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ORIGINAL PAPER

SAXS-data-based structural modeling of DNA–gadolinium complexes fixed in particles of cholesteric liquid-crystalline dispersions

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Abstract Structure of cholesteric liquid-crystalline dispersions (CLCDs) formed by double-stranded DNA molecules and treated with gadolinium salts was studied by small-angle X-ray scattering (SAXS). The obtained SAXS data open the way for structural modeling of these complexes to obtain a reasonable explanation for the correlated decrease in amplitude of an abnormal negative band in the circular dichroism (CD) spectra and the characteristic Bragg peak in the experimental small-angle X-ray scattering curves observed on treatment of CLCD by gadolinium salts. Model simulations of different kinds of structural organizations of the DNA–gadolinium complex were performed using novel SAXS data analysis methods in combination with several new, complementary modeling techniques, enabling us to build low-resolution three-dimensional structural models of DNA–gadolinium complexes fixed in CLCD particles. The obtained models allow us to suggest that a change takes place in the helical twist of quasinematic layers formed by these molecules at high concentrations of gadolinium salt. This change in the twist can be used to explain the experimentally observed increase in amplitude of an abnormal band in the CD spectra of DNA CLCD.

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Keywords Small-angle X-ray scattering · Modeling · DNA-based nanostructure · Circular dichroism

Abbreviations

CLCDs	Cholesteric liquid-crystalline dispersions
CD	Circular dichroism
SAXS	Experimental small-angle X-ray scattering

Introduction

Biological nanoconstructions are becoming increasingly popular as nanoscale functional materials with outstanding physicochemical properties, allowing their wide application in biotechnology and medicine. Among recently studied nanoconstructions, "rigid" particles of cholesteric liquid-crystalline dispersions (CLCDs) formed by double-stranded DNA molecules and treated with gadolinium salts are of special importance and interest due to their potential exciting applications in anticancer medicine (Yevdokimov and Sythev 2007, 2008). These rigid DNA nanostructures demonstrate new, unique properties resulting in two main effects: (1) amplification of the abnormal negative band in the circular dichroism (CD) spectra specific to the initial DNA CLCD, and (2) disappearance of the characteristic Bragg peak in the experimental small-angle X-ray scattering (SAXS) curves specific to spatially ordered double-stranded DNA molecules. These effects initially seem to be inconsistent and conflicting, and hence have to be explained. One of the most demonstrative methods to obtain a reasonable explanation is structural modeling of these complexes and comparison of scattering curves of the proposed model structures with experimental SAXS data.

Thus, the main goal of the present study is to build comprehensive structural models of the liquid–rigid transition in the state of double-stranded DNA molecules fixed in CLCD particles as a result of their interaction with gadolinium salt, and to compare the obtained information with the results of the applied investigation methods.

Experimental

Sample preparation and notations

The formation of the CLCDs based on double-stranded DNA, and its complexes with gadolinium, were described elsewhere (Yevdokimov and Sythev 2007, 2008; Salyanov et al. 2007; Yevdokimov et al. 2007).

The notations of all the samples and experimental parameters are presented in Table 1.

Samples were prepared for X-ray analysis by forming CLCD of initial DNA and CLCD of DNA–Gd^{3+} complexes in large (200–500 ml) volumes, recording their CD spectra, and then concentrating them by low-speed centrifugation (4,000 rpm, 30 min, 4°C; K23 centrifuge, Germany). Each pellet obtained by centrifugation was placed with a small amount of supernatant in a thin-walled (0.01 mm) quartz capillary of diameter 0.5 mm.

Characterization

The absorption spectra of the nucleic acids, the CLCD of the nucleic acids, and the CLCD of complexes (nucleic acid—gadolinium) were taken by using a Specord M-40 spectrophotometer (Germany), and the CD spectra were expressed as ΔA or $A_L - A_R$ value ($\Delta A \times 10^{-3}$ optical units), recorded by a portable SKD-2 dichrometer (manufactured by the Institute of Spectroscopy of the RAS, Troitzk, Moscow Region). In all cases, quartz cells with 1-cm optical path were utilized.

CD spectra, i.e., the dependence of measured ΔA or $A_L - A_R$ value (where the A value is the absorption for incident L- and R-circularly polarized light, respectively) upon λ, have been widely used since 1951 for comparison of properties of various DNA liquid-crystalline dispersions (see, for instance, De Vries 1951; Yevdokimov et al. 1992; Belyakov et al. 1996; Yevdokimov and Sythev 2008).

Table 1 Structural characteristics of the initial DNA CLCD particles, and on treatment by gadolinium

Samples	s_{max}, nm^{-1}	$\bar{d}$, nm	L, nm	$\Delta/\bar{d}$	A	S	r_t
DNA	1.9	3.2	21	0.12	0.52	0.0127	0
DNA/Gd_1	1.9	3.3	28	0.11	0.30	0.0057	0.082
DNA/Gd_2	1.9	3.3	30	0.10	0.22	0.0026	0.167
DNA/Gd_3	1.9	3.3	26	0.10	0.05	0.0005	0.33
DNA/Gd_4	1.9	3.3	24	0.10	0.02	0.0000	0.66

A amplitude of the first Bragg peak, relative units; S area under Bragg peaks, relative units; r_t the ratio of total $GdCl_3$ molar concentration to the molar concentration of the DNA bases

The morphology of the double-stranded DNA CLCD particles treated by $GdCl_3$ was examined using a commercial P47-SPM-MDT atomic force microscope (NT-MDT, Russia). To isolate these particles, the solution in which they were formed was filtered through a poly(ethylene terephthalate) (PETP) nuclear membrane filter with mean pore size of 0.15 μm (produced by the Institute of Crystallography of the RAS), which allows one to immobilize particles; the filter was flushed a few times with distilled water to remove nonspecifically absorbed $GdCl_3$ and dried in air for no less than 1 h (Yevdokimov and Sythev 2007).

Scattering experiments and data analysis

SAXS measurements were done on a laboratory AMUR-K diffractometer (Institute of Crystallography, Moscow) at wavelength of $\lambda = 0.1542$ nm in a Kratky-type (infinitely long slit) geometry, covering the momentum transfer range of $0.12\ nm^{-1} < s < 7.5\ nm^{-1}$ (here, $s = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle). The thickness of the sample (about 1 mm) varied along the length of the sample holder, so the irradiated volume could not be estimated and thus no absolute calibration was possible. The scattering curves were corrected for background scattering and primarily processed using standard procedures (Konarev et al. 2003; see also the EMBL website http://www.embl-hamburg.de/ExternalInfo/Research/Sax/ for program descriptions). All the scattering intensities discussed below are presented in relative units after normalization to zero sample absorption.

The repeat distances of the periodical motifs in the crystalline regions $\bar{d} = 2\pi/s_{max}$, corresponding to the peak position s_{max} on the scattering patterns, were calculated using the PEAK program (Konarev et al. 2003).

The mean long-range order dimension L (the size of crystallites) and the degree of disorder in the system $\Delta/\bar{d}$, were calculated from the following equations (Vainshtein 1966):

$$L = \frac{\lambda}{\beta_s \cos\theta}, \quad (1)$$

$$\Delta/\bar{d} = \frac{1}{\pi}\sqrt{\frac{\beta_s \bar{d}}{\lambda}}, \quad (2)$$

where β_s is the full-width at half-maximum intensity of a peak (in radians) observed at mean scattering angle of 2θ, and Δ is the mean-square deviation of distances between

neighboring, regularly packed structure motifs. Experimental errors of the determination of the parameters do not exceed 2%.

The volume distribution functions $D_V(R)$ of structural heterogeneities presented in the DNA specimen and in complexes of DNA with gadolinium were calculated using the indirect transform program GNOM (Svergun 1992). The program solves the integral equation

$$I(s) = (\Delta\rho)^2 \int_{R_{min}}^{R_{max}} D_V(R)\, m(R)\, i_0(sR)\, dR, \quad (3)$$

where R is the radius of a sphere, R_{min} and R_{max} are the minimum and maximum radii, respectively, $i_0(x) = \{[\sin(x) - x\ \cos(x)]/x^3\}^2$ and $m(R) = (4\pi/3)R^3$, are the sphere form factor and volume, respectively. The size distribution of the particles $N(R)$ is represented here in terms of the volume distribution function as $D_V(R) = m(R)N(R)$. In the computations of $D_V(R)$, the value of R_{min} was kept at zero; that of R_{max} was selected for each individual data set by successive runs with different values of this parameter.

Model simulations for two different kinds of structural organizations of the DNA molecules fixed in particles of the CLCD and treated with Gd salt were performed using the MASSHA program (Bernstein et al. 1977), a three-dimensional (3D) graphical system to display and manipulate atomic structures and low-resolution models. It is coupled with computational modules to allow interactive and automated rigid-body refinement against solution scattering data. The scattering amplitudes of the models were calculated by the CRYSOL programs (Svergun et al. 1995) for Protein Data Bank models (PDB) objects prepared by the MASSHA program. Space PDB models of simple bodies were created also by the program GEN_DAM_BODY (Konarev et al. 2003; see also the EMBL website http://www.embl-hamburg.de/ExternalInfo/Research/Sax/ for program descriptions).

Results and discussion

Comparison of the CD spectra with the SAXS curves for DNA CLCD treated with gadolinium salt

An abnormal negative band ($\lambda \approx 270$ nm) of the CD spectrum characteristic of the initial double-stranded DNA CLCD is changed as a result of treatment of this CLCD with $GdCl_3$.

The initial change is expressed as the decrease in the amplitude of the CD band (Fig. 1, curves 1 and 2).

According to theoretical calculations, the intense band in the CD spectrum (Fig. 1, curve 1) located in the absorption region of the nitrogen bases of the DNA

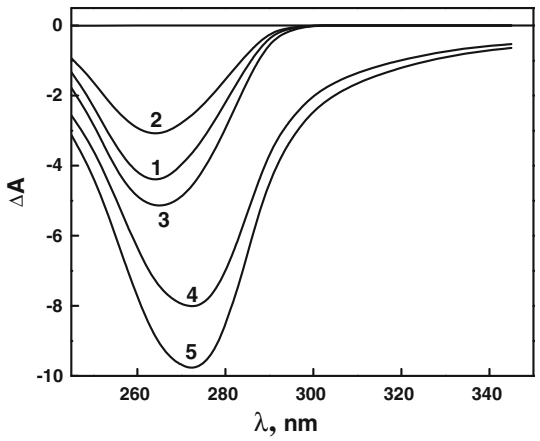

Fig. 1 CD spectra of the double-stranded DNA CLCD in the absence (*curve* 1) and in the presence of $GdCl_3$ in solution (*curves* 2–5): 2, $r_t = 1.65$; 3, $r_t = 16.5$; 4, $r_t = 32.7$; 5, $r_t = 95$. $\Delta A = A_L - A_R$ ($\times 10^{-3}$ opt. units). 0.3 M NaCl; 170 mg/ml PEG; 10 μg/ml DNA; $\Delta A = A_L - A_R$ ($\times 10^{-3}$ opt. units); r_t is the ratio of total $GdCl_3$ molar concentration to the molar concentration of the DNA nitrogen bases

molecule is direct evidence for the formation of the liquid-crystalline dispersion characterized by helically twisted structure (Keller and Bustamante 1986; Kim et al. 1986; Belyakov et al. 1996). Besides, theoretical calculations show that the appearance of this band unequivocally testifies to the macroscopic twist of neighboring "quasinematic" layers formed by DNA molecules in CLCD particles (Belyakov et al. 1996). In order to stress this peculiarity, the term "cholesteric liquid-crystalline dispersion" (CLCD) was used to signify these particles (Bouligand 1978; Belyakov et al. 1996). The negative sign of the band in the CD spectrum (Fig. 1, curve 1) proves the left-handed cholesteric twist of these layers formed by the right-handed DNA molecules (B-form) in these particles. The theoretical and experimental data (Belyakov et al. 1996; Gottarelli and Spada 1994; Yevdokimov et al. 1992) were used to identify the factors determining the sign and amplitude of the intense band in the CD spectrum of the DNA CLCDs. The most important points are as follows:

(1) The amplitude of the intense band in the CD spectrum depends on the size (diameter) of the CLCD particles and increases with their growth.
(2) The amplitude of the intense band depends on the helical pitch (P) of the twist of neighboring "quasinematic" formed by DNA molecules in the cholesteric structure. The smaller the pitch of the helical structure, i.e., the greater the angle between neighboring quasinematic layers of DNA molecules in the cholesteric structure, the greater the amplitude of the band in the CD spectrum, and vice versa.
(3) The sign of the intense band in the CD spectrum of DNA CLCD in the absorption region of its

chromophores (nitrogen bases) depends on the angle of their inclination with respect to the long axis of the DNA molecule.

(4) The amplitude of the band in the CD spectrum depends on the anisotropy of the DNA molecule, and negligible changes in the properties of the DNA secondary structure are accompanied by diminishing of the amplitude of the intense band in the CD spectrum.

Hence, the formation of the layered, spatially twisted structure of neighboring DNA molecules in particles of CLCDs as a result of phase separation in polymer-containing solutions is accompanied by the appearance of a specific, intense band in the CD spectrum in the region of the DNA nitrogen bases absorption. To stress the principal differences between molecular and "structural" circular dichoism, the term "abnormal band" was used to signify this band in the CD spectrum (Belyakov et al. 1996).

Figure 1 demonstrates as well that treatment of the DNA CLCD with gadolinium salt is accompanied by additional alteration of the abnormal band in the CD spectra. The gadolinium concentration dependence of the amplitude of the abnormal band (ΔA_{270}) in the CD spectrum of the DNA CLCD particles is shown in Fig. 2. One can see that the amplitude of the abnormal band in the CD spectrum of particles of the CLCD is changed, albeit slightly, at low concentrations of $GdCl_3$ (r_t below 0.5). It is important to note that, under these conditions, noticeable alterations in the CD spectra of the linear double-stranded DNA treated by gadolinium salts were observed (Haertle et al. 1981; Gruenwedel and Cruikshank 1991; Rosetto and Nieboer 1994; Li et al. 2003; Salyanov et al. 2007). Figure 2 shows unequivocally that low concentrations of gadolinium ions can induce alteration of the secondary structure of DNA molecules, even when fixed in the content of particles of CLCD.

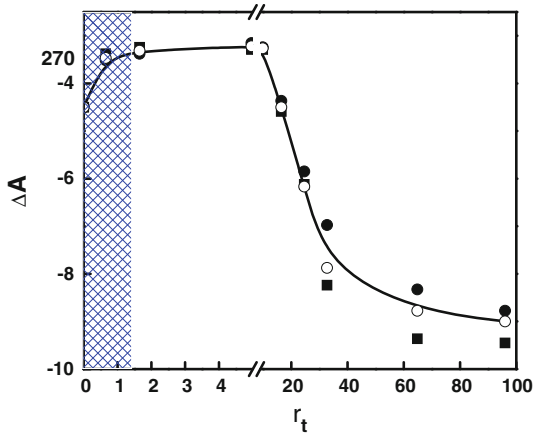

Fig. 2 Dependence of the amplitude of the abnormal negative band in the CD spectra of the CLCD formed by DNA–Gd^{3+} complexes upon r_t value. C_{DNA} = 10 μg/ml; C_{NaCl} = 0.3 M; C_{PEG} = 170 mg/ml; ΔA (λ = 270 nm) = $A_L - A_R$ ($\times 10^{-3}$ opt. units); r_t is the ratio of total $GdCl_3$ molar concentration to the molar concentration of the DNA nitrogen bases. The blue region in the figure is a region of the concentration of Gd^{3+} used for SAXS measurements

It can be assumed that binding of gadolinium ions to double-stranded DNA molecules, even when packed in particles of the cholesteric liquid-crystalline dispersion at low concentrations, leads to breaking of the homogeneity of the secondary structure of DNA. "Modified" nucleic acid molecules are separated into alternating fragments differing in conformations (e.g., B–Z–B–B–Z–Z, etc. for the DNA). The existence of fragments with the B-form in the modified DNA is confirmed by the fact that planar antibiotics (daunorubicin, mitoxantrone) are incorporated between pairs of bases of the modified DNA in particles of the cholesteric liquid-crystalline dispersion of the complexes of the DNA with gadolinium.

If the fragments of neighboring molecules of the complexes of the DNA with gadolinium, or even all molecules packed in particles of the cholesteric liquid-crystalline dispersion, acquire an inhomogeneous secondary structure, we can expect that the translational order of these fragments (molecules) will be broken and the small-angle reflection on X-ray patterns must disappear.

From Fig. 2 one can see as well that quite a sharp change in amplitude of the abnormal band in the CD spectrum of the CLCD of the DNA–Gd^{3+} occurs when there is large excess of gadolinium cations in the solution ($r_t > 20$), and its maximum is shifted by 10 nm toward longer wavelengths.

Hence, it was interesting to model the structural changes of DNA on the supramolecular level and to measure X-ray patterns of the DNA CLCD treated with different concentrations of gadolinium salt.

Here one can recall the following. It is well known that molecular motions in classical low-molecular compounds are free, and hence the corresponding X-ray scattering peaks are almost absent. Theories describing the appearance of abnormal optical activity of low-molecular cholesterics assume that sufficient conditions for abnormal optical activity are "orientation" ordering of chromophores of molecules in quasinematic layers of molecules and their helical ordering. These theories usually do not connect the mode of crystallographic packing of neighboring low-molecular compounds and the orientation order of their chromophores in the liquid-crystalline structure. However, in either case, the appearance of abnormal optical activity requires dense chromophore packing.

The experimental scattering curves from the CLCD formed by initial DNA molecules and their complexes with gadolinium salt are shown in Fig. 3 as a function of Gd concentration. Corresponding structural characteristics of

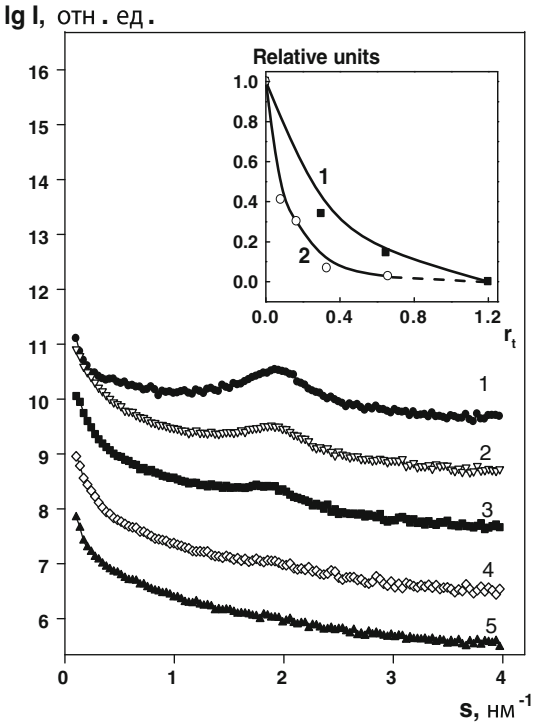

Fig. 3 Experimental SAXS patterns from the DNA CLCD (*curve* 1) and DNA CLCD particles treated with different concentration of Gd salt (*curves* 2–5). Successive curves are displaced one logarithmic unit down for better visualization. In the insert: Comparison of CD and SAXS data: (1) dependence of the relative ($\Delta A_{max} - \Delta A/\Delta A_{max}$) amplitude of the abnormal negative band of the CD spectra of the CLCD formed by (DNA–Gd^{3+}) complexes upon r_t value; and (2) dependence of the amplitude of the Bragg peaks (A) of the CLCD formed by DNA–Gd^{3+} complexes upon r_t value (Table 1)

samples, calculated from the scattering data using the PEAK program, are presented in the Table 1. The most important observation is that all structural changes of the DNA molecules in the CLCD detected by SAXS occur while the concentrations of the gadolinium salt are very low (Table 1 and Fig. 2, blue region). At $r_t = 0.66$ the characteristic Bragg peak has completely disappeared (Fig. 3, curve 5). In the region of low gadolinium concentration, a dependence of the relative ($\Delta A_{max} - \Delta A/\Delta A_{max}$) amplitude of the abnormal negative band of the CD spectra of the CLCD formed by DNA–Gd^{3+} complexes upon r_t value and a dependence of the amplitude of the Bragg peaks (A) of the CLCD formed by DNA–Gd^{3+} complexes upon r_t value (Table 1) demonstrate similar behavior (insert to Fig. 3). Therefore, the hypothesis that the parameters of the secondary structure of DNA in interaction with gadolinium are violated finds confirmation in the analysis of the SAXS spectra.

However, further increase of the concentration of the gadolinium salt does not change the profiles of the experimental SAXS curves. Thus, comparison of Figs. 1–3 shows that there are two effects characteristic of particles of the cholesteric liquid-crystalline dispersion of the DNA–gadolinium complexes: first, decrease in amplitude of the abnormal CD band, accompanied by disappearance of the maximum in the X-ray scattering curves for these complexes; and second, that the amplification of the abnormal band in the CD spectrum occurs at saturating concentrations of gadolinium cations bonded to the DNA and is not accompanied by change in the X-ray parameters of the DNA–gadolinium complexes.

To reveal causes of these effects, it is necessary to take into account the following. As shown previously (Yevdokimov et al. 2005; Yevdokimov and Sythev 2008), when Gd cations are bonded to linear double-stranded DNA molecules, the shape of the circular dichroism spectrum of these molecules changes sharply at $r_t \approx 0.5$. This change occurred due to breaking of the regular homogeneous character of the secondary structure of the DNA. Such breaking can be associated with the B $\rightarrow$ Z conformational transition and this is maximal at $r_t \approx 0.5$, despite the packing of the DNA molecules in particles of the cholesteric liquid-crystalline dispersion.

As one can see from the Fig. 3 and Table 1, all SAXS patterns are characterized by Bragg peaks at $s = 1.9$ nm^{-1}, and by scattering at very small angles (central scattering). The characteristic Bragg peak in the scattering patterns reveals the presence of some ordered, quasicrystalline regions in the samples, with spacing of about 3.3 nm, which is characteristic of the interhelical distance between closely packed initial DNA molecules (B-form), as reported by Belyakov et al. (1996). The shape of the Bragg peaks is typical of systems with quasicrystalline zones coexisting with disordered bicontinuous phases.

The presence of the central scattering indicates the formation of nanoscale structural heterogeneities in the samples, which is correlated with the formation of particles of DNA cholesteric liquid-crystalline dispersions (CLCDs) (Yevdokimov and Sythev 2007; Yevdokimov and Sythev 2008; Yevdokimov et al. 2008).

As is obvious from Table 1, general structural characteristics of the specimens such as the peak position s_{max} of the scattering curves, the repeat distances of the periodical motifs in the crystalline regions ($\bar{d} = 2\pi/s_{max}$), the mean long-range order dimension L (average size of crystallites), and the degree of disorder in the system ($\Delta/\bar{d}$) practically do not change. This indicates that the overall internal structure of the ordered regions of the closely packed DNA molecules remains constant. However, consecutive decrease in the areas under Bragg peaks after treatment of DNA CLCD with gadolinium salt results in a diminishing number of ordered quasicrystalline regions in the whole system. Thus, one can assume that the interaction between gadolinium cations and DNA molecules destroys their

parallel orientation and leads to formation of disordered regions.

Volume distributions of structural heterogeneities for the initial DNA CLCD and DNA CLCD treated with gadolinium salt

Volume distribution functions $D_V(R)$ of structural heterogeneities present in the CLCD DNA particles were calculated by the GNOM program (Svergun 1992) and are shown in Fig. 4.

All samples demonstrate bimodal distributions, with the first fraction corresponding to scattering objects characterized by sizes of about 5–6 nm, and a wide maximum with dimensions from about 10 to 50 nm. The first fraction of the distribution reflects a cross-section of molecular ensemble of the DNA molecules in quasicrystalline regions, while the second one reflects their length. The average sizes in the second fraction of the size distribution profiles correlate well with the mean value of L (i.e., the average size of crystallites) shown in Table 1. According to the shapes of the Bragg peaks in the experimental scattering curves, they do not reflect strong crystalline constitution of the molecular ensembles of the DNA molecules in the CLCD particles, but reveal the coexistence of some fraction of quasicrystalline regions with disordered bicontinuous phases. The extent of disorder in the system ($\Delta/\bar{d}$) represented in Table 1 is characteristic of such kinds of structural organization. Therefore, one can suggest a speculative model of the internal structure of the DNA CLCD particles presented in Fig. 5.

One of the most demonstrative ways to confirm any speculative suggestions is to build molecular models of the complexes and compare calculated scattering from the model structures with the experimental SAXS data, which is considered below.

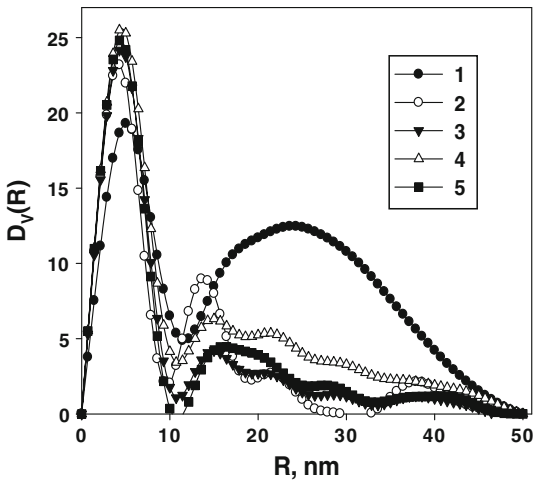

Fig. 4 Volume distribution functions for case 1 (*curve* 1) and case 2 (*curves* 2–5, corresponding to increasing gadolinium concentration in the samples)

Structural modeling of ordering DNA molecules and DNA complexes with high concentration of gadolinium ions in particles of CLCD

First of all, let us consider the possible behavior of a layered structure of DNA CLCD at high concentration of gadolinium salt. At saturating gadolinium concentrations, when the negative charges of the phosphate groups of the DNA are neutralized by positively charged gadolinium cations according to the data obtained by various physical methods, an additional process can occur. First, the gadolinium ions neutralizing the negative charges of the phosphate groups of the DNA make particles of the cholesteric liquid-crystalline dispersion insoluble. Indeed, in the presence of large excess of gadolinium ions, these ions, displacing sodium ions, are bonded to the phosphate groups of the DNA. It is worth noting that, when Gd^{3+} ions are bonded to polyphosphates, poorly soluble Gd polyphosphate is formed (solubility product of about 10^{-12} M). Since double-stranded DNA molecules have polyphosphate nature, these molecules in the presence of saturating gadolinium concentrations become poorly soluble in polyethylene-glycol salt aqueous solutions. Under these conditions, a stable spatial structure of dispersion particles can be formed, and the presence of poly(ethylene glycol) is not required to stabilize the structure of particles of the cholesteric liquid-crystalline dispersion. Moreover, gadolinium ions, neutralizing the charges of the phosphate groups of the DNA, create an excess positive surface charge on particles of the cholesteric liquid-crystalline dispersion, and aggregation of particles of the cholesteric liquid-crystalline dispersion becomes impossible.

Nonuniform distribution of gadolinium ions over the surface of DNA molecules results in a heterogeneous secondary structure, i.e., an irregular attraction between the fragments of the neighboring DNA molecules, fixed initially at about 3.3 nm (Table 1). The physical origin of this attraction mediated by a strong correlation interaction is related to either electrostatic or entropic contributions. In addition, such interactions are, probably, influenced by the interlocking Gd^{3+} ions, referred to as "counterion cross-links." Gd ions, when present at high concentration, are capable of overcompensating the DNA charges, and DNA charge inversion can take place, inducing a change in the spatial structure of the CLCD. Indeed, the separation of chains of the DNA molecules in the content of particles of the CLCD is impossible for steric reasons. The synergetic effects of local alterations of the secondary structure and charge inversion of the DNA molecule change the mode of spatial packing of these molecules in the particles of

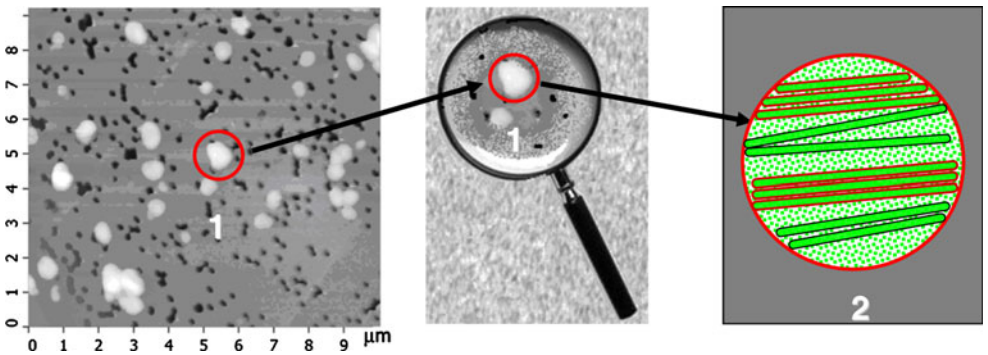

Fig. 5 Schematic presentation of the inner structure of CLCD particles: (1) two-dimensional (2D) AFM image of the DNA CLCD particles; and (2) the inner structure of one layer formed by DNA molecules in the CLCD particle with quasicrystalline (ordered) regions characterized by average lengths of about 25–30 nm and by a cross-section of the molecular ensembles of the DNA molecules of about 5–6 nm

CLCD. Under conditions of dense packing, the electrostatic forces between DNA molecules, which are intimately connected to the helical nature of the charge distribution, are large enough to overcome intrinsic forces that stabilize the initial cholesteric structure specific to B-form DNA. This can alter the parameters of the spatial structure of the DNA CLCD.

Indeed, it is not excluded that, after local changes in the DNA secondary structure induced at low extent of Gd^{3+}-cation interactions with the DNA, an additional process closely related to the change in mutual orientation of neighboring DNA layers in the particles of the CLCD takes place. Therefore, one can suppose that there are specific reasons responsible for the amplification of the abnormal band in the CD spectrum of the DNA CLCD.

In the framework of the theory (Belyakov et al. 1996) that describes the abnormal optical properties of the particles of the initial CLCD formed by DNA molecules, one can expect that there are two main reasons responsible for amplification of the abnormal band in the CD spectra. Taking into account that separation of chains of the DNA molecules in the content of particles of the CLCD is impossible for steric reasons (Grasso et al. 1991, 1992), the first reason is the increase in the diameter of the initial CLCD particles induced by interaction with gadolinium ions. The second reason is the change in the inner parameters of the cholesteric structure of the DNA CLCD as a result of interaction of gadolinium ions with these molecules.

The results of AFM measurements (Yevdokimov et al. 2008) show that the mean size of particles is (4.5–5.0) $\times$ 10^2 nm, i.e., the mean diameter of the DNA CLCD particles after treatment with gadolinium is comparable to the mean diameter of initial DNA CLCD particles. Hence, one can suppose that neutralization of phosphate groups of DNA molecules fixed in the content of CLCD by the rare-earth ions is not accompanied by alteration in the mean size of the CLCD particles. This is important, because it suggests that the average packing density of the DNA molecules in particles of CLCD of the DNA–gadolinium complex is similar to the packing density of the DNA molecules in particles of the CLCD formed from the initial molecules. This result further implies that the mean concentration of chromophores (DNA nitrogen bases) retained in particles of the CLCD of the DNA–gadolinium complex is high, and sufficient to maintain the high optical activity of liquid-crystalline particles, meaning that the first explanation for the amplification of the abnormal band in their CD spectra must be rejected. Thus, both CD and SAXS data lead to the necessity of the assumption that, besides a mechanism of changing of the secondary structure of DNA, there is a change of the twist of the layers at high concentration of gadolinium.

Modeling these possible structural changes we have used two alternative approaches. The first is a model of sample bodies generated using the GEN_DAM_BODY program without consideration of the peculiarities of the secondary structure of the DNA molecule. We used a long cylinder with diameter 2.3 nm and length of about 40–50 nm as a model of the initial DNA molecule. These cylinders were located to correspond to the periodical motifs in the crystalline regions $\bar{d} \approx 3.0$–3.3 nm. Two possible structural changes affecting the scattering curves were considered: disorder of parallel DNA molecules packaging, and bending of the DNA rods at the sites of location of Gd ions in the structure of DNA–Gd complexes. The best fit to the experimental scattering curve from the initial DNA in the particles of the cholesteric liquid-crystalline dispersions was obtained for the ensemble of slightly nonparallel cylinders, as shown in Fig. 6a. The scattering curve calculated by the CRYSOL program for the ordering of cylinders demonstrates the Bragg peak (curve 2) corresponding to the repeating distances with the periodical motifs $\bar{d} = 2\pi/s_{\max}$ in the model. The positions

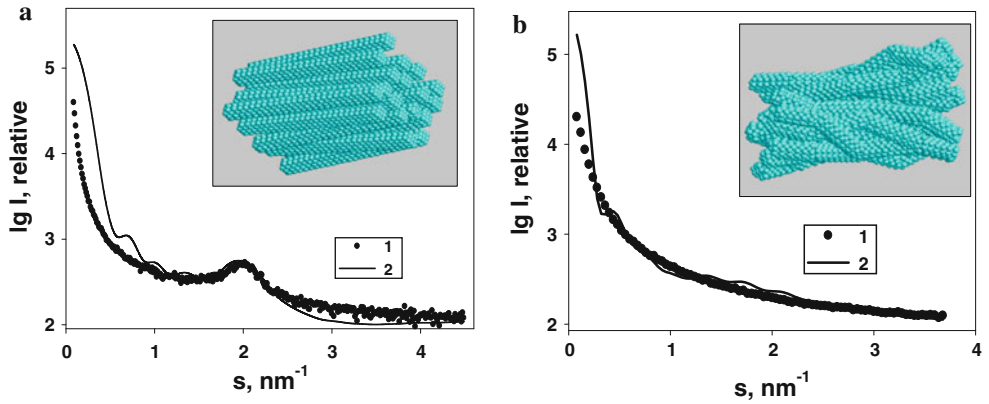

Fig. 6 Comparison of experimental SAXS curves for the initial DNA CLCD particles (*circles*) and a simulated curve from a conglomerate of parallel rods with low degree of disorder (*solid line*) for (a) highly ordered DNA packing, and (b) disordered DNA packing. Insets: PDB models of the packages. Diameter of the rod is 2.3 nm, length is 40–50 nm, and the distance between the centers of the rods is about 3.0–3.3 nm

of the experimental Bragg peak and that modeled coincide with good accuracy. However, the best fit for the DNA–Gd complexes could be achieved only by using the model of bending DNA rods, as demonstrated in Fig. 6b. All intermediate conditions of the complexes show the same consistent pattern: with increasing disorder, decrease of the amplitude of the peaks up to the point of their complete disappearance is observed (these particular cases not presented herein).

The second step in the modeling was to use the real DNA atomic structure to consider possible effects of secondary structure of the individual DNA molecules on the modeled scattering curves. This structure was obtained form the RCSB Protein Data Bank (PDB code 1bna for the double stranded DNA, B-form) and is shown in Fig. 7.

This structure was used in the MASSHA program to construct DNA blocks of slightly nonparallel molecules with length of about 40–50 nm (a), and that of bending DNA of the same length (b), shown in Fig. 8 in two perpendicular orientations.

The sizes of DNA blocks were chosen to agree with the sizes determined by GNOM analysis and according to the real length of DNA macromolecules in the complex (Yevdokimov and Sythev 2008). The scattering curves from these structures were calculated by the CRYSOL program, and obtained patterns are presented in Fig. 9 along with experimental scattering curves corresponding to the adequate DNA complexes in the particles of cholesteric liquid-crystalline dispersions.

These results practically coincide with those obtained when modeling using simple bodies (cylinders), where we did not consider the peculiarities of the internal DNA structure. This means, on one hand, that the changes in the DNA conformation that appeared under interaction with gadolinium salt are manifested on a level that can be

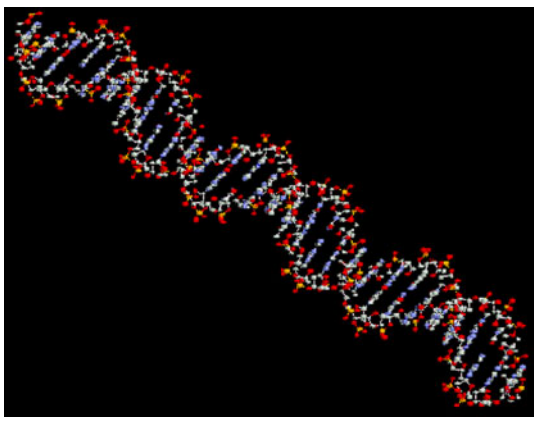

Fig. 7 Atomic structure of DNA molecule (B-form, fragment)

investigated by CD and other optical methods but that cannot be detected in detail by SAXS. On the other hand, structural models built on the basis of SAXS data have to consider bending and twisting of DNA macromolecules caused by interaction with gadolinium salt, as the best fits to the experimental scattering profiles from the DNA–Gd complexes could be achieved only by using such a model. Hence, Gd cations can modify the properties of neighboring DNA molecules, distorting their shapes. As a result, the structure of the ordered regions is altered, which is manifested by the disappearance of the characteristic Bragg peak in the experimental small-angle X-ray scattering profiles specific to the ordered DNA molecules. Besides, the interaction of Gd cations with the DNA molecules results in a high degree of neutralization of the negative changes of DNA phosphate groups, which leads to increase of interaction between the fragments of neighboring molecules DNA with the altered structure and is accompanied by structural liquid–rigid transformation of packing of the

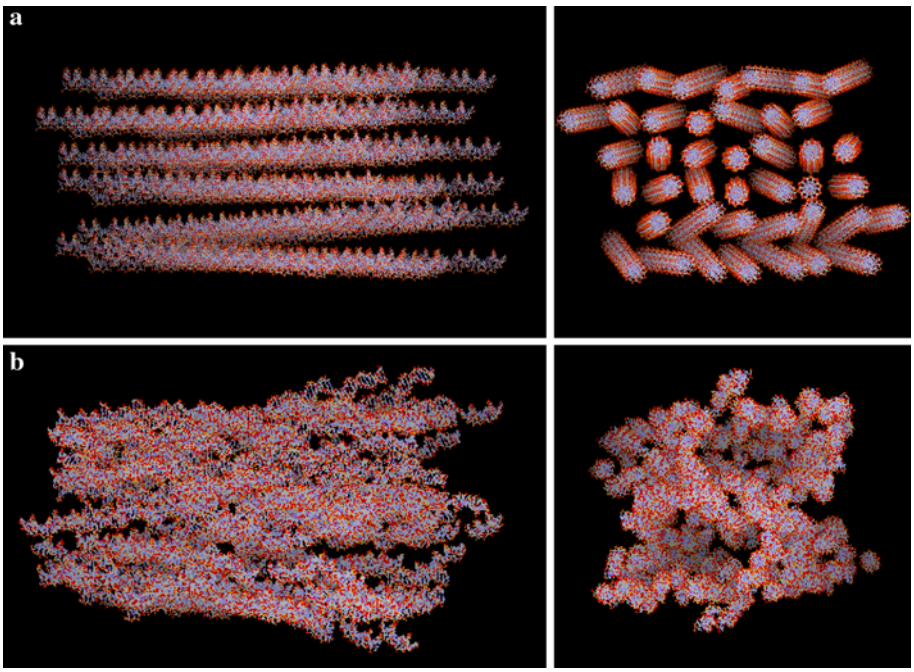

Fig. 8 Atomic structures of DNA blocks consisting of slightly nonparallel molecules (**a**) and that of the bending DNA (**b**) in two perpendicular orientations

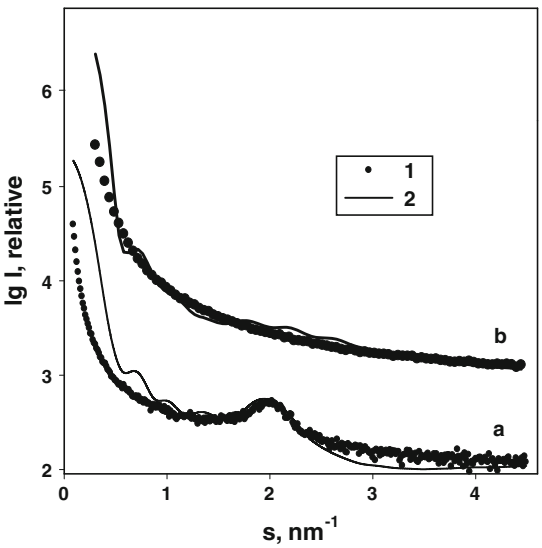

Fig. 9 Comparison of experimental SAXS curves for the initial DNA CLCD particles (*circles*) and simulated curves for the DNA blocks based on the real atomic DNA structures (*solid line*) for *a* highly ordered packing, and *b* disordered packing

DNA molecules and (on the supramolecular level) by alteration of the spatial structure of the particles of the DNA cholesteric liquid-crystalline dispersion.

Therefore, the experimental results described herein confirm the assumption that treatment of particles of DNA CLCD by gadolinium salt leads not only to the appearance of modified secondary structure of the DNA molecules packed in the quasinematic layers, but is also accompanied by alteration of the helical twisting layers of the cholesteric structure, i.e., it can facilitate the change in the cholesteric pitch, *P*, of the spatial structure of the DNA CLCD.

Conclusions

The apparent contradiction between the experimental CD data and those from SAXS has been resolved by corresponding structural modeling of the DNA–gadolinium complex. The data obtained in this work demonstrate that gadolinium cations interacting with double-stranded DNA molecules induced structural changes in the DNA–gadolinium complexes on two different levels. Firstly, this interaction is accompanied by change of the parameters of the secondary structure and by spontaneous changes in the helical twist of these molecules, as manifested by significant amplification of the abnormal negative band in the CD spectra. Secondly, strong attraction between neighboring DNA molecules at the Gd binding sites leads to distortion of the parallel packing of the DNA molecules and to their bending, expressed on the supramolecular level, which can be investigated by SAXS. Thus, joint use of circular dichroism and small-angle X-ray scattering allowed us to build a consistent spatial model of the DNA complex with gadolinium that satisfied the experimental data and considered the main changes expressed on the different structural levels.

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References

Belyakov VA, Orlov VP, Semenov SV, Skuridin SG, Yevdokimov YuM (1996) Comparison of calculated and observed CD spectra of liquid crystalline dispersions formed from double-stranded DNA and from DNA complexes with coloured compounds. Liq Cryst 20:777–784

Bernstein FC, Koetzle TF, Williams GJB, Meyer EF Jr, Brice MD, Rodgers JR, Kennard O, Shimanouchi T, Tasumi M (1977) The Protein Data Bank: a computer-based archival file for macromolecular structures. J Mol Biol 112:535–542

Bouligand Y (1978) Liquid crystalline order in biological materials. In: Blumstein A (ed) Liquid crystalline order on polymers. Academic, New York, pp 262–297

De Vries H (1951) Rotary power and other optical properties of certain liquid crystals. Liq Cryst 4:219–226

Gottarelli G, Spada GP (1994) Application of CD to study of some cholesteric mesophases. In: Nakanishi K, Beroua N, Woody RW (eds) Circular dichroism. Principles and applications. VCH, New York, pp 105–119

Grasso D, Fasone S, La Rosa C, Salyanov V (1991) A calorimetric study of different thermal behaviour of DNA in the isotropic and liquid-crystalline states. Liq Cryst 9:299

Grasso D, Gabriele-Campisi R, La Rosa C (1992) Microcalorimetric measurements of thermal denaturation and renaturation processes of salmon sperm DNA in gel and liquid-crystalline phases. Thermochimica Acta 199:239

Gruenwedel DW, Cruikshank MK (1991) Changes in poly [d(T − G)] × d(C − A)] chirality due to Hg(II)-binding: circular dichroism (CD) studies. J Inorg Biochem 43:29

Haertle T, Augustyniak J, Guschlbauer W (1981) Is Tb^{3+} fluorescence enhancement only due to binding to single stranded polynucleotides? Nucl Acids Res 9:6191

Keller D, Bustamante CJ (1986) Theory of interaction of light with large inhomogeneous molecular addregates. II. Psi-type dichroism. J Chem Phys 84:2972–2980

Kim M, Ulibarri L, Keller D, Maestre MF, Bustamante C (1986) The psi-type dichroism of large molecular aggregates. III. Calculations. J Chem Phys 84:2981–2989

Konarev PV, Volkov VV, Sokolova AV, Koch MHJ, Svergun DI (2003) PRIMUS: a Windows PC-based system for small-angle scattering data analysis. J Appl Crystallogr 36:1277–1282

Li L, Yang J, Wu X, Sun C, Zhou G (2003) Study of the co-luminescence effect of terbium-gadolinium-nucleic acids-cetylpyridine bromide system. J Luminescence 101:141

Rosetto FE, Nieboer E (1994) The interaction of metal ions with synthetic DNA: induction of conformational and structural transitions. J Inorg Biochem 54:167

Salyanov VI, Evseev AI, Popenko VI, Gasanov AA, Dembo KA, Kondrashina OV, Shtykova EV, Yevdokimov YuM (2007) Gadolinium complexes of linear and liquid-crystalline DNA. Biophysic 52:288

Svergun DI (1992) Determination of the regularization parameter in indirect-transform methods using perceptual criteria. J Appl Crystallogr 25:495

Svergun DI, Barberato C, Koch MHJ (1995) CRYSOL—a program to evaluate X-ray solution scattering of biological macromolecules from atomic coordinates. J Appl Cryst 28:768

Vainshtein BK (1966) Diffraction of X-rays by chain molecules. Elsevier, Amsterdam-London-New York

Yevdokimov YuM, Sythev VV (2007) Nanotechnology and nucleic acids. Open Nanosci J 1:19–31

Yevdokimov YuM, Sythev VV (2008) Principles of the design of nanostructures with nucleic acid molecules as building blocks. Russ Chem Rev 77:193–204

Yevdokimov YuM, Skuridin SG, Lortkipanidze GB (1992) Liquid-crystalline dispersions of nucleic acids. Liq Crystals 12:1–16

Yevdokimov YuM, Skuridin SG, Nechipurenko YuD, Zakharov MA, Salyanov VI, Kurnosov AA, Kuznetsov VD, Nikiforov VN (2005) Nonconstruction based on double-stranded nucleic acids. Int J Biol Macromol 36:103–115

Yevdokimov YuM, Salyanov VI, Kondrashina OV, Gasanov AA, Shtykova EV, Dembo KA (2007) Rare-earth-cation-induced change in the cholesteric twisting of neighboring nucleic acid molecules. J Exp Theor Phy 104(N):3–499

Yevdokimov YuM, Salyanov VI, Shtykova EV, Dembo KA, Volkov VV, Spirin PV, Slusheva AS, Prassolov VS (2008) A transition in DNA molecule's spatial ordering due to nano-scale structures changes. Open Nanosci J 2:17–28