Regulation of the spatial organization of DNA molecules in particles of liquid-crystalline dispersions by means of biologically active compounds

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Using the "structural conflict" method based on differences in the spatial organization of a liquid-crystalline dispersion (LCD) of DNA—polycation complexes and the DNA cholesteric LCD, it was shown that in a solvent with specific properties, the spatial structure of the LCD of DNA—polycation complexes can be controlled by biologically active compounds (BAC) that cause either dissociation of the DNA—polycation complex or destruction of the polycation molecules. In both cases, the cholesteric structure of LCD typical of the initial uncomplexed DNA molecules is recovered. The recovery of cholesteric LCD of DNA was proved by circular dichroism spectroscopy and confirmed by X-ray diffraction and polarization microscopy. The fact that spatial restructuring of LCD of the DNA—polycation complexes is induced by BAC in low concentrations opens up the possibility for using particles of these dispersions as sensing elements (sandwich-type biosensing units) of biosensor devices. A new method for determination of heparin based on the use of LCD of the DNA—polyconidine complexes was developed. The minimum heparin concentration determined in the sample equals ~0.4—0.5 μg mL^{-1}.

Key words: DNA, polycation, complexation, liquid-crystalline dispersions of DNA, circular dichroism, biosensor.

Linear double-stranded DNA molecules with low molecular weight ($<1 \cdot 10^6$ Da) form liquid-crystalline dispersions (LCD) with natural and synthetic polycations in water—salt solutions.[1,2] The LCD particles formed in solutions with different ionic strengths are characterized by different packing densities of DNA—polycation complex molecules.[3] A comparison of the data from X-ray diffraction analysis of the liquid-crystalline phases of DNA—polycation complexes formed under different conditions[3] with the phase diagram that reflects the polymorphism of liquid-crystalline phases of linear double-stranded DNA molecules[4] has shown[5] that a hexagonal LCD exists for 0.15 mol $L^{-1} \leq C_{NaCl} < 0.4$ mol L^{-1} and a cholesteric LCD exists for 0.4 mol $L^{-1} \leq C_{NaCl} < 0.55$ mol L^{-1}. These data suggest structural polymorphism of the LCD of DNA—polycation complexes.

The liquid-crystalline type of packing of the DNA—polycation complex molecules in particles of a hexagonal dispersion formed at $C_{NaCl} < 0.4$ mol L^{-1} together with the lack of abnormal optical activity suggested the possibility of controlling the packing of DNA molecules by means of biologically active compounds (BAC).

The purpose of this work was to demonstrate the possibility of controlling the pattern of structural organization of the LCD of DNA—polycation complexes by means of BAC and to design, based on the particles of these dispersions, a new type of biosensors with optical properties changing under the action of practically significant substances of different chemical nature.

Experimental

A DNA sample from chicken erythrocytes (Reanal, Hungary) with the molecular mass of ~$(0.3—0.7) \cdot 10^6$ Da was purified additionally by a reported procedure[6] and depolymerized. The native state of the depolymerized DNA sample was checked by the hyperchromic effect (~30—35%) that accompanies acid denaturation of double-stranded nucleic acids.[7] The DNA concentration in water—salt solutions was determined by spectrophotometry using the known[5] molar extinction coefficient of DNA ($\varepsilon_{259} = 6600$ L mol^{-1} cm^{-1}). A poly(ethylene glycol) sam-

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ple (PEG, molecular mass 4000 Da, Ferak, Germany) was used as received. A sample of the quaternary ammonium salt of a monodisperse conidine oligomer with the degree of polymerization of 25 (polyconidine, molecular mass 4042 Da) synthesized and characterized at the Institute of Immunology of the RF Ministry of Health was used as received.

A water—salt solution of polyconidine (4 mg mL^{-1}) was prepared by dissolving a weighed portion of the substance in 10^{-2} *M* Na phosphate buffer (pH ~7.0) containing 0.15 *M* NaCl.

LCD samples of the DNA—polyconidine complexes were prepared by adding the polyconidine solution to a water—salt solution (0.15 *M* NaCl in 10^{-2} *M* Na phosphate buffer) of DNA (C_{DNA} = 30 μg mL^{-1}, V_{DNA} = 3 mL) with continuous stirring. The resulting complexes were characterized by the *r* value, which is the ratio of the molar concentration of positively charged groups of the polyconidine molecules to the molar concentration of DNA nucleotides.

An individual protamine sample, stellin B[8] isolated from stellate sturgeon (*Acipencer stellatus*) gonads and characterized at the Chair of Natural Compounds of the Department of Chemistry of the M. V. Lomonosov Moscow State University was used as received.

The concentration of stellin B in the initial water—salt solution (0.15 *M* NaCl in a 10^{-2} *M* Na phosphate buffer, pH ~7.0) was equal to 1 mg mL^{-1}.

LCD of the DNA—stellin B complexes were obtained by adding a water—salt solution of stellin B with continuous stirring to a water—salt solution of DNA (V_{DNA} = 3 mL, C_{DNA} = 40 μg mL^{-1}). The resulting complexes were characterized by the *r** value, which represents the ratio of the molar concentrations of the basic amino acid residues in the stellin B molecule to the molar concentration of the DNA nucleotides.

Prior to the formation of LCD of the DNA—polyconidine and DNA—stellin complexes, all initial solutions were filtered through membrane filters (Millipore, USA) with a pore diameter of 0.8 μm to remove possible mechanical impurities.

Heparin (Serva, Germany) and trypsin (Sigma, USA) were used as received.

The cholesteric LCD of DNA used as the reference was formed according to a reported procedure[9] by mixing equal volumes of water—salt solutions of DNA and PEG.

Absorption spectra were recorded on a Specord M-40 spectrophotometer (Germany) and the CD spectra were obtained on an SCD-2 portable dichrometer.[10] In all cases, rectangular quartz cells with 1-cm optical path were used.

The CD spectra were plotted as the dependence of the difference ($\Delta\varepsilon$) of the molar extinction coefficients of the left-hand- (ε_L) and right-hand-polarized (ε_R) light on the wavelength (λ).

The preparation procedure of the cholesteric liquid-crystalline DNA plase and the liquid-crystalline phases of the DNA—polyconidine and DNA—stellin B complexes for X-ray diffraction analysis and polarization microscopy and the instrumentation used were described in detail previously.[5,11,12]

Results and Discussion

"Structural conflict" of the liquid-crystalline dispersion of the DNA—polycation complex in a solvent with particular properties. If an LCD formed from a DNA—polycation complex (Fig. 1, *a*) in a water—salt solution with a moderate ionic strength (in particular, at $C_{NaCl} < 0.4$ mol L^{-1}) is placed in a polymer-containing solvent (Fig. 1, *b*) that has exclusion properties and specifies, due to the osmotic pressure a different (larger than 25 Å) distance between the neighboring DNA molecules, then the two possible structural (and, hence, optical) states conflict with each other. On the one hand, rigid optically active DNA molecules tend to form a cholesteric LCD possessing an abnormal optical activity in this solvent. On the other hand, the presence of polycationic cross-links hampers the packing of DNA molecules typical of cholesteric liquid crystals. Therefore, the LCD of DNA—polycation complexes placed in a polymer-containing solvent should not possess abnormal optical activity. Meanwhile, under these conditions, DNA molecules without polycationic cross-links can undergo spontaneous restructuring resulting in the formation of a cholesteric LCD.

Thus, the removal in one or another way of polycations (including those that form intermolecular cross-links) from the DNA—polycation complex should be accompanied by an increase in the distance between neighboring DNA molecules (d_{Bragg} ~35 Å) and by their spatial restructuring, which finally results in the formation of a cholesteric LCD having abnormal optical activity.

Spatial reorganization of DNA molecules in particles of LCD of the DNA—polycation complex upon competitive removal of polycation molecules from this complex.

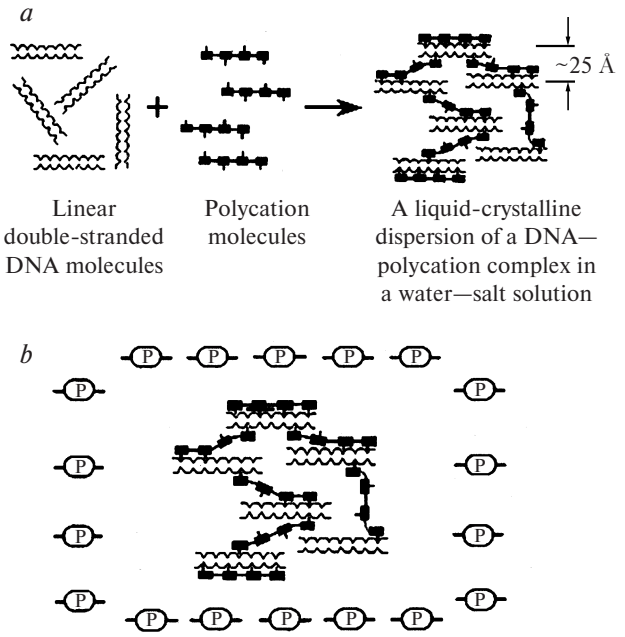


Fig. 1. Formation of an LCD of the DNA—polycation complex in a water—salt solution (*a*) and schematic presentation of the LCD of the DNA—polycation complex in an aqueous polymeric solution (*b*); the average distance (25 Å) between the DNA molecules in the DNA—polycation complex particles is indicated.

The removal of polycations from the DNA—polycation complex may be attained, for example, by adding a polyanion able to compete with DNA molecules for the polycation that forms both intra- and intermolecular complexes with DNA (Fig. 2, *a*). Therefore, two features are important for the polyanion. First, the association constants of the polyanion—polycation complex should exceed the association constant of the DNA—polycation complex; second, the association constant and the formation rate of the polyanion—polycation complex should not decrease appreciably on going from a water—salt solution to a polymer-containing solvent. When a polyanion—polycation pair that complies with these requirements has been chosen, the addition of a polyanion into the polymer water—salt solution containing the LCD of the DNA—polycation complex should be accompanied by removal of a fraction of the polycations from the DNA—polycation complex to form a polyanion—polycation complex that is more stable under these conditions. Upon removal of polycationic cross-links, DNA molecules acquire ability to be packed in the way typical of the initial linear double-stranded DNA molecules in this solvent. In particular, in a polymer-containing solution where the distance between the neighboring DNA molecules (d_{Bragg}) specified by its osmotic pressure equals ~35 Å, the DNA molecules should form a cholesteric LCD. In this case, an intense negative band located in the absorption region of DNA nitrogen bases should be expected to appear in the CD spectrum.[13]

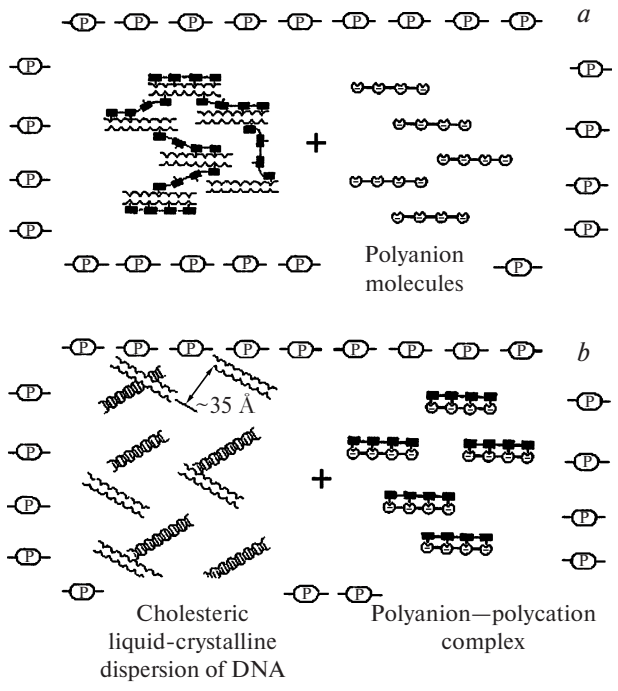
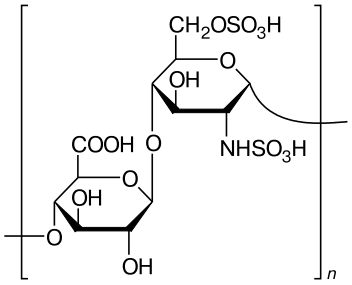


Fig. 2. Schematic presentation of a water—polymer solution containing an LCD of the DNA—polycation complex after addition of a polyanion (*a*) and spatial restructuring of DNA molecules in particles of the LCD of the DNA—polycation complex resulting in the formation of a cholesteric LCD (*b*).

As the polyanion, here we used heparin. Heparin is an acidic mucopolysaccharide; its linear polysaccharide chains with the molecular mass of 5000—30000 Da consist of sulfurated glucuronic acid and glucosamine residues.[14]



The heparin molecule contains a considerable number of negatively charged sulfate and carboxy groups; therefore, it is the strongest natural polyanion able to form complexes with many proteins and synthetic compounds bearing an overall positive charge. In addition, polyconidine shows a distinct affinity to heparin;[15] hence, heparin can compete with DNA molecules for the complexation with polyconidine.[16]

To verify this hypothesis, the LCD of DNA—polyconidine complexes formed in a water—salt solution (0.15 *M* NaCl, r = 0.91) was placed into a water—salt solution of PEG (C_{PEG} = 170 mg mL^{-1}, 0.225 *M* NaCl), and heparin was added.

Figure 3 shows the CD spectra of water—salt solutions of DNA (*1*) and LCD of DNA—polyconidine complexes (*2*) and their PEG-containing water—salt solutions before (curves *3* and *4*, respectively) and after addition of heparin (curves *5* and *6*, respectively). It can be seen that the LCD of the DNA—polyconidine complex does not possess abnormal optical activity in either water—salt solution (curve *2*) or PEG-containing solution (curve *4*).

In accordance with the above assumption, the addition of heparin to the LCD of the DNA—polyconidine complex in a water—salt solution of PEG gives rise to a strong negative band in the CD spectrum (see Fig. 3, curve *6*). The band amplitude is close to that of the negative band typical of the cholesteric LCD formed from the initial uncomplexed linear double-stranded DNA molecules (curve *3*). Note that the addition of heparin to a solution of PEG containing cholesteric LCD particles formed from the initial linear DNA molecules does not affect its abnormal optical properties (*cf.* curves *3* and *5* in Fig. 3).

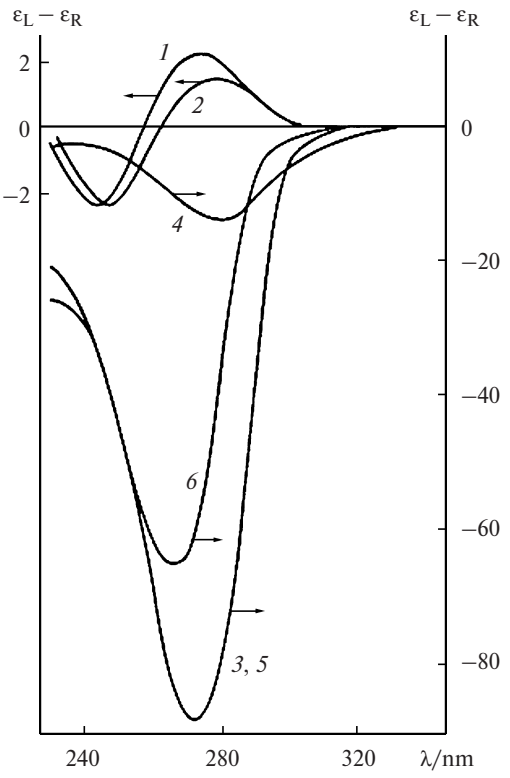


Fig. 3. CD spectra of water—salt solutions (0.15 *M* NaCl in 10^{-2} *M* Na phosphate buffer) DNA (*1*) and the DNA—polyconidine complex (*2*, $r = 0.91$) and their PEG-containing water—salt solutions (C_{PEG} = 170 mg mL^{-1}, 0.225 *M* NaCl in 10^{-2} *M* Na-phosphate buffer) before (curves *3* and *4*, respectively) and after (curves *5* and *6*, respectively) treatment with heparin (mass ratio heparin/polyconidine = 0.998). Here and below *r* is the ratio of the molar concentration of the positively charged groups of polyconidine molecules to the molar concentration of the negatively charged phosphate groups of DNA in solution.

An increase in the band amplitude in the CD spectrum of the LCD of DNA—polyconidine complex after the addition of heparin indicates that upon the formation of the heparin—polyconidine complex, some of the polyconidine molecules (including those cross-linking the neighboring DNA molecules) are removed from the DNA—polyconidine complex. Upon removal of polyconidine cross-links, DNA molecules acquire ability to be packed in the way typical of linear DNA molecules, in particular, in the cholesteric LCD particles formed at C_{PEG} = 170 mg mL^{-1}. This means that treatment of the LCD of the DNA—polyconidine complex with heparin induces changes in the spatial arrangement of DNA molecules in the LCD particles.

To confirm this conclusion, we carried out X-ray diffraction analysis of the liquid-crystalline phases obtained upon low-speed centrifugation of the LCD of the DNA—polyconidine complex before and after treatment with heparin in a water—salt solution of PEG.

The X-ray diffraction data (Fig. 4) indicate that in the presence of heparin, not only the maximum in the scattering curve shifts to smaller angles (the average distance between the DNA molecules characterized by the d_{Bragg} value increases from 26.74 to 32.93 Å) but the curve is also broadened (*cf.* curves *1* and *3* in Fig. 4). Curve *2* almost does not differ from the reference X-ray diffraction curve for the cholesteric liquid-crystalline phase of DNA (curve *3*). The observed changes in the X-ray diffraction properties of the liquid-crystalline phase of the DNA—polyconidine complex can be interpreted in terms of the views on its restructuring under the action of heparin.

This conclusion is also confirmed by the optical textures of thin films of the liquid-crystalline phase of the DNA—polyconidine complex before and after its treatment with heparin (Fig. 5): instead of a nonspecific texture (*a*), a fingerprint texture (*b*) typical of cholesteric liquid crystals is observed.[4,17]

Thus, treatment of LCD particles formed from the DNA—polyconidine complex with a physiologically significant polyanion, *viz.*, heparin, which is accompanied by removal of some of the polyconidine molecules from the initial complex, induces spatial restructuring of the DNA molecules in the dispersion particles in a PEG-containing solution.

An important conclusion that follows from the above data is that the spatial organization of DNA molecules in LCD particles can be controlled by BAC.

Spatial restructuring of DNA molecules in particles of the LCD of the DNA—polycation complex upon enzymatic hydrolysis of polycations. While developing the hypothesis

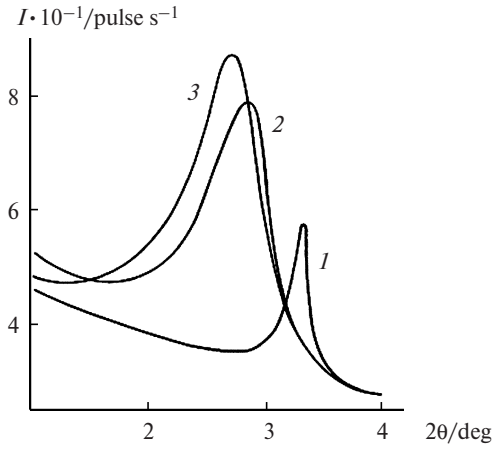


Fig. 4. Small-angle X-ray diffraction curves for the liquid-crystalline phase of the DNA—polyconidine complex ($r = 0.91$) before (*1*) and after (*2*) treatment with heparin (mass ratio heparin/polyconidine = 0.998); C_{PEG} = 170 mg mL^{-1}, 0.225 *M* NaCl in 10^{-2} *M* Na phosphate buffer; (*3*) X-ray diffraction curve for classical cholesteric liquid-crystalline DNA phase formed in a PEG-containing water—salt solution (C_{PEG} = 170 mg mL^{-1}, 0.3 *M* NaCl in 10^{-2} *M* Na phosphate buffer).

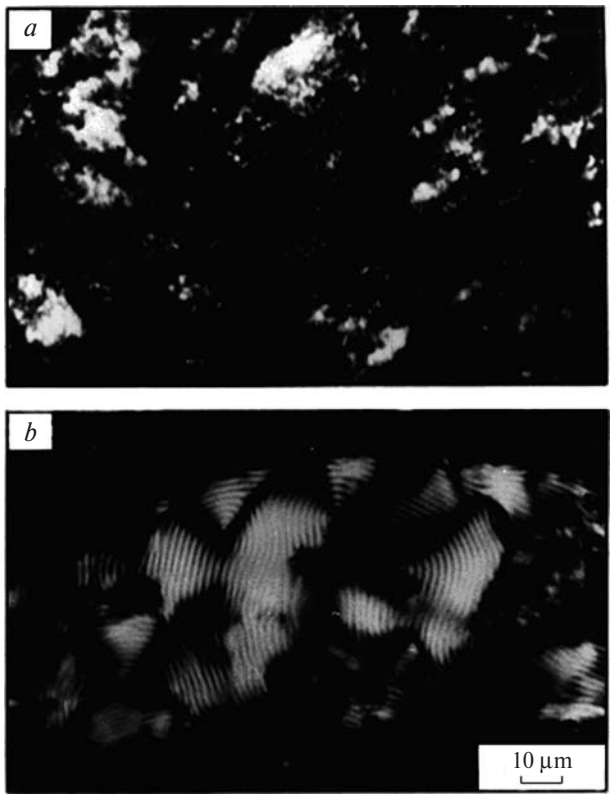


Fig. 5. Polarized-light views of optical textures of thin films (~20 μm) of the liquid-crystalline phase of the DNA—polyconidine complex (r = 0.91) before (*a*) and after (*b*) treatment with heparin (mass ratio heparin/polyconidine = 0.998); C_{PEG} = 170 mg mL^{-1}, 0.225 *M* NaCl in 10^{-2} *M* Na^+ phosphate buffer.

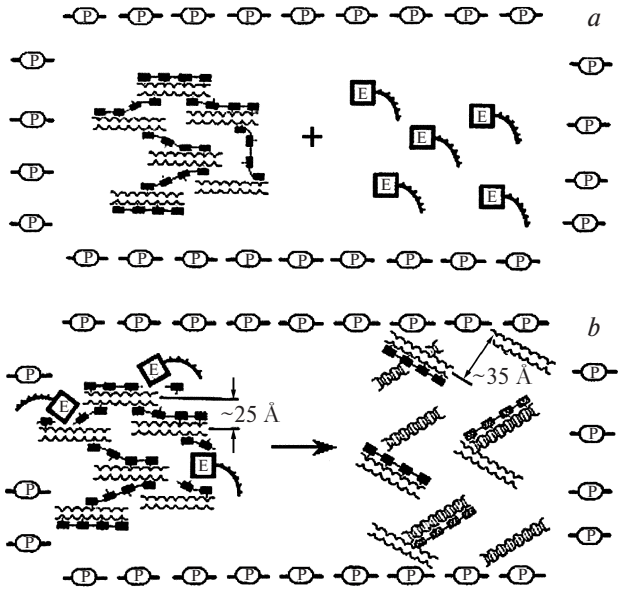


Fig. 6. Schematic presentation of a water—polymer solution containing an LCD of a DNA—polycation complex with addition of BAC able to induce degradation of the polycation (*a*) and spatial restructuring of DNA molecules in particles of the LCD of the DNA—polycation complex resulting in the formation of a cholesteric LCD (*b*).

of the possibility of controlling the spatial packing of DNA molecules in the LCD particles of the DNA—polycation complexes by means of BAC, one more approach can be proposed. In accordance with the strategy considered above (see Figs 1 and 2), this includes the following stages:

(1) the formation of an LCD of the DNA—polycation complex in a water—salt solution with a moderate ionic strength (see Fig. 1, *a*);

(2) transfer of the LCD of the DNA—polycation complex into a polymer-containing solvent providing conditions for the formation of the cholesteric LCD of DNA (see Fig. 1, *b*);

(3) addition, to the polymer-containing solution containing the LCD of the DNA—polycation complex, of a BAC able to induce destruction of the polycations rather than their competitive displacement from the DNA—polycation complex (Fig. 6, *a*).

In this case, of interest are natural polycations, in particular, stellins, which are relatively small nuclear proteins (protamines) rich in basic amino acids from the stellate sturgeon gonads; their chemical structure and physicochemical properties are well studied.[8,18] Protamines are known to be readily destroyed upon enzymatic hydrolysis.[8,18] In addition, protamines bind to DNA and form intermolecular cross-links thus participating in *in vivo* formation of the spatial arrangement of the sex cell chromatin.[19] Finally, in water—salt solutions with moderate ionic strength ($\mu \leq 0.15$), protamines, in particular stellins B, induce condensation of DNA, which affords LCD devoid of abnormal optical activity.[20]

To verify this hypothesis, the LCD of the DNA—stellin B complex formed in an 0.15 *M* NaCl solution (r^* = 0.6) was placed into a water—salt solution of PEG (C_{PEG} = 170 mg mL^{-1}, 0.225 *M* NaCl) and treated with trypsin.

The CD spectra of this dispersion before (curve *1*) and after addition of the enzyme (curve *2*) are compared in Fig. 7, *a*. No intense band is present in the CD spectrum of the LCD of the DNA—stellin B complex in the PEG-containing solution.

In accordance with the scheme shown in Fig. 6, *b*, a strong negative band appears in the CD spectrum of the LCD of the DNA—stellin B complex following its treatment with trypsin (Fig. 7, *a*, curve *2*). The appearance of this band attests to enzymatic hydrolysis of the stellin cross-links resulting in the change in the mode of spatial packing of DNA molecules accompanied by the formation of a spatial helical twist of the quasinematic DNA layers in the dispersion particles. This means that upon enzymatic hydrolysis of the stellin cross-links, DNA molecules realize the mode of spatial packing that is typical of DNA molecules in the cholesteric LCD particles formed

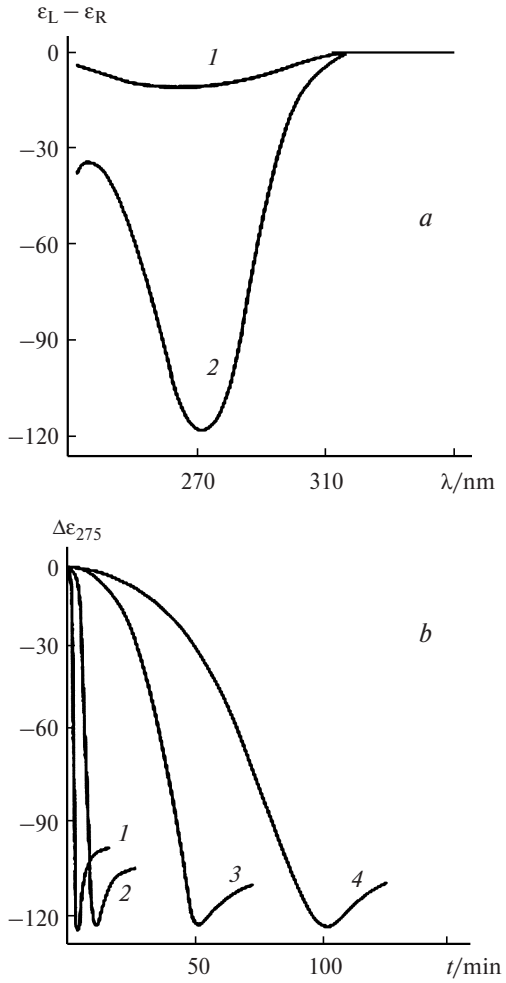


Fig. 7. *a*. CD spectra of an LCD formed from the DNA—stellin B complex (r = 0.6) before (*1*) and after (*2*) treatment with trypsin; *b*. Amplitude of the CD band (λ = 275 nm) of the LCD of the DNA—stellin B complex (r^* = 0.6) *vs*. duration of treatment with trypsin with concentration of 10^{-11} (*1*), 10^{-12} (*2*), 10^{-13} (*3*), 10^{-14} mol L^{-1} (*4*); C_{PEG} = 170 mg mL^{-1}, 0.225 *M* NaCl in 10^{-2} *M* Na phosphate buffer. Here and below r^* is the ratio of the molar concentration of the basic amino acid residues in the structure of stellin B to the molar concentration of DNA nucleotides.

at C_{PEG} = 170 mg mL^{-1}. The conclusion about the restructuring of the LCD of the DNA—stellin B complex is also confirmed by data from X-ray diffraction and polarization microscopy.

Thus, cleavage of the stellin cross-links with trypsin in a water—salt solution of PEG results in a change in the spatial structure of the LCD of the DNA—stellin B complex. In addition, the appearance of an intense negative band in the CD spectrum of LCD of the DNA—stellin B complex after treatment with trypsin also indicates that the presence of PEG in the solution and high packing density of DNA molecules in the dispersion particles do not inhibit trypsin. However, the addition of a trypsin inhibitor, diisopropyl phosphorofluoridate, into the solution completely suppresses the above-mentioned changes, which was detected by CD spectroscopy, X-ray diffraction analysis, and polarization microscopy.

Note (see Fig. 7, *b*) that the spatial restructuring of the LCD formed from the DNA—stellin B complex occurs at low trypsin concentration (~10^{-14} mol L^{-1}) and can also be induced by other hydrolytic enzymes such as pronase P and thrombin.[12]

The observed restructuring of the LCD of DNA—stellin B complexes under the action of proteolytic enzymes suggests a decompactization mechanism for sex cell chromatin. It is believed that after ovum fertilization with a sperm cell, trypsin-like proteinase actively participates in liberation of DNA from protamines.[21,22] Apparently, as in the case of the LCD formed from the DNA—stellin B complexes, it is the protamine cross-links that are the first to be hydrolyzed *in vivo* to give a loose nucleoprotamine structure. This favors the subsequent more complete degradation of protamines and their replacement by histons.

Thus, the presence of PEG and high packing density of DNA molecules in LCD particles of DNA—polycation complexes, on the one hand, do not inhibit the BAC targeting at the polycations incorporated in the DNA—polycation complexes, and, on the other hand, do not prevent (bio)chemical reactions involving these compounds, thus providing the control of the spatial arrangement of the LCD of DNA—polycation complexes using BAC.

The fact that the spatial restructuring of the LCD formed from the DNA—polycation complexes is induced by BAC in low concentrations attests to the possibility of using, in principle, this system for practical purposes as the sensing element (of sandwich type biosensors) in biosensor devices, which allow one to detect the presence and concentration of BAC that cause dissociation or disintegration of the DNA—polycation complex in the sample.

Sandwich-type biosensors based on liquid-crystalline dispersions of the DNA—polycation complexes. In sandwich-type biosensors, the polycations that cross-link the neighboring DNA molecules serve as the targets for BAC.

It was shown above that the removal of polycationic cross-links from the LCD of the DNA—polycation complex is induced by rather low concentrations of heparin (see Fig. 3) or trypsin (see Fig. 7, *b*) and, in a PEG-containing solution, it is accompanied by transition of DNA molecules into the cholesteric liquid-crystalline state, which is characterized by the presence of an abnormal band in the CD spectrum in the absorption region of nitrogen bases (λ ~270 nm). The appearance of the abnormal band in the CD spectrum can be used as the analytical criterion indicating that the system contains BAC that cause, in particular, enzymatic hydrolysis of the polycations or their competitive displacement from the DNA—polycation complex.

Thus, the polycations play a key role in the functioning of sandwich-type biosensors. Therefore, the polycations used to design this type of biosensors should possess the following physicochemical properties:

(1) the polycation should not have substantial intrinsic absorption in the UV region;

(2) polycations should not only neutralize the negative charges of the DNA phosphate groups, but be also extended enough for the formation of intermolecular cross-links between the neighboring DNA molecules;

(3) the interaction of the polycation with DNA should not cause noticeable changes in the secondary structure parameters of this macromolecule;

(4) polycations should incorporate reactive groups serving as the sites of attack by the BAC that is to be determined.

It is evident that the above-noted physicochemical properties of the polycation may allow various types of design of sandwich-type molecular biosensors.

As an example, below we present experimental results that describe the analytical potential of the sandwich type biosensor designed on the basis of LCD of the DNA—polyconidine complexes.

According to the general strategy (see Figs 1 and 2), the LCD of the DNA—polyconidine complex was placed into a water—salt solution of PEG and treated with heparin.

In Fig. 8, the CD spectrum of the initial LCD of the DNA—polyconidine complex (curve *1*) is compared with the CD spectra of the same dispersion (curves *2*—*8*) recorded after addition of heparin in different concentrations. The addition of heparin gives rise to a negative band with the maximum at λ ~280 nm in the CD spectrum of the LCD of the DNA—polyconidine complex, the band amplitude increasing with an increase in the heparin concentration in the solution.

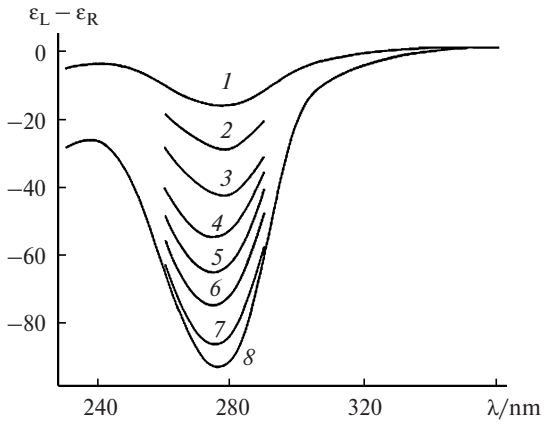


Fig. 8. CD spectra of a PEG-containing water—salt solution of the LCD of the DNA—polyconidine complex (r = 0.91) at different heparin concentrations: 0 (*1*), 0.2 (*2*), 0.4 (*3*), 0.6 (*4*), 0.8 (*5*), 1.0 (*6*), 1.4 (*7*), and 2.5 μg mL^{-1}(*8*); C_{PEG} = 170 mg mL^{-1}, 0.3 *M* NaCl in 10^{-2} *M* Na phosphate buffer.

The dependence of the amplitude of the band at λ = 280 nm in the CD spectrum of the LCD of the DNA—polyconidine complex on the heparin concentration (Fig. 9) shows that $\Delta\varepsilon_{280}$ is directly proportional to the heparin concentration in the concentration range from 0 to 1.2—1.3 μg mL^{-1}.

This dependence between the value of the optical signal generated by the LCD of the DNA—polyconidine complex in the PEG-containing water—salt solution after the addition of heparin and the heparin concentration allows one to use the straight section of the curve (see Fig. 9) as the calibration plot suitable for high-accuracy determination of low (<1 μg mL^{-1}) concentrations of heparin in a sample.

Thus, the intense negative band in the absorption region of DNA nitrogen bases in the CD spectrum in combination with direct proportionality between the amplitude of this band and the concentration of heparin added can be used to detect and determine low concentrations of heparin in a solution (liquid). Note that determination of very low (physiological) concentrations of heparin is important for practical health care, because changes in the physiological level of heparin in patient blood are often indicative of a preclinical stage of pathological conditions, mainly immune diseases.

On the basis of the above results, a fast and accurate method for heparin determination was developed and patented. The minimum concentration of heparin that can be determined in a liquid (solution) is ~0.4—0.5 μg mL^{-1}.

Practical implementation of the above-discussed potential of the bioanalytical system that includes a dichrograph and a sandwich-type biosensor based on LCD of DNA—polycation complexes depends on the solution of the following two problems:

(1) additional stabilization of LCD particles of the DNA—polycation complexes by means of advanced phys-

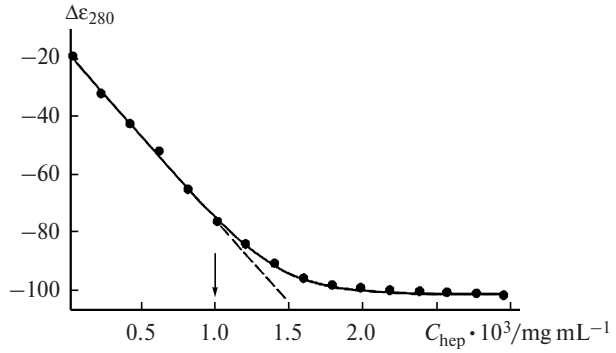


Fig. 9. Amplitude of the negative band in the CD spectra (r = 0.91) of PEG-containing water—salt solution of the LCD of the DNA—polyconidine complex *vs.* heparin concentration (C_{hep}); C_{PEG} = 170 mg mL, 0.3 *M* NaCl in 10^{-2} *M* Na phosphate buffer. The arrow marks the heparin concentration below which the direct proportionality holds between the $\Delta\varepsilon_{280}$ value and heparin concentration.

Fig. 10. Appearance of the portable dichrometer designed for the solution of analytical problems using biosensors based on the DNA LCD.

icochemical methods and biotechnological approaches, for example, by immobilization in an optically isotropic synthetic polymeric matrix permeable for low-molecular-weight compounds, *i.e.*, development of a film-type biosensor;

(2) design of a portable transducer of the optical signal generated by the LCD of the DNA—polycation complex.

Extensive research along these two lines is now in progress.

The appearance of the SCD-2 portable dichrometer designed for solving analytical problems using the "test tube" type of sandwich biosensors based on LCD of the DNA—polycation complexes is shown in Fig. 10. A test batch of these dichrometers (10 items) with the state certification was manufactured at the Experimental Plant for Scientific Instrument Making of the RAS (Chernogolovka, Moscow Region) based on the laboratory prototype designed at the Institute of Spectroscopy of the RAS (Troitsk, Moscow Region). It is noteworthy that the CD spectra of the LCD of DNA and DNA—polycation complexes given in the present work were also recorded by means of this instrument.

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References

1. S. G. Skuridin, V. K. Rybin, I. I. Zainev, M. Ya. Rozkin, V. S. Efimov, and Yu. M. Yevdokimov, *Dokl. Akad. Nauk SSSR*, 1991, **316**, 489 [*Dokl. Chem. USSR*, 1991 (Engl. Transl.)].
2. S. G. Skuridin, V. K. Rybin, A. T. Dembo, N. S. Badaev, and Yu. M. Yevdokimov, *Dokl. Akad. Nauk SSSR*, 1990, **311**, 980 [*Dokl. Chem. USSR*, 1990 (Engl. Transl.)].
3. S. G. Skuridin, V. S. Efimov, A. T. Dembo, and Yu. M. Yevdokimov, *Dokl. Akad. Nauk*, 1999, **365**, 400 [*Dokl. Chem.*, 1999 (Engl. Transl.)].
4. F. Livolant and A. Leforestier, *Prog. Polym. Sci.*, 1996, **21**, 1115.
5. S. G. Skuridin, Zh. G. Gulyaeva, and Yu. M. Yevdokimov, *Mol. Biologiya*, 2006, **40**, 1064 [*Mol. Biol.*, 2006, **40** (Engl. Transl.)].
6. J. Marmur, *J. Mol. Biol.*, 1961, **3**, 208.
7. N. K. Kochetkov, E. I. Budovskii, E. D. Sverdlov, N. A. Simukova, M. F. Turchinskii, and V. N. Shibaev, *Organicheskaya khimiya nucleinovykh kislot* [*Organic Chemistry of Nucleic Acids*], Eds. N. K. Kochetkov, E. I. Budovskii, Plenum Press, London—New York, 1971, Part 1, p. 224.
8. E. P. Yulikova, V. K. Rybin, and A. B. Silaev, *Khimiya Prirod. Soedin.*, 1979, **5**, 700 [*Chem. Nat. Compd.*, 1979, **5** (Engl. Transl.)].
9. Yu. M. Yevdokimov, S. G. Skuridin, and G. B. Lortkipanidze, *Liquid Cryst.*, 1992, **12**, 1.
10. O. N. Kompanets, *Usp. Fiz. Nauk* [*Physics Uspekhi*], 2004, **174**, 686 (Engl. Transl.).
11. B. Ya. Khatsernova, S. A. Silaeva, V. A. Golenchenko, S. G. Skuridin, I. I. Zainev, M. Ya. Rozkin, Yu. M. Yevdokimov, V. K. Rybin, L. V. Grigorova, and V. S. Efimov, *Mol. Biologiya*, 1991, **25**, 1308 [*Mol. Biol.*, 1991, **25** (Engl. Transl.)].
12. S. G. Skuridin, J. Hall, A. P. F. Turner, and Yu. M. Yevdokimov, *Liquid Cryst.*, 1995, **19**, 595.
13. Yu. M. Yevdokimov, S. G. Skuridin, S. V. Semenov, V. I. Salyanov, and G. B. Lortkipanidze, *Biofizika* [*Biophysics*], 1998, **43**, 240 (in Russian).
14. M. D. Mashkovskii, *Lekarstvennye sredstva (posobie dlya vrachei)* [*Medicines. Handbook for Doctors*], Ed. 12, Meditsina, Moscow, 1993, Vol. **2**, p. 79 (in Russian).
15. A. V. Nekrasov, T. Z. Berestetskaya, V. S. Efimov, and O. V. Chernova, *Khim.-Farm. Zh.*, 1982, 1211 [*Pharm. Chem. J.*, 1982 (Engl. Transl.)].
16. V. S. Efimov, S. B. Seredinin, O. V. Chernova, V. L. Grishin, T. Z. Berestetskaya, and A. V. Nekrasov, *Khim.-Farm. Zh.*, 1983, 55 [*Pharm. Chem. J.*, 1983 (Engl. Transl.)].
17. Yu. M. Yevdokimov, S. G. Skuridin, and V. I. Salyanov, *Liquid Cryst.*, 1988, **11**, 1443.
18. E. P. Yulikova, V. K. Rybin, and A. B. Silaev, *Bioorgan. Khimiya*, 1979, **5**, 5 [*Sov. J. Bioorg. Chem.*, 1979, **5** (Engl. Transl.)].
19. P. Suau and J. A. Subirana, *J. Mol. Biol.*, 1977, **117**, 909.
20. V. K. Rybin and S. G. Skuridin, *Izv. Akad. Nauk SSSR. Ser. Fiz.* [*Bull. USSR Acad. Sci., Ser. Phys.*] 1991, **55**, 1828 (in Russian).
21. N. K. Das and C. Barker, *J. Cell Biol.*, 1976, **68**, 155.
22. Y. Marushige and K. Marushige, *Biochim. Biophys. Acta*, 1975, **403**, 180.

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