PRAMANA
— journal of
physics

Vol. 65, No. 4
October 2005
pp. 723–729

Investigations on the liquid crystalline phases of cation-induced condensed DNA

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Abstract. Viral and nonviral condensing agents are used in gene therapy to compact oligonucleotides and plasmid DNA into nanostructures for their efficient transport through the cell membranes. Whereas viral vectors are best by the toxic effects on the immune system, most of the nonviral delivery vehicles are not effective for use in clinical system. Recent investigations indicate that the supramolecular organization of DNA in the condensed state is liquid crystalline. The present level of understanding of the liquid crystalline phase of DNA is inadequate and a thorough investigation is required to understand the nature, stability, texture and the influence of various environmental conditions on the structure of the phase. The present study is mainly concerned with the physicochemical investigations on the liquid crystalline transitions during compaction of DNA by cationic species such as polyamines and metallic cations. As a preliminary to the above investigation, studies were conducted on the evolution of mesophase transitions of DNA with various cationic counterion species using polarized light microscopy. These studies indicated significant variations in the phase behaviour of DNA in the presence of Li and other ions. Apart from the neutralization of the charges on the DNA molecule, these ions are found to influence selectively the hydration sphere of DNA that in turn influences the induction and stabilization of the LC phases. The higher stability observed with the liquid crystalline phases of Li–DNA system could be useful in the production of nanostructured DNA. In the case of the polyamine, a structural specificity effect depending on the nature, charge and structure of the polyamine used has been found to be favoured in the crystallization of DNA.

Keywords. Liquid crystalline DNA; alkali metal ions; DNA condensation; nanostructured DNA.

PACS Nos 61.30.-v; 71.20.Dg; 87.14.Gg; 81.16.Rf

1. Introduction

DNA condensation is the collapse of extended DNA chains into compact, orderly particles and this has received considerable attention in recent years due to its biological importance in DNA packaging in virus heads as well as in the

development of gene delivery vehicles [1–9]. A major requirement for gene therapy is the efficient transport of DNA through the cell membrane [1–3,8]. The first step in the packaging of DNA in nonviral gene delivery vehicles is the compaction of DNA to nanoparticles [1–3,8]. Multivalent cations such as polyamines, positively charged polymers, and peptides are known to provoke condensation of DNA to nanoparticles that appear as rods, toroids, or spheroids under the electron microscope [1–5,7–9]. Toroidal condensates are highly organized form of DNA and a recent study indicates the organization of DNA in a columnar hexagonal array in toroids [10]. As the DNA is concentrated (>1 mg/ml), the molecules spontaneously undergo unidirectional ordering (the solution starts to become birefringent under polarized light) and transform into liquid crystals of the 'cholesteric' type, which transforms into the 'columnar hexagonal' phase at higher concentrations [7,9]. The supercoiled DNA spontaneously organizes into the liquid crystalline phase to minimize the macromolecular excluded volume [11]. The liquid crystalline properties might be affected by the counterion layer (to neutralize the highly charged anionic polyelectrolyte nature of DNA) which determines the effective axial ratio and the excluded volume [12]. So, counterion neutralization is essentially required for the induction and stabilization of liquid crystalline DNA.

The induction of liquid crystalline phase has been achieved by earlier works mainly using low molecular weight fragmented DNA. Appropriate change of ionic concentration, pH and buffers might generate the same in high molecular weight DNA also. In the present work a comprehensive investigation of the LC phases of high molecular weight DNA in the presence of alkali metal and alkaline earth metal ions has been made and compared with those provoked by polyamines of varying structures.

2. Experimental part

Calf-thymus DNA was purchased from Worthington Biochemical (Freehold, NJ) and dissolved in 10 mM Na cacodylate buffer (10 mM Na cacodylate, pH 7.4, and 0.5 mM EDTA) at a concentration of 25 mM DNA phosphate (8.28 mg/ml). The weight average molecular weight of the DNA was 6×10^6. The observed A_{260}/A_{280} ratio of the DNA solution was 1.88, indicating that the DNA was free of protein contamination. The DNA sample was dialysed extensively against the Na cacodylate buffer. The concentration of calf-thymus DNA was determined by measuring the absorbance at 260 nm and using the molar extinction coefficient (ε) of 6600 M^{-1} cm^{-1}. The DNA concentration of 25 mM was selected for the present series of experiments because liquid crystalline phase transitions could be observed with this concentration of high molecular weight DNA in the presence of polyamines. For studies with the metal ions, DNA was dissolved in 0.1 M NaCl (pH 7). The dissolved DNA was then dialysed 4 times against NaCl (0.1 M). The final concentration of DNA was 7.143 mg/ml, determined from 260 nm absorbancy, assuming an extinction coefficient of 6600 $M^{-1}cm^{-1}$. Analytical grade LiCl, NaCl, KCl, RbCl, CsCl, $MgCl_2$, $CaCl_2$, $SrCl_2$ and $BaCl_2$ were used for the experiments.

DNA and polyamine solutions were stored at 4°C. The time of storing DNA/polyamine solutions before incubation has no effect on the nature of

liquid crystalline structures adopted by the DNA. The solutions were homogeneous at the start of our experiments.

Spermidine·3HCl, spermine·4HCl and N^4-methyl spermidine were purchased from Sigma Chemical Co. (St Louis, MO).

Polarizing microscopy

DNA was precipitated either directly on the glass slide or in an Eppendorf tube and centrifuged to sediment the precipitate for observation. The DNA precipitate was spread over the glass slides with a cover slip and sealed with a neutral solution of polystyrene and plasticizers in toluene to prevent dehydration of the sample. The preparations were observed under polarizing light or phase contrast light in a Nikon Optiphot Polarised Light Microscope equipped with a Nikon camera. All the preparations were optically negative (i.e. when the stage was rotated in a clockwise manner, the texture shift or extinction of disinclination lines occurred in a counter-clockwise direction) and showed negative birefringence (textures in black and white instead of a coloured pattern). After observing the initial phase appearance at 30°C, the preparations were incubated at 37°C for extended time periods to observe the phase changes until crystallization or complete darkening (isotropization) occurred.

For observations with metal ions, the solution was mixed with DNA, allowed to equilibrate for 2 h at 30°C, and then 20 μL of each metal ion complexed DNA solution was sandwiched between a clean microscopic glass slide and a cover slip, and the cover slips were then sealed with a neutral solution of polystyrene and plasticizers in toluene to prevent dehydration of the sample. The samples were then incubated at 37°C for 2–3 h. The slides were observed under a Nikon Optiphot Polarised Light Microscope equipped with a Nikon camera. Photographs of the prominent and distinct phases were taken.

3. Results and discussion

Although, in general, oligonucleotides and plasmids are used in gene transfer studies, high molecular weight DNA was selected for the present study because the LC behaviour was established mainly with low molecular weight fragmented DNA and it would be interesting to probe the feasibility of generating high molecular weight LC DNA. Alkali and alkaline earth metal ions are considered for the present study as some of them are essentially required as counterions along with vectors used in the preparation of DNA nanoparticles and also because a systematic study on their LC behaviour has not been reported. Our studies indicate that all the alkali metal ions used in the present study irrespective of their sizes provoke high molecular weight DNA to give highly birefringent anisotropic domains suggesting a supramolecular ordering of DNA molecules with polymorphous behaviour. It is interesting to note that the initial precholesteric blue phases, which is a transition from isotropic to cholesteric phase, are not observed in the present case except in the case of Li ions. This might be attributed to the ability of polyamines to

directly order the DNA molecules to the simple twist configuration of the more stable cholesteric phase. Two main phases, cholesteric and columnar hexagonal, either separately or in coexistence with variations in local conditions are observed. This is characterized by highly birefringent domains of oily streaks of finely divided textures with fingerprint patterns as observed by earlier workers on condensation of DNA by multivalent cations [6,7,9]. The presence of tear shaped defects attest to the formation of the cholesteric phase.

Typical photographs of the LC phases of DNA in the presence of metal ions and polyamines are shown in figures 1–4. According to Bloomfield *et al* [6] and Livolant and co-workers [7] in the case of condensation of DNA by multivalent cations or by dehydration, the formation of the hexagonally ordered columnar phase occurs at higher DNA concentration than that of cholesteric phase. In our study, it is interesting to note that the phase change occurred as a time dependent phenomenon. The cholesteric and hexagonal phases coexist in equilibrium over a concentration range whose width and boundaries depend on the polyamine concentration. The molecules are unidirectionally aligned with a lateral hexagonal order. Fan-shaped textures, which might have been formed from the original supple textures can be seen in the columns. The columnar hexagonal phase showed typical patterns of flower-like/dendrite domains whose homeotropic alignment prevented further analysis. Polyamines induce DNA precipitation which can be controlled to get nanoparticles.

Table 1 shows that all alkali metal ions, except Li^+, induced the liquid crystalline phase at a critical concentration of 0.89 mg/ml. However, liquid crystalline transition of DNA occurred only at a concentration of 3.75 mg/ml in the presence of 1 M of LiCl. It appears that up to a concentration of 3.75 mg/ml of DNA, liquid crystallinity of DNA is blocked by Li^+.

The observed differences between Li^+ and other alkali metal ions can be explained on the basis of the higher water of hydration of Li^+. An unusual stability was

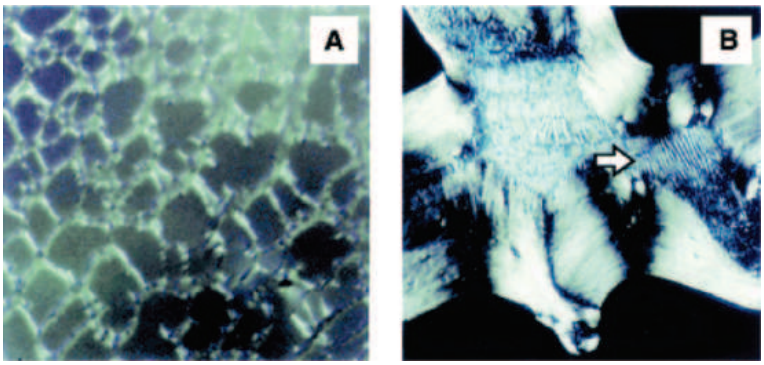

Figure 1. Liquid crystalline phase transitions of calf thymus DNA in the presence of spermidine. (**A**) Control. Calf thymus DNA solution (25 mM in a buffer containing 10 mM Na cacodylate, pH 7.4, and 0.5 mM EDTA) at 37°C (100 ×). (**B**) DNA (20 mM) mixes with 40 mM spermidine and incubated on a glass slide for 12 h at 37°C (200×). Cholesteric fingerprint texture is seen (marked by the arrow).

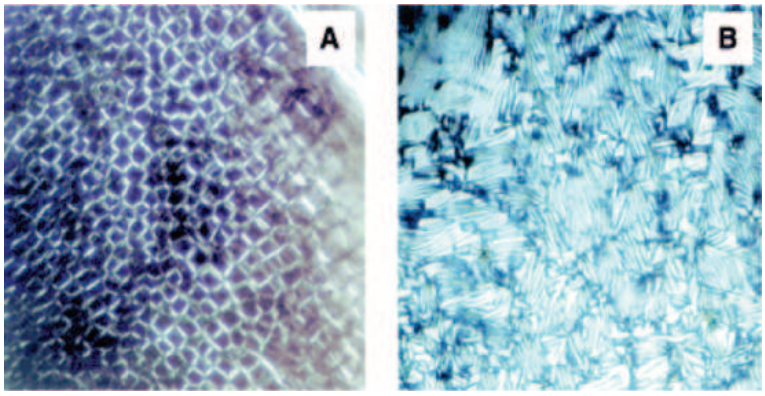

Figure 2. Liquid crystalline phase transitions of calf-thymus DNA in the presence of spermine. (**A**) DNA (20 mM in Na cacodylate buffer) mixed with 1 mM spermine and incubated on a glass slide at 22°C for 20 min (100×). The typical planar cholesteric phase is observed. (**B**) The glass slide in (A) was incubated for 10 h at 37°C and viewed through the plate under crossed polars (200×). Fingerprint texture with antiparallel grain boundaries is found.

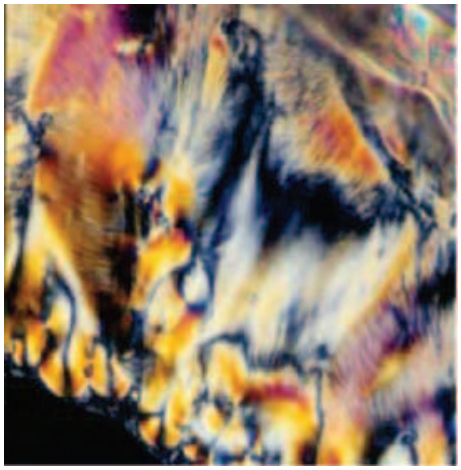

Figure 3. The biphasic texture with cholesteric fingerprint pattern and columnar fan-like texture of Li–DNA stable for two months. Magnification 10×.

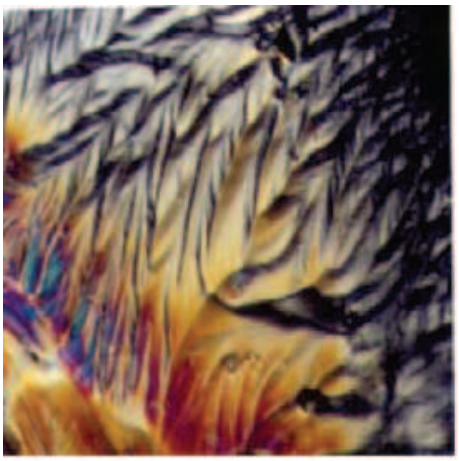

Figure 4. Columnar hexagonal herringbone pattern of Cs–DNA after 7 h of incubation at 37°C. Magnification 10×. Concentration of DNA dissolved in NaCl (pH 7) is ∼4 mg/ml.

observed for the liquid crystalline phases of DNA in the presence of Li^+ (stable upto two months compared to a few hours to few days in the cases of other metal ions, figure 3) and the phase transition from cholesteric to more ordered, columnar phase might be due to the complexation behaviour of Li^+ ion to DNA and the water

retaining capability of Li^+. We measured the time of transition from cholesteric to columnar phase which was found to be dependent on the size of the ion and the level of hydration. The transition is faster at high metal ion concentrations and when the size of the ion is larger. Thus, in the cases of Rb^+ and Cs^+, the transition occurred within few hours ($\sim$7 h) giving rise to textures typical of columnar hexagonal phase (figure 4) and the phases remained stable for 1–2 days. A behaviour similar to this phase transition was found in the case of DNA condensation in the presence of spermine, spermidine and N^4-methyl spermidine. As the charge density increased in the cases of spermine, spermidine and its N^4-methyl derivative, the transition from cholesteric to columnar phase took only a few hours for spermidine, whereas the time taken for transformation was around 12–48 h in the case of spermine and its N^1-acetyl derivative.

Our earlier work carried out with a series of structurally related cationic polyamines showed a structural specificity effect depending on the nature, charge and structure of the polyamine used. We also found that the increase in charge density decreased the critical concentration of the polyamines needed for the formation of LC phase.

4. Conclusions

The present studies show that monovalent and divalent cations as well as multivalent cations such as polyamines induce and stabilize liquid crystalline DNA exhibiting both cholesteric and columnar hexagonal phases above a critical concentration. Polyamines induce DNA precipitation which can be controlled to get nanoparticles. A structural specificity effect depending on the nature, charge and structure

Table 1. Liquid crystalline textures obtained when DNA concentration was varied keeping metal ion concentrations fixed.

DNA conc. (mg/ml)	LiCl (1 M)	NaCl (1 M)	KCl (1 M)	RbCl (1 M)	CsCl (1 M)
7.14	Nematic schleiren textures	Cholesteric planar	Cholesteric and columnar hexagonal	Cholesteric and herring-bone like texture	Iridiscent cholesteric Texture
3.57	Isotropic	Cholesteric	Cholesteric oily streak and finger-print pattern	Columnar hexagonal turning to crystal phase	Cholesteric, columnar hexagonal
1.79	Isotropic	Cholesteric	Cholesteric	Columnar hexagonal turning to crystal phase	Cholesteric
0.89	Isotropic	Isotropic	Isotropic	Isotropic	Isotropic
0.45	Isotropic	Isotropic	Isotropic	Isotropic	Isotropic
0.22	Isotropic	Isotropic	Isotropic	Isotropic	Isotropic

of the polyamine used has been found to be favoured in the crystallization of DNA. The size of the cation influences time of transition from cholesteric to columnar hexagonal phases. Although Mg is believed to block LC formation totally, we have observed LC phases under certain conditions. An unusual stability observed for the liquid crystalline phases of DNA in the presence of Li^+ might be due to the complexation behaviour of Li^+ ion to DNA and the water retaining capability of Li^+. Li as counterions can (of course after ascertaining its safety aspects) therefore be used in the preparation of stable DNA nanoparticles when used along with suitable polymer vectors.

Acknowledgements

Thanks are due to DST, New Delhi for providing funds under the new nanotechnology initiative.

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