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ORIGINAL PAPER

Re-entrant cholesteric phase in DNA liquid-crystalline dispersion particles

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Abstract In this research, we observe and rationalize theoretically the transition from hexagonal to cholesteric packing of double-stranded (ds) DNA in dispersion particles. The samples were obtained by phase exclusion of linear ds DNA molecules from water-salt solutions of poly(ethylene glycol)—PEG—with concentrations ranging from 120 mg ml^{-1} to 300 mg ml^{-1}. In the range of PEG concentrations from 120 mg ml^{-1} to 220 mg ml^{-1} at room temperature, we find ds DNA molecule packing, typical of classical cholesterics. The corresponding parameters for dispersion particles obtained at concentrations greater than 220 mg ml^{-1} indicate hexagonal packing of the ds DNA molecules. However, slightly counter-intuitively, the cholesteric-like packing reappears upon the heating of dispersions with hexagonal packing of ds DNA molecules. This transition occurs when the PEG concentration is larger than 220 mg ml^{-1}. The obtained new cholesteric structure differs from the classical cholesterics observed in the PEG concentration range 120–220 mg ml^{-1} (hence, the term 're-entrant'). Our conclusions are based on the measurements of circular dichroism spectra, X-ray scattering curves and textures of liquid-crystalline phases. We propose a qualitative (similar to the Lindemann criterion for melting of conventional crystals) explanation of this phenomenon in terms of partial melting of so-called quasinematic layers formed by the DNA molecules. The quasinematic layers change their spatial orientation as a result of the competition between the osmotic pressure of the

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solvent (favoring dense, unidirectional alignment of ds DNA molecules) and twist Frank orientation energy of adjacent layers (favoring cholesteric-like molecular packing).

Keywords Liquid-crystalline ds DNA dispersion particles · Circular dichroism · Abnormal optical activity · X-ray scattering curves · Textures · Hexagonal packing of ds DNA molecules in dispersion particles · Quasinematic layers · Cholesteric packing of ds DNA molecules in dispersion particles · Hexagonal → cholesteric phase transition

1 Introduction

Various DNA phases obtained by dissolution of lyophilized double-stranded (ds) DNA samples (of high and low molecular masses) in water-salt solution (with an adjustment of concentration with a buffer solution) have been known since 1961 [1–7]. An alternative method to prepare ds DNA phases is to increase the ds DNA concentration by ultrafiltration through a membrane whose pore size allows passage to water and ions but not to ds DNA molecules [8–10]. Many works in this field have been dedicated to the problem of packing ds DNA molecules in structures (phases) formed by such methods [11–14]. The performed studies proved the formation of ds DNA liquid-crystalline (LC) phases. Bulk ds DNA phases resemble viscous solutions in which neighboring DNA molecules are orientationally ordered, while keeping their ability to slide relative to each other. Upon the increase of the linear ds DNA concentration in water-salt solution, the isotropic solution transforms (via either blue phases or precholesteric stages) into the cholesteric LC phase. Under poly(ethylene glycol)—PEG—osmotic pressure compression the hexagonal densely packed structure becomes favorable and more concentrated phases can be considered even as true crystals [15–19].

The phase exclusion of the low molecular mass, rigid, linear, ds DNA (molecular mass $<1 \times 10^6$ Da) is realized under intensive mixing (stirring) of equal volumes of water-salt solutions containing PEG with the water-salt solutions containing a very low (5–10 μg ml^{-1}) concentration of ds DNA at room temperature. As a result, the formation of the liquid-crystalline dispersions (LCDs) of these molecules takes place [20, 21]. The principal scheme of ds DNA dispersion formation in water-salt PEG-containing solution is shown in Fig. 1. The factors that affect the efficiency of ds DNA phase exclusion have been determined earlier [20, 21]. The particles of the low molecular mass ds DNA dispersions are microscopic droplets of concentrated DNA solution, which cannot be taken in hand. A liquid mode of packing ds DNA molecules in particles of LCD prevents their immobilization on the surface of a membrane filter.

The mode of ds DNA packing in dispersion particles can differ from the mode typical of bulk LC phases due to the 'size effect' [22, 23]. Surprisingly this question (on the mode of ds DNA molecules packing in dispersion particles obtained at the phase exclusion from water-salt polymeric solutions) does not seem to have been addressed experimentally so far.

In this paper, we answer this question by comparing the circular dichroism (CD) spectra of LCD particles, X-ray scattering curves and textures of LC phases formed from these particles. Our goal is to determine the type of ds DNA molecules packing in the LCD particles in PEG-containing solutions. We show that the result crucially depends on the osmotic pressure of the solvent. The phase exclusion of ds DNA molecules from water-salt PEG solutions at room temperature results in the formation of LCD particles with a cholesteric or hexagonal mode packing of these molecules. We demonstrate that when the dispersion particles with the

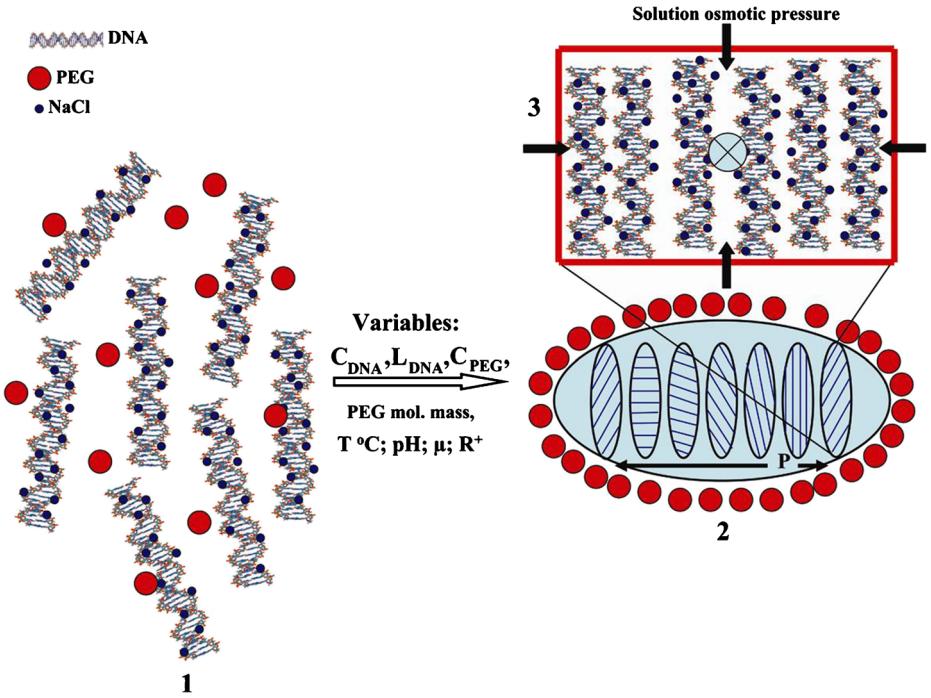

Fig. 1 Schematic diagram of the formation of liquid-crystalline dispersion as a result of phase exclusion of linear ds DNA molecules [molecular mass $(6–8) \times 10^5$ Da)] from PEG-containing water-salt solutions. 1. Initial ds DNA molecules in a PEG-containing solution (PEG molecules are shown by filled red circles) at C_{PEG} below the critical value. 2. Particle of the DNA liquid-crystalline dispersion (encircled by an oval) formed at C_{PEG} above the critical value. (**P** is the pitch of the helical twist of the spatial structure of a dispersion particle.) 3. A quasinematic layer formed by ds DNA molecules (the cross at the center denotes the rotation axis of the helical structure). [Note that DNA molecules in a quasinematic layer are located at a distance, which changes from 2.5 to 5.0 nm depending on the osmotic pressure of the solution (thick red frame and dark arrows are symbols of osmotic pressure of a PEG-containing solution)]

hexagonal packing of ds DNA molecules are heated, it results in a hexagonal → cholesteric phase transition. The transition is accompanied by the appearance of abnormal optical activity in these particles. Our results are rationalized by means of a concept of orientationally ordered quasinematic layers formed by ds DNA molecules, with a parallel alignment in the hexagonal structure. These layers can adopt a twisted configuration upon a temperature increase; and as a result of this process, a spatial helicoidal structure (the cholesteric phase) is formed.

2 Experimental

2.1 Preparation of ds DNA LCDs

Calf thymus depolymerized ds DNA (Sigma, USA) with a molecular mass of ~$(0.6–0.8) \times 10^6$ Da after additional purification was used. DNA concentration in the water-salt solutions was determined spectrophotometrically using the known value of the molar extinction coefficient ($\varepsilon_{max} = 6{,}600\ M^{-1}\ cm^{-1}$).

A PEG sample (Serva, Germany; molecular mass of 4,000 Da) was used without additional purification.

An initial water-salt solution of PEG (0.3 M NaCl, $C_{PEG} = 600$ mg ml^{-1}) was prepared by dissolving weighed portions of NaCl and PEG in 0.002 M Na^+ phosphate buffer (pH ~ 7.0).

DNA LCDs in water-salt solutions with different PEG concentrations (120 mg ml$^{-1} \leq C_{PEG} \leq 300$ mg ml^{-1}) were prepared according to the phase separation (condensation) technology described previously [21]. According to this method, equal volumes of water-salt solutions, one of which contained the DNA and the other the PEG (concentration of the DNA and PEG solution were twice as high as than the desired final value) were mixed and intensively stirred for 1 min. The resulting mixture was left at room temperature for 1 h to complete the formation of the DNA LCD.

The DNA concentration in the studied PEG-containing solutions was 30 μg ml^{-1}.

2.2 Optical measurements

The CD spectra were recorded using an SCD-2 portable dichrometer (produced by the Institute of Spectroscopy of the Russian Academy of Sciences, Troizk, Moscow). The CD spectra were represented as being dependent on the difference between the absorption intensities of left- and right-handed polarized light [ΔA; $\Delta A = (A_L - A_R)$] and on the wavelength (λ) [24].

In all cases, rectangular quartz cells (Hellma 100 QS, Germany) with an optical length of 1 cm were used.

The temperature in the temperature-controlled compartment of the dichrometer ranged from 20 to 80 °C and was set by the program 'Temperature control', which is part of the dichrometer SCD-2 software. After setting the required temperature, a cell containing 2 ml of DNA LCD was heated for 10 min in a temperature-controlled compartment and the registration of a CD spectrum in a wavelength range of 220 (250)–350 (600) nm was processed.

2.3 Theoretical calculations of the CD spectra of ds DNA LCD particles

To describe the peculiarities of ds DNA molecule packing in LCD particles we used their CD spectra. According to theory [24, 25], these spectra provide very useful and robust global information about the mode of ordering ds DNA molecules within many spatially distributed, independent, small-size particles (in contrast to microscopic observation of thin layers of LC ds DNA phases, which are much more sensitive to experimental detail) [19, 21].

To compute the CD spectra of ds DNA LCD particles under various conditions, the theoretical approach [25] was used. This approach is based on the theoretical consideration of electromagnetic wave absorption by large molecular aggregates (in our case, ds DNA LCD particles) [26, 27]. To study the optical characteristics of this structure, a LCD particle can be divided into cells and each cell can be considered as a single efficient chromophore. These efficient chromophores, which are absorbing dipoles, have an ordering that is similar to a cholesteric helix with a pitch P.

Following the method from [26, 27] we express the electric field at the point where a particular chromophore occurs as a superposition of the field of incident light and the electric fields that are generated by induced dipoles of all other chromophores of the system. As a result, we derive a self-consistent system of linear equations [25–27] for the field amplitudes. Then, the full extinction cross-section needed to compute the circular dichroism is determined by the optical theorem and we end up (after rotational averaging) with the expression below for

the experimentally measured difference between rotation-averaged cross-sections corresponding to left- and right-hand polarized light:

$$\langle\sigma_L\rangle - \langle\sigma_R\rangle = 4\pi k \sum_i \sum_j j_1(kr_{ij})\,\mathbf{a}_{ij}\cdot\hat{\mathbf{r}}_{ij}$$

where $j_1(kr_{ij})$ is a spherical Bessel function of the first kind of order 1 and $\mathbf{r}_{ij} = \mathbf{x}_j - \mathbf{x}_i$, $r_{ij} = |\mathbf{r}_{ij}|$ $\hat{\mathbf{r}}_{ij} = \mathbf{r}_{ij}/r_{ij}$

The vectors $\mathbf{a}_{ij}$ are written in the form:

$$\mathbf{a}_{ij} = \left[\left(\mathrm{Im}\mathbf{A}^{ij}\right)_{32} - \left(\mathrm{Im}\mathbf{A}^{ij}\right)_{23},\ \left(\mathrm{Im}\mathbf{A}^{ij}\right)_{13} - \left(\mathrm{Im}\mathbf{A}^{ij}\right)_{31},\ \left(\mathrm{Im}\mathbf{A}^{ij}\right)_{21} - \left(\mathrm{Im}\mathbf{A}^{ij}\right)_{12}\right].$$

where $\mathbf{A}^{ij}$ are 3×3 matrix blocks of matrix $\mathbf{A}$ (see details in [25]) and each block describes mutual polarizability and electromagnetic interaction between the *i*th and *j*th chromophores.

We use the expression above to compute the circular dichroism of ds DNA LCDs as a function of the relevant physical parameters, namely, the cholesteric pitch (P), the particle size (D), etc.

2.4 Structure analysis of ds DNA LC phases

For structure analysis and polarizing microscopy, we used pellets (~5 mg) of the LC phases obtained by slow-speed sedimentation (5,000 rev. min^{-1}, 40 min, 4 °C; centrifuge K-23, Germany) of the volumes of solutions containing LCD particles formed as a result of DNA molecule condensation ($C_{DNA} = 30$ μg ml^{-1}) at different PEG concentrations (120 mg $ml^{-1} \le C_{PEG} \le 300$ mg ml^{-1}).

The measurements of small-angle X-ray scattering (SAXS) curves were performed on an Amur-K laboratory diffractometer (Special Design Section, Shubnikov Institute of Crystallography of the Russian Academy of Sciences, Moscow) using Kratky-type (infinitely long slit) geometry in the range of the momentum transfer $0.12 < s < 10.0$ nm^{-1}, where $s = (4\pi\sin\theta)\,\lambda^{-1}$, 2θ is the scattering angle and $\lambda = 0.1542$ nm is the X-ray wavelength [28]. The experimental data were normalized to the incident beam intensity and then corrected for collimation errors according to a conventional procedure [29]. (These obtained results are presented in Fig. 3a).

Synchrotron SAXS measurements were performed as well at the DICSI station of the Kurchatov Synchrotron Radiation Source in Moscow, Russia equipped with 2D MAR165 CCD. The scattering intensity [I(s)] was recorded in the range of the momentum transfer $0.4 < s < 10$ nm^{-1} and $\lambda = 0.162$ nm is the X-ray wavelength. The measurements were carried out in a temperature range from 20 °C to 80 °C. (The obtained results are presented in Fig. 6 and in Table 1).

Experimental SAXS data were preliminarily processed using the PRIMUS program, and Bragg peaks on the SAXS curves were analyzed using the PEAK program [30].

The computer programs, SAXS data processing [31] and the standard parameters used, i.e., the d value (the repeating distances of the periodical motifs in the crystalline regions ($d = 2\pi/s_{max}$), corresponding to the peak position (s_{max}) on the scattering patterns (i.e., the mean distance between close-packed DNA molecules or the mean interhelical distance [29]). The mean long-range order dimension L (the size of crystallites); and the degree of disorder in the system Δ/d (Δ is the mean-square deviation of distances between neighboring, regularly packed structure motifs; d - the mean distance between close-packed DNA molecules) have

Table 1 Structural characteristics of the phase formed via low-speed sedimentation of DNA LCD particles measured at four different temperatures

Temperature, °C	s_{max}, nm^{-1} (± 0.1 nm^{-1})	d, nm (± 0.1 nm)	L, nm (± 3.0 nm)	Δ/d (± 0.01)
20	2.52	2.50	100	0.06
40	2.47	2.54	104	0.05
60	2.41	2.60	113	0.05
80	2.38	2.64	118	0.05

Conditions of DNA LCD particle formation: $C_{DNA} = 30$ μg ml^{-1}, $C_{PEG} = 300$ mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer; $T = 20$ °C

Note: $\mathbf{s_{max}}$ - the wave vector

d - the periodicity of the structure (the interhelical distance)

L - the crystallite size

Δ/d - the degree of disorder [Δ - the mean square deviation of distances between neighboring regularly packed structural elements; **d** - the periodicity of the structure (the interhelical distance)]

been described elsewhere [29–31]. Experimental errors of the determination of these parameters do not exceed 2%.

Thin layers of DNA LC phases were studied using a polarization microscope (Carl Zeiss Jena, Germany). A sample of DNA LC phase together with a small volume of the corresponding PEG-containing water-salt solution (~20 μl) were placed in a thermostatted quartz microcell (thickness is 20 μm) [32–34].

3 Results

3.1 Formation of ds DNA LCDs as a result of phase exclusion of molecules from PEG solutions and properties of these dispersions

Double-stranded DNA LCDs are formed as the result of phase exclusion when water-salt solutions of DNA are mixed with water-salt solutions of a synthetic, water-soluble, chemically neutral polymer, i.e., PEG (Fig. 1).

The evaluation of ds DNA LCD particle dimensions by means of various methods (low-speed centrifugation, ultraviolet (UV) light scattering, dynamic light scattering, etc.) demonstrates that the mean diameter of formed LCD particles is close to 500 nm and that one particle contains approximately 10^4 DNA molecules [20, 21]. These results were recently confirmed by a direct experimental size estimation of specially created rigid ds DNA dispersion particles immobilized on the surface of a nuclear membrane filter [35].

One can stress that the most useful method to determine the character of ds DNA molecule packing within small-sized particles at their small concentration (30 μg ml^{-1}) in PEG-containing solutions is circular dichroism [36].

The experimentally measured CD spectra of initial, linear, ds DNA molecules (B-form) and ds DNA LCDs in water-salt PEG-containing solution ($C_{PEG} > C^{cr.}_{PEG}$) are presented in Fig. 2a (curves 1–5, respectively). PEG is an optically inactive, isotropic compound [37] that does not possess the CD spectrum. Curve 1 in Fig. 2a is typical of the linear ds DNA B-form. The measured value of molecular dichroism ($\Delta\varepsilon$) is equal to about 2.0–2.4 $M^{-1}cm^{-1}$ [38]. (The

amplitude of the band typical of the B-form measured in the optical scale for registration of the CD spectrum of ds DNA LCD is equal to $(180–220) \times 10^{-6}$ optical units. This band is practically 'invisible' in the optical scale used for registration of the CD spectra of ds DNA LCDs).

In an interval of PEG concentrations up to 110 mg ml^{-1} , the shape of the CD spectra is unchanged, which shows that ds DNA in water-salt PEG-containing solutions conserves the parameters of the linear B-form. This was confirmed by the results of SAXS [38] of ds DNA pellets obtained under these conditions.

The increase in PEG concentration in an interval from 120 mg ml^{-1} to 170 mg ml^{-1} is accompanied by phase exclusion of ds DNA from PEG-containing water-salt solutions and the appearance of an intense negative band in the CD spectrum in the region, where the DNA nitrogen bases absorb ($\lambda_{max} \sim 270$ nm, curves 2–4). The growth of the amplitude of this band (without a change in shape of the CD spectra) is conditioned by a rise in the number of ds DNA dispersion particles (from 0 up to ~100%) in solution.

The formation of DNA dispersions in PEG solutions with C_{PEG} 170–200 mg ml^{-1} results in a decrease in the amplitude of the band at λ_{max} 270 nm in the CD spectra (curve 5).

Figure 2b shows the dependence of the amplitude of the intense negative band in the CD spectra in a PEG interval from 110 до 220 mg ml^{-1}, i.e., at $C^{cr.}_{PEG} < C_{PEG} < C^{limit}_{PEG}$. One can see that in domain (**I**), the phase exclusion of ds DNA molecules is accompanied by the appearance of an intense negative band in the CD spectrum in the region where the DNA nitrogen bases absorb. [Under the conditions used, the single-stranded (or denatured) DNA does not condense and cannot go into the composition of dispersion particles [39].]

The formation of DNA dispersions in PEG solutions with $C_{PEG} > 220$ mg ml^{-1} does not result in the appearance of an intense band in the CD spectrum (Fig. 2b, domain **II**), despite the fact that under these conditions, the concentration of dispersion particles is constant and equals ~100%.

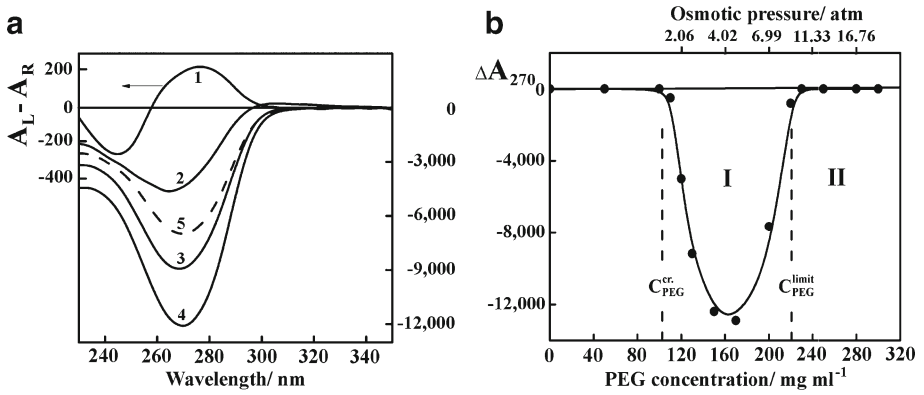

Fig. 2 **a** CD spectra of the water-salt solution of linear B-form DNA (curve 1, left ordinate) and DNA LCDs formed in water-salt solutions with different concentrations of PEG (curves 2–5, right ordinate): 1 - $C_{PEG} = 0$, 2 - $C_{PEG} = 120$ mg ml^{-1}, 3 - $C_{PEG} = 130$ mg ml^{-1}, 4 - $C_{PEG} = 170$ mg ml^{-1}, 5 - $C_{PEG} = 200$ mg ml^{-1}. $C_{DNA} = 30$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. $\Delta A = (A_L - A_R) \times 10^{-6}$ optical units, $L = 1$ cm, $T = 22$ °C. **b** Dependence of the amplitude of the band in the CD spectra of DNA and DNA LCDs ($\lambda = 270$ nm) upon PEG concentration. $C_{DNA} = 30$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. $\Delta A_{270} \times 10^{-6}$ optical units; $L = 1$ cm; $T = 22$ °C. $\mathbf{C_{PEG}^{cr.}}$ and $\mathbf{C_{PEG}^{limit}}$ – the 'critical' and 'limit' PEG concentrations, which are necessary for the existence of the DNA cholesteric LCDs, are shown

(Note that a conventional aggregation of DNA molecules can be accompanied by an appearance of low-intensity bands in the CD spectra of these aggregates but the reasons for the appearance of these bands remain unclear [40].)

Hence, in Fig. 2b, it is possible to define two domains (**I** and **II**) of PEG concentrations, in which formed ds DNA dispersion particles are discriminated by their optical properties.

In Fig. 3a, as an example, the small-angle X-ray scattering curves for two phases that were formed as a result of the low-speed sedimentation of the ds DNA dispersion particles obtained under different PEG concentrations are demonstrated. The inset in Fig. 3a shows the dependence of the d value upon C_{PEG}. In domain **I** (120 mg ml^{-1} ≤ C_{PEG} ≤ 220 mg ml^{-1}), the d value changes from 3.8 nm to 2.8 nm, whereas in domain **II** (220 mg ml^{-1} ≤ C_{PEG} ≤ 320 mg ml^{-1}), the d value decreases only insignificantly (from 2.8 to 2.4 nm).

The thin layer of ds DNA phases formed in domain **I** possesses classical fingerprint textures (Fig. 3b), typical of the cholesteric LC phases formed as a result of increase in ds DNA concentration in water-salt solutions [12, 17, 19, 41].

Thus, one can say that in domain **I**, the formation of ds DNA LCDs results in an appearance of an intense band in the CD spectra of these dispersions. (According to theory [24, 25], an intense band in the CD spectrum of ds DNA dispersions testifies, as a minimum, to the spatially twisted packing of DNA nitrogen bases in dispersion particles.) The ordered packing of neighboring ds DNA molecules in the structure of LCD particles followed from results of SAXS and their spatial twist results follows from ds DNA fingerprint texture.

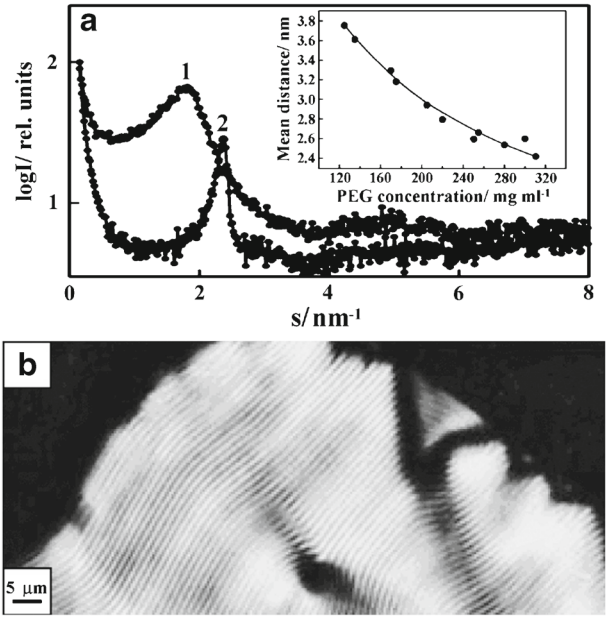

Fig. 3 Experimental small-angle X-ray scattering curves from phases formed via low-speed sedimentation of DNA LCD particles. Conditions of DNA LCD particle formation: 1 - C_{PEG} = 170 mg ml^{-1}, 2 - C_{PEG} = 300 mg ml^{-1}. C_{DNA} = 30 μg ml^{-1}; 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. Inset: the dependence of the mean distance between DNA molecules (d) in liquid crystalline phases upon PEG concentration. **a** Experimental curves of small-angle X-ray scattering by liquid-crystalline phases formed via low-speed sedimentation of DNA LCD particles. **b** Fingerprint texture typical of the DNA classical cholesteric liquid-crystalline phase. C_{PEG} = 170 mg ml^{-1}; 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. Bar corresponds to 5 μm

Conditions of domain **II** (C_{PEG} = 220–300 mg ml^{-1}) correspond to a regime of close packing of ds DNA molecules [41], i.e., to parallel ordering of neighboring ds DNA molecules. Under these conditions, the thin layers of ds DNA LC phases formed as a result of the low-speed sedimentation of the ds DNA dispersion particles possess a non-specific texture with 'black-bent-lines' [20]. (One can note that the packing of the ds DNA molecules in a hexagonal LC phase is not accompanied by the appearance of abnormal optical activity [19, 42].)

Hence, in domain **I**, the LCD particles formed from ds DNA molecules at room temperature possess a structure not only with ordered packing of neighboring ds DNA molecules but, as a minimum, the spatially twisted packing of DNA nitrogen bases. The mode of ordering of ds DNA molecules in the LCD particles in domain **II** significantly differs from that in domain **I** and corresponds, more probably, to their hexagonal packing.

3.2 The effect of temperature on the optical properties of ds DNA LCDs

It is well-known (and can be expected) that the heating of ds DNA LCD particles obtained in PEG domain **I** results in a decrease in the abnormal band amplitude in the CD spectra up to a zero value. This process depends on the concentration of PEG and takes place at temperatures that are much lower than the so-called 'melting temperature' of the ds DNA molecules secondary structure, i.e., DNA denaturation [21]. [Note that the 'melting temperature' of ds DNA molecules in used solutions (0.3 M NaCl + 0.002 M Na^+ phosphate buffer (pH ~ 7.0) + PEG) is close to 93–95 °C [21].] The peculiarities of this process have been carefully investigated in a whole series of papers [20, 21, 43]. Despite the increase in temperature, the osmotic pressure of the PEG-containing solution [44, 45] in domain **I** remains high [log $\pi \sim 7$ (cm^{-2}) or 1.35–10.33 atm] [21]. Double-stranded DNA molecules cannot 'leave' the limited physical volume of LCD particles, and separation of ds DNA chains in LCDs is impossible for steric reasons [46–49]. The disappearance of the abnormal band amplitude in the CD spectra of LCDs formed in domain **I** corresponds to the transition to a structure with a distorted location of the neighboring DNA molecules within the LCD particles. This is known as 'CD melting' of the spatial structure of LCD [21].

As for the DNA dispersion particles formed in domain **II**, the problem of spatial organization of the DNA molecules in these particles and the problem of the effect of temperature on the properties of these particles were not investigated until now.

In Fig. 4a, the changes in the amplitudes of the band in the CD spectra of ds DNA LCDs (λ = 270 nm) formed in the water-salt solutions with different PEG concentrations (220 mg $ml^{-1} \leq C_{PEG} \leq$ 300 mg ml^{-1}) upon the temperature increase are compared. One can see that the heating of dispersions formed at $C_{PEG} \geq$ 220 mg ml^{-1} (domain **II**) is accompanied by an unexpected optical effect, i.e., an intense negative band in the CD spectra arises for all ds DNA dispersions, which did not possess such a band at room temperature (Fig. 2b). This band suggests a formation of a twisted structure with the orientationally helically ordered DNA nitrogen bases, as a minimum.

Therefore, the DNA dispersions obtained at high PEG concentrations can be transformed, by a rise in the solution temperature, into a new structure that is characterized by an intense band in the CD spectra. Also, the value of the amplitude of this band ($\Delta\mathbf{A}_{270}$) for these dispersions is essentially higher than that of the values shown in Fig. 2b.

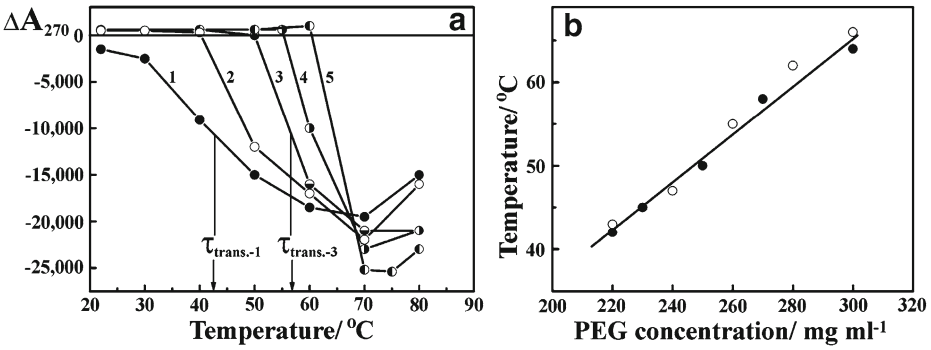

Fig. 4 **a** Dependence of the amplitude of the abnormal band in the CD spectra of DNA LCDs ($\lambda = 270$ nm) formed in water-salt solutions (0.3 M NaCl + 0.002 M Na^+ phosphate buffer) with different PEG concentrations on the temperature. 1 - $C_{PEG} = 220$ mg ml^{-1}, 2 - $C_{PEG} = 240$ mg ml^{-1}, 3 - $C_{PEG} = 260$ mg ml^{-1}, 4 - $C_{PEG} = 280$ mg ml^{-1}, 5 - $C_{PEG} = 300$ mg ml^{-1}. $C_{DNA} = 30$ μg/ml. $\Delta A_{270} \times 10^{-6}$ optical units; $L = 1$ cm. *Arrows* show the mean values of transition temperature ($\tau_{trans.}$) of DNA LCDs from the optically inactive to optically active state for two different condition sets. **b** Dependence of the value $\tau_{trans.}$ versus PEG concentration. Different points relate to two series of independent experiments

The sharp increase in the optical activity of the DNA dispersion particles by a temperature rise, which looks like a phase transition, represents by itself univocal evidence of a change in the spatial structure of the DNA dispersion particles.

From our experimental data we can define the average temperature at which the dispersion particle transforms from an optically inactive state into an optically active state as τ_{trans}. Figure 4b demonstrates that the higher the PEG concentration in the solution, the higher the value of τ_{trans}.

An intense negative, abnormal band in the CD spectra of ds DNA LCDs, which is induced by a temperature rise from 22 to 80 °C (Fig. 5, curve 2), does not go back, by cooling of the solutions from 80 °C to 22 °C (Fig. 5, curve 3), to the values that were specific to the initial packing of the DNA molecules in the dispersion particles (Fig. 5, curve 1). Such behavior seems to be quite natural for first-order phase transitions, at which the existence of metastable structures (and hysteresis) is possible. For the realization of the phase transition [that is, the

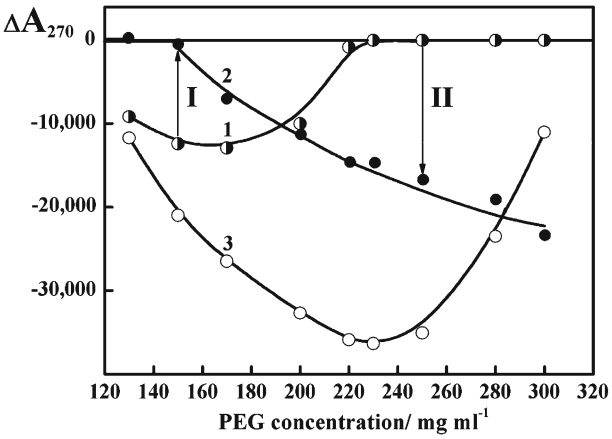

Fig. 5 Dependence of the amplitude of the abnormal band ($\lambda = 270$ nm) in the CD spectra of DNA LCDs formed at room temperature (curve 1), heated to 80 °C (curve 2) and cooled to room temperature (curve 3) on PEG concentration. $C_{DNA} = 30$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. $\Delta A_{270} \times 10^{-6}$ optical units; $L = 1$ cm. Symbols **I** and **II** correspond to various PEG domains; *arrows* show the direction of the CD band amplitude change at temperature increase

transition from a metastable to a stable (the deepest minimum of the free energy) state], the system, due to thermal fluctuations, should overcome the potential barrier separating the metastable and stable structures. It requires the formation of a critical germ (nucleus) of a new phase and its further growth (the growth can basically occur due to the diffusion of the molecules and not demanded thermo-activated processes).

Thus, at the heating of the LCD particles, an initial cholesteric packing of the DNA molecules results in the disappearance of their spatial helical structure and is accompanied by a decrease in their optical activity to zero values. On the contrary, the heating of LCD particles with parallel ordering of neighboring ds DNA molecules is accompanied by the appearance of optical activity in these particles.

3.3 Effect of temperature on small-angle X-ray parameters of the DNA phase formed at $C_{PEG} = 300$ mg ml^{-1}

Taking into account the behavior of ds DNA LCD particles formed in domain II, the SAXS curves of the DNA phases obtained from these particles were measured at different temperatures. Figure 6 demonstrates, for example, the SAXS curves of the ds DNA phase formed at $C_{PEG} = 300$ mg ml^{-1}. All X-ray parameters used for the description of scattering curves are presented in Table 1.

Comparison of these data allows one to say that the temperature rise is accompanied by a very small displacement of the SAXS curve maximum and very small changes of d values, i.e., the mean distance between close-packed DNA molecules. The cooling of the formed DNA phase to room temperature (~20 °C) results in restoration of the X-ray scattering curve maximum and other X-ray parameters typical of the initial phase.

Hence, despite significant temperature alteration in abnormal optical activity of ds DNA LCD particles formed at $C_{PEG} = 300$ mg ml^{-1}, the density of ds DNA packing in these particles remains practically unchanged.

3.4 Constancy of ds DNA B-form at hexagonal packing of molecules in PEG-containing solution

The state of the ds DNA secondary structure under conditions of high PEG concentration ($C_{PEG} = 300$ mg ml^{-1}) was checked by application of an 'external chromophore' approach based on the theoretical consideration of the peculiarities of the CD spectra well-known for its

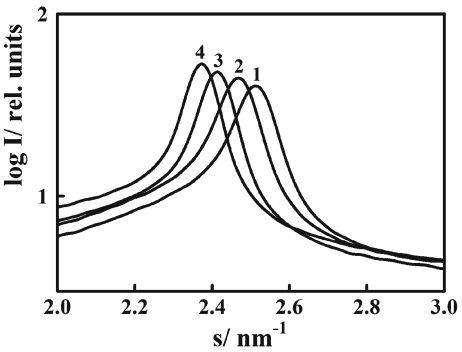

Fig. 6 Experimental small-angle X-ray scattering curves from the phase formed via low-speed sedimentation of DNA LCD particles measured at four different temperatures. 1–20 °C, 2–40 °C, 3–60 °C, 4–80 °C. Conditions of DNA LCD particle formation: $C_{DNA} = 30$ μg ml^{-1}, $C_{PEG} = 300$ mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer; $T = 20$ °C

low-molecular mass cholesteric liquid crystals [50–54]. This approach was developed for the case of ds DNA LCD particles [55] and recently modified [24, 25]. It takes into account both the twisted mode of ds DNA molecule packing and the results above concerning properties of dispersion particles. This theory allows one to describe and predict many optical peculiarities of ds DNA LCD particles.

Because linear, rigid ds DNA molecules contain chromophores (nitrogen bases), theoretical calculations [55] show that the appearance of the abnormal band in the CD spectrum (Fig. 2a) univocally testifies to the macroscopic (cholesteric) twist of neighboring nitrogen bases in the content of LCD particles. In the case of the ds DNA B-form, nitrogen bases are rigidly fixed at 90° with respect to the long axis of the ds DNA molecule. Hence, the twisted structure of DNA nitrogen bases means the twisted location of neighboring, linear, rigid ds DNA molecules. In order to stress this peculiarity, the term DNA cholesteric liquid-crystalline dispersions (CLCDs) or DNA cholesterics was used to signify these particles [35, 36, 55].

It is well-known that external compounds ('external chromophores' with an absorption band that does not coincide with the absorption band of nitrogen bases) can be specifically fixed and oriented with respect to the long axes of ds DNA molecules. For instance, 'external chromophores' can be rigidly fixed if there is intercalation between nitrogen bases of DNA B-form. Only in the case of the formation of CLCD from DNA molecules with 'external chromophores' in their composition should we expect the appearance of an additional abnormal CD band in the absorption region of these compounds.

As an 'external chromophore' we used a monomeric cyanine intercalating dye, SYBR Green I (SG) [57], which is highly selective for the native B-form of ds DNA molecules with a regular secondary structure. SG possesses an absorption band at $\lambda \sim 500$ nm.

Figure 7 demonstrates the obtained results. Curve 1 is the CD spectrum of ds DNA LCD formed at $C_{PEG} = 250$ mg ml^{-1} at room temperature. [We used $C_{PEG} = 250$ mg ml^{-1} but not 300 mg ml^{-1} in order to see the highest optical effect (see Fig. 5, curve 3)]. In accordance with the results shown in Fig. 2b, an abnormal band is absent in the region of adsorption on nitrogen bases ($\lambda \sim 270$ nm). The treatment of this LCD with SG (Fig. 7, curve 2) does not result in the appearance of a band in the visible region of the CD spectrum. In agreement with theoretical predictions [36], this result is obvious because under used conditions the mode of DNA molecule packing in LCD particles corresponds to a hexagonal one. However, the increase in temperature to 40 °C is accompanied by the appearance of two abnormal negative bands in the

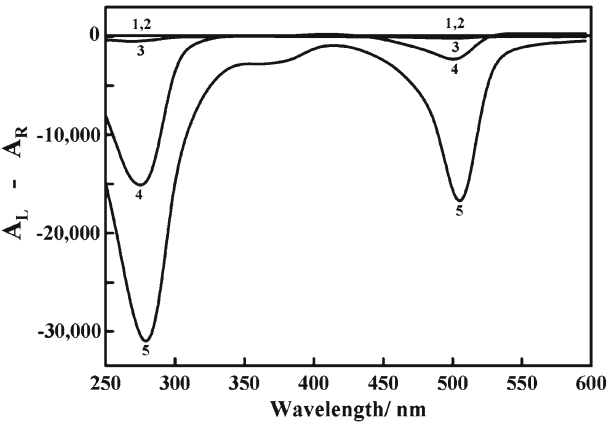

Fig. 7 CD spectra of DNA LCD treated with SYBR Green (curves 2–5), measured at different temperatures. 2–22 °C, 3–40 °C, 4–80 °C, 5–80 °C → 22 °C. $C_{DNA} = 30$ μg ml^{-1}, $C_{PEG} = 250$ mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. $C_{SG} = 0.781 \times 10^{-5}$ M. 1 – the CD spectrum of the initial DNA LCD (22 °C, control). $\Delta A = (A_L - A_R) \times 10^{-6}$ optical units, $L = 1$ cm

CD spectrum (curve 3). One occurs in the absorption region of the DNA nitrogen bases ($\lambda \sim 270$ nm) and the other lies in the absorption region of SG chromophores ($\lambda \sim 500$ nm). The shown CD spectrum indicates that the orientation of SG molecules coincides with the orientation of the nitrogen base about the DNA axis, and SG molecules intercalate into DNA so that the angle between the SG molecule and the long axis of the DNA is ~90°. The identical signs of the two bands in the CD spectrum mean that SG molecules are fixed in a twisted (cholesteric) structure formed by DNA molecules [36, 55]. The increase in temperature to 80 °C leads to the amplification of the amplitudes of both bands in the CD spectrum (curve 4). One should bear in mind that the abnormal band in the absorption region of DNA ($\lambda \sim 270$ nm) reflects a cholesteric quality; the more twisted the DNA molecules packing is, the more intense the band. Also, the cooling of the heated LCD to room temperature (curve 5) does not lead to restoration of the initial CD spectrum typical for hexagonal DNA LCD (curve 1).

Taking into account that SG is selective for the native B-form of ds DNA one can conclude that Fig. 7 demonstrates that ds DNA molecules preserve this form under phase exclusion from solutions with high PEG concentration and all temperatures used.

4 Discussion

The data presented in Fig. 2b define two domains (**I** and **II**) of PEG concentrations. In these domains, the dispersion particles are distinguished by the character of the spatial packing of DNA molecules.

Analyzing our experimental observations (in particular, an appearance of an intense negative band in the CD spectrum), we should bear in mind the following.

First, the polymer (PEG) is not included in the content of the formed particles [8, 46, 58–61].

Second, the size (diameter) of the ds DNA LCD particle is determined by a fine balance between the free energy of these particles and their surface free energy [56, 60–63]. The bulk free energy of an LCD particle tends to increase the particle size. The surface free energy (which depends on the surface tension between the condensed phase) tends to decrease the interface separating the DNA-rich phase and the isotropic solution. This competition suggests the existence of a critical size of LCD particles below which they are unstable or do not form at all.

Third, the packing density of ds DNA molecules in the particles of a dispersion prepared by mixing DNA and PEG solutions is determined by the lateral interaction between neighboring DNA molecules at the moment of their close approaching [21]. The distance between ds DNA molecules is determined by a balance between repulsive intermolecular forces and the compressing osmotic pressure of the PEG solution. The minimization of the excluded volume of neighboring linear, rigid, ds DNA molecules induces the parallel, unidirectional (nematic-like) alignment of ds DNA molecules.

Figures 2b and 3 (see Inset) show that when the water-salt solutions of PEG and ds DNA are mixed the osmotic pressure of the PEG solution causes the compression (condensation) of the DNA molecules and formation of dispersion particles [62]. The ds DNA molecule packing in the dispersion particles is determined not only by lateral interactions between the adjacent molecules at the moment of their close approaching in the PEG solution but also by the properties of this solution, though the PEG molecules do not enter into the composition of the formed dispersion particles [56, 61, 63].

Fourth, the comparison of the structural parameters of the ds DNA LC phases with the parameters of the LC phases formed as a result of increase in ds DNA concentration in water-salt solutions [12, 17, 19, 41] allows one to assume two modes of ds DNA molecule packing. Namely, the orientationally twisted (cholesteric-like) packing of ds DNA molecules and unidirectional (nematic-like) alignment of ds DNA molecules.

Accepting the scheme of ds DNA LCD particle formation (Fig. 1), we can conclude that the maximal density of ds DNA molecules in the LCD particles can be achieved at their hexagonal packing. Such packing can be illustrated by the structure shown in Fig. 8. This structure is not a true crystal, because there are disordered water molecules between the ds DNA molecules. The ds DNA molecules possess some disorder around their positions; they can slide and bend with respect to each other, as well as rotating around their long axis. This corresponds to the liquid character of their packing. Additionally, the fluctuation of the DNA secondary structure represents by itself the factor breaking the crystallinity of the hexagonal packing. Therefore, the hexagonal packing corresponds to only a short-range translation (and long-range, nematic-like, orientation) order. In this structure (as well as in the case of a hexagonal phase that is formed as a result of an increase in the DNA concentration in a water-salt solutions) and following the authors of previous works [64, 65], we admit the existence of three kinds of orientationally ordered molecular layers according to the three main directions of the hexagonal structure (one series is marked by the arrows in Fig. 8). Double-stranded DNA molecules are oriented in a plane of these layers, whose thickness is of the order of the inter-molecular

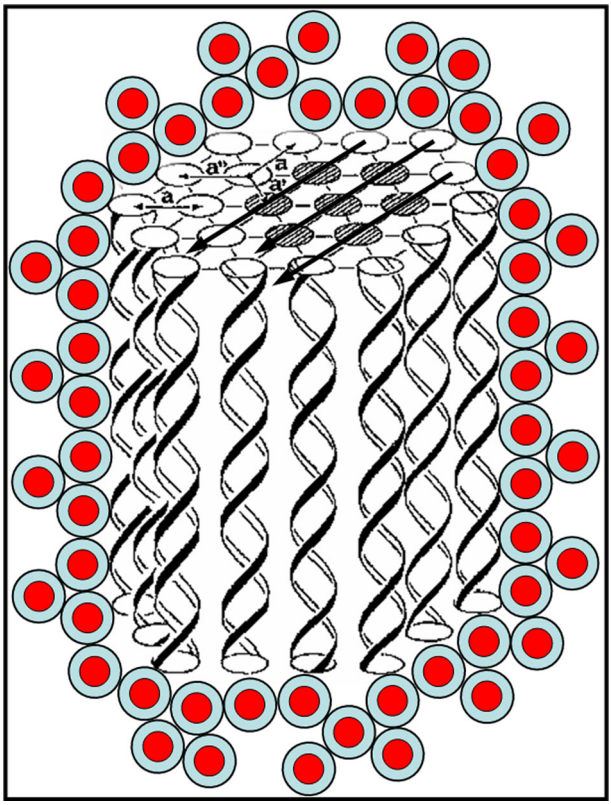

Fig. 8 Hypothetical scheme of hexagonal ds DNA molecule packing in dispersion particles formed in PEG-containing solution. (Dark spots are PEG-molecules outside closely packed DNA molecules) (See text above this Fig.)

spacing. In order to fit experimentally the measured size of LCD particles (500 nm), the neighboring ds DNA molecules (with a mean length of about 340 nm) must be not only ordered but some of them must be shifted along the long axes of ds DNA molecules. Simple evaluations show that these molecules form a spatial structure consisting of approximately 100 orientationally (but not positionally) ordered layers with 100 ds DNA molecules per layer [36]. (By analogy with classical cholesteric liquid crystals, each layer in the structure (Fig. 8) is termed after [66] as a quasinematic layer.)

It is worth stressing that a similar layered structure (Fig. 8) has been discussed also for the spatial structure of the chromosomes of dinoflagellates, based on an investigation of their cross-sections [67–72]. The comparision results of the textures and the CD spectra of DNA LC objects are presented in [42, 52] and the physical modeling of the DNA cholesteric structure in [65].

Fifth, the ds DNA molecules possess several levels of chirality (helical secondary structure of ds DNA molecules, helical distribution of counter-ions near the DNA surface as well as asymmetry of C-atoms in sugar residues). Therefore, chiral and anisotropic properties of ds DNA molecules [73–79] favor helical twisting of the adjacent DNA molecules that are packed into the particles of the dispersion. This results in a macroscopic twist in the orientation of ds DNA molecules with a characteristic spatial pitch (P) [19, 21]. Hence, unidirectional alignment of the ds DNA molecules competes with the tendency of these molecules to form a spatially twisted structure, or a cholesteric structure of the LCDs (CLCDs).

The formed cholesteric is ‘colored’ because DNA molecules contain ‘chromophores’ (nitrogen bases) that absorb UV irradiation. Various approaches have been used to describe the optical properties of the CLCD particles of many compounds, including nucleic acid molecules [50–54]. In the case of ds DNA CLCD, theory [25, 36] predicts the appearance of an intense band in the CD spectrum located in the region of absorption of the ds DNA chromophores (nitrogen bases).

It is well-known [52] that the CD spectra of isolated nitrogen bases or individual, initial, linear ds molecules of nucleic acids are characterized by a low-intensity band. The magnitude of the molecular circular dichroism ($\Delta\varepsilon$ in $M^{-1}cm^{-1}$), i.e., the physical constant for the isolated nitrogen bases, can be calculated theoretically. The calculations show that the alterations of the ds DNA secondary structure (denaturation, transition between conformations such as B-, A-, C- or Z-forms, or even the formation of unordered aggregates of DNA molecules) are accompanied by a change in the $\Delta\varepsilon$ value within 1–7 M^{-1} cm^{-1} [40]. However, the intense band in the CD spectrum of CLCD particles formed by ds DNA molecules is related mainly to the structural parameters of these particles but not with the fine details of the secondary structure of individual ds DNA molecules. This band reflects a so-called structural circular dichroism [24, 36]. In order to stress the difference between molecular and structural circular dichoism, we utilize the term ‘abnormal band’ for an intense band in the CD spectrum [24]. The measured abnormal negative band in the CD spectra (Fig. 2a, curves 2–5) univocally testifies to the left-handed twist of the spatial LC structure formed by ds DNA molecules. Bearing this in mind, we use the amplitude of this band in the CD spectra, expressed simply as an experimentally measured ΔA value (in optical units), to characterize the molecular packing.

Finally, the increase in PEG concentration is accompanied by the disappearance of an abnormal band in the CD spectra and also by a decrease in the distance between the DNA molecules (Fig. 3a, see Inset).

A few additional issues should also be mentioned.

A. The osmotic pressure of the PEG-containing solution plays a very important role [41, 46, 63, 77–79] in the stabilization of the spatial structure of ds DNA LCD particles formed under various conditions. Due to constant (fixed) osmotic pressure determined by PEG concentration, the spatial structure of DNA LCD particles in a PEG-containing solution is sterically limited ('frozen') [21]. Nevertheless, DNA molecules retain some diffusion degrees of freedom, determining a liquid character of the packing mode of DNA molecules in quasinematic layers. Therefore, the constant osmotic pressure of the PEG-containing solution defines the permanent spatial structure of LCD particles and, hence, the fixed distance between neighboring linear ds DNA molecules in these objects. With a high PEG concentration of the water-salt solution, the ds DNA dispersion particles are still formed, yet their abnormal CD band disappears. In this case, the packing density of ds DNA molecules corresponds to their packing in a hexagonal LC phase [12, 19], which lacks abnormal optical activity. In other words, a certain limit of PEG concentration in the solution (C^{limit}_{PEG}) causes the transition from a cholesteric mode of packing of neighboring ds DNA molecules in particles to hexagonal packing. This transition is accompanied by the unwinding of the helical spatial structure of particles and, consequently, by a decrease in the value of amplitude (ΔA_{270}) of an abnormal CD band of these particles to its full disappearance (Fig. 2b, domain **II**).

Note that at high PEG concentration and at room temperature, ds DNA LCD can demonstrate a low-intensity band in the CD spectra ($\Delta \mathbf{A}_{270} \sim (550–1{,}100) \times 10^{-6}$ optical units) of unknown origin (this value was ignored in the optical scale used for registration of the abnormal band). The results of the X-ray study of unoriented samples of phases, formed as a result of sedimentation of LCD particles formed at a PEG concentration of 300 mg ml^{-1}, suggests that ds DNA molecules remain in the B-form [38]. Our control of the ds DNA secondary structure under high PEG concentration (Fig. 7) confirms that DNA molecules conserve the B-form under these conditions.

B. The increase in the temperature of the PEG-containing solution is accompanied by a decrease of its viscosity. The leaving of water molecules from the PEG-polymeric chain results in a decrease of the osmotic pressure of the solution [44, 45]. Under these conditions, the diffusive mobility of neighboring DNA molecules in LCD particles increases. This results not only in a change in the character of interaction of the neighboring DNA molecules but also in their packing.

Figure 4a shows the change in the amplitude of abnormal negative bands in the CD spectra of DNA dispersions, formed at PEG concentrations ≥220 mg/ml (domain **II** in Fig. 2b) as the temperature increases. It can be seen that the temperature rise of PEG-containing solutions is accompanied by the appearance of an abnormal band. This means that when the dispersion particles with hexagonal packing of ds DNA molecules (and therefore, not having abnormal optical activity) are heated, a transition into another structural state characterized by abnormal optical activity takes place.

C. Cooling of the PEG-containing solution to room temperature increases the viscosity of the solution and the osmotic pressure of the solution compressing the spatial structure of the ds DNA LCD particles grows [44, 45]. Under these conditions, the diffusive mobility of ds DNA molecules in LCD particles decreases.

Points **A–C** mean that the change in PEG concentration, i.e., osmotic pressure of a solution, allows one to modify the compression (close approach) of neighboring DNA molecules in dispersion particles. So, the spatial structure of ds DNA LCD particles can be regulated both by the PEG concentration in the solution and its temperature.

The above experimental results and observations can be qualitatively rationalized in terms of the classical Lindemann criterion [80] for the melting of crystals. However, since we are interested in the transition from hexagonal to cholesteric molecular packing, the Lindemann criterion should be modified:

(i) The hexagonal structure of the DNA molecules is not a crystal with long-range translational and orientational orders. It possesses only the short-range translational order combined with nematic-like long-range orientational order. However, the translational order correlation length $\xi^{\mathbf{hex}}$ is larger than the characteristic molecular scale $\boldsymbol{l}$ on the order of 10–20 nm.

(ii) We are not dealing with complete melting, i.e., with transformation of the hexagonal structure into an isotropic liquid state. We observed the phase transition from hexagonal to cholesteric packing with long-range cholesteric-like (i.e., twisted) orientational order and short-range translational order with its correlation length $\xi^{\mathbf{ch.}}$ on the order of $\boldsymbol{l}$.

Let us summarize the ideology behind the Lindemann melting criterion. Upon the heating of a conventional crystalline structure, the amplitudes of the molecular thermo-fluctuational displacements $\mathbf{u}$ increase. According to the Lindemann criterion, when the mean squared amplitude of the displacements $\langle \mathbf{u}^2 \rangle$ becomes on the order of some fraction of the average inter-molecular distance $\boldsymbol{l}$, the system under consideration behaves as a liquid. Two essential comments are in order here:

– there is a small (1/10–1/30) numeric prefactor in the Lindemann criterion. The number (often referred to as Lindemann's number) is not a universal quantity; it depends on the system details. The solid-like behavior (i.e., small vibrations around static equilibrium positions) becomes unstable with respect to melting into liquid when the amplitudes of the vibrations are still much smaller than the average inter-molecular distance.
– the periodicity of the crystalline lattice is not a mandatory requirement for the Lindemann criterion. It works also for short-range translationally ordered systems provided that a typical inter-particle distance $\boldsymbol{l}$ exists. Therefore, the criterion can be applied to the case of the hexagonal DNA packing we are interested in.

With all the above said, let us reformulate the classical Lindemann criterion:

$$\left\langle \mathbf{u}^2(\mathbf{T_m}) \right\rangle \Big/ l^2 \approx \mathbf{10^{-2}} \tag{1}$$

(where $\mathbf{T_m}$ is the melting temperature) as a condition that the loss of the elastic energy becomes comparable with the gain of the entropy. This form of the Lindemann criterion reads (up to numeric factors and per unit volume) as:

$$\mathbf{1} \Big/ \mathbf{2}(\lambda) \left\langle \mathbf{u}^2 \right\rangle \Big/ l^2 \approx \mathbf{k_B T} \Big/ l^3 \tag{2}$$

(where λ is Young's elastic modulus). Both criteria (1) and (2) are equivalent.

The advantage of form (2) is that it can be applied to our case, i.e., hexagonal → cholesteric packing structural transition. We need only two modifications in Eq. (2):

-(a) In the cholesteric structure (unlike isotropic liquid), the orientational ordering remains long-range (although twisted). Therefore, the entropic gain for the transition from a hexagonal into a cholesteric structure is smaller than that for complete melting.
-(b) However, there is additional orientational Frank energy gain because in the nematic-like ordered hexagonal structure, the orientational Frank energy is larger than in the naturally twisted cholesteric structure. The energy (per unit volume) is:

$$\Delta E_{Fr} \approx 1 \Big/ 2K_{22}q_0^2 \tag{3}$$

[where $\mathbf{K_{22}}$ is the Frank twist modulus and $\mathbf{q_0} = \pi/\mathbf{P}$ ($\mathbf{P}$ is the cholesteric spiral pitch)].

Application of the modified (see above) Lindemann approach for the case of LCD DNA particles suggests that at a certain temperature characteristic of a PEG-containing solution, the amplitude of the displacements and rotation diffusion of neighboring ds DNA molecules in hexagonal phase (hexagonal structure, Fig. 8) reaches a critical value. Hexagonal packing becomes labile and in the absence of other external influences, the system would like to transform to the isotropic state. However, in our case, the transition of ds DNA molecules into the isotropic state is impossible due to steric reasons (because of PEG osmotic 'compression'). Hence, we can expect that instead of complete 'melting' of the hexagonal structure, another phase transition can happen. Unlike the isotropic state, the stable, spatial, helically twisted (cholesteric) structure (with an abnormal band in the CD spectrum) can be formed even at relatively high PEG osmotic pressure. The physical reason for such an incomplete 'melting' transition is that a partial orientational entropy loss (due to the transition to an orientationally ordered cholesteric structure) is compensated for by the contribution to the free energy associated with the orientation elasticity of chiral molecules of DNA. Indeed, since the DNA molecules themselves (and the main forces of interaction between them) are non-centrosymmetric (chiral), their parallel ordering is disadvantageous in terms of the orientation (Frank) elastic energy. In parallel (nematic-like) orientation of chiral molecules of DNA, the system orientation elastic energy penalty can be estimated as $\mathbf{K_{22}/P^2}$, where $\mathbf{K_{22}}$ is the twist Frank constant and $\mathbf{P}$ is an optimal pitch of the cholesteric packing of chiral molecules.

(One can add that the considered model of the temperature-induced hexagonal → cholesteric phase transition in the case of ds DNA LCD has similarities with the model of the concentration-induced hexagonal → cholesteric phase transformation as a result of the twist of 'hexagonal domains' in the liquid-crystalline phase [81].)

To check this hypothesis, we performed theoretical calculations of the CD spectra of ds DNA LCD particles under various conditions [25].

In Fig. 9, a few theoretically calculated CD spectra are presented. Curve 1 is the CD spectrum typical of classical cholesteric LCD [21] with the following experimental parameters: P value is 2500 nm; diameter (D) of particles is 550 nm; distance (d) between quasinematic layers is 3.5 nm; C_{DNA} used for formation of CLCD particles is 30 μg ml^{-1}. First of all, one can see that the calculated CD spectrum contains a negative band. The negative sign of this band in the CD spectrum found in theoretical computations agrees with the sign of band that is observed experimentally (Fig. 2b). Secondly, the intensity (ΔA) of this band is high and it practically coincides with the experimentally measured band in the CD spectrum of the ds DNA LCDs (Fig. 2b) after their formation in PEG-containing solution ($C_{PEG} = 170$ mg ml^{-1}). (These facts confirm the validity of our theoretical computations). Finally, the twist angle between quasinematic layers is equal to 0.5°.

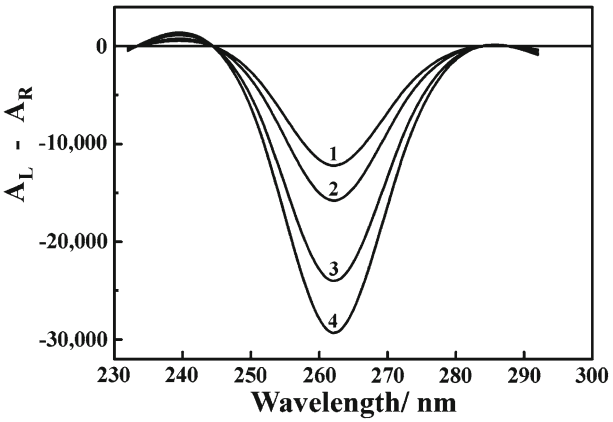

Fig. 9 Theoretically computed CD spectra of ds DNA cholesteric LCD particles: curve 1 – CD spectrum for classical ds DNA cholesteric LCD ($d = 3.5$ nm, $P = 2{,}500$ nm, $\varphi = 0.5°$); curve 2 – CD spectrum for model artificial ds DNA cholesteric LCD ($d = 2.5$ nm, $\varphi = 0.5°$); curve 3 - CD spectrum for model artificial ds DNA cholesteric LCD ($d = 2.5$ nm, $\varphi = 1.0°$); curve 4 - CD spectrum for model artificial ds DNA cholesteric LCD ($d = 2.5$ nm, $\varphi = 1.5°$). D = 550 nm, $C_{DNA} = 30$ μg ml^{-1}, $\Delta A = (A_L - A_R) \times 10^{-6}$ optical units, $L = 1$ cm. φ - the twist angle between neighboring quasinematic layers

Curve 2 in Fig. 9 presents, as an example, the theoretically calculated CD spectrum for the model, artificial cholesteric, which is formed as a result of a simple decrease of the distance (d) between the quasinematic layers from 3.5 to 2.5 nm (with other unaltered parameters), i.e., as a result of simple compression of the initial cholesteric structure. One can see that the intensity (ΔA) of the abnormal negative band in this case is only a little bit higher in comparison to the first case (curve 1). This result might suggest an increase in the distance between ds DNA molecules in dispersion particles with a hexagonal structure (initial d value equals 2.5 nm and at distance, equals 3.3 nm) at heating. But, this change in distance between the quasinematic layers could result only in a minor increase in the ΔA value (compare curves 2 and 1).

However, such an interpretation of changing Δ**A** values upon heating of ds DNA LCDs contradicts the experimentally measured CD spectra of these dispersions (Figs. 4 and 5).

Additionally, the performed experiments show (Table 1) that the heating of the ds DNA LC phase formed at $C_{PEG} = 300$ mg ml^{-1} is accompanied by only a minor increase in distance between ds DNA molecules.

Hence, the increase in the distance between ds DNA molecules packed in dispersion particles with the hexagonal ordering of these molecules cannot explain the results presented in Figs. 4 and 5.

Thus, there can be another reason for the increase in the abnormal band amplitude in the CD spectra. Indeed, the theoretical computations showed that by fixing the distance between quasinematic layers of ds DNA molecules in dispersion particles ($d = 2.5$ nm, Fig. 9) and increasing the twist angle between these layers from 0.5° to 1.5° (curves 2–4), it results in a big rise in the amplitude (ΔA) of the abnormal negative band. The computed value is close to the experimentally measured ΔA value shown in Fig. 5 (curve 3) after the heating of ds DNA LCDs and their subsequent cooling to room temperature.

In our experiments, we detected the appearance of an abnormal band in the CD spectra when heating the LCD particles to 80 °C [with a hexagonal mode of DNA molecule packing

with an experimentally measured distance (d) between the quasinematic layers of about 2.5 nm (Table 1)] and their subsequent cooling to 20 °C. The results shown in Fig. 5 suggest the formation of a new (re-entrant) cholesteric structure with different initial classical cholesteric parameters. Thus, we see that upon heating the LCD particles, the hexagonal packing of adjacent quasinematic layers of DNA molecules is transformed into the cholesteric phase. We also observe that the higher the osmotic pressure of solution, the greater temperature needed to switch the hexagonal → cholesteric phase transition.

Figure 10 shows the theoretically calculated dependence of the abnormal negative band amplitude (ΔA_{270}) upon the pitch (P) of hypothetical cholesteric structures. As seen from Fig. 10, the ΔA_{270} value increases when decreasing the cholesteric pitch value until it reaches a certain value (comparable with the incident radiation wavelength). Then, the instantaneous distribution of the electric field intensity vector approximately coincides with the directions of absorbing dipoles. The ΔA_{270} value sharply decreases with a further decrease in the P value. (Detailed discussion of this effect is beyond the scope of this paper.)

Point 1 (green) in Fig. 10 corresponds to real parameters of the initial 'classical' cholesteric [21]. However, point 2 (red) shows the high value of th abnormal negative band amplitude (ΔA_{270}) measured experimentally after heating and cooling of the DNA LCD particles with hexagonal packing of quasinematic layers (i.e., ΔA_{270} equals ~ $36{,}500 \times 10^{-6}$ optical units). This point corresponds to the cholesteric structure with a P value equal to about 500 nm. Unfortunately, it is practically impossible by our optical method to detect the 'fingerprint' texture of such a cholesteric.

The high value of the amplitude of an abnormal negative band in the CD spectrum of the DNA LCDs at their heating (or cooling) is related to a decrease in the P value and a corresponding increase in the twist angle between the quasinematic layers existing in the particles of these LCDs [25]. Hence, in agreement with Eq. 3, the stabilization of the twisted structure of a newly formed cholesteric phase is, indeed, realized due to an increase of the twist angle between the quasinematic layers existing in the particles of these LCDs.

Comparison of Fig. 4a with Fig. 5 demonstrates a dual role for temperature in the formation process of a new cholesteric DNA phase. On the one hand, the temperature initiates the formation of the germ of a new phase and its growth until it reaches the critical size for the formation of a new twisted structure of the DNA dispersion particles. On the other hand, the

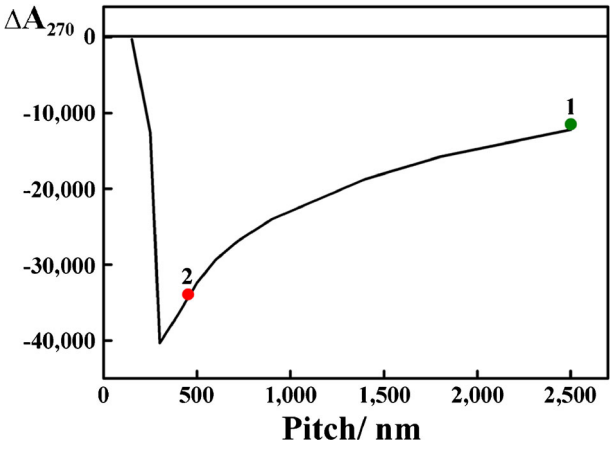

Fig. 10 Theoretically calculated dependence of the abnormal negative band amplitude (ΔA_{270}) upon the pitches (**P**) of hypothetical ds DNA cholesterics. Point 1 (*green*) corresponds to the real parameters of ds DNA classical cholesteric. Point 2 (*red*) corresponds to the P value that is equal to about 500 nm. $C_{DNA} = 30$ μg ml^{-1}, $\Delta A_{270} \times 10^{-6}$ optical units, $L = 1$ cm

temperature increases the thermal fluctuations of adjacent quasinematic layers and can prevent their exact spatial ordering within the structure of these particles. The perfectly twisted spatial fixation of these layers (a sort of freezing of the DNA molecules in these layers) and, hence, DNA chromophores (that is, nitrogen bases), can be reached as the temperature decreases to room temperature. The data shown in Fig. 5 favor this hypothesis. Figure 5 (curve 3) illustrates that upon cooling of the system, the amplitude of the abnormal negative band (in the DNA nitrogen bases absorption region) increases.

Therefore, we conclude that both factors, the osmotic pressure of the solution and its temperature, determine the formation of LCD particles with different modes of DNA molecule packing. Evidently the mode of DNA molecule packing in the particles of dispersions depends not only on the energetic but also on the kinetic factors [82], which is why, e.g., after the hexagonal → cholesteric phase transition, a drop in the temperature of the solution to room temperature will not result in an immediate restoration of the initial hexagonal packing. This is typical for first-order phase transition hysteresis behavior. Our results show that the higher the temperature of the PEG solution, the stronger the tendency of the quasinematic layers formed by chiral ds DNA molecules to not have hexagonal but rather cholesteric packing.

5 Conclusions

The results obtained in this work can be rationalized in terms of the orientationally ordered quasinematic layers of ds DNA molecules with a parallel alignment in the hexagonal structure. These layers can adopt a twisted configuration upon a temperature increase, and, as a result of this process, the spatial helicoidal structure (the cholesteric phase) is formed. The packing mode of the adjacent quasinematic layers of DNA molecules in dispersion particles, obtained at the phase exclusion of these molecules from water-salt solutions of polymers, is determined not only by the osmotic pressure of the solution but also by its temperature.

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