Liquid crystalline phases in concentrated aqueous solutions of Na\(^+\) DNA

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ABSTRACT

Concentrated aqueous saline solutions of short (146-base-pair) DNA fragments suddenly become turbid and iridescent when the DNA concentration is slightly increased or the temperature is decreased. Microscopic examination through crossed polarizing filters shows that turbidity and iridescence is due to formation of a liquid crystalline DNA phase similar to cholesteric liquid crystals formed by other semirigid, but nonelectrolyte, chiral polymers. Several distinct textures of the liquid crystalline phase or phases are observed depending on DNA concentration, temperature, and method of sample preparation. Textures observed include spherulites with Maltese crosses, striated and highly colored ribbons, whorls of periodic interference fringes, and colored flakes. The liquid crystalline DNA phase coexists in metastable equilibrium with the isotropic phase over a relatively narrow temperature/concentration range—approximately 175–250 mg/ml and 25–62°C (limit of measurements). At higher concentrations and temperatures above ~25°C, the solutions appear fully liquid crystalline. When concentrated solutions are cooled below room temperature, crystals form due to precipitation of supporting electrolyte. A partial phase diagram is reported for the isotropic → liquid crystal → crystal transitions of solutions of DNA in buffered saline (2 M Na\(^+\)). The general features of this phase diagram and the critical DNA volume fraction for formation of the anisotropic phase are consistent with the observed and theoretically predicted phase behavior of rodlike or semirigid nonelectrolyte polymers.

Rill et al. (1) found that concentrated solutions of short, relatively homogeneous-length DNA (~500 Å, from nucleosome cores) undergo a sudden phase change to a liquid crystal-like state, characterized by appearance of turbidity and iridescence and by decreases in intensities of several NMR resonances, with slight increases in DNA concentration or decreases in temperature. Brian et al. (2) noted a turbid phase near the cell bottom upon equilibrium sedimentation of similar DNA solutions and attributed this phenomenon to a liquid crystalline phase (3). Ordering was also noted in x-ray-scattering studies of concentrated solutions and gels of high molecular weight DNA (4, 5). Formation of DNA liquid crystals in aqueous solutions of simple electrolytes has not been examined in detail, however. Here I report microscopic observations of a cholesteric liquid crystalline phase formed in aqueous saline solutions of DNA and present a partial phase diagram for transitions of such solutions between isotropic and liquid crystalline phases. These phase transitions, which occur with only Na\(^+\) as the DNA counterion, are distinct from the collapse of DNA at low ionic strengths that is caused by heavy metals, polycations, or organic solutes (6–11). Spontaneous ordering of DNA solutions at near physiological concentrations is of fundamental significance to considerations of mechanisms of DNA packing in vivo and may be related to the behavior of other semirigid biopolymers.

METHODS

Preparation and Characterization of DNA. DNA was isolated from nucleosome cores obtained by digestion of calf thymus chromatin with micrococcal nuclease after removal of histone H1 (1). Polycrylamide gel electrophoresis in denaturing 12% and non-denaturing 6% gels, with Hpa II restriction endonucleases and pUC9 DNA as markers, showed that the median DNA length was 146 base pairs (bp), with >90% of the DNA between 140 and 170 bp. Approximately 5% of the DNA was “tight” dinucleosome size (280–320 bp), 1% was >340 bp, and <1% was smaller than 120 bp. Isolated DNA was precipitated from 0.3 M sodium acetate with 3 volumes of cold ethanol, washed twice with cold 80% ethanol, dried in vacuo, and then dissolved in small volumes of 90 mM NaCl/5 mM sodium cacodylate/3 mM sodium azide/1 mM disodium EDTA, final pH 6.8. DNA isolations were carried out in buffered 50 mM EDTA, and at least 1 mM EDTA was included in all subsequent buffers to eliminate heavy metal binding to DNA. Glass-distilled water and analytical grade reagents were used for all solutions.

Microscopy. Samples, either in a 2-mm-path-length cuvette or between glass slides separated by ~20-μm (Handi-wrap film) or ~80-μm (laminating plastic) spacers and sealed with wax, were viewed with a Leitz Ortholux II microscope equipped with an Orthomat-W camera system and white-light illumination. Polarizing filters (Hoya Optical, Tokyo, Japan) were placed on the light stage and above the objective and oriented to achieve maximal extinction in the absence of sample, unless stated otherwise. Slides were cleaned by soaking in concentrated nitric acid and washing with 1% sodium bicarbonate followed by distilled water. Partial alignment of the liquid crystalline phase was achieved by rubbing slides 50 times with a Kimwipe along the long axis prior to sample application. Alignment is presumed to be caused by development of static charge on the slide.

Phase Transitions. These were detected by monitoring changes in transmittance at 600 nm with temperature using a Cary 219 UV/visible spectrophotometer with thermistor accessory and thermostatically monitored cell compartment. Samples were contained in a 2-mm-path-length, Teflon-stoppered cuvette. Temperature changes were controlled using a Lauda refrigerated circulating bath and a Yellow Springs Instruments temperature programmer typically set at a heating (cooling) rate of 0.1°C/min. Sample concentrations were varied by adding 50-μl aliquots of buffer to the cuvette. Uniform solutions were achieved by warming the cuvette to 50–55°C for a minimum of 4 hr, with occasional mixing by repeated inversion. The most concentrated samples were carried through 2 or 3 slow warming/cooling cycles over a period of 2–3 days, to assure reproducible behavior, before the reported data were acquired. To prevent DNA denatur-

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Abbreviation: bp, base pair(s).

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ation, temperature was never permitted to exceed 65°C. The integrity of DNA was examined after over 30 heating/cooling cycles. Electrophoresis under denaturing conditions showed that the DNA size distribution was unchanged from that of the original sample and similar to that observed on nondenaturing gels. Thermal denaturation of the DNA in 50 mM NaCl/10 mM Tris-HCl, pH 7.6/1 mM Na2EDTA occurred sharply with a $T_m$ of 80.4°C and a hyperchroomicity of 42%, demonstrating that the DNA was fully double-stranded. A small amount of precipitate formed when the stock solution was first warmed to 65°C. This precipitate, possibly a trace of residual protein or denatured A+T-rich DNA fragments, did not interfere with subsequent measurements and represented <0.1% of the total sample mass.

DNA concentrations were determined from the 258 nm absorbancies of dilutions of 50-μl aliquots of sample dispensed with a positive-displacement micropipette (Drummond, Broomall, PA), assuming $E_{1%}$ = 200. The accuracy of these measurements was estimated as ±5% by weighing successive aliquots on a Cahn microbalance.

RESULTS

Observations made below are best described in the context of established behavior of semirigid polymers. Macromolecular ordering is entropically driven and can occur despite repulsive interactions to maximize free volume. Theories of two-component nonelectrolyte solutions of rodlike or semirigid polymers predict several solution states depending on solvent strength, temperature, solute axial ratio, and concentration (2, 3, 12–18). A fully anisotropic, nematic, or cholesteric liquid crystalline phase is predicted at a high critical-volume fraction (depending approximately inversely on axial ratio) of rodlike solute. At slightly lower concentrations, isotropic and anisotropic phases are approximately equal in chemical potential and coexist in a metastable, biphasic state. The predicted phase behavior has been observed for several homopolymers in nonaqueous solvents (3, 19–24). For example, Robinson in 1956 described the cholesteric morphology of the fully anisotropic phase of poly(benzyl-L-glutamate) in dimethylformamide, and of spherulites of the anisotropic phase suspended in the isotropic phase, as observed microscopically through crossed polars (19, 20). Miller and coworkers (20–23) have shown that the phase behavior of poly(benzyl-L-glutamate) agrees well with predictions of Warner and Flory (18).

Concentrated solutions were prepared by dissolving nucleosome-core DNA (sodium form) in a small volume of buffer saline. Solutions with DNA concentrations of 240 and 267 mg/ml remained turbid and iridescent after several days at room temperature, in agreement with previous observations (1). Microscopic examination through crossed polars showed that turbidity was due to a liquid crystalline phase and not gross aggregation or failure to dissolve DNA particles. Different “textures” were observed, depending on sample concentration, history, and thickness. The 240-mg/ml sample, when placed between an 80-μm spacer on a stroked glass plate (see Methods), contained banded spherulites and broad, striated, and multicolored “ribbons” oriented along the stroking axis. Ribbons were surrounded by a dark, nonbirefringent background and interconnected by thinner ribbons (Fig. 1A). Spherulites with Maltese crosses were observed when this sample was placed on an unrubbed slide without coverslip (not shown, but see Fig. 4). The more concentrated sample (267 mg/ml) between 80-μm spacers produced a kaleidoscope of colors in patches with generally blurred outlines. Superimposed on these patches were whorls of periodically spaced lines (Fig. 1B). Striations, but not colors, were observed when samples were viewed without polarizers. Light reflected from both slides was highly iridescent, changing color with changes in reflection angle.

![Fig. 1. DNA liquid-crystal textures viewed microscopically through crossed polarizing filters. All samples were multicolored. Images were photographed in color but printed in black and white. (A) Liquid crystalline ribbons obtained when 240-mg/ml sample prepared by warming was cooled to room temperature and placed on stroked plates separated by an 80-μm spacer. (B and C) A more concentrated sample (267 mg/ml) prepared as in A. The flaky texture in C was obtained after specimen B was cooled to 3°C and then warmed. (Magnification ×84 in A and C; ×107 in B.)](image-url)
These observations show that DNA forms a cholesteric liquid crystalline phase similar to that of poly(benzyl-L-glutamate) and other chiral, semirigid polymers. At the lower concentration, the solution is biphasic. The cholesteric character of the latter phase is indicated by (i) spherulite formation, (ii) parallel striations due to periodic refractive index changes across cholesteric planes, (iii) iridescence due to wavelength- and angle-dependent, Bragg-type reflections from the planes, and (iv) stepped changes in transmittance of light through crossed polarizers with temperature for the 188-mg/ml solution.

The temperature and concentration dependencies of DNA phase changes were examined quantitatively by monitoring changes in transmittance at 600 nm due to scattering. Upon cooling to ~2°C, all samples became opaque, but not iridescent. Transmittance changes were subsequently monitored as samples were slowly heated and then cooled (Fig. 2). Three distinct phases were observed—the opaque phase, a turbid phase, and a clear phase. At all concentrations the opaque phase dispersed abruptly upon heating to 20–30°C, with a mid-transition temperature decreasing with decreasing DNA concentration (Figs. 2 and 3). Above this transition temperature, the most concentrated samples transmitted light weakly and transmittance increased gradually with temperature. The turbid phase passed light through crossed polarizers (Fig. 2) and exhibited large, positive circular dichroism from 350 to 700 nm (not shown). Phase transitions were reversed by slow cooling, but with reproducible hysteresis. Transition to the opaque phase occurred about 20°C below the dissolution temperature. Samples that were turbid at high temperatures became progressively more turbid with decreasing temperature, and hysteresis was modest (≤5°) above 30°C.

The opaque and iridescent phases were identified by microscopic examination of the cuvette at different transition stages. Textures of the most concentrated samples heated just above the first transition temperature were characteristic of a fully cholesteric phase. Upon further heating the sample became biphasic, developing numerous small, clear patches in an opaque-white, slightly iridescent network. Convective turbulences disrupted the network and caused streaming birefringence. After resting, the previously disturbed solutions appeared uniformly turbid, and microscopic examination revealed spheroidal globules with multicolored surface patches (Fig. 4A). Upon cooling these samples became uniformly more turbid and highly iridescent due to formation of small, well-defined spherulites (Fig. 4B).

The nature of the opaque phase was revealed when samples were heated to ~1°C above the mid-transition temperature and then slowly cooled by 3–4°C. Large crystals grew from the cuvette surfaces (not shown). Most crystals were needle-like, but a few appeared rhombic or triclinic. A preliminary x-ray diffraction pattern obtained from a single large, rhombic-appearing crystal by A. H.-J. Wang (Massachusetts Institute of Technology) was typical of a small inorganic salt. Subsequent analysis by flame emission spectroscopy showed that the 267-mg/ml DNA solution contained 2 M Na+ in excess of the nucleotide concentration. Excess sodium originated from sodium acetate used in the DNA precipitation. This higher-than-expected salt concentration affects quantitative, but not qualitative, interpretation of the phase behavior, since the primary effect of increased ionic strength is to decrease the effective DNA radius (see below). Concentrated DNA samples exhaustively dialyzed against buffered 0.2 M NaCl retained the liquid crystalline phase; hence high ionic strength is not required. In the
following discussion the sodium ion concentration in these samples is assumed to be 2 M. Effects of deviations from this concentration are small because the effective DNA radius changes modestly for sodium concentrations greater than 1 M (2).

The observations provide the basis for a partial phase diagram describing transitions of DNA solutions between isotropic and anisotropic states (Fig. 3). The steep, moderately temperature-dependent boundary (solid line) between isotropic and biphasic (isotropic plus liquid crystalline) states is qualitatively consistent with theory (2, 12–18) and the behavior of poly(benzyl-l-glutamate) in good organic solvents (19–24). The present data are not sufficient to justify a rigorous comparison with available theories, but calculations of the DNA volume fraction required to observe the anisotropic phase are in good agreement with the behavior of solutions of rodlike particles predicted by the lattice-statistics treatment of Flory and coworkers (12, 13, 16–18). This theory cannot be applied directly because the effective radius of DNA plus counterion—and hence the effective volume fraction and axial ratio—depend on ionic strength. Brian et al. (2) applied scaled particle theory to model the DNA-plus-electrolyte system by a one-component system of effective spherocylindrical solute particles with hard-core radii determined by the interparticle distance where repulsive interactions are near kB T. This approach successfully describes the concentration and ionic-strength dependencies of the osmotic pressures of concentrated, isotropic DNA solutions. The effective radius determined for Na+ DNA in 2.0 M NaCl by Brian et al. (2) is 11.7 Å, and the corresponding axial ratio (length/diameter) for 146-bp DNA is 21. By comparison, the effective radius determined in 1.0 M Na+ is 13.0 Å and the axial ratio is 19. The density increment (ρp/ρC2) for dilute solutions of DNA in 2.0 M NaCl is 0.377 (25). Appearance of the anisotropic phase upon cooling occurred at ~62°C for a DNA concentration of 250 mg/ml, and at 25°C for a concentration of 175 mg/ml. Assuming that DNA in 2 M Na+ can be modeled by a hard spherocylinder using the above values for the effective DNA radius and density increment in 2 M Na+, the critical volume fractions corresponding to these DNA concentrations are 0.40 and 0.28, respectively. The former value is in good agreement with the critical volume fraction of 0.364 calculated by Flory and Ronca (16) for athermal solutions of rods with an axial ratio of 20.

The decrease in critical DNA volume fraction with temperature is similar to the behavior of poly(benzyl-l-glutamate) and other semirigid polymers. The implication of attractive interactions in the liquid crystalline DNA phase seems to contradict the conclusion of Brian et al. (2) that net interactions between DNA rods are repulsive at all distances. A contradiction does not necessarily follow, however, since Warner and Flory (18) have shown that slight anisotropy of dispersion interactions between polymer segments is sufficient to significantly lower the critical volume fraction for phase separation. For rods of axial ratio = 20, the predicted critical volume fraction decreases from the athermal limit of 0.36 to 0.27 when the anisotropic energy is ~1.5 kcal per segment (18). The effective segment length in the lattice treatment is the true length divided by the axial ratio, or about 7 bp for double-stranded DNA; hence very small anisotropic interaction energies between base pairs may be sufficient to explain the observed decrease in critical volume fraction with temperature. At least two types of anisotropic interactions might influence the ordered DNA phase: (i) normal dispersion interactions due to the greater polarizability of bases in directions perpendicular to the helix axis and (ii) electrostatic "dispersion" interactions due to fluctuations of the counterion atmosphere. The latter mechanism is suggested by the observed DNA orientation in strong electric fields due to polarization of the counterion atmosphere (26).

A fully liquid crystalline phase is expected at high concentrations of rods. Examination of the most concentrated samples just above the crystal dissolution temperature suggested existence of fully liquid crystalline phase (Fig. 1C), but residual isotropic phase might not be detected microscopically (21–24). The broken line in Fig. 3 indicates the approximate temperature, above the crystal dissolution temperature, at which an inflection was observed in the increase in transmission. This line is regarded tentatively as a boundary between the biphasic and liquid crystalline regions. Magnetic resonance or other techniques are required to unequivocally establish this boundary.

**DISCUSSION**

The results show that DNA spontaneously forms an ordered, liquid crystalline phase or phases similar to mesophases of rodlike or semirigid nonelectrolyte polymers. DNA concentrations required for ordering are high but well within the physiological range. Further, critical volume fractions for transition to polymer liquid crystalline phases decrease with increasing chain length up to a limit related to the persistence length (1, 2, 12, 13). It has been shown previously that increasing the DNA length to 437 bp decreases the critical concentration to approximately 90 mg/ml at room temperature (1). DNA packing ratios (ratio of initial to final longest dimension) in vivo range from several hundred to one in

**FIG. 4.** Microscopic textures of DNA phases obtained during heating/cooling cycles described in the legends to Figs. 2 and 3. The 188-mg/ml solution was viewed directly in the cuvette through crossed polars and photographed on Kodak Plus-X pan (black-and-white) film. (A) Globules (with multicolored patches) formed by heating moderately above the crystal dissolution temperature. (B) Spherulite clouds formed after heating above the clearing temperature and then cooling to room temperature. (×110.)
phages, bacterial nucleoids and sperm heads; to 40–60 to 1 in interphase chromatin fibrils (27). DNA concentrations corresponding to these packing ratios are extremely high. For example, the packing ratio of 520:1 found in T-even phage heads corresponds to an 80% solution of DNA. This concentration is also approached in sperm heads. Local DNA concentrations in interphase nuclei, though not so extreme, are still a few tens of milligrams per milliliter. The multiple mechanisms evolved for packaging DNA in vivo presumably reflect various compromises between requirements for economy of space and facility of gene information readout.

There is much interest in describing the mechanisms and work involved in DNA packaging in vivo. Discussions are often couched in terms of properties of DNA in dilute solutions (extreme chain stiffness and high excluded volume). These properties are consequences of both the base-stacked, double-helical structure and the polyanionic, self-repulsive character of DNA. Basic proteins are predominantly responsible for DNA charge neutralization in vivo. Dense folding of DNA in dilute solutions cannot be explained solely in terms of charge-shielding, however; hence basic proteins are often viewed as playing active, complex roles in driving DNA folding. Such views fail to take into account the fact that DNA packing in vivo takes place at high concentrations of DNA and other macromolecules. Under these conditions arguments based on the thermodynamic and statistical behavior of DNA in dilute solutions are not strictly appropriate.

DNA, like other semirigid polymers, can spontaneously form stable (or metastable), concentrated, partially ordered phases in the absence of attractive intramolecular interactions or obvious condensing agents such as multivalent cations. Favorable segment–segment interactions serve to augment, but not replace, the influence of chain stiffness on polymer ordering (12, 13, 18). Proteins and polypeptides may therefore serve primarily to direct the natural ordering process along a pathway consistent with functional requirements.

Studies of phase transitions of defined-length DNA fragments at different ionic strengths may provide a basis for extending current theories of semirigid polymer behavior to polyelectrolytes, including other biopolymers. In addition, the availability of aligned liquid crystals of DNA may permit new applications of methods employing polarized radiation to structural studies of DNA and DNA complexes with small molecules.

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