensity, $I_T$, and NOE were essentially constant in the concentration range (79 to 193 mg/ml) below the critical concentration. The (NOE-suppressed) integrated signal intensity and the NOE both dropped about 80% at 305 mg/ml (above the critical concentration), and the $I_T$ increased ~30%. The $^{31}$P linewidth increased linearly over the range from 79 to 193 mg/ml, and appeared to remain constant from 193 to 305 mg/ml (Fig. 5). Increasing DNA concentration had an interesting effect on the inorganic phosphate resonance. A single sharp resonance was observed at 79 mg/ml. As concentration was increased, a second, broader upfield resonance appeared, and the intensity of the original sharp peak decreased.

**Changes in Macroscopic Solution Properties**—Several DNA Raman lines are sensitive to conformation and differ in the A, B, C, and Z DNA forms (21, 22). Of particular interest are the bands assigned to phosphate stretching and bending modes, which occur at 875 and 1091 cm$^{-1}$ for B form DNA. Raman spectra from 700 to 1200 cm$^{-1}$ obtained for 147-np DNA at concentrations above and below the phase transition were almost indistinguishable and were identical with that reported for B form DNA (21).

**DISCUSSION**

**The DNA Phase Change**—We have observed that short, double-stranded DNA samples undergo a spontaneous phase change at high concentrations, in the absence of condensing agents such as heavy metals, polycations, or neutral polymers. Changes in macroscopic solution properties, e.g. increased viscosity and appearance of opalescence (birefringence) suggest molecular ordering in the concentrated phase. Preliminary, low-angle, x-ray scattering data support this conclusion. A similar phase change was described recently by Brian et al. (15), who observed a sharp discontinuity (due to scattering) in sedimentation equilibrium patterns of concentrated solutions of comparably sized DNA. As indicated by Brian et al., this phase change is related operationally and in theory to the well studied collapse of DNA to an anisotropic state upon addition of neutral polymer (e.g. polyethylene glycol) and is analogous to formation of cholesteric liquid crystals by anisotropic small molecules. In such cases “the helicoidal progression in the direction of the helix axes through the cholesteric phase with a pitch larger than the wavelength of the light accounts for the macroscopic turbidity of the phase” (15).

Spontaneous ordering of highly asymmetric, noninteracting molecules to yield liquid crystals is well known. As described first by Onsager (10), ordering is predicted from excluded volume effects, expressed in terms of viral coefficients (10–12) or lattice-site occupation probabilities (14). Put simply, the solution free energy is minimized by alignment of the rods. A two-dimensional analogy can be made to the most efficient arrangement of rods floating on a pond.

Ordering of electrostatically repulsive DNA rods may seem paradoxical. Repulsions can be accounted for, however, by defining the axial ratio in terms of the true radius plus an incremental radius where the exponentially decreasing electrostatic potential is greater than $\kappa T$, where $\kappa$ is the thermal Debye length. This approach, incorporated in scaled particle theory, successfully describes DNA nonideality at concentrations below the phase transition (at ionic strengths sufficient for effective charge screening) and predicts a phase change at high concentrations (15). Increasing ionic strength reduces the effective radius, hence it should increase the critical concentration. We have not yet examined this effect.

Phase changes of solutions of rodlike particles have been treated theoretically in several ways (reviewed in Ref. 15). All theories predict an inverse dependence of critical concentration on axial ratio (for large ratios), as observed for DNA. Predictions of the critical concentration (expressed as volume fraction) differ considerably, however. Calculation of the critical volume fraction is not straightforward because the counter-ion atmosphere must be included. Short DNA molecules can be modeled as spherocylinders with an effective volume

$$V_n = \frac{4}{3} \pi a^3 + \pi a^2 L$$

where $a$ is effective radius and $L$ is length = (number of nucleotide pairs) $\times 3.4 \times 10^{-8}$ cm for B form DNA (15). The effective hard sphere radius of DNA determined by Brian et al. (15) for $C_i = 0.2$ M Na$^+$ is 21 Å, in good agreement with theoretical calculations of Stigter (23). The effective radius of DNA at $C_i = 0.1$ M Na$^+$ used here may be slightly larger.

Critical volume fractions calculated from Equation 1 for 147- and 234-np length DNA for an effective radius of 21 Å were 0.82 and 0.54, respectively, at 25°C. These values are significantly higher than the corresponding values of 0.54 and 0.38 predicted by the equation of Flory (14). Critical volume fractions predicted by other treatments are significantly smaller than those of Flory (see Fig. 8 of Ref. 15). The discrepancies between calculated and predicted critical volume fractions are not due to errors in length, since changes of ±20 bp altered volume fractions calculated according to Equation 1 by <2%. Reasonable agreement with the critical volume fractions predicted according to Flory were obtained for an effective radius of 3 Å. Agreement with the critical volume fraction calculated according to Onsager (10) or Lasher (13) was obtained only by setting the effective radius nearly equal to the true DNA radius (9.8 Å, see Ref. 15), a physically unreasonable result. An effective radius of 3 Å, though smaller than determined at lower DNA concentrations, is still larger than the effective radius of 13 Å reported for DNA at $C_i = 1.0$ M Na$^+$ (15). Additional data are required to determine if counter-ion atmosphere contraction is a feature of the phase transition and to readily compare theoretical approaches.

It is important to note that the phase change observed here is distinctly different from the “bundling” of DNA fibers at low ionic strengths described recently by Mandelkern et al. (24). Besides different ionic strength requirements, these processes differ in response to chelating agents and in fundamental thermodynamics. Bundling is inhibited by chelating agents and facilitated by heavy metals and an increase in temperature (i.e. bundling is driven by a positive change in entropy) (24). Formation of the anisotropic phase at high DNA concentrations occurs in the presence of chelating agent and is driven

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by a negative change in enthalpy (opposed by a slight negative change in entropy).^2

The favorable enthalpy change of the anisotropic DNA phase transition is surprising since DNA interactions are usually repulsive. At high concentrations, however, strong competition must occur between DNA and added cations and anions for limited water of solvation. A small negative enthalpy change can be rationalized in terms of rearrangement of solvent structure and DNA ion atmosphere to maximize electrostatic and dipolar interactions, with a consequent decrease in total entropy. Ordering of the ion atmosphere is suggested by the splitting and increase in linewidth of the inorganic phosphate resonance in concentrated DNA solutions (Fig. 5). More NMR studies of this and other solution components (e.g. Na') may provide insights into aspects of the ordering process.

Intermediate States of DNA Interactions—DNA motional dynamics monitored by 3C and 31P NMR were strongly concentration-dependent. Dynamic effects are expected at high DNA volume fractions. The surprising results are observations of major differences in dynamics at various DNA sites with increasing concentration. Effects on dynamics were primarily observed as significant decreases in signal intensities. As described below, losses of integrated signal intensities can occur only if 

\[ T1 \] and \n
\[ T2 \] values of the affected resonances are greatly changed, i.e. if motions of the respective sites are greatly slowed. Since intensities, particularly of C1', C3', and C5' resonances, decreased without concomitant changes in linewidths (related to \n
\[ T2 \] values) or NOEs at concentrations far below the phase transition, we must conclude that 147-np DNA existed in two or more states over the entire concentration range studied. Similar effects were observed for 234- and 437-np DNA over a range from 6 to 60 mg/ml (data not shown).

An intermediate, partly ordered state is predicted by theories of solutions of rodlike particles (10-14). As described by Flory (14), transition to a fully anisotropic phase should occur only when its free energy is well below that of the isotropic phase, i.e. when the actual volume fraction exceeds the predicted critical volume fraction. A metastable biphasic region, in which isotropic and anisotropic phases coexist and the concentration in the anisotropic phase slightly exceeds that in the isotropic phase, is predicted in a narrow range of the phase diagram about the critical concentration (14). Such biphasic regions have been observed for poly-γ-benzyl-a,L-glutamate (26) and other polymers, and intermediate aggregated states of DNA have been suggested (27). In addition, many small anisotropic molecules undergo multiple transitions from less to more ordered liquid-crystalline phases, preceded by formation of partially ordered clusters or nucleation centers (28).

Large changes in C3' and C1',4' integrated intensities for 147-np DNA occurred from 79 to 131 mg/ml, well below the apparent critical concentration. Likewise, Brian et al. (15) observed a discontinuity, interpreted as a phase change, in the sedimentation equilibrium pattern of similar size DNA at an apparent concentration of 102 mg/ml. Effective DNA volume fractions at concentrations of 79 to 131 mg/ml calculated from Equation 1 for an effective radius of 21 Å are 0.37 to 0.61, in a range where phase changes are predicted (14). Thus it is reasonable to assume that solutions of 147-np DNA at concentrations between ~70 and 180 mg/ml contained a biphasic mixture. Local density and charge fluctuations presumably give rise to local anisotropic DNA clusters in a manner similar to precrystallization nucleation of small molecules. Such clusters are likely to be variable in size and degree of organization and to have a transient existence.

This model forms a self-consistent basis for describing the effects of increasing concentration on motional dynamics at different carbon sites in 147-np length DNA. In the fully isotropic phase, all DNA sites undergo rapid internal motions. Intermolecular interactions are sufficient to slow motions of the exocyclic, backbone-linked C5' carbon in approximately 50% of the DNA apparently occur at concentrations as low as 6.5 mg/ml. Motions of base and sugar ring carbons are not affected at concentrations from 6.5 to 46 mg/ml and hence are uncoupled from C5' motions. Above 46 mg/ml, in the expected biphasic range, motions of all sugar and base carbons except C2' become frozen in an increasing fraction of DNA, presumably due to formation and growth of large clusters. In the true anisotropic phase, motions of all carbons except C2' are essentially frozen.

C2' ring carbon motions clearly are much less sensitive to intermolecular interactions than are motions at other DNA sites. Modest line broadening of the C2' resonance in the biphasic region suggests only slight slowing or reduction in amplitude of C2' motions. Even above the critical concentration, C2' must have significantly more mobility than other DNA carbons in at least half of the DNA molecules. At present we cannot determine if loss of C2' intensity at the highest concentration is due entirely to homogenous line broadening of all resonances, or if some resonances are unobserved due to extreme broadening.

The Nature of DNA Dynamics Monitored by 3C and 31P NMR—The pattern of effects of DNA interactions on motions at different sites is consistent with configurational properties of the polynucleotide chain. The concept that B form DNA undergoes high frequency longitudinal (bending) and torsional oscillations with periods considerably shorter than the persistence length (~500 Å or 147 np) is now established from extensive dynamics measurements and theoretical treatments (reviewed in Ref. 29, and 30). In accord with this view, we have found that an extensive 3C NMR relaxation data set obtained by us for sugar and base carbons of 120-160-base pair length DNA over a wide range of frequencies is generally (excepting C2' data only) best fit by a model which assumes that C-H vectors undergo rapid (~nanosecond) motions restricted to lie within cones with half angles of approximately 20-25°. These rapid motions are superimposed on slow, axially symmetric overall tumbling. Simple anisotropic rotational models or jump models used to interpret more limited 3C and 31P NMR data (1-4, 31-33) do not fit our multi-field data set.

The conic diffusion model is a physically reasonable description of motions resulting from a combination of local longitudinal and torsional helix deformations. Bending motions will be severely restricted in ordered DNA phases because of the necessity for efficient packing. In the framework of the conic diffusion model, restrictions of bending motions will reduce the cone half-angle, thereby increasing the predicted \n
\[ T1 \] values and linewidths and decreasing NOEs. The precise magnitudes of these effects at specific DNA sites will depend on geometric factors, on the frequency distributions and relative RMS angular displacements associated with bending versus torsional deformations, and on any additional motions that are uncoupled to periodic deformations. At present, little is known about the relative contributions of bending and torsional

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1. Senechal et al. (25) have also described ordering of solutions of ~170-nm long poly(A)poly(U) duplexes. This ordering was observed at low ionic strengths in the absence of chelating agents, and therefore probably is most closely related to the bundling described by Mandelkern et al. (24).

2. Loss of signal would result from immobilization in a liquid crystalline phase, with or without alignment induced by the NMR magnetic field.
motions to DNA dynamics monitored by NMR methods. Several proposed models have assumed that torsional contributions to the $^{31}$P $T_1$ and NOE are minimal, (2-4, 31-33) but Allison et al. (30) have recently argued convincingly for major torsional contributions.

In general, interpretations of $^{31}$P relaxation data are obscured by uncertain contributions from spin diffusion, various $^1$H dipolar interactions, chemical shift anisotropy, and perhaps, chemical shift anisotropy-dipolar cross relaxation terms. The potential for obtaining very different pictures of DNA dynamics from $^{31}$P and $^{13}$C NMR data is illustrated by our observation that the $^{13}$C NMR spectrum of DNA in nucleosome cores is almost unobservable, indicating that DNA motions are strongly restricted, yet the integrated intensity, linewidth, and $T_1$ of the $^{31}$P resonance of the same sample were nearly the same as observed for protein-free 147-np DNA. We therefore conclude that, at best, $^{31}$P NMR measurements yield a very incomplete representation of DNA dynamics.

Since protonated carbons are relaxed almost exclusively by defined dipolar $^3$H coupling, relaxation data can be interpreted in a straightforward manner. Loss of C5' intensity in the biphasic region could be due to a large increase in $T_1$, from $\sim0.25$ s (causing spin saturation at the 2-s pulse repetition rate employed) and/or a precipitous decrease in $T_2$, causing extreme broadening. Allison et al. (30) have argued that $T_2$ of the DNA $^{31}$P resonance is determined by slow overall molecular motions. If these arguments applied generally, disappearance of C5' resonance intensity would be due simply to the slower rotational rate of DNA in clusters. However, the same argument should apply to all resonances. The observation that C2' and C5' resonance intensities are not affected in the biphasic region demonstrates that rapid internal motions, and not overall rotation, principally determine $T_1$ for protonated carbons.

Decreases in C5' and other resonance intensities in the biphasic region can therefore be attributed primarily to losses in internal motions. Small contributions of overall motion to $T_2$ values of C2' and the phosphate may be reflected by the modest increases in linewidths observed in the biphasic region. Insofar as formation of anisotropic phases or clusters should profoundly diminish bending, but not torsional deformations, we conclude that $^{13}$C $T_2$ values and NOEs of DNA are principally determined by some combination of coupled, high frequency bending deformations and uncoupled motions.

Site-specific dynamics variations with concentration also identify certain features of uncoupled motions in DNA. Loss of bending deformations should strongly affect motions of backbone atoms. Pseudorotation and other sugar ring motions need not be eliminated, but the number of allowed configurations will be reduced. This behavior was observed. Prime examples are the freezing of exocyclic backbone C5' motions at low concentrations, and retention of significant ring C2' motions above the critical concentration. In addition, at intermediate concentrations in the expected biphasic region the backbone C3' carbon intensity dropped significantly, approaching the intensity of C5'. The effect of concentration on the other backbone carbon, C4', was obscured by overlap of resonances, but the intensity decrease of the composite C1',C4' band in the biphasic region was about half that observed for C3', suggesting that C4' motions were also suppressed.

We have noted that the conic diffusion model does not fit multi-field relaxation data for C2' as adequately as for data of the other carbons in double-stranded DNA.\(^2\)

Deviations from the model indicate that C2' motions include conic diffusion plus another fast motional component. We therefore conclude that restricted sugar ring motions involving rapid, large C2' excursions can occur without coupling to backbone motions. Such motional components are reasonable since C2' is the only doubly protonated sugar ring carbon.\(^6\) Likewise, pseudorotations of all ring and base carbons can occur without concomitant C5' motions.

Relationships to DNA Packing Conformations—The above observations pertain to current questions about mechanisms of DNA packing in vivo, and the apparent differences in B form DNA pitch in solution versus in fibers. Although we have primarily examined phase changes of 147-np DNA, we have noted that increasing the DNA length reduces the concentrations required to observe the biphasic region and anisotropic phase formation. Analogous condensation of long, semi-rigid (worm-like) random polymers is predicted from extensions of the theory of Flory (35) and has been observed for synthetic polymers (26, 35). Clearly, the intrinsic rigidity of DNA, and consequent excluded volume effects, are sufficient to guarantee collapse and ordering of DNA at high concentrations occurring in vivo.

Passive folding may occur in viruses and/or other simple organisms, but DNA in eukaryotic chromatin is coiled by histone proteins into discrete, 110-Å diameter, bead-like units (nucleosomes). Higher order folding of nucleosomes into ~250-Å diameter fibrils appears to involve supercoiling of the beaded fiber, not side-by-side association (reviewed in Ref. 36). A major function of histones may be prevention of DNA clustering at the first and second levels of DNA folding. Since the 250-Å fibrils are also stiff chains, ordering due to excluded volume effects could be important in the higher order nuclear organization of chromatin.

There is debate about the number of base pairs per turn of B form DNA determined in solution using superhelical DNAs (10.4-10.5 np) (37, 38) and the number determined by x-ray diffraction studies of DNA fibers and oriented gels (10.0 np) (34, 39). This debate centers about effects of intermolecular interactions on DNA conformation in fibers, on one hand, and the validity of measurements made in solution, on the other. Although our NMR measurements do not directly determine the DNA winding angle, we have shown that DNA conformational dynamics are strongly influenced at concentrations far below those in fibers or gels obtained by swelling oriented fibers (34). The low concentration conformation monitored with supercoiled DNA is a statistical average over many states allowed by bending and torsional deformations, and other less coupled motions. The conformation monitored by x-ray diffraction is also statistically determined, but bending and other motions are significantly restricted, i.e. only a subset of configurations allowed at low concentrations is sampled at high concentrations. Since statistical averaging to yield the same mean winding angle for states allowed at low and high concentrations would be extremely fortuitous, differences in winding angles should be expected. In fact, a change in DNA pitch seems to occur in DNA bundles described by Mandelkern et al. (24).

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\(^2\) R. L. Rill and P. H. Hilliard, Jr., submitted for publication.
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