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Multiplicity of 're-entrant' cholesteric structures in DNA liquid-crystalline dispersions

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Contents

Abstract. **Information about properties of liquid-crystalline dispersions of DNA molecules formed as a result of their phase exclusion is systematized. The influence of temperature and osmotic pressure on the structure of these dispersions has been elucidated in the framework of the concept of 'quasinematic' layers of orientationally ordered DNA molecules in dispersion particles. A new hexagonal → 're-entrant' cholesteric packing phase transition of DNA molecules discovered by the authors is described, taking into account the generalized Lindemann criterion. The multiplicity of the 're-entrant' phases and their structure are shown to depend on the characteristics of DNA and water-polymer solutions.**

Keywords: DNA, liquid-crystalline dispersions, circular dichroism, textures, intercalators, 're-entrant' phases, Lindemann criterion

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Dedicated to the memory of O N Kompants, the creator of portable dichrometers that allowed new experimental data to be obtained

1. Introduction

The packing of double-stranded (DS) DNA molecules in liquid-crystalline (LC) phases and liquid-crystal dispersions (LCDs) remains in the focus of much experimental and theoretical work.

It has been known since 1961 that the dissolution of a sample of a lyophilized high molecular weight DNA preparation and alteration of its concentration using a buffer solution are accompanied by neighboring molecules being drawn together and the formation of condensed phases. Historically, the cholesteric [1] and hexagonal [2–7] phases were among the first to be described.

An alternative method for preparing DNA phases consists in increasing the concentration of DNA by ultrafiltration of the solution through a membrane with a pore size that allows water molecules and salt ions but not DS DNA molecules to pass through [8, 9]. The phases of DS DNA obtained by raising its concentration in aqueous-salt solutions are essentially viscous liquors in which neighboring DNA molecules are orientationally ordered and preserve the ability to slide relative to each other.

A large number of specialized reviews and experimental studies are devoted to the problem of packing DS DNA molecules in phases formed at different concentrations [10–14]. The packing of DS DNA molecules in phases has been investigated using electron (and cryoelectron) microscopy, as well as polarization microscopy of thin layers of these phases [15–17]. To determine the distance between DS DNA molecules in the formed phases, an X-ray diffraction analysis was used. It demonstrated that adjacent DNA molecules are

orientationally ordered and spaced 2.5–5.0 nm apart on average, i.e., orientationally ordered phases are similar to *crystals*. According to the results of a texture analysis of thin layers of these phases, DNA molecules are mobile, i.e., phases retain the properties of a *liquid* (see, for example, [18–20]). As a result of these studies, the term "liquid-crystalline phases of DS DNA" was coined, and it became possible to clarify details of the packing of DNA molecules in LC phases observed upon a rise in DNA concentration. A cascade of the sequences of DNA phases looks like this: isotropic phase → (blue phase?) → cholesteric phase → columnar phase → crystalline phase [20, 21].

It has also been shown that mixing a DS DNA solution with solutions of some hydrophilic polymers, such as polyethylene glycol (PEG), polyacrylic acid, or polyvinylpyrrolidone, leads to phase exclusion of DNA. Since the experiments by L Lerman (1971), this process has been called psi (ψ)-condensation (psi is the acronym for polymer-salt-induced) [22–25]. This method of phase exclusion can be implemented, depending on two parameters: namely, the molecular mass (MM) of DNA and its concentration, manifested as intramolecular or intermolecular condensation of these molecules.

In the first case, the phase exclusion of DS DNA molecules with a very high molecular weight ($\geqslant 10\times10^6$ Da) realized at a very low DNA concentration leads to the formation of single DNA particles consisting of one molecule [23, 26]. Toroidal particles of DS DNA formed when sparing the destruction of the heads of some bacteriophages were first discovered in [27]; later, such particles were observed under different conditions [28].

Notwithstanding modern theoretical approaches [29, 30] to the description of intramolecular DNA condensation, there is no universal theory describing the formation of toroidal DNA particles.

In the second case, the phase exclusion (condensation) of low molecular weight linear DNA (molecular weight $< 1\times10^6$ Da) from aqueous-salt solutions of PEG [31, 32] or a mixture of PEG with mineral oil [33] at room temperature is accompanied by the aggregation of neighboring molecules and the formation of dispersion particles (Fig. 1)

Dispersion particles are "microscopic droplets of a concentrated DNA solution" [34]. From the biological point of view, the particles of DNA dispersions make up a model system which simulates the packing of DNA molecules in the simplest biological objects. Such packing is observed in the heads of bacteriophages, bacterial nucleoids, chromosomes of dinoflagellates, and sperm nuclei of some animals (horses, rabbits) and humans [35], which are actually dispersed systems of microscopic size with an ordered but versatile DNA packaging. The retention of high reactivity pairs of nitrogenous bases in DNA molecules present in the 'quasinematic' layers of dispersion particles provides a basis for the creation of sensitive elements (biosensors) of analytical systems for the identification of biologically active and chemically meaningful compounds interacting with these bases [36]. Finally, the transition from DNA LC phases to dispersion particles poses the question of how DS DNA molecules are packed in these particles.

While the packing features of DNA molecules in LC phases are amenable to investigation by a variety of experimental methods, the study of dispersions implies dealing with DNA particles of a small finite size instead of massive LC systems. Due to the so-called finite size effect [37], the physicochemical properties of dispersion particles can differ significantly from those of bulk LC phases. In such a 'limited' geometry, a number of metastable structures can exist [38], first and foremost because the surface properties of particles can affect their spatial structure. The answer to the question of how DNA molecules are packed in dispersion particles is not of merely theoretical interest; it also requires the use of highly specialized research methods to be obtained [38].

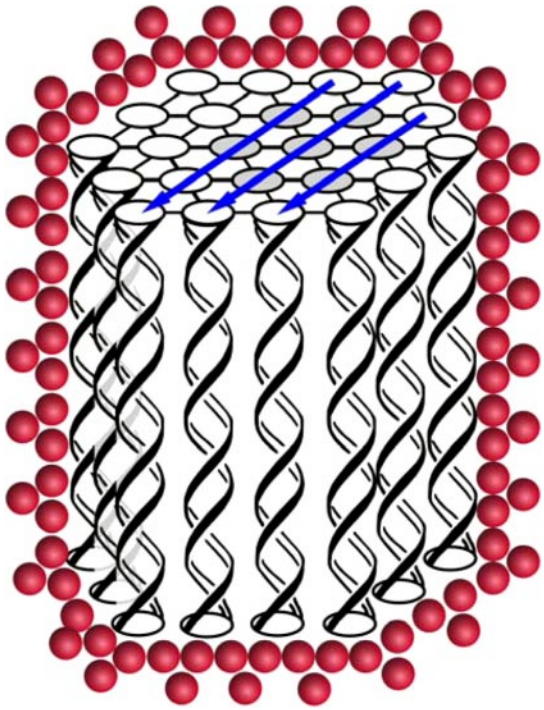

Figure 1. (Color online.) Hypothetical scheme of hexagonal packing of linear DS DNA molecules in a dispersion particle formed in an aqueous-salt solution of PEG. Red balls are PEG molecules. Arrows indicate quasinematic layers of DNA molecules.

With this in mind, the authors of Ref. [39] undertook an analysis of possible ways of packing DS DNA molecules in LCD particles formed in PEG-containing solutions with high osmotic pressure. Reference [40] reports the appearance of a new DNA phase, which we called the 're-entrant' cholesteric phase.

The objective of this review is to generalize the results of experimental and theoretical studies on the properties of DNA-containing LCD particles formed under different conditions upon phase exclusion of these molecules from aqueous polymer solutions, as well as to systematize new data suggesting the formation and multiplicity of 're-entrant' cholesteric structures in DNA dispersion particles.

2. Formation and properties of DNA dispersion particles at room temperature

Particles of dispersions formed at room temperature as a result of phase exclusion of semi-rigid linear DS DNA molecules from aqueous-salt solutions of PEG are characterized by the following features.

(1) A theoretical estimation of the particle size in dispersions shows that at a DNA molecular weight of less than 1×10^6 Da the mean particle diameter is close to 500 nm, and one particle contains about 10^4 DNA molecules [41, 42]. This

estimate is confirmed by the results of direct measurements of the size of 'solid' (gel-like) particles of DNA immobilized on a nuclear membrane filter [43].

(2) The polymer (PEG) is not included in the resulting particles of DNA dispersions [44–47]. The osmotic pressure of a PEG-containing solution plays an important role in stabilizing the structure of the particles of DNA dispersions formed under different conditions [48–51]. The aforementioned particle size of DNA dispersions is a result of the balance between the total free energy of the particles and their surface free energy [52]. The competition between the volumetric free energy of a dispersion particle (which tends to increase the particle size) and its surface free energy (depending on surface tension between DNA-rich particles and the surrounding PEG solution and tending to reduce the surface between the DNA-rich phase and the surrounding solution) [52, 53] suggests a critical size of dispersion particles below which they are either unstable or not formed at all.

(3) Complete separation of chains of DNA molecules in a dispersed particle and their folding into single statistical coils are impossible for steric reasons [54, 55]. At a constant osmotic pressure, the spatial structure of DNA dispersion particles determined by the PEG concentration is sterically limited ('frozen').

(4) The orientational order parameter (S) of nucleic acid molecules in dispersion particles lies in the range of $\sim 0.6-0.8$ [56], which indicates a pronounced tendency toward retention of a constant general spatial structure of dispersion particles. The large value of the order parameter means that DNA molecules tend to order in a common direction in spite of orientational and conformational fluctuations and structural defects,

(5) Packing density of DNA molecules in dispersed particles (the mean distance between DNA molecules) is determined by the balance of forces of intermolecular repulsion of neighboring molecules and the compressive osmotic pressure of PEG at the moment of their approach. The screening of DNA charges and osmotic pressure of the PEG solution accelerate the convergence of DNA molecules and their orientational nematic-like ordering. The combination of such conditions as the minimization of the free volume of DS DNA molecules and the need for the most dense packing of the largest number of DNA molecules in a small volume of a dispersed particle accounts for the tendency of these molecules toward a parallel (nematic, orientational) arrangement inside the particle. The higher the osmotic pressure of a PEG-containing solution, i.e., the higher the concentration of PEG in the solution, the higher the packing density of DNA molecules in the dispersion particles formed during phase exclusion of these molecules.

(6) A high local concentration of linear semi-rigid DNA molecules is achieved in the particles. For example, at $C_{\mathrm{PEG}} = 170$ mg ml^{-1}, the local concentration of linear semi-rigid DNA molecules (C_{DNA}) in dispersion particles is 299 mg ml^{-1}; at $C_{\mathrm{PEG}} = 300$ mg ml^{-1}, it is approximately 600 mg ml^{-1}.

3. Hexagonal packing of DNA molecules in dispersion particles

Obviously, the maximum packing density of DNA molecules in dispersion particles upon phase exclusion of these molecules from polymer-containing solutions is achieved when they are hexagonally packed [57]. Such a packing is illustrated by the structure shown in Fig. 1. It can be conjectured, following the opinions of the authors of [20, 58, 59], that this structure (like the macroscopic hexagonal phase formed by DS DNA molecules in aqueous salt solutions) has three types of 'layers' built from the molecules identically ordered in space [60, 61] which correspond to the three main axes of the hexagonal structure (one series is shown in Fig. 1). These layers are characterized by a nematic (one-dimensional) order in the orientation of linear DNA molecules. These molecules lie in the plane of layers located at a distance D, the thickness of which is approximately equal to the distance between these molecules. The fact that the orientation of molecules in each layer is similar to that in nematic liquid crystals gave reason to use the term 'quasinematic layer' for its designation [21, 62, 63]. We shall use this term in further discussions.

The above mode of packing is noncrystalline, since a layer of water molecules is retained between DNA molecules that can move relative to one another while each rotates around its long axis. (When molecules rotate freely about their long axes, the detailed molecular packing structure in the LC phase is 'lost' [64].) Moreover, fluctuations in the structure of semi-rigid DNA molecules relative to the vertical axis leading to its undulation, u, [59, 65, 66] also contribute to breaking crystalline 'rigidity' of the hexagonal packing [67].

All these factors are responsible for the 'liquid' character of DNA packing.

Taking into account possible local deviations from the three-dimensional periodic packing scheme [68, 69], the hexagonal packing of DNA molecules manifests itself in the diffraction pattern of small-angle X-ray scattering in the form of a single broad peak (reflex), indicating only local ordering of these molecules [57, 68, 69].

Thus, phase exclusion of semi-rigid linear DS DNA molecules from a polymer-containing solution at room temperature leads to the formation of dispersions in particles of which DNA molecules are packed in the form of quasinematic layers characterized by a 'short-range' positional order in the arrangement of these molecules. Quasinematic layers can also exist in the hexagonal packing of DNA molecules.

4. Cholesteric packing of DNA molecules in dispersion particles

The anisotropic and chiral properties of DS DNA molecules (their helical structure, the helical arrangement of counterions, asymmetry of C-atoms in sugar residues) account for the tendency of these molecules toward spinning inside particles, i.e., the formation of a spatially twisted phase [59].

The scheme of fixation of DNA molecules in an LCD particle (see Fig. 1) and the aforementioned high value of the orientational order parameter suggest that a spatial twist of neighboring molecules is possible only when adjacent layers of DNA molecules rotate in space.

If the interaction between distantly spaced DS DNA molecules can be described as that between homogeneously charged rods, the so-called chiral interaction determined by both anisotropic (chiral) properties of DNA molecules themselves and the chirality of intermolecular interactions begins to contribute to the interaction upon a reduction in the intermolecular distance to 5.0 nm (or less) that occurs as the PEG osmotic pressure increases [70].

The main effect of chirality is that chiral molecules do not tend to pack in parallel with their neighbors; rather, they form a small angle with respect to them, i.e., chirality promotes the

macroscopic twisting of molecules with a certain characteristic pitch [71].

This means that the packing of DNA molecules in dispersion particles is associated with the competition between their nematic ordering (parallel arrangement) inside the particles under the effect of PEG compressive osmotic pressure and twisting in space due to the chirality of the molecules. At a certain combination of osmotic pressure and temperature of the solution, the anisotropic and chiral contributions to the total energy of interaction between DNA molecules lead to their 'long-range' orientational ordering, i.e., a spiral spatial (cholesteric) twist of the layers of DNA molecules packed in dispersed particles [72].

It should be borne in mind that, if DNA molecules in adjacent layers of a dispersion particle rotate about their axes independently of each other, then their chiral interactions can be averaged and result in the vanishing of the total effect. The behavior of the nearest neighbor DNA molecules must be correlated to ensure a noticeable manifestation of the effect of chiral interaction in the system under consideration [73].

Because chiral interaction decreases with increasing distance between molecules faster than basic (nonchiral) electrostatic forces, chiral effects become significant at short distances between DNA molecules when they 'feel' each other's chirality.

A minimum energy is reached upon a mutual turn of neighboring DNA molecules through a certain angle, i.e., chirality promotes macroscopic twisting of the molecules with a characteristic spatial pitch P. Under these conditions, a spatially twisted (cholesteric) structure of DNA dispersion particles forms, with the distance between the molecules ranging from 5.0 to 3.0 nm [20, 35]. The P value is about 2500 nm, which corresponds to the twist angle of the adjacent layers of DNA molecules in a dispersion particle on the order of 0.7° [20, 21, 41].

The magnitude and the sign of chiral interaction can depend on both small changes in the DNA structure [74] and temperature [72].

The cholesteric packing of DNA molecules in LCD particles manifests itself as a 'fingerprint' texture in the phases formed during the concentration of DNA dispersion particles as a result of low-speed centrifugation.

5. Circular dichroism spectra of DNA dispersions

Unambiguous evidence of the formation of a spatially twisted structure of DNA dispersion particles was obtained using the ideology of the method of the 'external chromophore' introduced into the cholesteric LC phases of synthetic compounds [75–78].

This method reveals, in a certain arrangement of external chromophore molecules in the cholesteric phase, an intense line in the absorption band of this chromophore in the circular dichroism (CD) spectrum [79–81].

In the molecular structure of DS, DNA includes nitrogenous bases (chromophores that absorb in the ultraviolet (UV) region of the spectrum) rather rigidly fixed with respect to the long axis of these molecules (the bases are inclined at an angle of approximately 90°).

The formation of a layer of adjacent DNA molecules means that nitrogenous bases are built (introduced) into it, and the resulting structure is 'colored'.

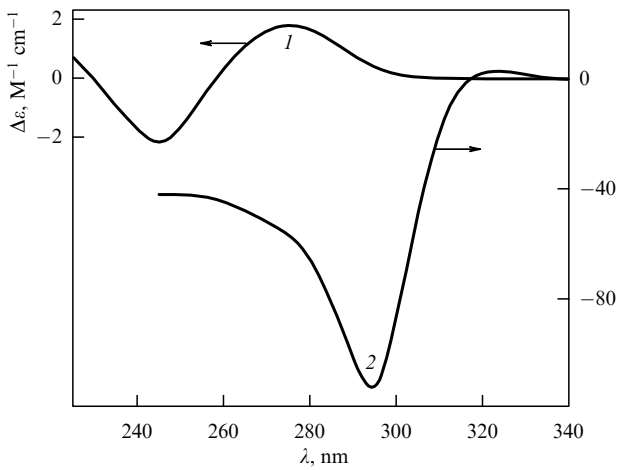

Figure 2. CD spectra of an aqueous-salt (0.3 M NaCl) solution of linear B-form DS DNA (curve *1*, left ordinate axis) and a thin layer (≈ 20 μm) of the cholesteric LC phase of DNA (curve *2*, right ordinate axis). Molar circular dichroism showing difference in the absorption of left (A_L) and right (A_R) circularly polarized light $\Delta\varepsilon = (A_L - A_R)/(CL)$, where C is concentration of DNA [M, or mole l^{-1}], L is thickness (optical path length) in the rectangular quartz cuvette [cm]. A_L and A_R are measured in optical units.

This finding is confirmed by the fact that, for a certain geometry of polarized light beam propagation [82, 83] through some regions of thin layers of the cholesteric DNA phase, an unusual band is observed in CD spectrum (Fig. 2, curve *2*). It should, of course, be added that, in thin films of LC phases, it is, in principle, difficult to achieve the exact position of the cholesteric phase with respect to the light beam. A homogeneous orientation (without defects) of such a phase is observed only in exceptional cases and only in very small areas of the film. This circumstance affects the position and amplitude of the intense band in the CD spectrum [83]. Nevertheless, the emergence of this band in the CD spectrum in the absorption region of DNA chromophores (nitrogenous bases) indicates a spiral spatial packing of DNA molecules in the cholesteric phase [84].

A theory that takes into account the ideology of the external chromophore method, the 'long-range' (orientational) order in the arrangement of nitrogenous bases in the layers formed by low-molecular-mass DNA molecules (MM less than 1×10^6 Da), and the spatial spiral twisting of these layers in dispersion particles, predicts the appearance of an intense band in the CD spectrum of dispersions [85]. In the case of helical (i.e., cholesteric) packing of neighboring DNA molecules in the resulting particles, the intense band in the CD spectrum must be located in the absorption region of the nitrogenous bases [85].

Figure 3 compares, by way of example, CD spectra of an aqueous-salt solution of linear B-form DNA (Fig. 3a) and the theoretically calculated CD spectrum of cholesteric LCD DNA (Fig. 3b). It shows that the formation of DNA dispersion does indeed lead to the appearance of an intense band in the CD spectrum in the UV region (in the absorption region of nitrogenous bases). Because pairs of nitrogenous bases are rigidly fixed with respect to the long axis of DNA molecules, the helical packing of nitrogenous bases is possible only when neighboring DNA molecules in dispersion particles form a spatial helically twisted (cholesteric) structure.

Without going into the particulars of the description of the details of the DNA CD spectra theory [85], it is worth

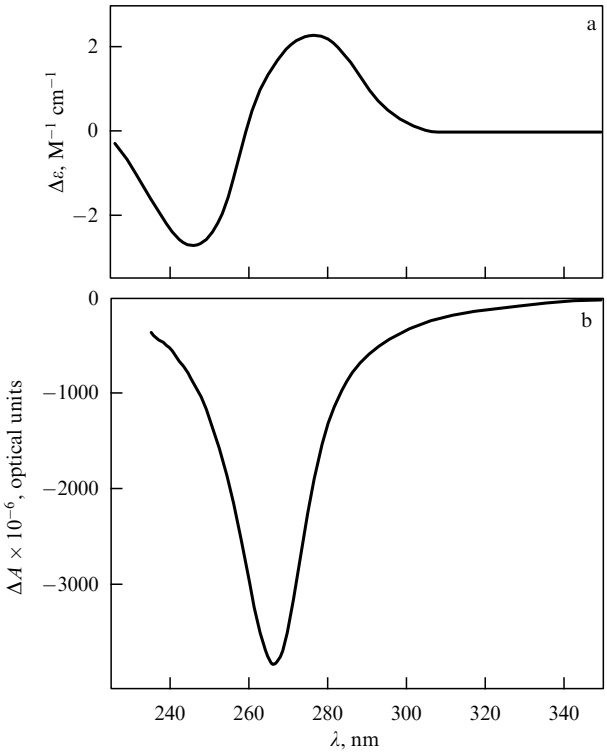

Figure 3. CD spectrum of an aqueous-salt (0.3 M NaCl + 0.002 M Na^+-phosphate buffer) solution of linear B-form DS DNA (a) and calculated CD spectrum of cholesteric LCD DS DNA (b). $C_{DNA} = 10$ μg ml^{-1}, pitch of the spirally twisted structure of DNA dispersion particle is $P = 2500$ nm, its diameter $D = 500$ nm. $\Delta A = A_L - A_R$, $L = 1$ cm.

singling out those results of the calculation that are most relevant in the context of this review.

The amplitude of the band in the CD spectrum (Fig. 3b) depends (given that the DNA secondary structure and properties of nitrogenous bases are constant) on the concentration of dispersion particles and their *structural parameters* (DNA molecular weight, helical twist pitch of the spatial structure, particle size, etc.) [86]. This intense band reflects the so-called *structural* circular dichroism [80] and is characterized by the magnitude of the band amplitude ΔA (in optical units) in the CD spectrum. To emphasize the fundamental difference between molecular circular dichroism that reflects properties of isolated nitrogenous bases or nitrogenous bases present in noncondensed linear DNA molecules and structural CD, the term 'abnormal band' was introduced to denote intense bands in the CD spectra of particles with cholesteric packing of DNA molecules regardless of their sign [87]. Also, the term abnormal optical activity is used to describe the properties of dispersed particles.

Calculations made it possible not only to clarify the conditions (the size of dispersion particles, the pitch of the helical structure, etc.) under which an abnormal band can theoretically appear in the CD spectra of dispersions in the absorption region of DNA chromophores but also to determine the properties of a water-polymer solution at which it can be registered experimentally.

Specifically, Fig. 4 illustrates the dependence of the amplitude of the abnormal band on the diameter of dispersed particles obtained upon phase exclusion of low-molecular-mass DNA molecules (MM less than 1×10^6 Da) from an aqueous salt solution of PEG. Figure 5 shows its dependence on the pitch of the cholesteric spiral of such particles.

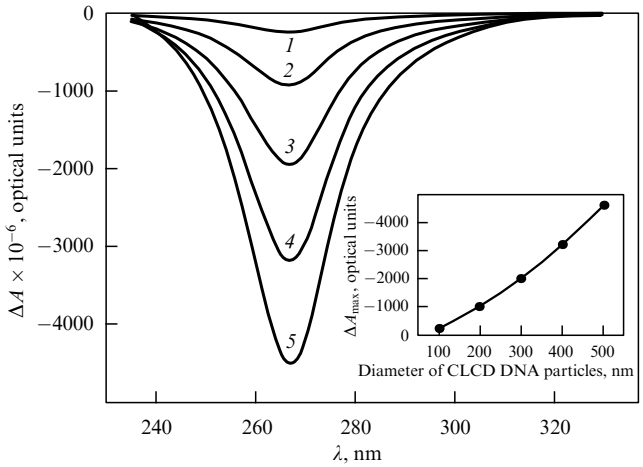

Figure 4. Calculated CD spectra of cholesteric LCD DNA with differently sized particles (diameter $D = 100$, 200, 300, 400, and 500 nm) (curves *1–5*, respectively). $C_{DNA} = 10$ μg ml^{-1}, $P = 2500$ nm, $L = 1$ cm. Inset: dependence of amplitude (ΔA_{max}) of abnormal negative band in the CD spectrum of cholesteric LCD on the size (diameter) of particles of DNA dispersions.

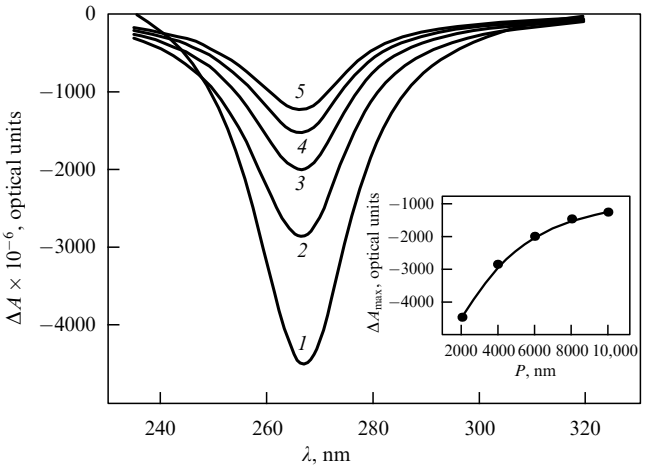

Figure 5. Calculated CD spectra of cholesteric LCD DNA, particles of which differ in the pitch of the cholesteric helix: $P = 2000$, 4000, 6000, 8000, and 10,000 nm (curves *1–5*, respectively). $C_{DNA} = 10$ μg ml^{-1}, $D = 500$ nm, $L = 1$ cm. Inset: dependence of amplitude (ΔA_{max}) of abnormal negative band in the CD spectrum on pitch P of the spatially twisted structure of cholesteric LCD DNA.

In addition, the theory predicted the appearance of an additional anomalous band in the absorption region of compounds positioned in a certain way between nitrogenous base pairs of DNA.

Figure 6 shows, by way of example, the theoretically calculated CD spectra of dispersions obtained by phase exclusion of DNA molecules forming a complex with the colored antibiotic, daunomycin (DAU). DAU molecules can integrate between DNA base pairs. In the calculations, it was assumed that the main absorption band of DAU chromophores is located in the visible region of the spectrum ($\lambda_{max} \sim 500$ nm).

Evidently, the phase exclusion of DNA molecules bound to DAU at a low concentration of the antibiotic is accompanied by two optical effects: first, the abnormal negative band in the absorption region of DNA nitrogenous bases does not change; second, an additional band appears in the absorption area of DAU chromophores in the visible region

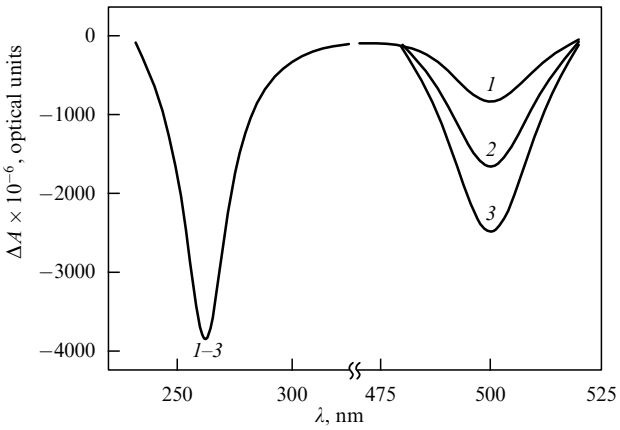

Figure 6. Calculated CD spectra of cholesteric LCD composed of DNA molecules bound to antibiotic daunomycin (curves *1–3* correspond to different concentration C_a of antibiotic: $C_{a3} > C_{a2} > C_{a1}$). $C_{DNA} = 10\ \mu g\ ml^{-1}$, $D = 350$ nm, $P = 2500$ nm, $L = 1$ cm.

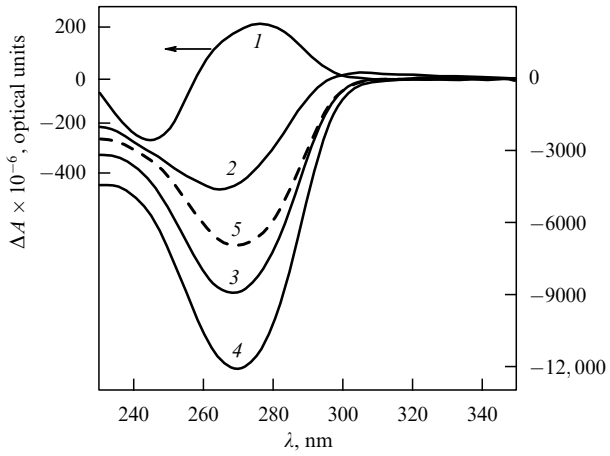

Figure 7. Experimental CD spectra of an aqueous-salt solution of linear B-form DNA (curve *1*, left ordinate axis) and LCDs obtained by means of phase exclusion of DS DNA molecules (MM DNA $\leqslant 1 \times 10^6$ Da) from aqueous-salt solutions with different PEG concentrations (curves *2–5*, right ordinate axis): *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}, $C_{DNA} = 30\ \mu g\ ml^{-1}$, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $L = 1$ cm, $T = 22\,^\circ$C.

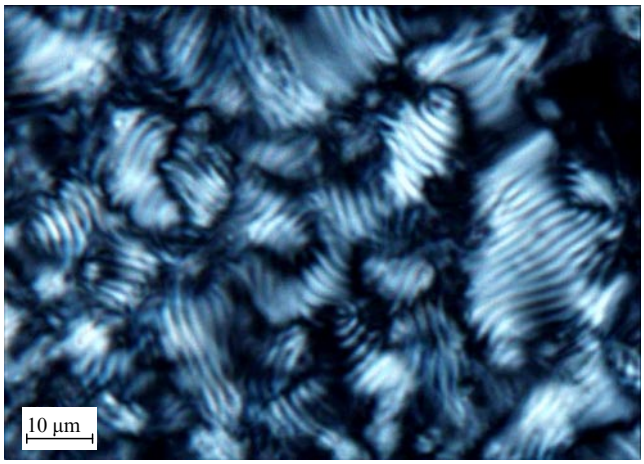

Figure 8. Optical texture of a thin layer of cholesteric LC phase obtained by low-speed centrifugation of particles of low-molecular-mass DNA dispersion (DNA MM $\sim (0.2-0.3) \times 10^6$ Da) formed in an aqueous salt solution of PEG. $C_{DNA} = 30\ \mu g\ ml^{-1}$, $C_{PEG} = 170$ mg ml^{-1}, PEG MM = 4000 Da, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer.

of the CD spectrum. The appearance of this band in the CD spectrum with the sign coinciding with that of the abnormal band in the absorption region of nitrogenous bases means that DAU molecules not only are bound to DNA molecules in a complex but are also oriented in this complex with respect to the axis of the cholesteric structure of DNA dispersion particles at the same angle ($\sim 90^\circ$) as the pairs of nitrogenous bases. This is possible only in the case of embedding (intercalation) of DAU molecules between DNA nitrogenous base pairs. Finally, the amplitude of the band in the DAU absorption region depends on the concentration of the antibiotic bound to DNA molecules. This opens up the possibility of determining DAU concentration using the circular dichroism method.

Thus, the registration of CD spectra makes it possible not only to elucidate the nature of the spatial packing of DNA molecules in dispersion particles but also to determine the mode of arrangement of different compounds on DNA molecules in these particles.

Generally, the results of theoretical calculations of CD spectra indicate that rigid anisotropic DNA molecules in dispersion particles realize their potential tendency toward spatially twisted (cholesteric) packing. Therefore, for DNA LCD particles with an abnormal band in the CD spectrum, the name *cholesteric liquid-crystalline dispersion* (CLCD) is used.

It is of interest to compare the results of theoretical calculations of CD spectra with experimentally measured spectra.

Figure 7 compares a CD spectrum of the linear form of DNA (curve *1*) with experimental CD spectra of LCDs formed as a result of phase exclusion of low-molecular-mass DNA from aqueous-salt solutions of PEG of different concentrations (curves *2–5*). It can be seen that, under almost all the conditions used, the formation of DNA dispersions is accompanied by the appearance of an abnormal negative band in the CD spectrum in the absorption region of nitrogenous bases. According to the theory [88], the appearance of such a band indicates that adjacent DNA molecules in dispersion particles form a spatial helically twisted (cholesteric) structure.

The formation of DNA dispersion particles with cholesteric molecular packing is also evidenced by the fact that a thin layer of the phase obtained by sedimentation of DNA dispersion particles has an optical 'fingerprint' texture (Fig. 8) characteristic of the cholesteric phase [84].

A decrease in the amplitude of the negative abnormal band in the CD spectrum to zero upon a rise in the PEG concentration (see Fig. 7) testifies to the unwinding of the spatial spiral structure of dispersion particles [89].

Consequently, the registration of CD spectra in the absorption region of nitrogenous bases makes it possible to judge the state of the spatial structure of DNA CLCD particles. It should be noted that, in contrast to the usual aggregation of DNA molecules, which occurs at any length of these molecules and is not accompanied by the appearance of abnormal optical activity, CLCD is formed upon phase exclusion of DS DNA molecules only if their length exceeds 150 Å ($\sim$ 50 base pairs). In this case, the phase exclusion of high-molecular-mass DNA molecules (MM more than 10^6 Da) from aqueous-salt solutions of PEG is not accom-

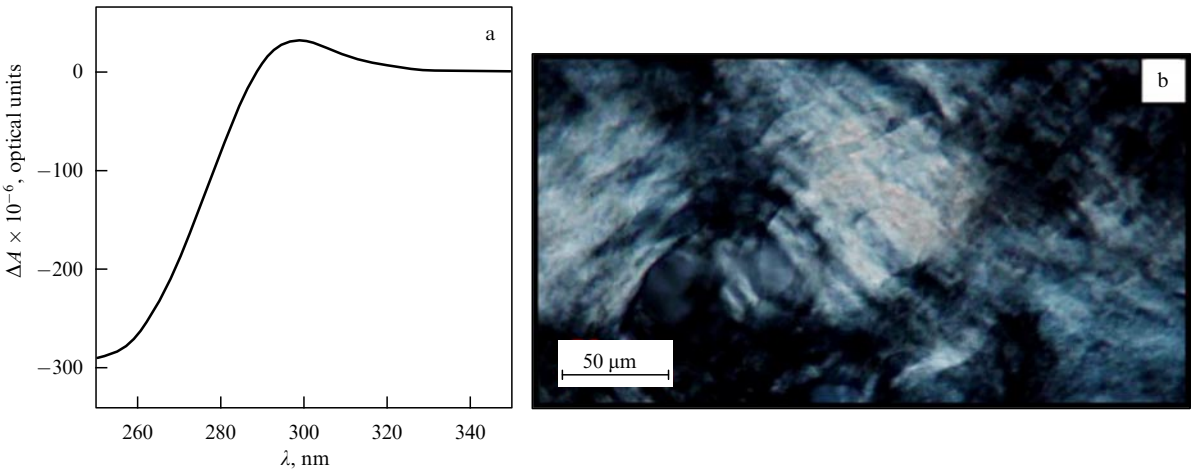

Figure 9. (a) Experimentally measured CD spectrum of LCD obtained by means of phase exclusion of high molecular weight ($\sim 13 \times 10^6$ Da) DS DNA molecules from an aqueous-salt PEG solution. (The very small amplitude of the band in the spectrum is perceived as a value close to zero when the spectrum is recorded under normal conditions.). (b) Optical texture of a thin layer of LC phase obtained by low-speed centrifugation of high-molecular-mass DNA dispersion particles formed in the aqueous-salt PEG solution. $C_{DNA} = 30$ μg ml^{-1}, $C_{PEG} = 170$ mg ml^{-1}, PEG MM 4000 Da, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer.

panied by the appearance of an intense band in the CD spectrum (Fig. 9a) or a 'fingerprint' texture, even if it leads to the aggregation of neighboring DNA molecules (Fig. 9b).

Finally, the recording of CD spectra of dispersions formed at different PEG concentrations gives evidence of the existence of DNA dispersions with different packings of these molecules in dispersion particles.

Figure 10 shows the dependence of the maximum amplitude of the negative abnormal band in the CD spectrum on the osmotic pressure of a PEG solution (PEG concentration). It can be seen that two packing modes are possible for semi-rigid linear DNA molecules at room temperature. At an osmotic pressure of the solution from 1 to 10 atm, there is a spatially twisted (cholesteric) packing of DNA molecules in dispersion particles (domain II region) which is readily detected from the appearance of an abnormal band in the CD spectrum in the absorption region of DNA nitrogenous bases. Such packing arises from the thin layer texture of the DNA phase formed as a result of the low-speed centrifugation of DNA particles (see Fig. 8). This is the 'fingerprint' texture characteristic of cholesterics.

Particles of dispersions formed at an osmotic pressure of more than 10 atm ($C_{PEG} \sim 240-300$ mg ml^{-1}, domain III region) have practically no abnormal band in the CD spectrum [20]. The absence of an abnormal band in the CD spectra of dispersions suggests that the packing of DS DNA molecules in dispersion particles formed at PEG concentrations corresponding to the of domain III region differs from the packing of these molecules in the region of domain II. In this case, the texture of a thin layer of the DNA phase formed as a result of the low-speed centrifugation of particles of DNA dispersion formed at a high concentration of PEG indicates the absence of 'fingerprints'. This texture contains only parts with optical anisotropy.

Thus, the recording of CD spectra shows that the phase exclusion of DS DNA molecules of low-molecular-mass (less than 1×10^6 Da) at room temperature from water-salt solutions of PEG leads to the formation of LCD particles that differ, depending on PEG osmotic pressure, in the way these molecules are packed.

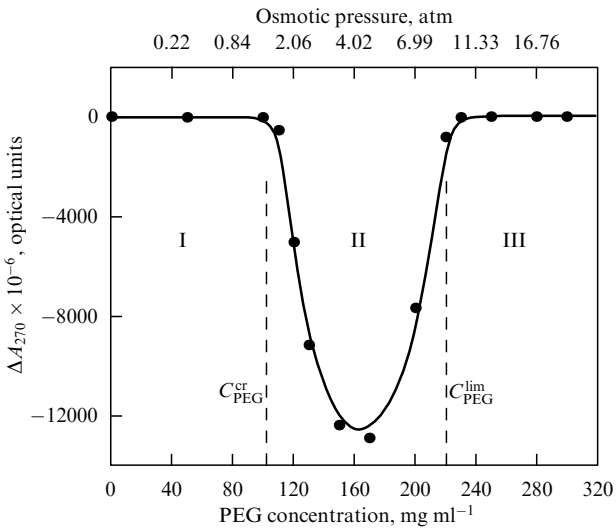

Figure 10. Dependence of measured amplitude ΔA_{270} of the abnormal band in CD spectra of DNA aqueous-salt solutions at wavelength $\lambda = 270$ nm on PEG concentration. Upper abscissa axis shows the values of osmotic pressure of PEG solutions, the concentrations of which are indicated on the lower abscissa axis. $C_{DNA} = 30$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer. $L = 1$ cm, $T = 22$ °C, C_{PEG}^{cr} and C_{PEG}^{lim} — critical and limit PEG concentrations determining the 'boundary' conditions for the existence of LCD differing in the mode of DNA packing (domain II region and domain III region).

Taking into account the diagram shown in Fig. 10, it can be inferred that the following sequence of phase transitions occurs upon phase exclusion of low-molecular-mass DNA molecules as PEG concentration in the solution increases at room temperature: isotropic phase (domain I region) → cholesteric phase (domain II region) → hexagonal phase (domain III region) of DNA.

6. Particles of DNA liquid-crystalline dispersions formed at elevated temperature

Figure 11 shows, as an example, a change in the CD spectrum of a DS DNA dispersion formed in the region of domain II (curve *1*, $C_{PEG} = 170$ mg ml^{-1}, osmotic pressure

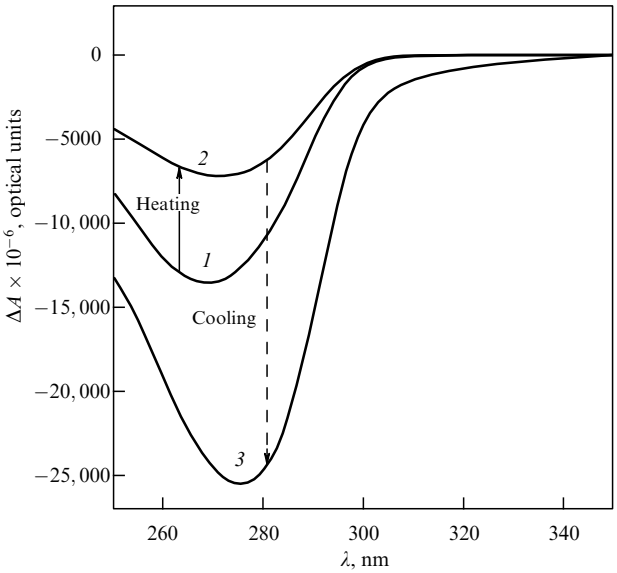

Figure 11. CD spectra of DNA cholesteric dispersion formed in the region of domain II (C_{PEG} = 170 mg ml^{-1}, curve *1*) upon heating (curve *2*) and subsequent cooling (curve *3*): *1* — 22 °C, *2* — 80 °C, *3* — 80 °C → 22 °C. C_{DNA} = 30 μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer, L = 1 cm. Solid and dashed arrows show change in amplitude of abnormal band in the CD spectrum of CLCD DNA upon heating and cooling, respectively.

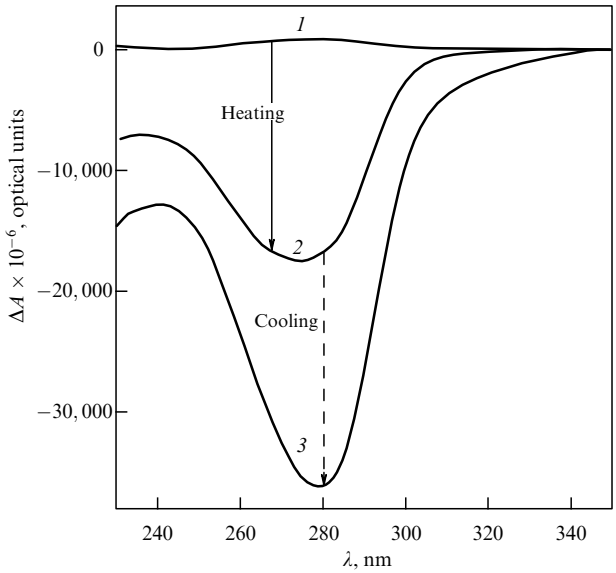

Figure 12. CD spectra of DNA dispersion formed in the region of domain III (C_{PEG} = 250 mg ml^{-1}, curve *1*) upon heating (curve *2*) and subsequent cooling (curve *3*): *1* — 22 °C, *2* — 80 °C, *3* — 80 °C → 22 °C. C_{DNA} = 30 μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer, L = 1 cm. Solid and dashed arrows show change in amplitude of abnormal band in the CD spectrum of CLCD DNA upon heating and cooling, respectively.

π = 4.6 atm) upon heating and subsequent cooling (curves *2* and *3* respectively). The well-studied process of the reduction in the abnormal band amplitude in CD spectra of cholesteric dispersions upon heating (cf. curves *1* and *2* in Fig. 11), called 'CD melting' [41], reflects the unwinding of the spatial spiral structure of dispersion particles.

The cooling of a dispersion is accompanied not only by the restoration of the abnormal band in the CD spectrum but also by an increase in its amplitude (curve *3*). This effect is consistent with the idea of improving the character of DS DNA packing in the spatial structure of dispersion particles as a result of 'thermal training' of LCDs [90, 91]. Such a change in the CD spectrum upon heating and cooling is characteristic of all dispersions formed in the region of PEG osmotic pressure corresponding to domain II.

By way of example, Fig. 12 shows a change in the CD spectra of a DNA dispersion with the hexagonal packing of molecules upon heating and cooling. This dispersion was formed in the range of PEG concentrations corresponding to domain III (C_{PEG} = 250 mg ml^{-1}, π = 12.43 atm). In this case, a rise in temperature is accompanied by an unusual optical effect [40, 92]: *for particles of a DNA dispersion that barely had an abnormal band in the CD spectrum at room temperature* (see Fig. 12, curve *1*), *this band appears* (see Fig. 12, curve *2*).

The shape of CD spectra for all dispersions formed in the region of domain III changes in a similar way upon heating. In this case, the amplitude of the abnormal band in the CD spectrum increases at a certain critical heating temperature (T^{cr}[° C]) [92] that depends on the PEG osmotic pressure (Fig. 13a, curves *1-1–3-1*). Figure 13b shows that, the higher the osmotic pressure of PEG, the greater the T^{cr} value at which the amplification of the abnormal band in the CD spectrum occurs when dispersed particles containing hexagonally packed DNA molecules are heated.

Figure 14 compares curves characterizing abnormal optical activity in all DNA LCDs formed in both domain II and III regions of PEG osmotic pressure upon heating (curve *2*) and cooling (curve *3*).

The phase diagram presented in Fig. 14 shows that a decrease in DNA LCD temperature is accompanied by different optical effects, depending on the PEG osmotic pressure [92]. At a pressure up to 10 atm, the magnitude of abnormal optical activity grows with cooling (see Fig. 14, curve *3*), whereas at a PEG osmotic pressure in excess of 10 atm a tendency toward partial restoration of the initial abnormal optical activity of LCDs is observed (see Fig. 14).

It should be noted that the general form of the phase diagram shown is typical of all semi-rigid linear DS DNA molecules (molecular mass $\leqslant 1 \times 10^6$ Da) used to obtain dispersions; however, the specific form of the diagram (the amplitude of the observed effect and the position of its minimum) depends on the molecular mass, nucleotide composition and concentration of DNA, and, apparently, the temperature history of the dispersion.

Since the abnormal band in the CD spectrum reflects the helical (cholesteric) packing of quasinematic layers of DS DNA molecules in LCD particles [88], it can be assumed that heating all particles with the initial hexagonal packing of DS DNA molecules (domain III) leads to the emergence of a new spatially twisted structure of these molecules [39]. We called this new structural state of DNA molecules in dispersion particles the 're-entrant' cholesteric state (see [40]) to emphasize that heating the particles of a dispersion with a hexagonal packing of DNA molecules results in a hexagonal to re-entrant cholesteric packing phase transition.

To recall, the 'classical' cholesteric state is realized when the average distance between DNA molecules is close to 35 Å [11, 41].

Because, in our case, an increase in osmotic pressure in the range of PEG concentrations corresponding to domain III is

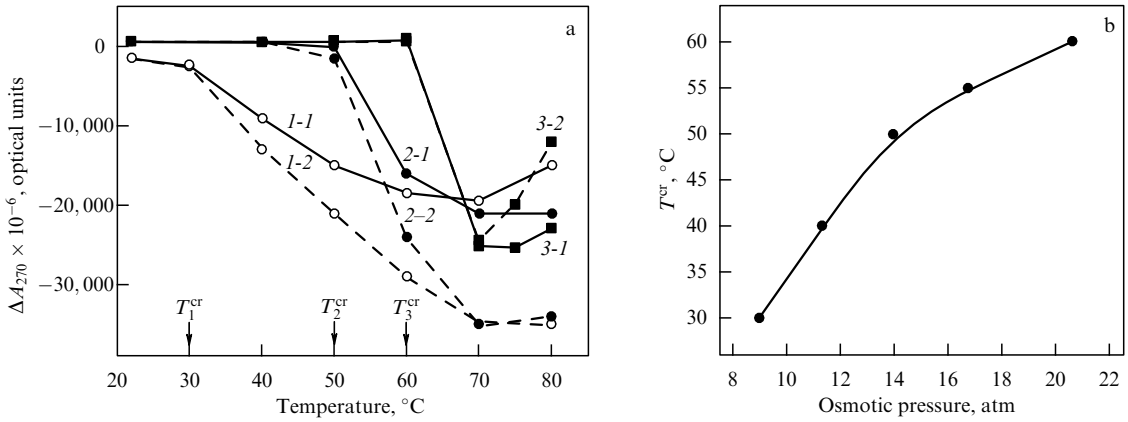

Figure 13. (a) Temperature dependence of amplitude of abnormal band ($\lambda = 270$ nm) in CD spectra of DNA LCD formed in the region of domain III in aqueous-salt solutions with different PEG concentrations (solid curves *1-1–3-1*). *1* — $C_{PEG} = 220$ mg ml^{-1}, *2* — $C_{PEG} = 260$ mg ml^{-1}, *3* — $C_{PEG} = 300$ mg ml^{-1}. Dashed curves characterize optical properties of DNA LCD formed at $C_{PEG} = 220$, 260, and 300 mg ml^{-1} (curves *1-2*, *2-2*, and *3-2*, respectively) after the heating → cooling cycle: 30 °C → 22 °C, 40 °C → 22 °C, 50 °C → 22 °C, 60 °C → 22 °C, 70 °C → 22 °C, and 80 °C → 22 °C. $C_{DNA} = 30$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer, $L = 1$ cm. T^{cr} is 'critical' temperature at which amplification of abnormal band in the CD spectrum begins upon DNA LCD heating. Arrows indicate critical temperatures values (T_{1-3}^{cr}) characteristic of DNA LCD formed at PEG concentrations of 220, 260, and 300 mg ml^{-1}, respectively. (b) Dependence of T^{cr} on osmotic pressure of PEG aqueous-salt solutions.

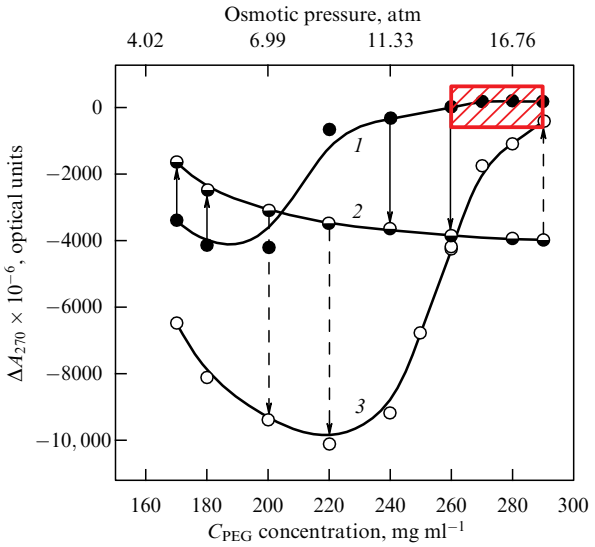

Figure 14. Dependence of amplitude ΔA_{270} of abnormal band ($\lambda = 270$ nm) in CD spectra of DNA LCD formed at room temperature in aqueous-solutions of PEG with different osmotic pressures (curve *1*), heated to 80 °C (curve *2*), and cooled to room temperature (curve *3*). $C_{DNA} = 10$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+ phosphate buffer, $L = 1$ cm. Solid and dashed arrows show changes in amplitude of abnormal band in the CD spectrum with an increase in temperature from 22 °C to 80 °C and its decrease from 80 °C to 22 °C, respectively. Shaded rectangle in the case of DNA particles with hexagonal packing denotes region of inaccurate determinations of small (close to zero) values of the band amplitude that depend on the 'history' of preparation of these particles.

accompanied not only by a decrease in the mean distance between DNA molecules within the range from 28 to 24 Å [40] but also by a possible distortion of the secondary structure of these molecules, it is necessary to check the degree of 'cholesterism' in the packing of DNA molecules in particles with a 're-entrant' structure; in other words, it is necessary as a minimum to determine the state of the secondary structure of these molecules under conditions of a high PEG concentration [93–95].

7. Intercalation of antibiotics between DNA base pairs in dispersion particles with different packing of molecules

The state of the secondary structure of DNA in dispersion particles formed at different osmotic pressures of the solution was verified from the result of intercalation (incorporation) of the anthracycline antibiotic, doxorubicin (DOX), between pairs of DNA nitrogenous bases. This antibiotic is an analog of DAU; the theoretically calculated CD spectra of DAU complexes with DNA molecules within cholesteric dispersion particles are shown in Fig. 6.

The structural components of anthracycline antibiotics include aglycone (anthracycline) and sugar residue [96–99]. The structure of the antibiotics was elucidated using chemical and physicochemical methods [97] and confirmed by direct chemical synthesis. These antibiotics are water-soluble low-molecular-mass compounds with four reactive oxygen atoms at positions 5, 6, 11, and 12 of aglycone (Fig. 15). Antibiotics of the anthracycline series have absorption bands in both the visible and UV regions of the spectrum [99], the first located within the wavelength range of 400–600 nm, the other in the 200–300 nm region, respectively.

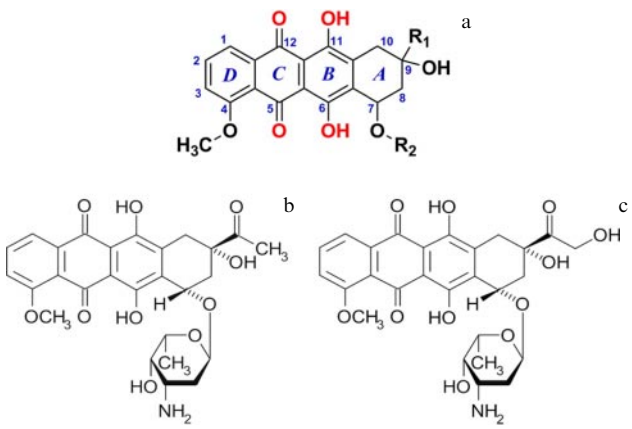

Figure 15. Structure of aglycone of antibiotics of the anthracycline group (a) and structural formulas of anthracyclines DAU (b) and DOX (c).

Transition moments in the molecules of anthraquinones and anthracyclines were calculated in Refs [100, 101]. It turned out that, in anthraquinone showing absorption bands with a maximum at 476 and 340 nm, the long-wavelength transition moment lies in the chromophore plane and is directed along the axis connecting the oxygen atoms, i.e., parallel to the long axis of the molecule, and is localized in the plane of the anthraquinone chromophore.

When DS DNA molecules are added to anthracycline antibiotics, the long-wavelength band shifts by 20–30 nm (depending on the structure of the antibiotic) towards longer wavelengths (bathochromic shift) and its intensity decreases (hypochromic effect) [97].

The hypochromic effect in the antibiotic absorption band in the presence of DS DNA reflects a reduction in polarity of the environment of the chromophore upon its binding to DNA associated with the incorporation (intercalation) of the flat chromophore portion between the pairs of nitrogenous bases of DNA molecules.

In the case of DAU and some other anthracycline antibiotics, their interaction with DNA is accompanied by quenching of luminescence [102], which indicates a change in their electronic state in the complex with DNA.

Important proof of the intercalation of anthracyclines is the unwinding of circular covalently closed supercoiled DNA during its interaction with antibiotics and dyes. The unwinding of circular supercoiled DNA during interaction with an antibiotic is accompanied by a change in its hydrodynamic characteristics and evidences the formation of an intercalation complex with DNA. The angle of unwinding of the double helix of a DNA molecule upon its interaction with DAU, iremycin, and violamycin B1 is approximately half the twist angle [103] characteristic of ethidium bromide (20°). The addition of anthracycline antibiotics to DNA increases its viscosity and decreases the sedimentation coefficient [104] due to enhanced contour length and rigidity of DNA molecules upon binding to the antibiotic.

The calculations reported in [104, 105] show that an increase in the contour length of DNA (ΔL) when it binds to anthracycline antibiotics amounts to 27–30%, an equivalent to 3.6–4.0 Å per molecule of the DNA-bound antibiotic. The enhancement of DNA rigidity upon binding one antibiotic molecule evaluated from a change in the persistent DNA length is 3.6 Å for DAU, 3.4 Å for adriamycin, and 3.0 Å for aclamycin, which exceeds the respective values of this parameter for acridine dyes. The increase in the contour length of DNA during its interaction with anthracycline antibiotics once again testifies to the intercalation of the antibiotics between DNA base pairs accompanied by partial unwinding of a DNA molecule and its elongation.

In a DNA-anthracycline complex, the plane of the antibiotic chromophore is roughly parallel to the plane of the DNA base pairs, i.e., perpendicular to the long axis of the double helix [100].

Figure 16 shows a model of DNA complexes with anthracycline antibiotics [100]. According to this model, an antibiotic chromophore is inserted between DNA base pairs from the side of the narrow groove of the double helix (Fig. 16a). The complex is stabilized due to interplanar (stacking) interactions of the planar ring system of the antibiotic with DNA bases located 'above' and 'below' the intercalation site, as well as a result of the electrostatic interaction of positively charged amino groups of the sugar residue of the antibiotic with a negatively charged phosphate group of DNA. The long axis of the intercalated antibiotic molecule turns out to be perpendicular to the line connecting adjacent DNA base pairs at the site of intercalation.

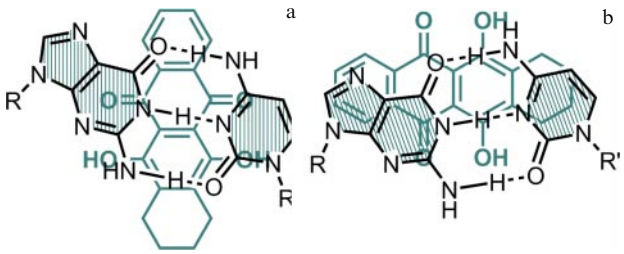

Figure 16. Schematic representations of intercalation (a) and 'external' (b) complexes between anthracycline aglycones and base pairs of a DS DNA molecule (view along the long axis of the DNA helix; DNA base pairs are shaded).

It can be concluded that the secondary structure of DNA must correspond to the physicochemical parameters of its B-form if an intercalation complex between anthracycline antibiotics and linear DS DNA molecules is to be formed. This is because the degree of hydration of B-form DNA, the distance between nitrogenous base pairs, etc. provide the conditions for unwinding the double helix and lengthening the DNA molecule that are necessary for the embedding of an intercalator molecule between the pairs of nitrogenous bases.

Anthracycline antibiotics are also capable of forming nonintercalation ('external') complexes with single-stranded polynucleotides [106], polyphosphates, and denatured DNA [107]. However, such complexes are significantly inferior to those formed by these antibiotics with DS DNA molecules in terms of strength. An analysis of the binding constants of anthracyclin — B-form DNA complexes [108, 109] confirmed the hypothesis of the formation of 'external' complexes with a lower binding constant than that of the classical intercalation complexes.

The presumed structure of an external complex is shown in Fig. 16b. Double-stranded RNA and polynucleotides with the secondary structure of the A-form do not produce intercalation complexes with anthracycline antibiotics [109, 110]. Nevertheless, these nucleic acids are capable of 'external' binding of anthracyclines due to the electrostatic interaction between the positively charged amino sugar of the antibiotic and the negatively charged phosphate group of the nucleic acid [112].

Thus, the above data show that, depending on the conditions (nature of the nucleic acid, ionic strength, degree of filling of the polymer with the ligand), the molecules of anthracycline antibiotics form either intercalation or external complexes with DS nucleic acid molecules; moreover, antibiotic molecules occupy a certain fixed position on these molecules.

Importantly, in the case of the formation of an intercalation complex with DS DNA molecules, oxygen atoms in positions 5, 6 or 11, 12 of anthracycline antibiotics become practically inaccessible to chemical reactions (especially for chelation), whereas the same atoms take part in various chemical reactions during formation of an external complex with molecules of both DS DNA and DS RNA.

Particles of dispersions obtained by phase exclusion of DNA molecules from aqueous-salt solutions with different concentrations of PEG were treated with the anthracycline antibiotic doxorubicin (DOX) taking into account the above

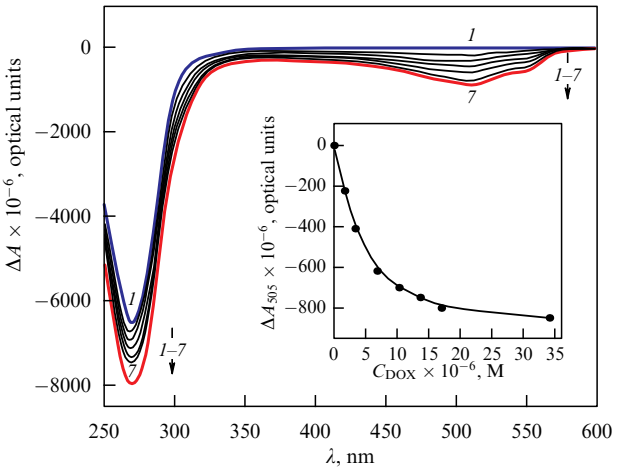

Figure 17. CD spectrum of a cholesteric dispersion of DNA treated with DOX. Curve *1* — CD spectrum of initial cholesteric dispersion heated from 22 °C to 80 °C and cooled to room temperature (22 °C). Curves *2–7* — CD spectra after treatment of cholesteric dispersion with DOX: *2* — $C_{DOX} = 1.72 \times 10^{-6}$ M, *3* — $C_{DOX} = 3.44 \times 10^{-6}$ M, *4* — $C_{DOX} = 5.17 \times 10^{-6}$ M, *5* — $C_{DOX} = 6.12 \times 10^{-6}$ M, *6* — $C_{DOX} = 17.18 \times 10^{-6}$ M, *7* — $C_{DOX} = 34.14 \times 10^{-6}$ M. $C_{DNA} = 10$ μg ml^{-1}, $C_{PEG} = 170$ mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $L = 1$ cm. Inset: dependence of band amplitude in the CD spectrum ($\lambda = 505$ nm) of DNA dispersion with cholesteric packing of molecules in particles ($C_{PEG} = 170$ mg ml^{-1}) treated with DOX on DOX concentration, $L = 1$ cm.

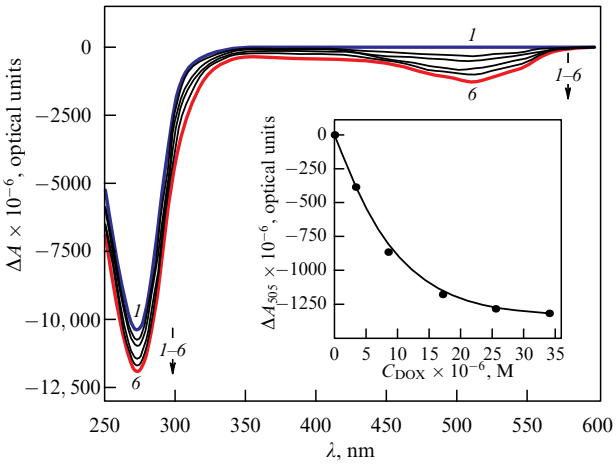

Figure 18. CD spectra of a dispersion of the 're-entrant' cholesteric phase of DNA treated with DOX: curve *1* — CD spectrum of dispersion of initial 're-entrant' phase obtained by heating to 80 °C and cooling to 22 °C of dispersion with hexagonal packing of molecules; curves *2–6* — CD spectra after treatment of the 're-entrant' phase with DOX: *2* — $C_{DOX} = 1.72 \times 10^{-6}$ M, *3* — $C_{DOX} = 3.44 \times 10^{-6}$ M, *4* — $C_{DOX} = 6.89 \times 10^{-6}$ M, *5* — $C_{DOX} = 13.74 \times 10^{-6}$ M, *6* — $C_{DOX} = 34.14 \times 10^{-6}$ M, $C_{DNA} = 10$ μg ml^{-1}, $C_{PEG} = 240$ mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $L = 1$ cm. Inset: dependence of band amplitude in the CD spectrum ($\lambda = 505$ nm) of DNA particles with a 're-entrant' cholesteric structure ($C_{PEG} = 240$ mg ml^{-1}) treated with DOX on the DOX concentration, $L = 1$ cm.

literature data. A dispersion of a 'classical' cholesteric formed upon phase exclusion of DNA molecules from an aqueous-salt solution with a PEG concentration of 170 mg ml^{-1} was used as a control.

For comparison, Fig. 17 (curve *1*) shows the CD spectrum of a cholesteric dispersion (after its thermal training, $C_{PEG} = 170$ mg ml^{-1}), the particles of which are formed from DNA molecules and then treated with DOX. In the visible region of the CD spectrum, an additional band appears in the absorption band of DOX, the negative sign of which coincides with the sign of the band in the absorption region of DNA nitrogenous bases, in excellent agreement with the theory [85]. The appearance of this additional band in the CD spectrum means that DOX molecules not only interact with DNA molecules but integrate (intercalate) between DNA nitrogenous base pairs [34]. The angle of inclination of DOX molecules practically coincides with that of the nitrogenous bases of DNA with respect to the axis of the cholesteric structure of dispersion particles.

The inset in Fig. 17 shows the dependence of the negative band amplitude in the CD spectrum of cholesteric DNA particles in the absorption region of DOX on the DOX concentration in the solution. This dependence shows that DOX molecules interact with DNA molecules until the DNA molecules are completely saturated with anthracycline.

Changes to CD dispersion spectra in the absorption regions of both DNA chromophores and the chromophores of the compound intercalating between nitrogenous base pairs shown in Fig. 17 are characteristic of cholesteric structures retaining the B-form parameters.

Figure 18 shows the CD spectrum of a DNA dispersion, the particles of which having the 're-entrant' cholesteric structure ($C_{PEG} = 240$ mg ml^{-1}) were also treated with DOX. This dispersion was obtained from particles with a hexagonal packing of DNA molecules by heating them to 80 °C and cooling to 22 °C. This process led to the appearance of an abnormal band in the CD spectrum, corresponding to the formation of a 're-entrant' cholesteric structure (see above).

Several facts are noteworthy.

First, in a dispersion of particles with a 're-entrant' structure, the amplitude of the band in the absorption region of nitrogenous bases is much greater than that in a classical cholesteric formed at $C_{PEG} = 170$ mg ml^{-1} (see Fig. 17).

Second, an additional band — the negative sign of which coincides with the sign of the band in the absorption region of DNA nitrogenous bases — appears in the absorption band of DOX in the visible region of the CD spectrum of the dispersion, the particles of which have a 're-entrant' cholesteric structure. The appearance of such an additional band suggests an interaction between DOX and DNA molecules. Moreover, the greater amplitude of the band in the absorption region of DNA chromophores corresponds to the higher value of the equilibrium band amplitude at $\lambda = 505$ nm.

Third, the dependence of the negative band in the CD spectrum in the DOX absorption region in particles with the 're-entrant' cholesteric structure (see inset in Fig. 18) on the DOX concentration shows that these molecules can integrate (intercalate) between the pairs of nitrogenous bases of DNA molecules until their complete saturation with anthracycline.

It should be borne in mind that, according to the results of an X-ray diffraction analysis [40], an increase in PEG osmotic pressure causes a noticeable decrease in the mean distance between DNA molecules in the formed structure. Theoretically, this decrease must lead to an enlargement of the abnormal band amplitude in the CD spectrum of DNA cholesteric dispersions [88].

Figure 19 compares the results of theoretical calculations for two conditional DNA dispersions with cholesteric packing of molecules differing in their parameters. It can be

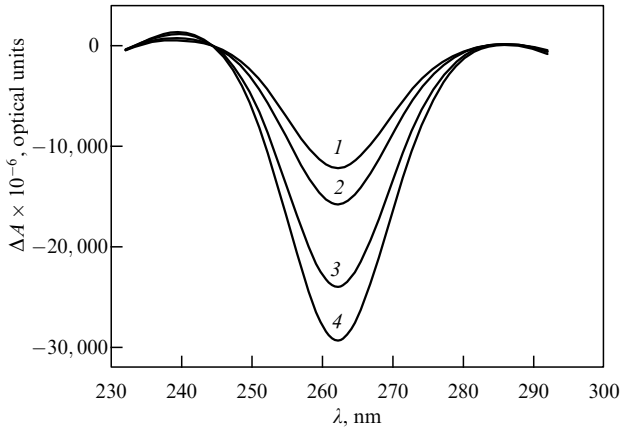

Figure 19. Calculated CD spectra of two conventional DNA cholesterics differing in average distance d between quasinematic layers and twist angle φ of these layers: *1* — $d = 3.5$ nm, $\varphi = 0.5°$, *2* — $d = 2.5$ nm, $\varphi = 0.5°$, *3* — $d = 2.5$ nm, $\varphi = 1.0°$, *4* — $d = 2.5$ nm, $\varphi = 1.5°$. $C_{DNA} = 30$ μg ml^{-1}, $L = 1$ cm.

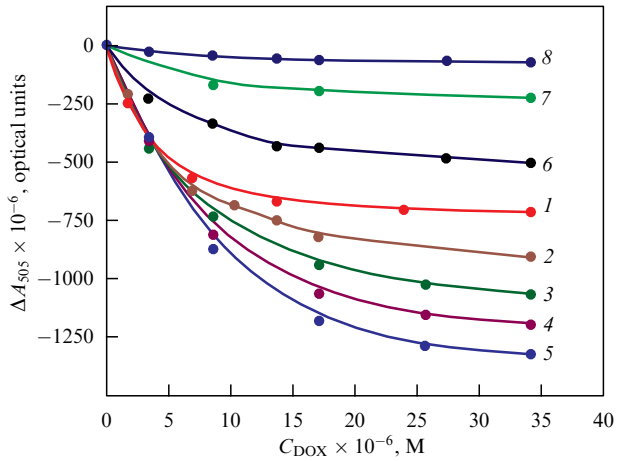

Figure 20. Dependences of band amplitude in the CD spectrum ($\lambda = 505$ nm) for particles of DNA dispersions with cholesteric and 're-entrant' cholesteric structures treated with DOX on DOX concentration: *1* — $C_{PEG} = 170$ mg ml^{-1}, *2* — $C_{PEG} = 180$ mg ml^{-1}, *3* — $C_{PEG} = 200$ mg ml^{-1}, *4* — $C_{PEG} = 220$ mg ml^{-1}, *5* — $C_{PEG} = 240$ mg ml^{-1}, *6* — $C_{PEG} = 260$ mg ml^{-1}, *7* — $C_{PEG} = 270$ mg ml^{-1}, *8* — $C_{PEG} = 280$ mg ml^{-1}. $C_{DNA} = 10$ μg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $L = 1$ cm.

seen that a decrease in the mean distance between DNA molecules forming the cholesteric structure from 35 to 25 Å leads to a noticeable increase in the abnormal band amplitude in the CD spectrum. Based on Fig. 19, it can be argued that a decrease in the distance between DNA molecules in dispersion particles (with the remaining parameters of the secondary structure of these molecules unaltered) due to a rise in PEG osmotic pressure should be accompanied by an increase in the amplitude of the abnormal band in the CD spectrum.

A comparison of Figs 18 and 19 shows that the optical properties of a 're-entrant' structure exceed those of the initial cholesteric. Because of the correlation between the bands in the absorption regions of DNA and antibiotic chromophores in the CD spectrum (with the secondary structure of DNA molecules being unchanged), a broadening of the band in the DNA chromophore region is accompanied by a growth of the analogous band in the absorption region of antibiotic chromophores in the visible region of the CD spectrum. Such a correlation is possible only if the parameters of B-form DNA molecules are preserved, as is necessary for DOX intercalation. Thus, dispersion particles with the 're-entrant' cholesteric structure formed from particles with a hexagonal packing of DNA molecules at $C_{PEG} = 240$ mg ml^{-1} retain the parameters of the B-form DNA molecules unaltered. A further rise in the PEG concentration is accompanied by a change in the properties of 're-entrant' cholesteric structures.

Figure 20 shows dependences of the band amplitude in the CD spectrum of dispersions in the absorption region of DOX chromophores on its concentration in the solution. These dispersions are formed at different PEG concentrations.

An analysis of the data presented in Fig. 20 revealed the following facts.

First, an increase in the PEG concentration from 170 to 240 mg ml^{-1} leads to an enlargement of the band amplitude in the absorption region of DOX chromophores, in excellent agreement with the theoretical concept holding that an increase in the PEG osmotic pressure is accompanied by a decrease in the distance between DNA molecules in dispersion particles and a corresponding increase in the abnormal band amplitude (see Fig. 19). In this case, there is a correlation between the enlargement of band amplitudes in the absorption regions of DNA chromophores and of the compound intercalating between DNA base pairs, with the secondary structure of the DNA remaining unchanged. Therefore, the broadening of the band in the CD spectrum in the DOX absorption region (see Fig. 20, curves *1–5*) reflects only a reduction in the distance between DNA molecules in the resulting dispersion particles with the 're-entrant' cholesteric structure. In such particles, the B-form of the DNA molecules is preserved.

Second, an increase in the PEG concentration to 280 mg ml^{-1} is accompanied by an additional decrease in the mean distance between DNA molecules in dispersion particles with a hexagonal molecular packing [40]. It is possible that under such conditions the properties of the quasinematic layers themselves begin to change. This results in a sharp decrease in the amplitude of the abnormal negative band ($\lambda = 270$ nm) in the CD spectra of both dispersions formed from such particles and those having the 're-entrant' cholesteric structure [94].

Due to the correlation between amplitudes of the abnormal bands in DNA and DOX absorption regions, the amplitude of the band in the absorption region of DOX should also decrease. Moreover, at a high packing density of DNA molecules in dispersion particles, parameters of the secondary structure of these molecules begin to change in parallel with the changes in the properties of the quasinematic layers. Under these conditions, the correct intercalation of DOX between DNA base pairs and the correct placement of DOX in the quasinematic layers of dispersion particles becomes impossible.

Therefore, when dispersions with a high packing density of DNA molecules are treated with DOX molecules, the shape of the band in the CD spectrum in the absorption region of DOX chromophores resembles that in the CD spectrum characteristic of a linear DNA molecule treated with anthracycline [113]. It becomes extremely difficult to determine the efficiency of intercalation of this anthracycline between DNA nitrogenous base pairs (from the width of the low-intensity band in the CD spectrum).

The data presented in Fig. 20 give reason to assume that heating initial particles of dispersions with the hexagonal packing of molecules results, depending on the mean distance between DNA molecules, in the formation of at least several families of particles differing not only in the ability to form 're-entrant' cholesteric structures but also in the ability to interact with anthracycline intercalators.

8. 'Re-entrant' cholesteric DNA structures and the Lindemann criterion

In 1910, F Lindemann proposed a fairly simple semi-empirical model of melting of crystalline materials [114]. Lindemann's main assumption was that these materials are capable of transition to a new structural state when the amplitude of thermal atomic vibrations (the degree of the mean square displacement of atoms, to be precise) exceeds some relatively small (approximately 0.1) fraction of the distance between nearest neighbors [115]. This fraction is termed the 'Lindemann number' (L). The empirical value of L for many simple crystals is about 10% of the interatomic distance [116]. In principle, the magnitude of the Lindemann number is *a priori* unknown; it depends on many factors, e.g., intermolecular (or interatomic) interaction and the structure and dimensions of the system [117, 118].

By itself, the Lindemann number does not allow predicting the properties of a new phase formed when the Lindemann criterion is satisfied.

As far as the case under consideration is concerned, it should be noted that the theory of melting of the hexagonal phase formed by semi-rigid DNA molecules is yet to be proposed. An important relevant study was carried out by T Odijk [65, 66] on the assumption that, in the case of a hexagonal packing of DNA molecules in quasi-nematic layers, these molecules are parallel to each other (Fig. 21). Under certain conditions, in the limit with relatively small fluctuations of the hexagonal structure, i.e., at $u \ll D$ [66], the phase state of the above structure can change and, according to Lindemann's semi-empirical criterion,

$$\frac{u_{\mathrm{m}}}{D_{\mathrm{m}}} = L\,,$$

where the subscript m means that the equality takes place on the melting curve of the hexagonal DNA phase and a new phase can arise.

Estimates of the Lindemann number for such a transformation of the hexagonal structure have been reported by different authors based on the results of experiments and theoretical calculations. $L = 0.122$ [66] (it should be noted that the hexagonal phase of DNA in [66] existed at a distance of 4.8 nm between the molecules, at variance with the known literature data [20, 35, 46]: $L = 0.73$ [119], $L = 0.48$ [120], $L = 0.13-0.18$ [121], $L = 0.098 \pm 0.003$ [14]).

One can use the data from Ref. [66] to estimate the Lindemann number from the modulus of the nematic orientational order parameter (S). According to these data, $L = (1-S)^{1/2}$. Taking into account the fact that, in the case of liquid crystals formed by different DS nucleic acid molecules, the order parameter is 0.6–0.8 [92], the value of L lies in the range of 0.16–0.04, in agreement with the above estimates.

Estimates of the Lindemann number substantially depend on the accuracy of determining the distance between the nearest neighbors (i.e., on the D value) and on molecular characteristics.

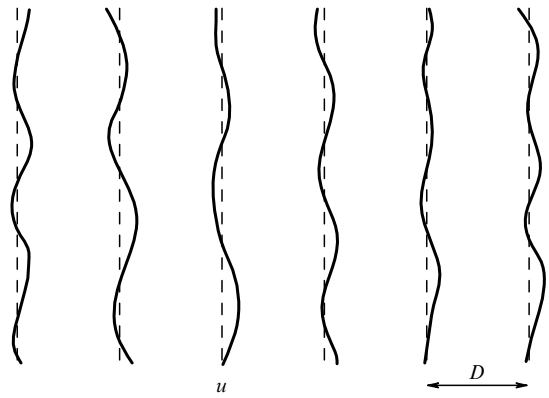

Figure 21. Schematic two-dimensional representation of a 'quasinematic' layer formed by weakly fluctuating semi-flexible DNA molecules. D — mean distance between neighboring DNA molecules, u — amplitude of flexural thermal fluctuation displacements of DNA molecules in the layer.

When a usual crystal structure is heated, the amplitudes of molecular thermal fluctuation displacements (u) increase. When the value of mean squared displacements $\langle u^2 \rangle$ becomes of the order of a certain fraction of the intermolecular distance l, the system displays properties of a fluid [122–124].

Two important additions are in order:

— as mentioned above, the Lindemann number is not a universal value; it depends on the symmetry and dimension of the system itself and characteristics of molecules that form it. Specifically, it was shown in the aforementioned work by T Odijk that the Lindemann number substantially depends on electrostatic repulsion, which significantly modifies the amplitude of molecular displacements;

— the crystal-like behavior (i.e., small displacements relative to the equilibrium position) becomes unstable and the transition to a liquid appears possible when the vibration amplitudes are still much smaller than the average distance between molecules.

To recall, a similar criterion for structural phase transformations can also be used for systems lacking in a long-range crystalline order. The criterion also makes it possible to describe structural changes in systems with short-range translational ordering (where distance l between molecules is conserved only on average).

The above means that the criterion is applicable in the case of hexagonal packing of DNA molecules. In this case, the classical Lindemann criterion can be reformulated as

$$\frac{\langle u^2(T_{\mathrm{m}})\rangle}{l^2} \approx 10^{-2}\,, \tag{1}$$

where T_{m} is the melting temperature, a condition that the loss of elastic energy become comparable to the gain in entropy. This form of the Lindemann criterion (up to numerical coefficients per unit volume) can be represented as

$$\frac{1}{2}\lambda\,\frac{\langle u^2\rangle}{l^2} \approx \frac{k_{\mathrm{B}}T}{l^3}\,, \tag{2}$$

where λ is Young's modulus and k_{B} is the Boltzmann constant.

Both criteria, (1) and (2), are equivalent.

The above experimental data (see Fig. 14) can be qualitatively explained using this generalized Lindemann criterion. Since the transition from the hexagonal packing of

DNA molecules to the cholesteric one is of interest, this criterion must be modified taking into account the following factors.

(1) The hexagonal structure of DNA molecules (see Fig. 1) is not a crystal with long-range translational and orientational ordering. In this structure, only a short-range translational order is possible in combination with a nematic-like long-range orientational ordering.

(2) In our case, we are not dealing with 'complete' melting, i.e., with the transition of DNA molecules from the hexagonal state to an isotropic one, but are observing a phase transition of a structure with a hexagonal packing into a structure with a long-range cholesteric-like orientational order and short-range translational ordering.

An advantage of formula (2) is that it can be applied in the case under consideration, i.e., to describe the structural transition observed upon heating LCD particles with a hexagonal packing of DNA molecules.

Only two modifications of equation (2) are needed:

(a) in a cholesteric structure, the long-range orientational order is preserved (although the structure is twisted), whereas an isotropic structure is lacking in such order. In other words, the gain in entropy upon the transition from a hexagonal to a cholesteric structure is smaller than in the case of complete melting of the hexagonal structure;

(b) however, there is an additional gain in the Frank orientational elastic energy, since, in a nematic-like ordered hexagonal structure, the Frank orientational elastic energy is higher than in a twisted cholesteric structure. This energy (per unit volume) is expressed as

$$\Delta E_{\mathrm{Fr}} \approx \frac{1}{2} K_{22} q_0^2 \,, \tag{3}$$

where K_{22} is Frank's constant of elasticity, $q_0 = \pi/P$ (P is the pitch of the cholesteric helical structure).

The use of the modified (see above) Lindemann approach in the case of DNA LCD particles means that, at a certain temperature of a PEG-containing solution, the amplitude of displacements and rotational diffusion of neighbouring DS DNA molecules in the hexagonal phase, i.e., the hexagonal structure (see Fig. 1), reaches a critical value. The hexagonal packing becomes mobile, and, in the absence of other external factors, the system tends to pass into an isotropic state.

However, in our case, the transition of linear DNA to an isotropic state and its folding into an isolated statistical coil are impossible for steric reasons (osmotic compression of the system by PEG) [54, 55]. At a constant osmotic pressure, the spatial structure of the particles in a DNA dispersion determined by the PEG concentration turns out to be practically 'frozen'. Therefore, it can be expected that a phase transition other than complete 'melting' of the hexagonal structure can occur. In contrast to the isotropic structure, a new stable spatial spirally twisted structure can arise even at a relatively high osmotic pressure of PEG, and the phase transition occurs before the hypothetical (unattainable) 'melting' temperature is established.

The physical reason for such incomplete 'melting' is that the partial loss of orientational entropy (due to the transition to an ordered helically twisted cholesteric structure) is compensated by the contribution to the free energy associated with the orientational elasticity of chiral DNA molecules. Indeed, because DNA molecules themselves (and the main forces of interaction between them) are noncentrosymmetric (chiral), their parallel ordering is disadvantageous

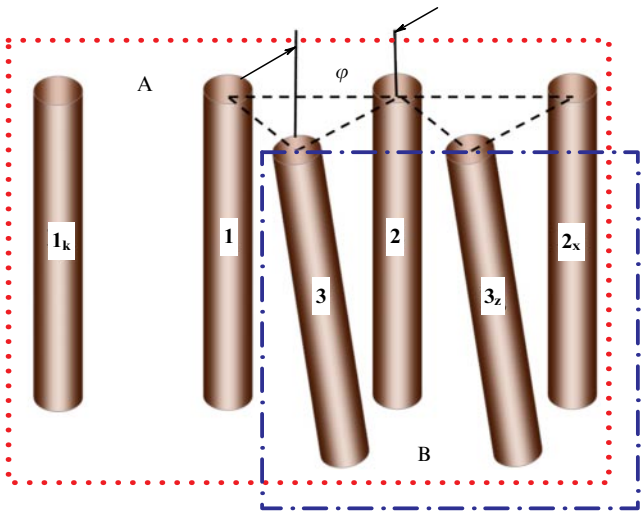

Figure 22. (Color online.) Schematic representation of 'triads'. A 'triad' is a combination of two DNA molecules (1 and 2) located in one 'layer' (layer A, shown by the red dashed line) and the third DNA molecule (3) rotated through angle φ in the parallel 'layer' (layer B, shown by the blue dashed line).

in terms of the Frank orientational elastic energy. With a parallel (nematic-like) orientation of chiral DNA molecules, the system loses in the orientational elastic torsion energy determined by the Frank elasticity constant K_{22} and the pitch (P) of the optimal cholesteric packing of chiral molecules.

Therefore, a new stable spirally twisted cholesteric spatial structure can arise from DS DNA molecules upon heating LCD particles with a hexagonal packing of neighbouring quasinematic layers at a temperature below the hypothetical (unattainable) 'melting' temperature even at a relatively high PEG osmotic pressure. Such a structure was called 're-entrant cholesteric' and the phase transition regarded as a transition from hexagonal to reversible cholesteric packing of DNA molecules within LCD particles [40]. (It can be added that the discussed model of temperature-induced hexagonal packing $\rightarrow$ cholesteric packing phase transition in the case of DNA LCD particles is in principle similar to the hexagonal $\rightarrow$ cholesteric packing phase transition resulting from the twisting of cholesteric domains in the macroscopic LC phase caused by changes in concentration [10]).

The simplest illustration of the observed optical effects is based on taking into account the theoretical results describing the formation of the cholesteric phase from DNA molecules [62, 125]. The authors suggested that triads of linear DNA molecules (Fig. 22) be used as building blocks determining the properties of this phase. The application of this model to the case of DNA LCD particles permits us to consider the following scenario of the transition from the hexagonal to the cholesteric structure.

Suppose that DNA molecules in the stable hexagonal structure shown in Fig. 1 actually form quasinematic layers. Let us arbitrarily designate two adjacent layers as layers A and B. DNA molecules in these layers are initially packed hexagonally. In principle, the hexagonal structure should 'melt' with increasing temperature of the solution and DNA molecules pass into an isotropic state. However, the application of the Lindemann criterion permits us to consider a totally different possibility. Upon heating under conditions of increased diffusion mobility of DNA molecules and reduced osmotic pressure of the PEG solution, the possibility of the

fluctuational displacement of neighbour molecules 1 and 2 (layer A) and the enhancement of their lateral interaction accompanied by their fixation at a certain distance in the plane (within one quasinematic layer) appears. An ensemble of these molecules forms. If molecules 1_k,1 or 2_x, 2, etc. from the same plane approach the ensemble, it leads only to an enlargement of the original ensemble. Under these conditions, molecule 3 (of the adjacent B layer) must 'feel' the combined chirality of the ensemble of molecules 1 and 2 differing from the chirality of initial molecules rather than the chirality of the spatial form of each molecule 1 and 2 (as in the initial hexagonal packing).

If the 'twisting' force between molecules 1 and 2 is initially absent, then the interaction with molecule 3 may give rise to a certain twist angle of the molecules, which gains importance [66, 73]. A change in the character of interaction between molecule 3 and the chiral ensemble of molecules 1 and 2 results in rotation of molecule 3 in space [34, 126]. A triadic structure appears (see Fig. 22) that provides the basis for the formation of the nucleus of a new phase.

The subsequent rotation of the entire B layer and, consequently, of the adjacent quasinematic layers leads to the growth of the nucleus up to a critical size necessary for the formation of a new phase from the metastable hexagonal phase. (In principle, the growth of a critical nucleus due to the diffusion of neighboring layers does not necessarily require additional thermoactivation processes.) The emerging new spirally twisted phase represents a DNA cholesteric and has an anomalous optical activity.

The above 'triadic' mechanism, together with the differences among the curves shown in Fig. 14, gives evidence of the dualism of the effect of temperature. Lindemann's criterion suggests the involvement of one more factor (besides the osmotic pressure of the solution), namely temperature, which determines the formation of LCD particles differing in the modes of packing DNA molecules. On the one hand, temperature causes the formation of a new phase nucleus and its growth. On the other hand, it increases fluctuations of neighboring quasinematic layers and prevents their ordered spatial arrangement in the structure of dispersion particles. Therefore, only a drop in temperature leading to a rise in the osmotic pressure of the solution that 'compresses' neighboring molecules and DNA layers while decreasing the diffusion mobility of DNA molecules facilitates not only an accurate spatial ordering of quasinematic layers of DNA molecules but also their 'freezing' within the newly formed cholesteric structure. This means that a decrease in temperature makes a transition to the original hexagonal structure practically impossible both for kinetic reasons and due to a change in the character of the interaction between neighboring DNA molecules.

The results presented in Fig. 14 can be regarded as a confirmation instance of the stated hypothesis. Indeed, this figure shows that a decrease in the LCD temperature leads to a noticeable increase in the anomalous band in the CD spectrum located in the region of absorption of DNA nitrogenous bases. Also, attention should be given to the following. If the viscosity (concentration) of a PEG solution at room temperature is such that it ensures a high diffusion rate of neighboring DNA molecules, the 'compressive' osmotic pressure not only leads to a lateral interaction of neighboring molecules 1 and 2 but also facilitates rotational displacement of molecule 3 (see Fig. 22). Under these conditions, a spatially twisted (cholesteric) structure of DNA dispersion particles appears (with the distance between DNA molecules ranging from 50 to 30 Å) [20, 35, 46]. However, with a rise in viscosity of the PEG solution (i.e., an increase in its osmotic pressure) when the diffusion rate of DNA molecules falls, the osmotic pressure provides only the lateral interaction of neighboring molecules 1, 2, and 3. Under these conditions at room temperature, the angle φ (see Fig. 22) between quasinematic layers tends to zero; upon its vanishing, the hexagonal packing of DNA molecules is realized.

To sum up, there are conditions under which neither anisotropic nor chiral properties of DNA molecules make a sufficient contribution to the free energy of molecule-molecule interaction, and their hexagonal packing is realized in LCD particles. In other words, the packing entropy of rigid linear DS DNA molecules exceeds the energy contribution due to their chiral properties [73]. However, the situation changes upon a change in temperature. Our results show that, the higher the temperature of a PEG-containing solution, the stronger the tendency of the quasinematic layers formed by chiral DNA molecules toward cholesteric rather than hexagonal packing.

9. Conclusion

The results of experimental and theoretical studies of the properties of DNA LCDs formed during phase exclusion of these molecules from aqueous polymer solutions are generalized and systematized. The influence of temperature on the structure of LCD particles is analyzed in the framework of the concept of quasinematic layers of orientationally ordered DNA molecules, taking into account the Lindemann criterion. Even small thermally induced fluctuations of DNA molecules can lead to a phase transition into a cholesteric-like structure (with inhomogeneous spatial twisting). Moreover, such flexural fluctuations can significantly increase the energy of intermolecular electrostatic repulsion (simply due to fluctuational convergence of charges), which also reduces the stability region of the nematic-like orientational structure of the hexagonal phase. Heating of dispersion particles with the hexagonal packing of DNA molecules alters the spatial orientation of neighboring quasinematic layers and is accompanied by a new hexagonal → 're-entrant' cholesteric packing phase transition of DNA molecules. The 're-entrant' cholesteric packing phase has anomalous physical properties (optical and texturographic).

The multiplicity of structural forms of new 're-entrant' cholesteric structures depends on the characteristics of DNA molecules themselves (average distance between molecules in dispersion particles, nucleotide composition, and molecular mass), the osmotic pressure of PEG solutions, and their temperature. It is manifested, in particular, as different abilities to interact with intercalating compounds (anthracyclines). The idea of the existence of 're-entrant' cholesteric DNA structures should be taken into account when considering the properties of thermotropic microorganisms.

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