

A Liquid Crystalline Phase in Spermidine-Condensed DNA

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ABSTRACT Over a large range of salt and spermidine concentrations, short DNA fragments precipitated by spermidine (a polyamine) sediment in a pellet from a dilute isotropic supernatant. We report here that the DNA-condensed phase consists of a cholesteric liquid crystal in equilibrium with a more concentrated phase. These results are discussed according to Flory's theory for the ordering of rigid polymers. The liquid crystal described here corresponds to an ordering in the presence of attractive interactions, in contrast with classical liquid crystalline DNA. Polyamines are often used *in vitro* to study the functional properties of DNA. We suggest that the existence of a liquid crystalline state in spermidine-condensed DNA is relevant to these studies.

INTRODUCTION

The condensation of DNA by polyamines has been extensively investigated (for a review, see Bloomfield, 1991). Depending upon the concentration and length of the DNA molecules, condensation leads to three main types of structures:

- 1) In extremely dilute solutions (about 1 $\mu\text{g/ml}$ or below), long DNA molecules undergo a monomolecular collapse.
- 2) In very dilute solutions (about 10 $\mu\text{g/ml}$), microaggregates form with short or long molecules and remain in suspension.
- 3) In dilute solutions (about 1 mg/ml), large aggregates are formed that sediment readily.

Experimental data reveal the presence of a local ordering in all these condensed structures. In particular:

- Very dilute solutions of high molecular weight DNA in the presence of the tetravalent cation spermine show circular dichroism spectra characteristic of a cholesteric helical supramolecular ordering of DNA (Becker et al., 1979) also called ψ -type CD spectra (Lerman and Allen, 1974; Maestre and Reich, 1980). However, these states are metastable (Becker et al., 1979)
- The structure of the aggregates formed by spermine or by the trivalent cation spermidine with high molecular weight DNA molecules has been investigated by x-ray diffraction (Schellman and Parthasarathy, 1984; Rau and Parsegian, 1992). A strong equatorial reflection is observed and corresponds to a 25.5 Å Bragg spacing. From these data, the authors assume a hexagonal lattice (crystalline or liquid crystalline) (Schellman and Parthasarathy, 1984). Several types of liquid crystalline structures have been suggested for these aggregates (Schellman and Parthasarathy, 1984; Damaschun et al., 1986), but their occurrence has not been established.

Experimental data are compatible with three types of equilibrium structures: (i) a crystal, (ii) a liquid crystal (iii), or a structure possessing the geometry of the liquid crystal but lacking fluidity (a pseudomorphose: Bouligand, 1969).

Our goal is to investigate the nature of the polyamine-condensed DNA states with short DNA molecules under such conditions and to verify their thermodynamic stability.

MATERIALS AND METHODS

DNA fragments were prepared by selective digestion of calf thymus chromatin with micrococcal nuclease (Strzelecka and Rill, 1987). To assess the polydispersity of the fragments, a sample was 5'-end-labeled with T4 DNA kinase using $\gamma^{32}\text{P}$ -ATP. The length of the fragments was determined by electrophoresis on polyacrylamide gels. Quantification of the radioactive bands was carried out on a PhosphorImager (Molecular Dynamics). In view of the polydispersity of the sample, DNA was further fractionated according to the method described by Lerman et al. (1976). Successive aliquots of absolute ethanol were added to the TE solution (10 mM Tris, 1 mM EDTA, pH 7.6) containing 5 mg/ml DNA and 0.3 M ammonium acetate. DNA molecules were sequentially precipitated according to their length, from longer to shorter fragments.

DNA solution (25 mg/ml) was extensively dialyzed against 40 mM NaCl in TE buffer. Condensation of DNA was induced by the addition of spermidine (3 HCl, Fluka) (stock solution 392 mM in TE buffer) to the DNA solution. In the experiments reported here, the final concentrations were 1 mg/ml DNA, 5.7 mM NaCl, 10 mM MgCl_2 , and 15.7 mM spermidine. The sample was vortexed, incubated at room temperature for 15 min, and centrifuged at $11,000 \times g$ for 7 min. The DNA pellet was recovered and deposited between slide and coverslip. After being sealed with DPX, a neutral solution of polystyrene and plasticizers in xylene (Fluka), to avoid any dehydration, the preparation was observed in a polarizing Optiphot Nikon microscope.

RESULTS AND DISCUSSION

Nature of the condensed states

Condensation of DNA by spermidine from a 1 mg/ml solution of short DNA fragments was studied under a wide range of salt and spermidine concentrations (4–200 mM NaCl and 1–150 mM spermidine, in the presence of 0 or 10 mM MgCl_2) (Pelta, 1993). The efficiency of the precipitation, quantified by the amount of DNA sedimented in

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the pellet, varies from 0 to 98% depending on the salt and spermidine concentrations. We are dealing here with a precipitation process inasmuch as a more or less important part of DNA segregates from the initial homogeneous solution and sediments into a pellet (or a precipitate), which corresponds to a condensed DNA phase. However, the analysis of the pellets in polarizing microscopy (when they contain at least 12% of the total amount of DNA in solution) reveals that the condensed DNA is not in an amorphous state. A strong birefringence shows a supramolecular ordering of the DNA molecules. The sample is fluid even though its viscosity may vary largely according to the ionic conditions under which precipitation occurred. Multiple phases are found and their relative amounts depend on the ionic conditions (paper in preparation). Two different birefringent phases are usually observed, either separately or in coexistence. They are presented in Fig. 1 for a given experimental point chosen here as a representative example. In this particular case, 90% of the DNA was recovered in the pellet. Textures with fingerprint patterns led us to recognize a cholesteric liquid crystal with a large helical pitch. Tear-shaped defects lines characteristic of this phase (Bouligand, 1974) are frequently observed (not illustrated). The phase is fluid and flows spontaneously. In addition, another phase limited to small "germs" is also observed. In these "germs," the intensity of the transmitted light is more intense, suggesting a higher packing density of molecules. Their aspect is reminiscent of the textures of DNA columnar hexagonal liquid crystal (Livolant et al., 1989), but they never coalesce to form large domains. We cannot ascertain the molecular fluidity of these "germs," which are carried by the flow. Their structure remains to be characterized by x-ray diffraction experiments. Samples remain stable for months.

In our experiments, the length of the fragments varies from 130 to 600 bp with 50% of 146 (± 7) bp. The polydispersity of the sample was large compared with the monodispersity required for theoretical analyses, as discussed below. Therefore, we reproduced these experiments with further size-fractionated DNA as described in Materials and Methods. The DNA fragment length was then comprised between 110 and 180 bp. The same structures are observed, which rules out the possibility of a partition of DNA fragments into the two condensed phases according to their length. In the range mentioned above, polydispersity does not appear to have a significant effect on our data.

Stability of the condensed phases

To determine whether these states were thermodynamically stable phases or metastable states, the following experiments were performed:

i) Instead of adding spermidine to the DNA saline solution, condensation of DNA was obtained by dilution with TE buffer of a concentrated and homogeneous spermidine and DNA solution (according to Becker et al., 1979). The final concentrations were the same as described in the first experiment. Dilution was either slow (progressive addition of 900 μl of TE buffer at a rate of 10 μl per mn) or fast (addition of the total volume of TE buffer at once). In both cases, the amount of DNA recovered in the pellet was equal to $90 \pm 3\%$, as in the first protocol. The nature of the pellet was the same as described above, with the two different birefringent phases apparently in the same proportions. For long DNA molecules, slow and fast dilution experiments yield different circular dichroism spectra of polyamine-condensed DNA (Becker et al., 1979). This demonstrates the metastability of

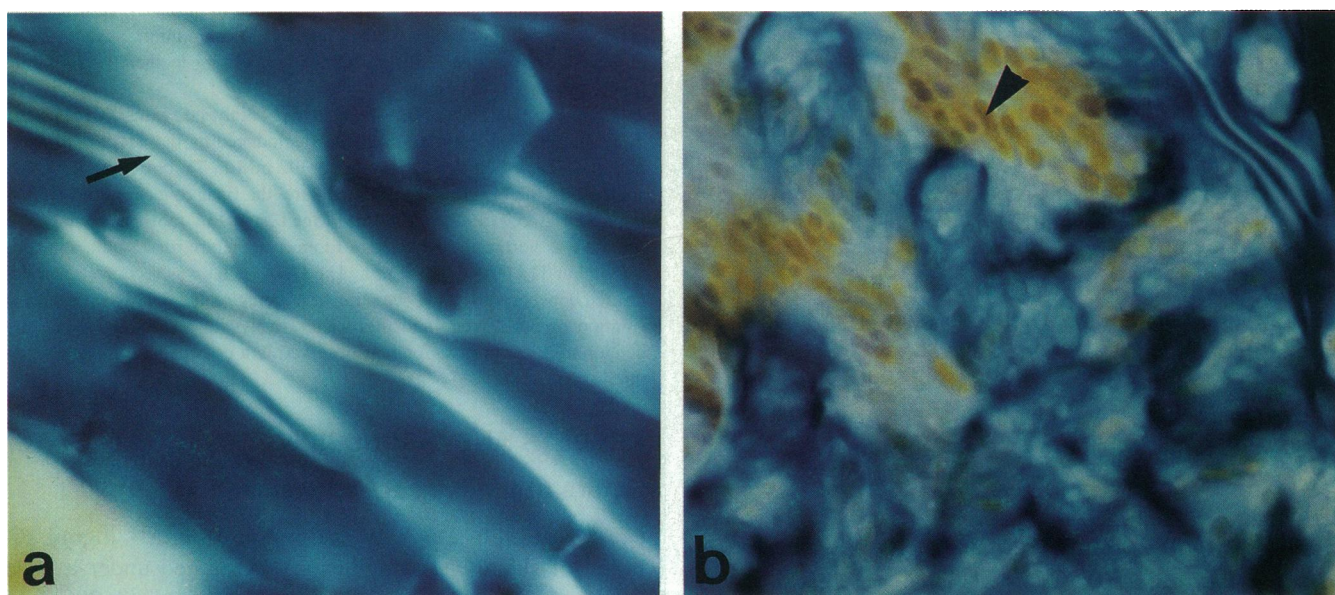


FIGURE 1 Aspects of the DNA aggregates observed between crossed polars in a polarizing Nikon Optiphot microscope. Cholesteric liquid crystal patterns with fingerprints (*arrow*) (*a*). "Germs" of a more concentrated phase (*arrowhead*) are also observed (*b*). Magnification 150x in *a* and 750x in *b*.

the states observed. With short DNA fragments, the textures are the same whatever the method used to reach the chosen final concentrations. These data suggest that we are dealing with stable phases.

ii) The DNA pellet was separated from the supernatant, dried down in vacuo for a few hours until it reached a solid consistency, and then resuspended in the supernatant solution. After one or two days of equilibration, the sample was shown to recover its initial structure and fluidity. In view of these two series of experiments, we may therefore assume that these phases are thermodynamically stable.

In summary, for the experimental point considered here, we have observed that condensation of short DNA fragments by polyamines leads to a triphasic phase system: a dilute isotropic solution (about 100 $\mu\text{g/ml}$) in equilibrium with two condensed birefringent phases of DNA. The liquid crystalline nature of one of these phases has been established. It is a cholesteric phase with a helical pitch much larger than the classical cholesteric helical pitch obtained with short fragments in NaCl solution (about 20 μm vs. 2.5 μm). The second phase may be either crystalline or liquid crystalline and remains limited to small "germs." It differs from the columnar hexagonal phase described in monovalent salt solution.

Short-range versus long-range supramolecular ordering in spermidine-condensed DNA

There are numerous data reporting condensation of DNA by polyamines. In dilute or extremely dilute solution, DNA molecules either collapse (when only a single long DNA molecule condenses on itself) or aggregate (when more than one molecule is involved) in the form of rods or toroids (Gosule and Schellman, 1978). These terms are chosen following the terminology given by Post and Zimm (1982). In our case, the use of short DNA fragments rules out the formation of collapsed structures. We are dealing with aggregation phenomena. However, in contrast with previous data, the aggregation is not restricted to microscopic domains such as toroids or rods but extends over long range distances, which allows the observation and characterization of these liquid crystalline phases. Our results may raise the question of the nature of the supramolecular organisation of DNA inside these microscopic aggregates. Could the toroids and rods be microdomains of a liquid crystalline phase? The question is open. Nevertheless, other parameters such as the length (and possibly circularity) of the DNA molecules used in these experiments undoubtedly introduce a number of constraints that modulate the self-organizing properties of short DNA fragments.

Interpretation of the results in the context of Flory's theory

From a theoretical point of view, our results can be discussed using Flory's theory for the ordering of rigid polymers (Flory, 1956). In this theory, the quality of the solvent is

described by the Flory-Huggins parameter χ , which measures the enthalpy of mixing (Flory, 1953). A schematic phase diagram of short rigid polymers in solution as a function of concentration c and χ is shown on Fig. 2. There are three regions: a pure isotropic phase (I) and a pure anisotropic phase (II) separated by a biphasic region (III). In the region of low χ (corresponding to good solvent conditions), the biphasic region is very narrow. When the solvent quality is is

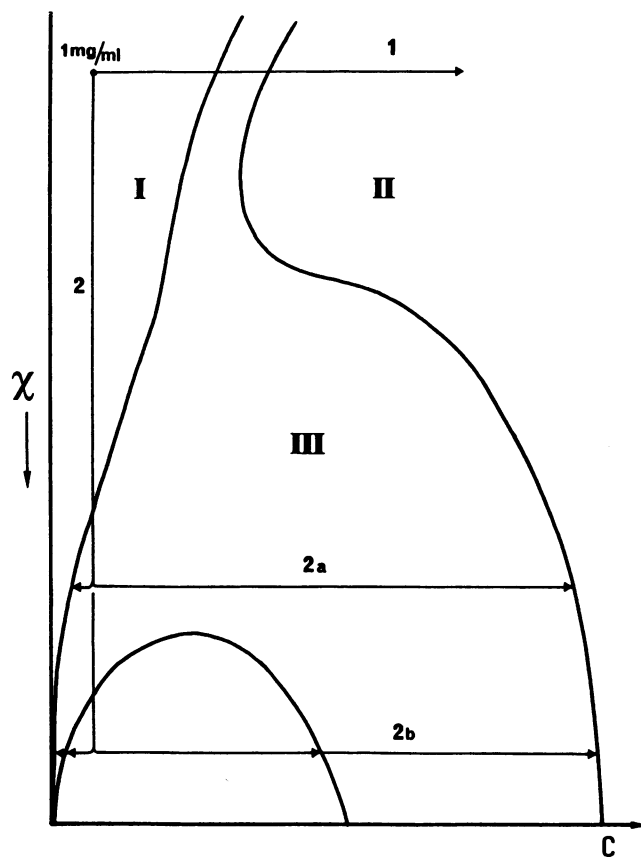


FIGURE 2 Simplified schematic phase diagram of rigid polymers in solution as a function of concentration c and χ (plotted as minus χ upwards). There are three regions: a pure isotropic phase (I) and a pure anisotropic phase (II) separated by a biphasic region (III). In the regions of low χ (corresponding to good solvent conditions), the appearance of the anisotropic phase occurs for relatively low concentrations of polymers and the biphasic region is very narrow. When the solvent quality is decreased, the biphasic region widens considerably and separates two regions that greatly differ in concentration. The concentration of the isotropic phase becomes very low, whereas that of the anisotropic phase is very high. The phase diagram is drawn for an axial ratio (L/d) of about 11.5 for 146 base pairs DNA fragments (Strzelecka and Rill, 1990). For this value, it is predicted to lack the critical and triple points expected for longer molecules (Khokhlov and Semenov, 1985; Warner and Flory, 1980). We superposed on this diagram the coexistence curve for the classical amorphous phase separation (Flory, 1953; Post and Zimm, 1982; Papkov, 1984). Path 1 illustrates the formation of liquid crystals in the presence of repulsive interactions, obtained by increasing DNA concentration. Path 2 corresponds to the experiments in which the overall DNA concentration is kept constant, and χ is increased by addition of spermidine (as reported here). This path may (b) or may not (a) cross the amorphous phase separation curve. The more concentrated phase is not represented on this diagram.

decreased, the biphasic region widens considerably and separates two regions that greatly differ in concentration. The concentration of the isotropic phase becomes very low, whereas that of the anisotropic phase is very high.¹

For DNA, the value of χ depends on the ionic composition of the solution. There are no available data giving the correspondence between χ and the composition of the solvent in mono-, di-, and trivalent cations. Our discussion, therefore, is of necessity qualitative

- In the presence of monovalent cations only, χ remains small and interactions between DNA molecules are net repulsive. Numerous experiments were performed in this part of the phase diagram (reviews in Yevdokimov et al., 1988; Rill et al., 1991; Livolant, 1991). There is no aggregation of the molecules and multiple liquid crystalline phases of DNA are obtained by increasing DNA concentration with a roughly constant low value of χ corresponding to good solvent conditions (*path 1* in Fig. 2). These experimental data were compared with Flory's theory by Strzelecka and Rill (1990). These liquid crystals can also be obtained by addition of an incompatible polymer to a dilute (a few mg/ml) DNA solution (Ψ -DNA; Lerman and Allen, 1974). Although the addition of the polymer corresponds to an increase in χ , in this particular case, the polymeric condensing agent is virtually excluded from the DNA-rich phase. The condensation results from the osmotic pressure exerted by the incompatible polymer, and the liquid crystalline phases are similar to those obtained by progressive dehydration.

- The addition of di- or trivalent cations creates a more complex situation. In the experiments reported here, the overall DNA concentration is kept constant and the solvent quality is decreased (increasing χ) by the addition of spermidine (*path 2* in Fig. 2). Then, aggregation may occur when approximately 90% of DNA charges are neutralized by counterions (Wilson and Bloomfield, 1979), according to Manning's condensation theory (Manning, 1978). Condensation involves a delicate balance between repulsive and attractive forces (review in Marquet and Houssier (1991) and Bloomfield (1991)). The electrostatic repulsion between DNA phosphates is by far the dominant thermodynamic barrier to DNA condensation. In our experiments, the addition of spermidine to the solution decreases the electrostatic repulsion between DNA molecules and, thus, increases the parameter χ . A more direct connection between Flory's theory and Manning's condensation theory remains difficult because in Flory's theory, the free energy is separated into two distinct terms: the entropy of mixing and the enthalpy of

mixing χ , whereas the Manning's condensation theory involves entropies of mixing of the counterions (in the condensed state and in the bulk solution) that cannot be directly compared with the enthalpic term χ .

In the experiments reported here, there are two possibilities: path 2a and path 2b. At this time, we do not know how to choose between them. If the quench is deep enough, we may enter the region of the diagram limited by the coexistence curve for the amorphous phase separation (Flory, 1953) (path 2b). Post and Zimm (1982) have proposed that this is the case for DNA condensation. In this case, the separation can proceed through the formation of two amorphous phases of different concentrations, which are metastable.

Polymer liquid crystals in poor solvent conditions

According to Flory's theory, in poor solvent conditions, a crystalline or a liquid crystalline phase is expected to coexist with a dilute isotropic one. To the best of our knowledge, this liquid crystalline state has not been observed experimentally with other rod-like polymers. Instead, a gelation process was reported with Poly- γ -benzyl-L-glutamate (PBLG) (Miller, 1978; Russo et al., 1987) and with the rod-like particle Tobacco Mosaic Virus (TMV) (Bernal and Fankuchen, 1941), which was not predicted by equilibrium theory.

With PBLG, which has been extensively studied, when an isotropic, biphasic, or ordered solution is brought into the wide biphasic region of the phase diagram, the solutions are always observed to form a transparent, mechanically self-supporting gel. These gels are reversible. The question of the molecular order in these gels remains unclear. An enormous variation in physical appearance, scattering power and contrast in the polarizing microscope has been reported (Russo et al., 1987). Usually, rods seem to be poorly aligned (Russo et al., 1987). Nevertheless, in particular conditions, "spongy" gels were occasionally observed in PBLG/toluene systems. Exceptionally, these gels were shown to present "a banded appearance similar to cholesteric liquid crystalline structure" (Russo et al., 1987).

With TMV, appropriate pH or ionic conditions also lead to a so-called gel that was never well characterized (Bernal and Fankuchen, 1941; Fraden et al., 1985).

Our observations of a liquid crystal state are in good agreement with theoretical predictions. It is nevertheless unusual to discover that a precipitate (or an aggregate) has a liquid crystalline nature. Indeed, the two terms implicitly suggest an amorphous state, whereas liquid crystalline phases are characterized by order and fluidity properties. The existence of a liquid crystalline state in poor solvent conditions requires a delicate balance of attractive and repulsive forces. Overall attractive forces are needed to induce precipitation from dilute solution and yet they must be weak enough to prevent 3D crystallization.

¹ The use of Flory's theory may be questioned in the case of DNA molecules. Indeed, this theory applies to rod-like molecules. Short DNA fragments are more accurately described as semi-flexible persistent polymers. For such molecules, Khokhlov and Semenov (1985) proposed an extension of Onsager's theory (Onsager, 1949) to treat attractive interactions (see van der Schoot and Odijk (1990) for a critical discussion of this approach). This theory leads to a phase diagram very similar to the one proposed by Flory.

Possible biological implications

In conclusion, we have observed a liquid crystalline phase in polyamine-condensed DNA. Polyamines are ubiquitous compounds involved in numerous cellular processes (Tabor and Tabor, 1984). They are often used in vitro for the study of the functional properties of DNA. They increase for instance the efficiency of replication (Schekman et al., 1974), transcription (Baeza et al., 1987), homologous pairing of DNA strands (Gonda and Radding, 1986), DNA renaturation (Christiansen and Baldwin, 1977; Sikorav and Church, 1991), cleavage by restriction enzymes (Pingoud et al., 1984; Srivenugopal et al., 1987; Oller et al., 1991), and catenation of DNA by topoisomerases (Krasnow and Cozzarelli, 1982). A correlation between aggregation of DNA and the stimulatory effect was demonstrated in several cases (Gonda and Radding, 1986; Krasnow and Cozzarelli, 1982), leading the authors to suggest that the aggregate was fluid. The liquid crystalline state reported here provides an experimental evidence in favour of the existence of such fluid aggregates. Whether a liquid crystalline state is also present under the conditions mentioned above remains to be established. DNA condensation by polyamines has also been considered from the point of view of prebiotic chemistry (Baeza et al., 1992). The existence of liquid crystalline phases could be relevant to such considerations.

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