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Nanoconstructions based on double-stranded nucleic acids

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Abstract

We describe the formation and properties of nanoconstruction that consists of the double-stranded DNA molecules located at distance of 35–50 Å in the spatial structure of particles of their cholesteric liquid-crystalline dispersions and cross-linked by artificial nanobridges. The resulting nanostructures possess the peculiar spatial and optical properties.

Keywords: Nanostructure; Nucleic acids; Molecular design; Liquid-crystalline dispersions; Chelate complexes; Nanobridges; Circular dichroism

1. Introduction: the creation of nanoconstructions based on ds nucleic acids

Nanodesign based on the double-stranded (ds) nucleic acids (NAs), i.e. directed creation of the three-dimensional, spatial constructions with the tailored properties,"building blocks" of which are dsDNA molecules or their complexes with biologically active compounds, is a topic of current theoretical and experimental interest [1–3]. The dsDNA (RNA) nanoconstructions (NaCs) are of significal practical importance, as a minimum, from two points of view. First, NaCs with adjustable spatial parameters can be used in bioelectronics and biosensorics [4,5]; second, the DNA NaCs can be used for the delivery of genotoxicants or biologically relevant compounds into eucaryotic cells.

The very possibility of using dsNA for formation of NaCs with controlled parameters is based on a few properties characteristic of NA molecules only:

(a) the short helical molecules of dsNA with lengths of the order of 100–1000 Å have a high local rigidity at standard solvent properties, that allows such molecules to be used as "building blocks" without change in their physical properties;
(b) flexible single-stranded NA not only recognizes a complementary strand but also hybridizes with it to form a strong complex; this causes a change in the spatial structure of the single-stranded NA and the formation of a rigid double-stranded molecule;
(c) creation of sticky ends in dsNA combained with an appearance of "branch-point", because of the presence of specific sequences of nitrogen bases in this structure, makes it possible to branch the resulting NaCs built thereof;
(d) in the case of rigid dsNA molecules, their properties and the character of intermolecular interaction under different conditions can be programmed, making it possible to tune the peculiarities of designed spatial constructions;
(e) nitrogen bases in the spatial NA constructions retain their capability not only to interact with different chemical substances or biologically active compounds but also to orient them with respect to the long axis of NA molecule, which imparts additional chemical reactivity to the whole construction.

Abbreviations: CD, circular dichroism; dsNA, double-stranded nucleic acid; DAU, anthitumor anthracycline antibiotic-daunomycin; LCD, liquid-crystalline dispersion; NaC, nanoconstruction; NA, nucleic acid

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To date, two main strategies have been described for designing NA nanostructures.

First strategy, which takes into account points "b–d" above and could be named conventionally as a successive design or step-by-step design, based on successive modification of initial NA molecule. According to approach suggested and developed by Seeman [6], newly-synthesized, rigid, ds fragments of DNA (polynucleotides) containing not only sticky ends but also particular base sequences were used as building blocks with adjustable properties. By the technique of oligonucleotide synthesis in combination with a set of restriction endonucleases and ligases used for cleavage and linking of desired sequences, the authors created nanostructures having the shapes of cube, octahedron, hitched octahedron, dodecahedron, etc., whose stiffening ribs are DNA molecules [7].

There are a few additional approaches, which may be called "supplementary" to the approach suggested by Seeman. One of such technique is based on the use of molecule composed of two single-stranded self-complementary oligonucleotides, the ends of which are bound to each other via a rigid chain of two *p*-(2-hydroxyethyl)-phenylethynyl-phenyl spacers linked to a tetrahedral carbon atom [8]. The hybridization of oligonucleotide fragments, which results in formation of ds structure, is accompanied by the emergence of a set of multi-arm star-shaped NaCs. The rigidity of the constructions is provided by alternation of hydrocarbon fragments in definite spatial conformation and ds oligonucleotides. Another technique by Niemeyer et al. [9] is based on the use of bis(biotinylated) DNA molecule and biotin-binding protein—streptavidin. This allows nanostructures of closed loop shape to be made using reactions of complex formation.

An approach to nanodesign used almost simultaneously by Mirkin et al. [10] and by Alivisatos et al. [11] uses as the building blocks the single-stranded NA linked to the particles of colloidal gold. The addition of "foreign" NA, whose base sequence is complementary to the initial NA, leads not only to the formation of rigid dsNA by hybridization, but also to the formation of a three-dimensional construction containing spatially ordered gold particles and dsNA fragments.

It should be noted that the issue of the practical application of NaCs created from a single dsNA molecule via step-by-step technology seems to be determined by the tasks to be solved by researchers. The retention of physicochemical properties of NA during the nanodesign opens the way to insert atoms or molecules of different compounds ("guest" molecules) into initial NA molecules or resultant NaCs. In particular, NaCs whose properties depend on the length of NA molecules and the size of inserted metal particles could be used as biosensing elements [5,12]. Moreover, the NaCs could be used in nanoelectronics. If three-dimensional ordering of single NaCs, i.e. their crystallization, will be achieved, it is likely that compounds poorly crystallizable under common conditions, which are introduced somehow into the NaC, could be crystallized within NaC. However, such an ordering itself is a complicated task, which is not solved to date.

Second strategy of creating NaCs containing dsNA molecules suggested by us earlier [13] takes into account points "a, d, e" above. This strategy *differs in principle* from all the above variants of the step-by-step strategy, because our strategy makes use of the liquid-crystalline dispersions (LCD), rather than single NA molecules, resulting from the phase exclusion of dsNA molecules from aqueous polymeric solutions. As a result of phase exclusion, rigid ds molecules of NA (or polynucleotides) form particles composed of about 10^4 molecules; each particle is about 5000 Å in size, which was evaluated by several experimental techniques (the low speed sedimentation, the UV-light scattering, the laser correlation spectroscopy, etc.) and confirmed by theoretical calculations [14]. According to the X-ray study, NA molecules are ordered in the particle at distances of 30–50 Å, i.e. they acquire the properties of a crystal, but molecules in the neighboring layers are mobile, i.e. they retain the properties of a liquid. Such combination of properties allows this structure to be called as "liquid-crystalline" (see reviews [14,15] and early references cited therein). The most important features of dsNA LCDs are well established now. First, LCDs exist under certain boundary conditions, which are determined, in particular, by solution ionic strength, by the value of osmotic pressure of aqueous polymeric solution, etc. The osmotic pressure, which depends on polymer concentration in solution, determines the distance between the NA molecules in a particle. Second, spontaneous constraint of diffusional degrees of freedom of neighboring NA molecules takes place upon phase exclusion. Third, the combination of geometrical and optical anisotropy of NA molecules causes each next layer formed by NA molecules in the structure of the liquid-crystalline particle (a so-called 'quasinematic layer') to be turned through a certain angle with respect to previous one, i.e. spatially twisted or a so-called "cholesteric structure" of the particle arises. Violation of the boundary conditions results in the disappearance of the spatial structure of particle. Fourth, because NA molecules contain chromophores (nitrogen bases absorbing in the UV-region of the spectrum), the resulting cholesteric may be named as "colored" cholesteric. Since the bases are virtually perpendicular to the long axis of NA molecules forming adjacent layers in the structure of the cholesteric, theory [16] predicts an appearance of an intense (abnormal) band in the circular dichroism (CD) spectrum in the bases absorption region, which is indeed observed experimentally. It should be noted that theory [16] imposes no limitations on the number of chromophores that could be introduced into the NA structure in the same manner, i.e. one could expect an appearance of the abnormal CD bands preferably for compounds intercalating between the NA base pairs. This means that there is an analytical "instrument" capable of monitoring the finest variations in the properties of NA molecules and cholesterics produced thereof. And finally, the chemical reactivity of NA molecules remains unchanged upon formation of the LCD particles; this opens

the way to purposeful alteration of the properties of these molecules.

Consideration of the above points reveals the fundamental possibility for spatial fixation of the neighboring, closely located and fairly low-mobile NA molecules by formation of nanobridges (crosslinks) between these molecules, i.e. it is possible to create NaC, whose properties can be specified in advance by controlling both the properties of NA molecules and solvent used. This means that our technology provides a possibility to create NaCs with preset properties. Besides, the use of the LCD particles for nanodesign automatically solves the problem of ordering both neighboring NA and guest molecules, which is not solved yet in the case of step-by-step strategy.

Thus, there is a possibility to use NA molecules within the LCD particles as building blocks with adjustable properties. At the first stage of our study, we inserted artificial nanobridges (crosslinks) with adjustable properties between NA molecules in LCD particles. The crosslinking of NA molecules by chelate complexes based on anthracycline antibiotics has been used to create NaCs. In this paper, we will focus our attention on the model (structure) of nanobridges between the neighboring NA molecules. For this purpose the optical properties of the nanoconstructions were analyzed, the number of metal ions in nanobridge was evaluated, the thermal stability of the nanoconstructions was measured, the preconditions for nanobridge formation were specified, and an attempt at a theoretical description of nanobridges was undertaken.

2. Materials and methods

The formation of NaCs based on dsNAs or synthetic polyribonucleotides was achieved by three-step procedure described in detail earlier [17–19]. In physicochemical sense, the initial system was composed of separate liquid-crystalline particles formed by dsNA molecules isotropically distributed in poly(ethylene glycol)–water–salt (NaCl) solution added with DAU and $CuCl_2$.

The absorption spectra were recorded on a spectrophotometer ("Specord M40", Germany) and the CD spectra were recorded by a portable dichrometer SKD-2 (manufactured by the Institute of Spectroscopy of the RAS, Troitzk, Moscow region). In all cases, the quartz cells with 1 cm optical path have been utilized.

The morphology of NaCs particles was examined using a commercial Atomic Force Microscope P47-SPM-MDT (produced by NT-MDT, Russia). To isolate NaCs, the solution in which they were formed was filtered through a poly(ethyleneterephtalate) nuclear membrane filter (diameter of pores 0.1–0.25 μm, produced by the Institute of Crystallography, RAS), that allowed us to immobilize DNA particles; filters were dried in air no less than 1 h.

To estimate the number of Cu^{2+} ions, included in a composition of nanobridge, the magnet moment of Cu^{2+} ions was determined. The dsDNA based NaC was centrifuged (5000 rpm; 40 min; 15 °C), the obtained deposit was flushed a few times by a distilled water to erase the extra amount of non-specifically absorbed $CuCl_2$ and utilized as a sample for the further analysis.

The magnetic properties of the NaC sample were measured by the superconducting interferometer device (SQUID-magnetometer) produced by Mendeleev University (Moscow) at magnetic field of 71.29 mT (712.9 Oe) at sample position. The NaC sample was characterized magnetically before and after magnetic filed introduction within temperature range from 4.2 K to room temperatures. The cooling to helium temperatures was carried out at zero magnetic field (ZFC), then a magnetic field was introduced, and temperature was increased to 100 K. An amount of quanta (M) of magnetic flux through NaC sample was measured continuosly during this procedure. The M value is proportional to the magnetic moment (P_m) of the sample used (in our case, Cu^{2+} ions play the role of the paramagnetic centers in the sample of NaC. These ions in d_9-state participate in the formation of chelate nanobridges with reactive oxygen atoms and possess non-zero magnetic moment [20]). The cooling to helium temperatures was repeated, but already at a non-zero magnetic field (FC). Again the amount of quanta (M) of magnetic flux through NaC sample was measured continuosly durind this procedure. The histoty of cooling did not influence the results obtained.

3. Results and discussion

3.1. The CD spectra of the dsDNA liquid-crystalline particles cross-linked via nanobridges

In Fig. 1, the CD spectra of an initial DNA cholesteric LCD (curve 1), this dispersion treated with DAU (curve 2),

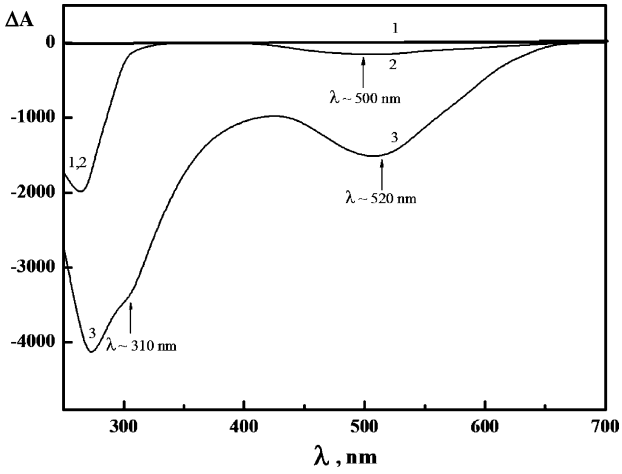

Fig. 1. The CD spectra of DNA cholesteric LCD (1), this dispersion treated by DAU (2) and then $CuCl_2$ solutions (3). $C_{DNA} = 5.5$ μg/ml; $C_{DAU} = 27.3 \times 10^{-6}$ M; $C_{Cu^{2+}} = 9.9 \times 10^{-6}$ M; $C_{PEG} = 170$ mg/ml; 0.3 M NaCl; 2×10^{-3} Na^+–phosphate buffer; pH 6.7. $\Delta A = A_L - A_R$ ($\times 10^{-6}$ opt. units).

and then by $CuCl_2$ solutions (curves 3) are compared. The formation of the DNA LCD particles results in an appearance of the intense band in UV-region of the spectrum (curve 1). The negative sign of the band in the CD spectrum ($\lambda \sim 270$ nm) of LCD of DNA indicates the left-handed twist of the spatial structure of particles of cholesteric LCD [17] resulting from phase exclusion of the right-handed DNA molecules. The amplitude of the band at $\lambda \sim 270$ nm remains practically unchanged at any reasonable DAU concentration added to the DNA LCD (curve 2). But, addition of DAU to the DNA LCD is accompanied by an appearance of a new band located in the absorption region of DAU ($\lambda \sim 500$ nm). The amplitude of this band is growing at increase of DAU concentration, reaching the maximal value at the DAU concentration, which corresponds to the maximal degree of the DNA saturation by DAU molecules, and does not alter upon further growth of DAU concentration (it is necessary to add that the amplitude of the band in the CD spectrum characteristic of DAU complex with linear ds DNA (not liquid-crystalline!) does not exceed a few units of ΔA). The negative sign of the band at $\lambda \sim 500$ nm, which similar to the sign of the band, reference for the initial cholesteric DNA LCD, shows that the orientation of DAU molecules in respect to long axis of the DNA helix is coinciding with orientation of the DNA base pairs. These results reflect an intercalation (insertion) of DAU molecules between base pairs of the DNA molecules, fixed at particular distance due to osmotic pressure a polymeric solvent used for the phase exclusion of the DNA molecules (one can add, that the reactive groups of DAU (keto-oxygen, *peri*-OH groups) become unavailable for chemical reactions upon intercalation).

It should be noted, that no substantial alterations in the CD spectrum in the DAU absorption region were observed, when cholesteric LCD composed of dsRNA molecules were treated with DAU. The lack of a strong CD band in the region of DAU absorption indicates, that in this case no intercalation complex is formed between DAU and dsRNA. Hence, in contrast to DNA, DAU molecules are located isotropically near the RNA surface (they can form only an "external" complex and these molecules are practically "invisible" in the CD spectrum).

An addition of $CuCl_2$ solution to the DNA LCD treated by DAU and having an equilibrium value of the amplitude of the band at $\lambda \sim 500$ nm results not only in a many-fold increase (amplification) of this band, but also a band located in the UV-region of the spectrum (curve 3) (the bands at $\lambda \sim 500$ nm and $\lambda \sim 300$ nm are characteristic of the CD spectrum of linear, isotropic DAU–Cu^{2+} complexes; this reflects the existence of low- ($\lambda \sim 500$ nm) and high- ($\lambda \sim 300$ nm) energy electronic transitions in DAU moieties [21–24] of the complexes. These two bands are maintained at the formation of a complex between the linear DNA and DAU [25]). Indeed, at addition of $CuCl_2$ not only amplification but also the change in the shape of the band in the UV-region (curve 3) of the CD spectrum is observed. In particular, the curve 3 in Fig. 1 has a "shoulder" at $\lambda \sim 310$ nm.

However, the most important is that similar changes took place in the CD spectrum of dsRNA LCD subsequently treated by DAU and Cu^{2+} ions [26], where intercalation of DAU is not possible at all due to sterical reasons [27].

Here, we stress again that DAU molecules located isotropically along neighboring NA molecules are practically "invisible" in the CD spectrum of the LCD. In addition, anisotropic location of DAU molecules, due to their intercalation between DNA nitrogen base pairs, does not induce the "extra-increase" of the band in the CD spectrum after addition of $CuCl_2$, because complex formation is not possible.

Hence, one can suppose that there is a quite different mechanism, which explains the "extra-increase" of the CD band after addition of $CuCl_2$. This mechanism does not need intercalation of DAU molecules between NA base pairs. Fact of the amplification of the bands of the dsDNA LCD, as well as dsRNA LCD, in the visible and in UV-regions shows, that under conditions used a part of DAU molecules is indeed located near DNA (or RNA) forming "external" (non-intercalative) complexes. The DAU molecules of "external" complexes are acceptable for chemical reaction [27–29], for instance, for formation of chelate complex with Cu^{2+} ions. The physicochemical properties of "external" complex are different from that of intercalation complex between DAU and DNA molecules (compare curves 2 and 3). Because the amplification of the optical properties of isotropically located chromophores is impossible, the results above mean: the DAU–Cu^{2+} complexes should be ordered near the surface of NA molecules in the content of the particles of cholesteric LCDs. Indeed, according to the theory [16], the amplification of optical properties is possible only for such chromophores, which are severely fixed in respect to the "director" of quasinematic layer of the cholesteric liquid crystal.

In Fig. 2, the dependences of the amplitudes of negative bands at $\lambda = 505$ nm (A) and $\lambda = 310$ nm (B) on concentration $CuCl_2$ are shown. It is evident, that despite various amplitudes of the bands, located in the visible and UV-regions of the spectrum, these curves are S-shaped, and the amplification of the bands begins *only after the achievement* of a "critical" (C^{cr}) concentration of copper ions in solution. It allows one to speak that the bands reflect the properties of the chromophore, which is being anisotropic at its location between DNA molecules in the structure of the LCD particles. Such chromophore is the complex of DAU–Cu^{2+}.

The many-fold amplification of the bands at $\lambda = 505$ and 310 nm indicates the appearance of an additional (as well as intercalation) type of anisotropic arrangement of DAU molecules in proximity to DNA molecules. The anisotropic arrangement of DAU–Cu^{2+} complexes, which differs from common intercalation of DAU and causes the amplification of the 505 and 310 nm bands in the CD spectrum of the NA LCD, could be explained by two different reasons. First, one may suppose that, owing to stacking interaction between DAU molecules this is caused by the formation of vertical stacks (*n*-mers) of DAU molecules *near* the NA surface in the structure of the LCD particles. This means that a shell of

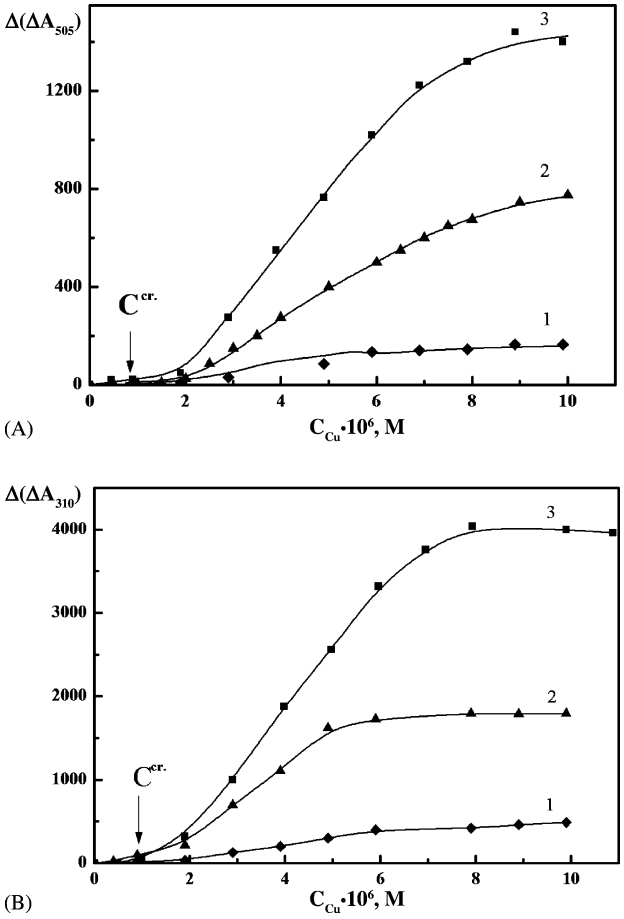

Fig. 2. The dependence of "extra-increase" $\Delta(\Delta A)$ of amplitude of the bands at $\lambda = 505$ nm (A) and $\lambda = 310$ nm (B) in the CD spectra of LCD formed by DNA–DAU complexes upon $CuCl_2$ concentration. (1) $C_{DAU} = 12.0$; (2) $C_{DAU} = 15.6$; (3) $C_{DAU} = 27.0 \times 10^{-6}$ M; $C_{DNA} = 5.5$ μg/ml; $C_{PEG} = 170$ mg/ml; 0.3 M NaCl; 2×10^{-3} Na^+–phosphate buffer; pH 6.7.

DAU appears in a proximity to the surface of NA molecule where a portion of DAU molecules is bridged by Cu^{2+} ions [28]. It is obvious that the direction of 'the vertical axis of the resulting structure of DAU *n*-mers coincides with the direction of the NA long axis'. Second, one may suppose that complexes DAU–Cu^{2+} are located between the neighboring NA molecules in such a way that they form nanobridges between these molecules. It should be noted that, in principle, the "beginning" of the nanobridge could be formed not only by DAU molecule forming the "external" complex with NA molecules [28,29] but also by Cu^{2+} ion chelating NA nitrogen bases [30]. The direction of the long axis of the nanobridges, formed by [DAU–Cu^{2+}] complexes, proves to be perpendicular to the direction of the long axis of NA molecules, although the orientation of DAU molecules is close to that of NA nitrogen base pairs.

The above-listed properties of LCD particles of NAs (Section 1) allow choosing between the variants of DAU–Cu^{2+} complexes disposition. In the case of the first assumption, the main factor stabilizing the liquid-crystalline structure of NA particles, even covered with DAU molecules, is still the osmotic pressure of the aqueous polymer solution. Consequently, violation of the boundary conditions, in particular dilution of aqueous polymer solution, which is accompanied by a decrease in the osmotic pressure, should result in the transition of NA molecules from liquid-crystalline into isotropic state. Isotropic NA solution is known to be devoid of abnormal optical activity. This means that in the first case the dilution should result in the disintegration of cholesteric structure of LCD and in the disappearance of abnormal optical activity. In the case of the second assumption, the osmotic pressure of solution is not the main factor affecting the character of packing NA molecules in LCD particle; in this case, the abnormal optical activity should persist even upon dilution, and the specific optical activity should remain constant. We have shown that multiple dilution of PEG-containing water–salt solution does not lead to an appreciable decrease in the specific abnormal optical activity of the DNA LCD treated by DAU and $CuCl_2$, meaning that the mutual orientation of neighboring NA molecules is not violated even outside the boundary conditions. This is possible only if neighboring NA molecules *are indeed cross-linked via nanobridges*, which stabilize the cholesteric structure of the LCD particles. Thus, the reason for the increase in the amplitude of bands located in different regions of CD spectrum of LCD of NA consecutively treated by DAU and $CuCl_2$ is the formation of nanobridges between neighboring NA molecules, i.e. the formation of the NA nanoconstruction.

Besides, we can suppose that at addition of copper ions the DNA LCD, not only DAU–Cu^{2+} nanobridges between DNA molecules are formed, but also the DNA secondary structure is, in part, "perturbed" as a result of interaction of copper ions with nitrogen base pairs. Hence, the amplitude of the band at $\lambda \sim 270$ nm related with the DNA chromophores, that "feels" the general state of the DNA secondary structure, could be influenced by various components participating in the nanobridge formation. This band can be utilized to check the fact of the presence of the nanobridged structure, but the use of this band for the description of fine details of mechanism of the "nanobridging" is, probably, non-correct, especially at high DNA concentration.

Thus, only the bands at $\lambda = 310$ and 505 nm caused by DAU chromophores should be used for the description of the concrete mechanism of the nanobridge formation.

3.2. The magnetometric evaluation of Cu^{2+} ions in nanobridge

In Fig. 3, the temperature dependence of experimental value, *M* (proportional to the magnetic moment (P_m)), measured for NaC sample is shown. Using the temperature dependence of the magnetic moment upon temperature, the magnetic susceptibility (χ) was calculated as:

$$\chi = \frac{P_m}{H} \quad (1)$$

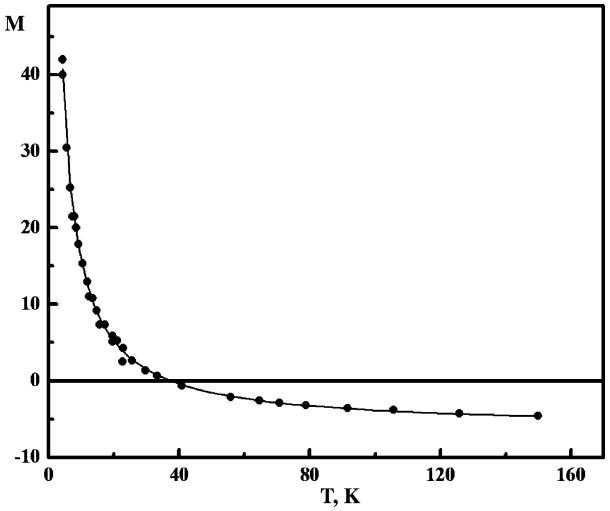

Fig. 3. The temperature dependence of experimental *M*-value produced by NaC sample in the magnetic field.

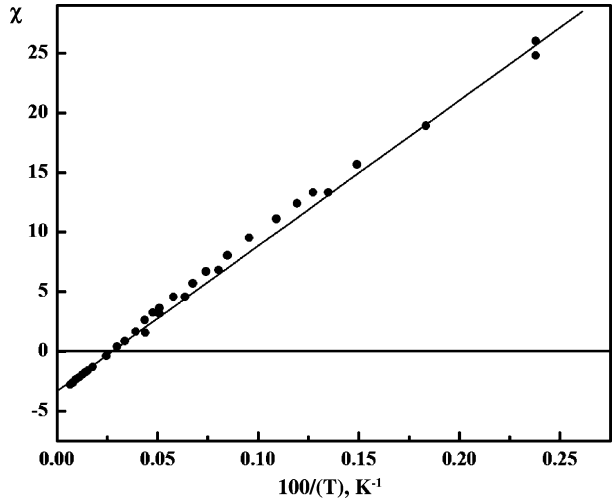

Fig. 4. The temperature dependence of magnetic susceptibility χ of NaC sample. χ value is expressed in CGS emu/Oe units ($\times 10^{-8}$).

where the H is the magnetic field value, that is equal to 712.9 Oe.

Fig. 3 shows that the experimental curve contains the contributions both from paramagnetic and diamagnetic centers. The paramagnetic centers are only Cu^{2+} ions. The basic diamagnetic contribution is caused totally by water molecules and DAU aromatic rings. At low temperatures the magnetic susceptibility is positive, whereas at high temperatures it is negative. Hence, the total magnetic moment is represented by two parts, i.e. the positive paramagnetic part, caused by Cu^{2+} ions, and the negative diamagnetic part, caused totally by both the presence of the "rest" water and benzene rings of DAU.

Two contributions into the total magnetic moment have been separated by mathematical processing of experimental temperature dependence of magnetic susceptibility using the Curie–Weiss equation with constant value of χ_0.

The dependence of χ(T) is well approximated by the well-known [31] analytical dependence shown in Fig. 4:

$$\chi = \chi_0 + \frac{C}{(T - T_C)} \tag{2}$$

where $C = (\mu_{eff})^2 \times N/(3 \times k)$ and N is the number of the paramagnetic centers Cu (d_9).

The calculated value of χ_0 was negative; this corresponds to the joint contribution from water molecules and daunomycin. The number of paramagnetic centers (N) was calculated, too. The value of the effective magnetic moment was estimated from ESR experiments. The g-factor at ambient temperature makes 2.09. One can estimate an effective magnetic moment of one Cu^{2+} ion in a NaC sample as:

$$\mu_{eff} = g\mu_B\sqrt{j(j+1)} = 1.82\mu_B \tag{3}$$

From temperature dependence of magnetic moment (Figs. 3 and 4) it is possible to evaluate the number of Cu^{2+} ions, being in d_9-state, a namely N, as:

$$N = \frac{3 \times k \times C}{(\mu_{eff})^2} = 1.96 \times 10^{18} \tag{4}$$

where $C = 1.47 \times 10^{-6}$ (CGS units) is the constant obtained from an approximation of the temperature dependence of magnetic susceptibility according to the Curie–Weiss law.

This allows us to perform a few practically important evaluations. Let's designate the mass of one DNA molecule of as m_{DNA}. In the sample used, the value of m_{DNA} was about 8×10^5 Da or 1.33898×10^{-18} g. The concentration of DNA in a solution, C_{DNA}, is 50 μg/cm^3 = 0.00005 g/cM^3; the volume of solution, from which a sample of DNA NaC was formed, is 80 cM^3. From these data, the total mass of DNA, M_{DNA}, is 0.0040 g.

This means that the number of DNA molecules in a sample, N_{DNA}, is equal to:

$$N_{DNA} = \frac{M_{DNA}}{m_{DNA}} = 2.98735 \times 10^{15} \tag{5}$$

Hence, each DNA molecule contains approximately:

$$\frac{N}{N_{DNA}} = 716\ Cu^{2+}\ \text{ions in}\ d_9\text{-state} \tag{6}$$

As the number of helical turns in the DNA molecule is equal to:

$$\frac{8 \times 10^5}{6.6 \times 10^3} = 1.2 \times 10^2 = 120, \tag{7}$$

from here it follows that on each turn of DNA helix 716/120 = 5.9 (∼6) copper ions are located.

One can suppose that all of these copper ions are participating in the formation of nanobridges within NaCs. In this case, each nanobridge, located in each helical turn between the neighboring DNA molecules, contains approximately six copper ions.

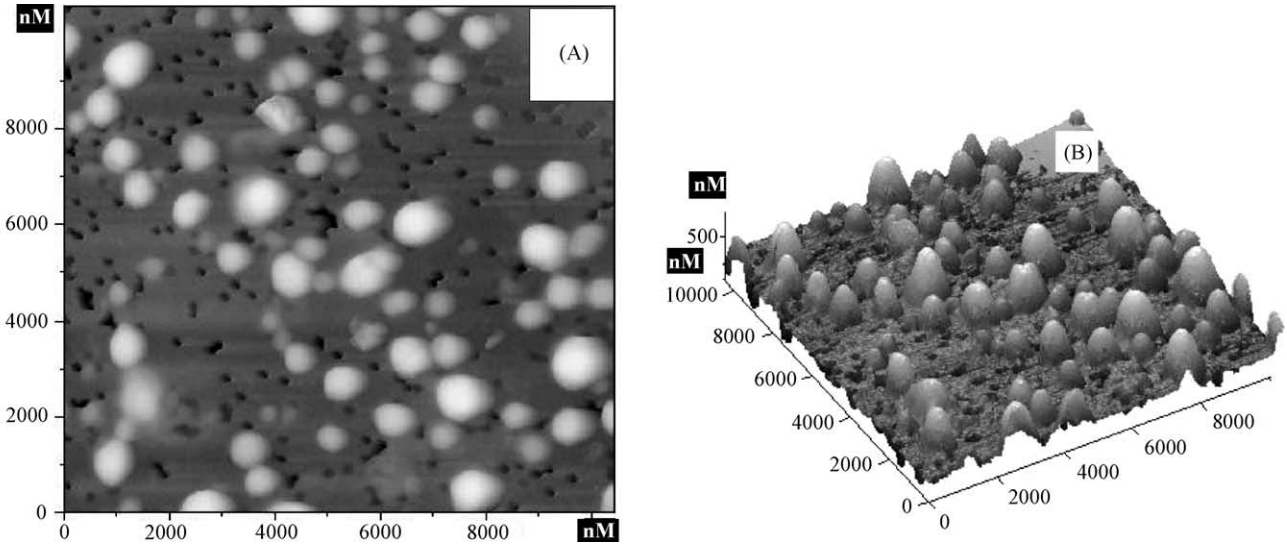

Fig. 5. 2D- and 3D-AFM images of the DNA NaCs immobilized onto the surface of the nuclear membrane filter (PETP) (A and B, respectively). The small dark spots correspond to pores in the filter ($D \sim 0.2\,\mu m$).

The emergence of nanobridges of [. . .–Cu^{2+}–DAU–Cu^{2+}–DAU–Cu^{2+}–. . .] between neighboring NA molecules can be expected to lead to the formation of spatial structure of NaCs. The stability of the NaCs is determined by number and properties of nanobridges rather then the properties of initial polymeric solution. This means that NaC would persist even in aqueous salt solution, which substantially facilitates its handling. Taking into account this circumstance, an opportunity is opened to visualize the particles of LCD of NA after their transformation into a spatially fixed NaCs.

3.3. Visualization of particles of nanoconstructions

Fig. 5 demonstrates, as an example, 2D- and 3D-images of the dsDNA LCD particles, treated sequentally with DAU and $CuCl_2$ solutions, and immobilized on nuclear membrane filter. The examination of size distribution of particles of NaCs obtained by direct measurent of 400 particles showed, the particles have a shape that reminds the prolate cylinder; and although their sizes vary from 4000 to 8000 Å, an average diameter is about 5000 Å. It should be noted, that this is the value obtained by direct mesurement of the dimensions of the LCD particles, in contrast to values, based on the results of previous indirect measurements of the DNA particles [14]. Thus, the size of the NaCs deposited on the nuclear filter in absence of the osmotic pressure of a solvent coincides with the size of the DNA particles in solutions with fixed stationary osmotic pressure [14]. Hence, as a result of formation of nanobridges between the neighboring DNA molecules, there appear the NaCs with fixed three-dimensional spatial structures. Although the number of the nanobridges can be insignificant, they can represent by themself the factor of stabilization of spatial structure of DNA NaCs.

Thus, for the first time, we visualized NaCs based on the particles of cholesteric LCD of DNA and obtained data characterizing the macroscopic parameters of these NaCs.

3.4. Temperature dependence of optical properties of nanoconstructions

In Fig. 6, the CD spectra of NaCs registered at their heating are shown. The amplitude of the abnormal band in visible region decreases practically up to zero, whereas the band in UV-region diminishes to the value conforming to the amplitude of the initial DNA LCD. This result shows, that under conditions of the fixed osmotic pressure of a solution (determined by PEG concentration) the cholesteric structure of the LCD remains unchanged during the heating.

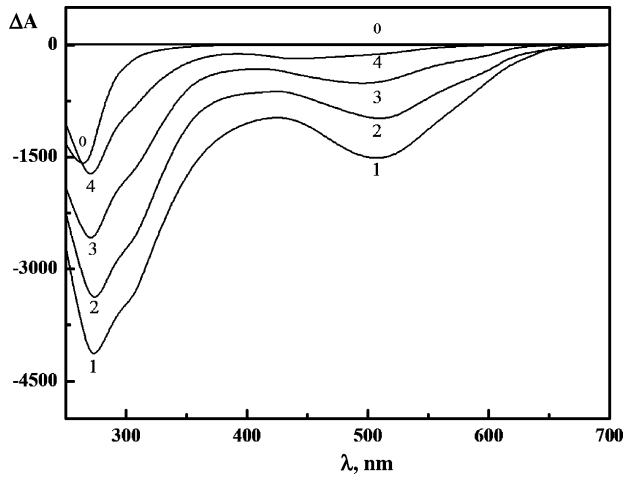

Fig. 6. The CD spectra of NaC at different temperatures. (1) −38 °C; (2) −56 °C; (3) −60 °C; (4) −68 °C; (0) the CD spectrum of the initial DNA LCD at room temperature; $C_{DNA} = 5.5\,\mu g/ml$; $C_{DAU} = 27.3 \times 10^{-6}$ N; $C_{Cu^{2+}} = 9.9 \times 10^{-6}$ M; $C_{PEG} = 170\,mg/ml$; 0.3 M NaCl; 2×10^{-3} Na^+–phosphate buffer; pH 6.7. $\Delta A = A_L - A_R$ ($\times 10^{-6}$ opt. units).

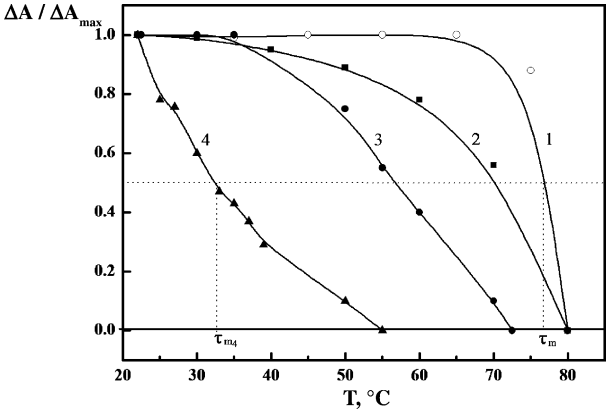

Fig. 7. The temperature dependences of relative changes in the amplitude of the band (λ = 310 nm) in the CD spectra of DNA LCD and NaCs. (1) DNA LCD; NaCs, (2) $C_{DAU} = 64.0$; $C_{Cu}{}^{2} = 20.0$; (3) $C_{DAU} = 27.3$, $C_{Cu^{2+}} = 9.9$; (4) $C_{DAU} = 15.0 \times 10^{-6}$ M, $C_{Cu^{2+}} = 5.0 \times 10^{-6}$ M; $C_{DNA} = 5.5$ μg/ml; $C_{PEG} = 170$ mg/ml; 0.3 M NaCl; 2×10^{-3} Na^{+}–phosphate buffer; pH 6.7.

In Fig. 7, the dependences of the relative values of the amplitudes of the bands in the CD spectrum of the cholesteric LCD of an initial dsDNA (curve 1) and NaCs, formed under various conditions on the basis of this LCD (curves 2–4), upon temperature are compared. Stability of cholesteric DNA LCD and stability of NaCs was characterized by the value of the "CD melting temperature" (τ_m), conditionally accepting that this is a temperature at which the amplitude of an abnormal band in the CD spectrum decreases on 50% [32]. Curve 1 in Fig. 7 shows that the cholesteric DNA LCD is "melted" in a narrow temperature range (∼5 °C) and the τ_m value of about 75 °C. The process of the "CD melting" of the DNA LCD particles reflects the change in the twist angle of their spatial structure; it does not accompanied by the dsDNA chains separation and, due to sterical reasons, the process of the "CD melting" of the DNA LCD is fully reversible [33,34]. The "melting" of NaCs occurs in the temperature range where the cholesteric structure of the LCD particles still persists. The τ_m values registered at λ = 505 nm and at 310 nm coincide each other (Fig. 8). Besides, the "CD melting" of NaCs registered by both bands depends on the concentration of both Cu^{2+} ions and DAU molecules in solution: the higher the concentration of any of these components of the nanobridges (at constant concentration of the other one), the higher τ_m value of NaCs. Fig. 7 is consistent with an increase in the number or in the thermodynamic stability of nanobridges formed between the DNA molecules. In addition, a similar character of the dependences of τ_m on Cu^{2+} and DAU concentration, i.e. upon the concentration of both "building" elements, speaks in favour of approximately equal amounts of Cu^{2+} ions and DAU molecules in the structure of nanobridges.

As distinct from cholesteric LCD of DNA, the cooling of solution, in which NaC was formed, results only in partial (by 30%) recovery of the initial abnormal optical activity. Such a decrease of ability of NaC to regenerate the initial structure reflects the perturbation of the DNA secondary structure.

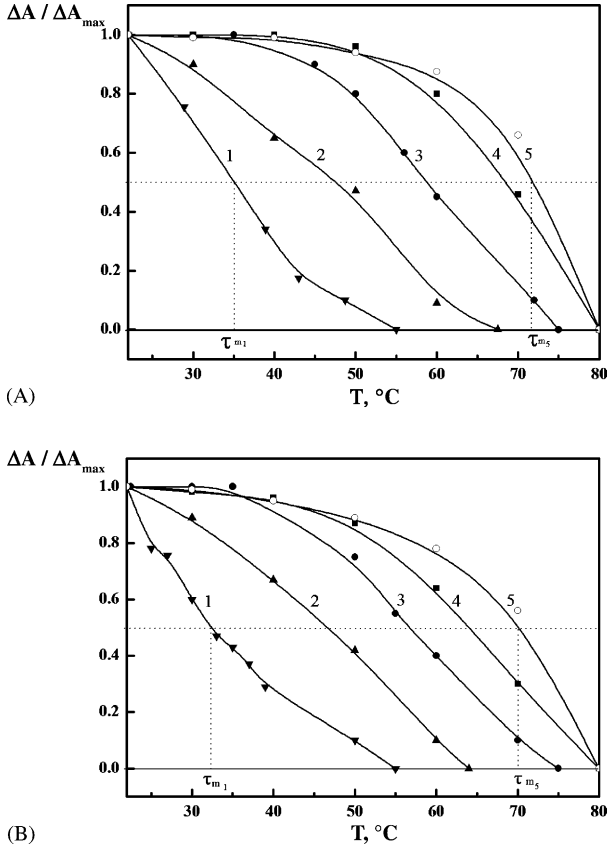

Fig. 8. The relative change in the amplitude of the bands (λ = 505 nm (A); λ = 310 nm (B)) in the CD spectra of NaCs vs. temperature at various concentrations of DAU and Cu^{2+} ions. (1) $C_{DAU} = 15.0$; $C_{Cu}{}^{2} = 5.0$; (2) $C_{DAU} = 21.0$; $C_{Cu^{2+}} = 7.5$; (3) $C_{DAU} = 27.3$, $C_{Cu^{2+}} = 9.9$; (4) $C_{DAU} = 45.0$; $C_{Cu^{2+}} = 15.0$; (5) $C_{DAU} = 64.0 \times 10^{-6}$ M; $C_{Cu^{2+}} = 20 \times 10^{-6}$ M; $C_{DNA} = 5.5$ μg/ml; $C_{PEG} = 170$ mg/ml; 0.3 M NaCl; 2×10^{-3} Na^{+}–phosphate buffer; pH 6.7.

This is consistent with the results above on possible interaction of Cu^{2+} ions with initial NA molecules during the heating [35–37]. This interaction is accompanied by a distortion of the parameters of the DNA secondary structure and this makes impossible the cholesteric ordering of a so-modified, neighboring DNA molecules.

The curves shown in Fig. 7 permit one an assumption to be made that the decrease in the abnormal optical activity of the DNA cholesteric and of NaCs based on DNA occuring at different temperatures may be caused by different reasons. First, it is not excluded that the decrease in the abnormal optical activity of NaC indicates that the spatial (twisted?) structure formed by the neighboring nanobridges located between DNA layers can disappear upon heating, and this process takes place at low temperature. Second, the difference in the "melting" temperatures for DNA cholesteric and NaC based on DNA reflects dissimilar processes. In particular, the "melting" of the DNA cholesteric is explained by the unwinding of cholesteric helical structure of LCD particle, whereas the "melting" of the NaC may be associated with dissociation of the nanobridges into components occurring in the core of intact DNA cholesteric upon temperature increase.

3.5. The prerequisites for formation of nanobridges between ds nucleic acids

3.5.1. The structure of anthracycline antibiotics

The analysis of more than 10 DAU analogs differing by the presence and position of substituents at the anthracycline aglycon showed [38] that the presence of four reactive atoms of oxygen in the 5, 6 and 11, 12 positions of aglycone is one of essential prerequisite for the amplification of optical activity upon building of nanobridges. Each Cu^{2+} ion in the bridge can form four bonds with coplanar oxygen groups. The absence of such a combination of oxygen group in nogalamycin leads to its inability to form a nanobridge with a spatial structure that provides for crosslinking of neighboring NA molecules. Iremycin, cinerubin A and aclacinomycin have reactive oxygen atoms in the 5, 6 and 11, 12 positions. However, no amplification of abnormal optical activity is observed in these cases. This result indicates that an additional condition for the formation of a nanobridge is indeed the formation of "external" complex, whose spatial position on the NA molecule and sterical structure provide the "beginning" and the "end" of nanobridge.

3.5.2. The "phasing" of NA molecules

To obtain nanobridges between NA molecules it is necessary to create, first of all, initiating ternary complex, for instance (DNA–Cu^{2+}–DAU) complexes on both neighboring NA molecules. This complex is formed by DAU molecule of "external" complex, and Cu^{2+}-ions chelating the NA nitrogen bases (N7 of guanin, mainly). Taking into account the stereochemisrtry of NA molecules, one can say, that the initiating ternary complex should be "immersed" in the NA groove and spatially fixed here. After formation of initiating complex, DAU molecules are acceptable for nanobridging (Fig. 9). The more the number of DAU molecules (and Cu^{2+} ions) in nanobridge, the higher the equilibrium constant of the bridge formation, i.e. the higher its stability.

Taking into account the stereochemical structure of DAU–Cu^{2+} chelate complex one can suppose that the rigid, flate nanobridges are formed only between the closest neighbor NA molecules. However, because of the helical structure of dsNA, in order to join the same (similar) chemical groups in the content of the neighboring NA molecules by the nanobridge with a fixed symmerty, it is necessary to turn the NA molecule "2" around its long axis on 180° in respect to molecule "1" (Fig. 9). This means, that nanobridging will

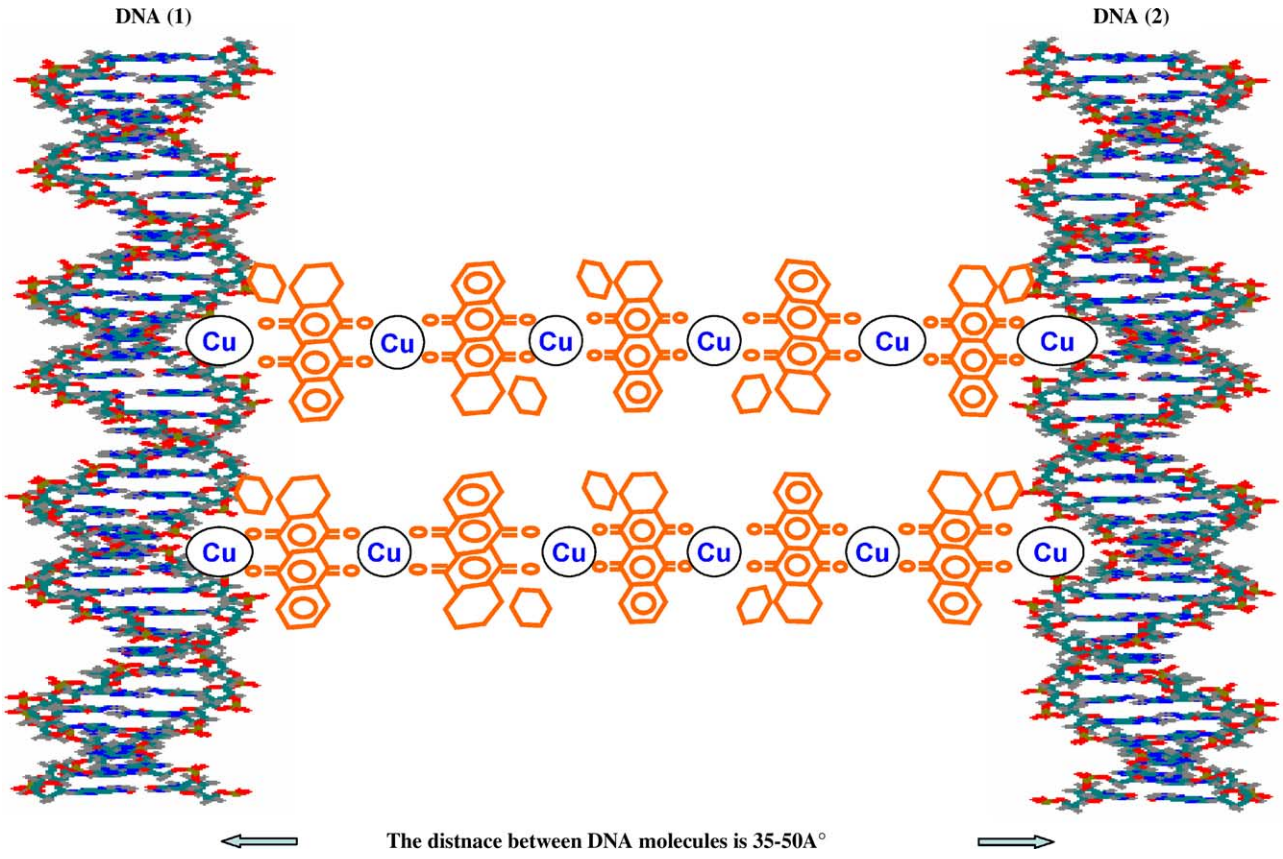

Fig. 9. The view of nanobridge between the two neighboring DNA molecules fixed in a quasinematic layer. For simpilicity, the spatial orientation of nanobridges is turned on 90° in respect to orientation of the DNA nitrogen bases. To form a flat, rigid nanobridge between DNA molecules, with account of fixed distance between DNA molecules and their helical symmetry, DNA molecule (2) is turned on 180° in respect to position of DNA (1).

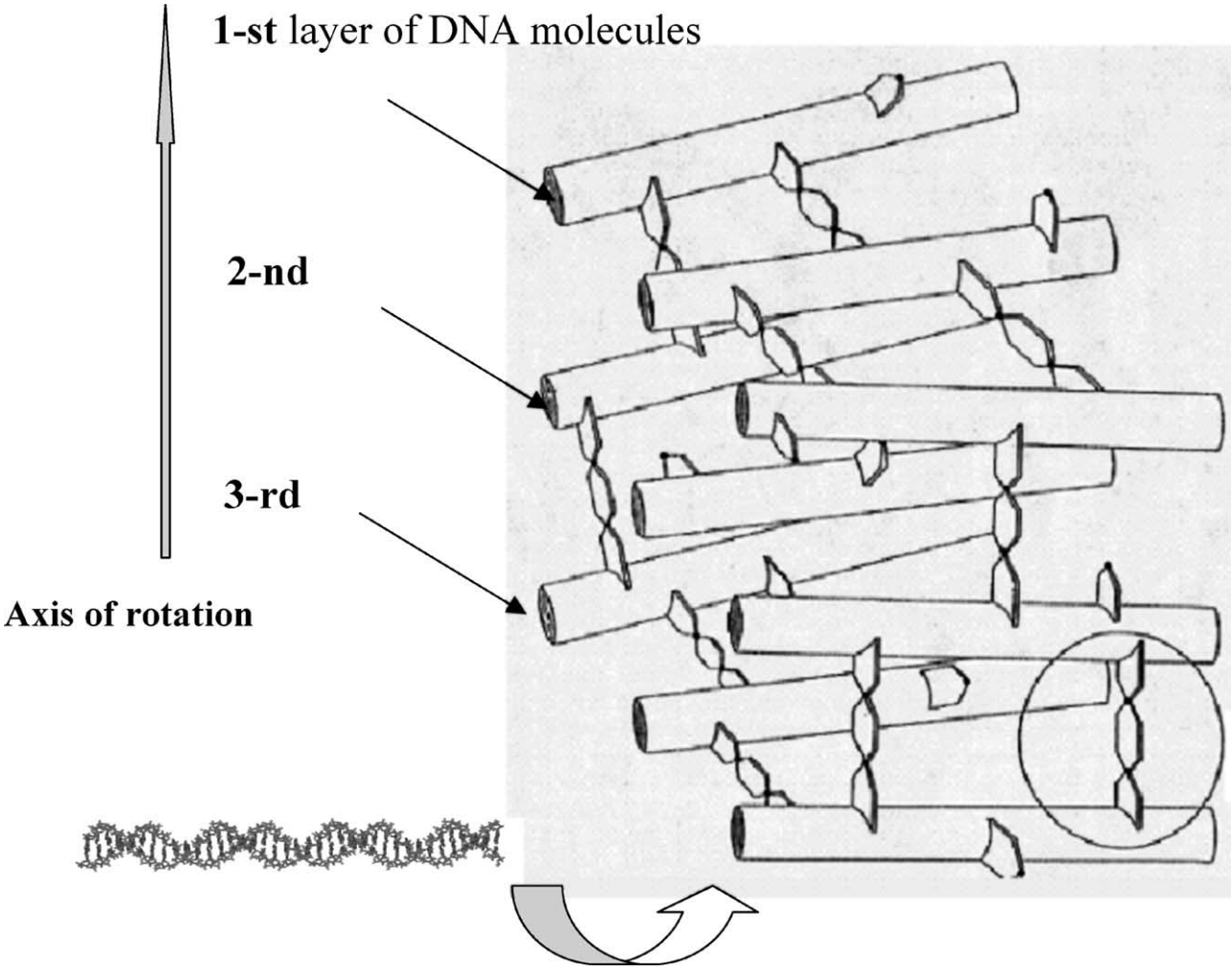

Fig. 10. A scheme of the three-dimensional nanoconstruction based on dsDNA molecules. For simplicity, only three DNA quasinematic layers are shown. DNA molecules are shown as rods. Circular insert indicates the nanobridge.

be met only at certain sterical positions of dsNA molecules within quasinematic layers of particles of LCD, when spatial adjustment of position of neigboring NA molecules (the "phasing" [39]) is spontaneously realized.

Hence, the "phasing" of NA molecules, is the second prerequisite for the formation of nanobridges.

3.5.3. The distance between dsNA molecules

The experimental result above shows that six Cu^{2+} ions and five DAU molecules exist in the content of nanobridge between neighboring NA molecules. The stereochemistry of dsNA molecules and symmetry of the nanobridge permits one to maintain, that a very important parameter related to NA molecules, i.e. the certain distance between axes of NA molecules, is essential for the formation of nanobridges (Fig. 9). This distance is directly determined by the osmotic pressure of a solvent. In our case, this distance is within a range of 35–50 Å [14,38]. Taking into account the symmetry and structure of nanobridges (Fig. 9), one can say, that for the sterical phasing of NA molecules certain distance between the ordered dsNA molecules in a quasinematic layer, and, hence, definite degree of diffusion fredom for both NA molecules should exist, which is enough for a rotation of molecules around their long axes. Very close packing of NA molecules (say, at distance about 25 Å) will restrict the sterical phasing and formation of nanobridges with the shown symmetry. Hence, the definite distance between dsNA molecules is a final prerequise for the nanobridge formation with symmetry shown in Fig. 9.

The prerequisites above show that the formation of nanobridges between neighboring dsNA molecules is a very delicate stereochemical process, which could be realized under rather strict conditions.

3.6. Hypothetical structure of nanoconstruction based on ds nucleic acids

The fact of existence of sufficiently stable structure, whose properties to a considerable extent are independent on the properties of aqueous polymeric solution allowed us to sug-

gest the following hypothetical structure of the particles of NaCs on the basis of cholesteric NA LCD (Fig. 10). Because all the NA molecules in the structure of the LCD particles are equal in their physicochemical properties, and, hence, in their abilities to form DAU–Cu^{2+} nanobridges, the hypothetical structure takes into account two modes of arranging of these nanobridges. According to the scheme, the DAU–Cu^{2+} should be located within NA quasinematic layers as well as between these layers. The resulting NaC is a three-dimensional structure where the diffusion mobility of neighboring NA molecules is sharply decreased; therefore, the structure lacks many properties characteristic of the liquid-crystalline dispersions of NAs. Indeed, X-ray diffraction analysis indicates a higher degree of crystallinity of the DNA NaCs as compared with the particles of DNA LCD [14,38]. There are a few principal differences between initial particles of the NA LCDs and NA NaCs. First, in contrast to the NA LCD, the structure of NaC is not "liquid-crystalline" any more; this is rigid, crystal-like, three-dimensional structure. Second, in contrast to the NA LCD, NaC has extraordi-

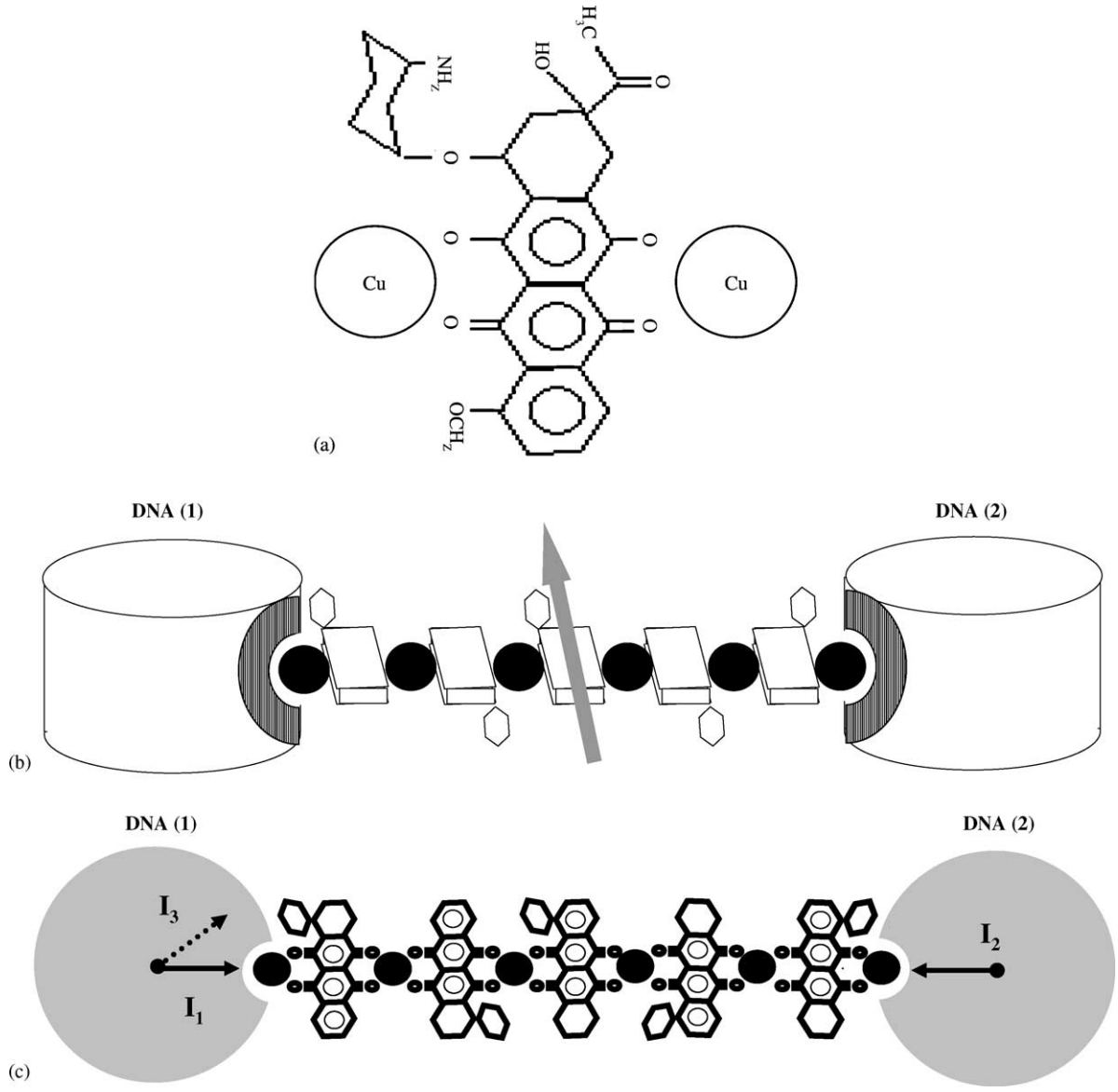

Fig. 11. The scheme of nanobridge between NA molecules used for theoretical calculations. (a) Chelate complex (Cu^2–DAU–Cu^{2+}). (b) Scheme of nanobridge. Each nanobridge is consisting of six Cu^{2+} ions (dark spots) and five DAU molecules. This picture shows that Cu^{2+} ion is immersed in the DNA groove due to ternary chelate complex (DNA–Cu^{2+}–DAU) formation. The second-order symmetry axis of nanobridge is shown by arrow. The nanobridge crosslinks the reaction centers of both NA molecules (shaded area). In this case, the spatial orientation of nanobridge coincides with orientation of the NA nitrogen bases. (c) Top view of NA molecules (1 and 2) and nanobridge between these molecules. The nanobridge starts at NA molecule (1), its direction from the potential Cu^{2+} ion-binding site is illustrated by vector I_1. NA molecule (2) is turned on 180° (shown by vector I_2) in respect to spatial position of NA (1). If Cu^{2+} binding site corresponds to the next base pair in the NA nucleotide sequence, then vector I_3, specific for a new nanobridge direction, is turned, because of the helical symmetry of NA, on 36°; thus making impossible the formation of the nanobridge between NA molecules.

nary high optical activity both in the UV- and visible regions of the CD spectrum. Third, NaC is consisting (probably) of two cholesterics. Along with a cholesteric composed of initial NA molecules, there is a possibility for formation of a cholesteric from the nanobridges located between neighboring NA layers. In fact, we have obtained a novel NaC that may be called as "cholesteric-in-cholesteric", because the first cholesteric is still formed by twisted in space NA layers, the second one-by nanobridges located between the NA layers (Fig. 10). Such a "cholesteric-in-cholesteric" structure attracts much attention. For instance, one can, probably, consider the difference in the melting curves (Figs. 7 and 8) as an indication on different thermal stability of these cholesterics. Theoretical analysis of the properties of such structures was started recently [40]; preliminary results show that the properties of cholesteric formed by nanobridges may not "follow" the properties of cholesterics formed by NA molecules.

In conclusion, one can say, that the NaCs, built by us, represent a new type of nanobiomaterial. The properties of this material depend on properties of nanobridges and they should be adjusted according to the requirements of the consumer.

Appendix A. Theoretical description of the creation of nanobridges between ds nucleic acid molecules resulting in the formation of nanoconstruction

The experimental results presented above provide a basis for the thermodynamic model of the formation of NaC. A thermodynamic model used by Nechipurenko earlier [39,41] is illustrated by Fig. 11.

Assume that at a constant pressure and temperature, the LCD particles, consisting of NA molecules with adsorbed DAU molecules and Cu^{2+} ions, are in equilibrium with free DAU molecules and copper ions in solution. Suppose, all nearest neighboring NA molecules, located in one and the same quasinematic layer of LCD, are "in-phase" [39]. This means that all of them are so phased relative to each other that nanobridges can be formed between the neighboring NA molecules (Figs. 9 and 11). The nanobridges are chelate complexes consisting of alternating DAU molecules and Cu^{2+} ions. One complete period of NA can be envisioned as a single reaction center. Each such center is able to bind a Cu^{2+} ion and to serve as a background for the nanobridge. Concerning the adsorption theory, one quasinematic layer, formed by NA molecules, can be considered as a set of simple equivalent matrices consisting of reaction centers. Within the framework of the proposed theory, the formation of NaCs is considered as "coordinated adsorption" on reactive centers. The "coordinated absorption" means that a nanobridge starting at the reactive center of one NA molecule is terminated at the reactive center of the nearest NA molecule owing to spatial phasing of neighboring NA molecules in the quasinematic layer [30,41].

Assume that Δf_1 and Δf_2 are the free energies of the DAU molecule and Cu^{2+} ion interaction with the NA reaction center, respectively (energy in this case will be measured in kT units). Let us designate as Δg free energy of the DAU molecule interaction with a Cu^{2+} ion in the nanobridge.

Following the Scatchard approach one can write the relation for a change of free energy of the system under consideration [30,42] and evaluate the equations for nanobridge formation:

$$\begin{cases} r_1 = \exp(-\Delta f_1)\, C_{\mathrm{f}}(1 - r_1 - r_2 - R), \\ r_2 = \exp(-\Delta f_2)\, C_{\mathrm{Cu^{2+}}}(1 - r_1 - r_2 - R), \\ R(1-R) = \exp(-2(\Delta f_2 + (n-1)\Delta g)) \\ \quad C_{\mathrm{f}}^{n-1}\, C_{\mathrm{Cu^{2+}}}^{n}\, (1 - r_1 - r_2 - R)^2. \end{cases} \tag{A.1}$$

where $C_{Cu^{2+}}$ is the concentration of free copper ions in solution, C_f the concentration of free DAU molecules in solution,

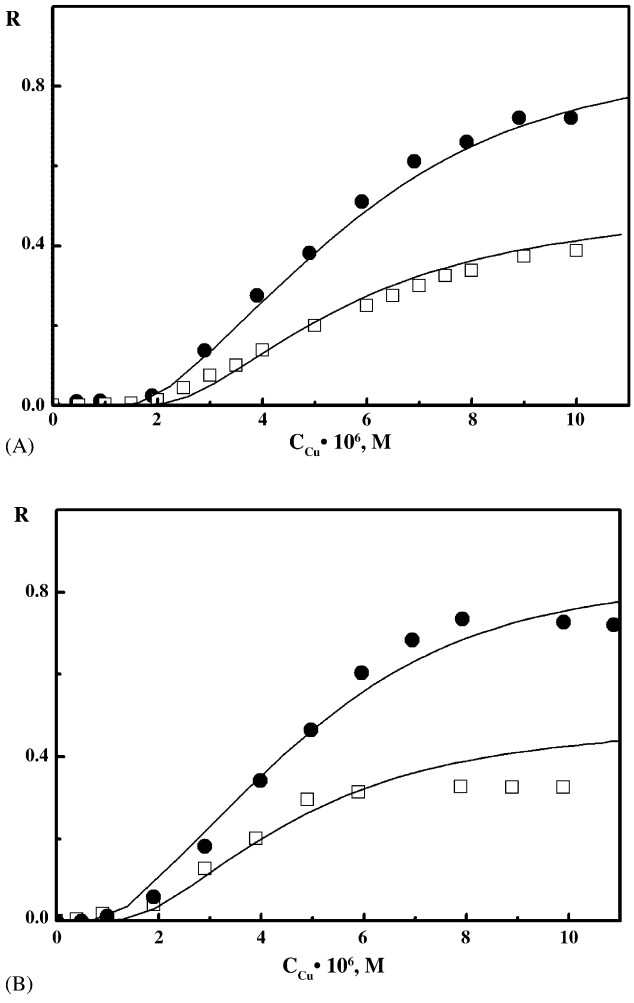

Fig. 12. The comparison of experimental points ((□) and (●)) and theoretical curves of the saturation degree (R) of DNA molecules by nanobridges at increase of Cu^{2+} ion concentration. $C_{DAU} = 15.6$ and 27.3×10^{-6} M ((□) and (●), respectively). The experimental points were measured by the amplitudes of the bands at 310 nm (A) and 505 nm (B) in the CD spectra and normalized with account of the maximal value of the bands at DAU concentration 60×10^{-6} M. The theoretical curves have been calculated according to Eq. (A.1), where n was equal to 6, $\Delta f_1 = 11.4$ kT, $\Delta g = 11.2$ kT and $\Delta f_2 = 13.8$ kT for shown above DAU concentrations.

r_1, r_2 the degrees of the saturation of the reaction centers with DAU molecules and Cu^{2+} ions, respectively, n the number of Cu^{2+} ions (in our case n was accepted to be six), $(n-1)$ the number of DAU molecules in nanobridge and R is the degree of the saturation of matrix with nanobridges.

Eq. (A.1) enables one to calculate the theoretical binding curves: knowing the energy of DAU binding to NA and the energy of formation of coordination complex between DAU molecules and Cu^{2+} ions in a nanobridge for any set of concentrations of free DAU molecules and free Cu^{2+} ions in solution one can calculate the degree, R, of the saturations of NA matrix with nanobridges.

To analyze the experimental data one needs to add the Eq. (A.1) with the material balance ratios, which take into account the binding DAU molecules and Cu^{2+} ions to NA, as well as a possible formation of DAU n-mers. In this case, one can combine the free concentrations of DAU and Cu^{2+} ions with their total concentrations in a solution.

By Eq. (A.1) we have performed calculations, which showed that in the region of Cu^{2+} ion concentration $\leq 10\,\mu M$ quantitative correlation can be achieved between the theoretical curves and the whole family of experimental results obtained at various DAU concentrations.

Special experiments were carried out, which revealed the maximal possible saturation of LCD particle with nanobridges at the DAU concentration in solution equals to $60\,\mu M$. We thought that the CD signal for this case corresponded to maximal filling $R = 1$ which made possible to normalize all experimental data and estimate the energy of interaction between the DAU molecule and Cu^{2+} ion as $\Delta g \approx 11\,kT \approx 6.6$ kcal/mol.

The results of calculation for the cases of $\lambda = 505$ and 310 nm (Fig. 12) demonstrate that theoretical curves fit the experimental data. A more precise estimation of the nanobridge formation energy requires independent data.

References

[1] R.F. Service, Science 277 (1997) 1036.
[2] N.C. Seeman, Trends Biotechnol. 17 (1999) 433.
[3] N.C. Seeman, Nature 421 (2003) 427.
[4] Yu.M. Yevdokimov, V.S. Bundin, M.A. Ostrovsky, Sensory Systems 11 (1997) 374 (in Russian).
[5] E. Katz, I. Willner, Angew. Chem. Int. Ed. 43 (2004) 6042.
[6] N.C. Seeman, J. Theor. Biol. 99 (1982) 237.
[7] J. Chen, N.C. Seeman, Nature 350 (1991) 631.
[8] J. Shi, D.E. Bergstrom, Angew. Chem. Int. Ed. 36 (1997) 111.
[9] C.M. Niemeyer, M. Adler, S. Gao, L. Chi, Angew. Chem. Int. Ed. 39 (2000) 3056.
[10] C.A. Mirkin, R.L. Leistinger, R.C. Mucic, J.J. Storhoff, Nature 382 (1996) 607.
[11] A.P. Alivisatos, K.P. Jonson, X. Peng, T.E. Wilson, C.J. Loweth, Nature 382 (1996) 609.
[12] B.H. Robinson, N.C. Seeman, Protein Eng. 1 (1987) 295.
[13] Yu.M. Yevdokimov, V.I. Salyanov, E. Gedig, F. Spener, FEBS Lett. 392 (1995) 269.
[14] Yu.M. Yevdokimov, S.G. Skuridin, G.B. Lortkipanidze, Liquid Cryst. 12 (1992) 1.
[15] Yu.M. Yevdokimov, Liquid Crystals and Their Practical Applications, Vol. 3, 2003, p.10 (in Russian).
[16] V.A. Belyakov, V.P. Orlov, S.V. Semenov, S.G. Skuridin, Yu.M. Yevdokimov, Liquid Cryst. 20 (1996) 777.
[17] Yu.M. Yevdokimov, V.I. Salyanov, F. Spener, M. Palumbo, Int. J. Biol. Macromol. 19 (1996) 247.
[18] Yu.M. Yevdokimov, V.I. Salyanov, B.V. Mchedlischvili, V.A. Bykov, A.V. Belyaev, S.A. Saunin, F. Spener, M. Palumbo, Nucleosides Nucleotides Nucl. Acids 19 (2000) 1355.
[19] Yu.M. Yevdokimov, V.I. Salyanov, M.A. Zakharov, Lab Chip. 1 (2001) 35.
[20] A. Wells, Strukt. Khim. 3 (1988) 224 (in Russian).
[21] R.N. Capps, M. Vala, Photochem. Photobiol. 33 (1981) 673.
[22] S.H. Etaiw, M.M.A. Sekkina, G.B. El-Hefnawey, S.S. Assar, Can. J. Chem. 60 (1981) 304.
[23] F.T. Greenaway, J.C. Dabrowiak, J. Inorg. Biochem. 16 (1982) 91.
[24] A. Moustatih, A. Garnier-Suillerot, Inorg. Chem. Acta 135 (1987) 17.
[25] E.J. Gabbay, D. Grier, R.E. Fingerle, R. Reimer, R. Levy, S.W. Pearce, W.D. Wilson, Biochemistry 15 (1976) 2062.
[26] V.I. Salyanov, E.I. Kats, Yu.M. Yevdokimov, Mol. Biol. 34 (2000) 461 (in Russian).
[27] J. Doskocil, I. Fric, FEBS Lett. 37 (1973) 55.
[28] H.D. Coble, H.F. Holtzclaw, J. Inorg. Nucl. Chem. 36 (1974) 1049.
[29] V. Malatesta, A. Gervasini, F. Morazzoni, Inorg. Chem. Acta. 136 (1987) 81.
[30] Yu.D. Nechipurenko, V.F. Ryabokon, S.V. Semenov, Yu.M. Yevdokimov, Biofisika 48 (2003) 635 (in Russian).
[31] R. White, Quantum Theory of Magnetism, second Ed., Springer-Verlag, Berlin, 1983, p. 88 (Chapter 3).
[32] Yu.M. Yevdokimov, T.L. Pyatigorskaya, N.A. Belozerskaya, Ya.M. Varshavsky, M. Becker, D. Zirver, Mol.Biol. 11 (1977) 507 (in Russian).
[33] D. Grasso, S. Fasone, C. la Rosa, V. Salyanov, Liquid Cryst. 9 (1991) 299.
[34] D. Grasso, R.G. Campisi, C. La Rosa, Thermochim. Acta 199 (1992) 239.
[35] M. Kaneko, E. Tsuchida, J. Polym. Sci. Macromol. Rev. 16 (1981) 397.
[36] Yu.P. Blagoi, V.L. Galkin, G.O. Gladchenko, S.V. Kornilova, V.A. Sorokin, A.G. Schkorbatov, Metallocomplexes of Nucleic Acids in Solutions, Naukova dumka, Kiev, 1991, p. 272 (in Russian).
[37] V.A. Sorokin, V.A. Valeev, G.O. Gladchenko, M.V. Degtyar, Yu.P. Blagoi, Biofizika 45 (2000) 773.
[38] Yu.M. Yevdokimov, V.I. Salyanov, L.V. Buligin, A.T. Dembo, E. Gedig, F. Spener, M. Palumbo, J. Biomol. Str. Dyn. 15 (1997) 97.
[39] Yu.D. Nechipurenko, S.A. Strel'tsov, Yu.M. Yevdokimov, Biofizika 46 (2001) 428 (in Russian).
[40] V.L. Golo, E.I. Kats, Yu.S. Volkov, V.I. Salyanov, Yu.M. Yevdokimov, J. Biol. Phys. 27 (2001) 81.
[41] Yu.D. Nechipurenko, M.A. Zakharov, V.I. Salyanov, Yu.M. Yevdokimov, Biofizika 47 (2002) 600 (in Russian).
[42] Yu.D. Nechipurenko, G.V. Gurskii, Biofizika 48 (2003) 773 (in Russian).