UDK 577.323:544.25:535:56:546:59

***Yu. M. Yevdokimov*[1], *S. G. Skuridin*[1], *V. I. Salyanov*[1], *V. I. Popenko*[1], *E. V. Shtykova*[2], *N. G. Khlebtsov*[3], *G. A. Shafeev*[4], *E. I. Kats*[5]**

GOLD NANOPARTICLES INFLUENCE DOUBLE-STRANDED DNA MOLECULES «RECOGNITION» AND PREVENT FORMATION OF THEIR CHOLESTERIC STRUCTURE

[1]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences,
Vavilov str., 32, 119991 Moscow, Russia. E-mail: yevdokim@eimb.ru
[2]Shubnikov Institute of Crystallography, Russian Academy of Sciences,
Leninsky ave., 59, 119333 Moscow, Russia. E-mail: viwopisx@yachoo.co.uk
[3]Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences,
Entuziastov ave., 13, 410049 Saratov, Russia. E-mail: nkhlebtsov@gmail.com
[4]Prokhorov General Physics Institute, Russian Academy of Sciences,
Vavilov str., 38, 119991 Moscow, Russia. E-mail: gashafeev@gmail.cov
[5]Landau Institute for Theoretical Physics, Russian Academy of Sciences,
Kosygin str., 2, 119334, Moscow, Russia. E-mail: kats@landau.ac.ru

The negatively charged gold nanoparticles (nano-Au) in solutions of high ionic strength interact with free, linear double-stranded (ds) DNA or synthetic poly(ribonucleotide) (poly(I)×poly(C) or poly(A)×poly(U)) molecules or with these molecules ordered in the spatial structure of the cholesteric liquid-crystalline dispersion (CLCD) particles formed as a result of their phase exclusion from solutions containing poly(ethylene glycol). However, the consequences of these interactions differ noticeably. If we first pretreate free ds nucleic acid (NA) molecules with nano-Au and then create dispersion, the orientationally ordered structures do not form. This leads to disappearance of abnormal band in the circular dichroism spectra at a very low concentration of added nano-Au. If we treat condensed ds NA, i.e. ds NA in the content of CLCD particles, with nano-Au, the orientational order remains stable. This order evolves (from cholesteric to untwisted, nematic-like) upon increase in nano-Au concentration. The preliminary measurements and evaluations show that the analogous spectral changes are also typical for palladium nanoparticles. The differences in nano-Au action on free and condensed ds NA molecules can result in various damaging effects in living cells and be accompanied by various genetic consequences.

Key words: *nanoparticles of noble metals; free double-stranded nucleic acids; circular dichroism of nucleic acid molecules; cholesteric liquid-crystalline DNA dispersions; cholesteric → nematic transition; aggregated DNA molecules.*

© Yevdokimov Yu. M, Skuridin S. G., Salyanov V. I., Popenko V. I., Shtykova E. V., Khlebtsov N. G., Shafeev G. A., Kats E. I., 2014

Ю. М. Евдокимов[1], С. Г. Скуридин[1], В. И. Салянов[1], В. И. Попенко[1],
Э. В. Штыкова[2], Н. Г. Хлебцов[3], Г. А. Шафеев[4], Е. И. Кац[5]

НАНОЧАСТИЦЫ ЗОЛОТА ВЛИЯЮТ НА «УЗНАВАНИЕ» ДВУХЦЕПОЧЕЧНЫХ МОЛЕКУЛ ДНК И ЗАПРЕЩАЮТ ФОРМИРОВАНИЕ ИХ ХОЛЕСТЕРИЧЕСКОЙ СТРУКТУРЫ

[1] Институт молекулярной биологии им. В. А. Энгельгардта РАН,
ул. Вавилова, 32, 119991 Москва, Россия. E-mail: yevdokim@eimb.ru
[2] Институт Кристаллографии им. А. В. Шубникова РАН
Ленинский пр-т, 59, 119333 Москва, Россия. E-mail: viwopisx@yachoo.co.uk
[3] Институт биохимии и физиологии растений и микроорганизмов РАН
Энтузиастов пр-т, 13, 410049 Саратов, Россия. E-mail: nkhlebtsov@gmail.com
[4] Институт общей физики им. А. М. Прохорова РАН,
ул. Вавилова, 38, 119991 Москва, Россия. E-mail: gashafeev@gmail.cov
[5] Институт теоретической физики им. Л. Д. Ландау РАН
ул. Косыгина, 2, 119334, Москва, Россия. E-mail: kats@landau.ac.ru

Отрицательно заряженные наночастицы золота (нано-Au) в растворах высокой ионной силы взаимодействуют со свободными, линейными молекулами двухцепочечной (дц) ДНК и синтетических полирибонуклеотидов (поли (I)×поли (C), поли(A×поли(U)), а также с молекулами этих биополимеров, упорядоченными в пространственной структуре частиц холестерических жидкокристаллических дисперсий (ХЖКД), образуемых ими в водно-солевых растворах, содержащих полиэтиленгликоль. Однако последствия этих взаимодействий заметно отличаются. Если сначала обработать дц молекулы нуклеиновой кислоты (НК) нано-Au, а затем из комплексов (НК-нано-Au) сформировать дисперсию, то ориентационно упорядоченная структура дц НК не образуется. Такие дисперсии не обладают аномальной оптической активностью, причем исчезновение интенсивной полосы в их спектрах кругового дихроизма происходит при очень низкой концентрации нано-Au. После обработки нано-Au конденсированных молекул дц НК (т.е. молекул НК в составе частиц ХЖКД) ориентационный порядок последних остается стабильным при всех использованных (в том числе и высоких) концентрациях нано-Au. Однако в этом случае пространственная структура частиц ХЖКД теряет свою спиральную закрутку и холестерик НК переходит в нематик. Предварительные результаты показывают, что аналогичные структурные изменения имеют место после обработки частиц ХЖКД НК наночастицами палладия. Различия в действии нано-Au на свободные и конденсированные молекулы дц ДНК могут приводить к разным повреждающим эффектам в живых клетках и сопровождаться разными генетическими последствиями.

Ключевые слова: *наночастицы благородных металлов, двухцепочечные нуклеиновые кислоты, круговой дихроизм нуклеиновых кислот, холестерические жидкокристаллические дисперсии ДНК, переход холестерик → нематик, агрегированные молекулы ДНК.*

1. Introduction

In recent years several review papers [1–3] were devoted to hybrid materials formed by nanoparticles dispersed in liquid-crystalline (LC) phases or lamellar structures of low molecular mass compounds. The most interesting aspect in this respect is liquid crystals because of competition between non-chiral nanoparticle aggregation and chiral cholesteric twisting. Such hybrid materials can combine typical electronic properties of metal nanoparticles (light absorption, electrical conductivity, magnetism, etc.) with characteristic properties of liquid crystals (fluidity, anisotropy, etc.).

The investigation of these materials has become the focus of intense worldwide research, motivated by the perspective of practical applications (display technology, drug delivery, information storage and so on). From a more fundamental point of view, this approach allows one to create materials with new, sometimes unexpected, properties.

It is noteworthy that many studies in this branch of nanotechnology deals with the properties of liquid crystals or lamellar phases doped using silver or gold nanoparticles (nano-Au) due to their exceptional optical properties [4–9]. Indeed, the chemical and physical properties of noble metal nanoparticles depend on their size, shape, structure, and dielectric environment [10, 11].

With regard to the liquid crystals made of high molecular mass biopolymers (e.g., of DNA molecules), such systems were handled with nano-Au. The latter investigations were started in 2010 [12], although properties of linear single-stranded (ss) and double-stranded (ds) DNA molecules containing nano-Au on their surfaces were reported in the pioneer contributions by Mirkin [13] and Alivisatos [14].

It is noteworthy that the physicochemical properties of spatially twisted (cholesteric) liquid-crystalline dispersions (CLCDs) of ds DNA reflect some properties of these macromolecules in biological objects such as chromosomes of primitive organisms (for instance, the chromosomes of the Dinoflagellate) and DNA-containing viruses [15, 16].

Hence, doping ds DNA CLCDs with nano-Au is of interest to both biologists and researchers in the area of nanotechnology.

From a fundamental point of view, understanding the current of physical and chemical mechanisms behind CLCDs + nano-Au properties are still remains limited. Specifically, the reason why linear ss DNA can effectively adsorb nano-Au whereas ds DNA does not, is still poorly investigated. Besides, there are set of papers [17–24] with opposite statements concerning the interaction of linear ds DNA molecules with nano-Au. These inconsistencies in the literature reflect the lack of a complete understanding of the fundamental interaction mechanism between nano-Au and DNA molecules.

The purpose of our study is to reveal the phenomena caused by the nano-Au action on ds DNA molecules. It seems constructive to pose a few questions and to answer them on a general level. Namely we tried to answer the following questions.

1) Whether nano-Au is coordinated with linear ds DNA molecules in colloid solutions?

2) Whether (linear ds DNA-nano-Au) complex can form CLCD in polymer-containing solution?

3) Whether nano-Au can interact with the ds DNA molecules assembled in particles of CLCDs and alter their spatial structure?

2. Materials and methods

2.1 Preparation of metal nanoparticles and determination of their average sizes

Colloid solutions containing nano-Au of different sizes were used in this study.

Solutions (hydrosols) containing nano-Au with the average diameters of 2, 5, and 15 nm were prepared by reduction of $HAuCl_4$ according to the previously described procedures. Specifically, 2 nm nano-Au with the number particle concentration of $C_N = 3.5\times10^{15}$ particle ml^{-1} were obtained by Duff method [25], 5 nm nano-Au ($C_N = 1.3\times10^{13}$ particle ml^{-1}) were fabricated according to the method described by Khlebtsov et al. [26]. Finally, 15 nm nano-Au ($C_N = 5\times10^{12}$ particle ml^{-1}) were synthesized according to Frens citrate protocol [27]. The numerical concentrations of all nano-Au samples were calculated using the material balance (provided that Au is reduced completely).

Pulsed laser ablation in water was used to prepare nano-Au and palladium nanoparticles (nano-Pd). Generation of metal nanoparticles via laser ablation of solid target in liquid was described in details elsewhere [28].

Bulk metallic target immersed into deionized water was ablated by laser irradiation. For generation of nano-Au ytterbium fiber laser with pulse duration of 70 ns, repetition rate of 20 kHz and pulse energy of 1 μJ at 1060–1070 nm was used. Focal spot on the target surface was about 50 μm in diameter with 207 mm F-Theta objective.

Nano-Pd were obtained by means of the first harmonics of Nd:YAG Fuego laser system (Time-Bandwidth) with a pulse duration of 10 ps, repetition rate of 200 kHz and pulse energy of 500 μJ at 1064 nm. Focal spot on the target surface was about 70 μm in diameter with 150 mm F-Theta objective. The laser focal spot was moved across the sample surface with scanning galvo mirror system at the speed of 1000 mm s^{-1}.

The ablation process lead to the reddish coloration of solution for nano-Au and brownish for nano-Pd [29].

All nanoparticles were negatively charged. Their ξ-potentials at neutral pH values were approximately – 40 mV. The average sizes (diameters) of the metal nanoparticles in stock preparations were verified by transmission electron microscopy (TEM). Micrographs of nanoparticles were obtained using a Jem-100CX electron microscope (Jeol, Japan).

2.2 *Reagents and preparation of ds NA CLCDs*

2.2.1 *Nucleic acids and chemicals*

A calf thymus depolymerized ds DNA (Sigma, USA) with a molecular mass of ~ $(0.6–0.8)\times10^6$ Da after additional purification was used. A synthetic ds poly(ribonucleotides) poly(I)×poly(C) (Sigma, lot 023K4032, USA; molecular mass ~ 0.3×10^6 Da) and poly(A)×poly(U) (Sigma, lot 066K4015, USA; molecular mass ~ 0.3×10^6 Da) was used without additional purification.

Ds nucleic acid (NA) concentrations in the water–salt solutions were determined spectrophotometrically using the known values of the molar extinction coefficients (ε_{max} = 6,600 M^{-1} cm^{-1} for DNA, ε_{max} = 4,900 M^{-1} cm^{-1} for poly(I)×poly(C) and ε_{max} = 5,740 M^{-1} cm^{-1} for poly(A)×poly(U)).

Poly(ethylene glycol) (PEG; Serva, Germany; molecular mass of 4,000 Da) and cyanine dye SYBR Green I (SG; Sigma, USA) samples were used without additional purification. SG concentration in the water-salt solutions was determined spectrophotometrically using the known value of the molar extinction coefficient (ε_{max} ~ 73,000 M^{-1} cm^{-1} [30]). The stock solution of SG was stored at 4 °C in the light-tight container.

2.2.2 *Formation of ds NA CLCDs*

Standard CLCDs of ds linear ds DNA or poly(ribonucleotide) molecules were prepared according to the technology of intensive mixing (stirring) of PEG-containing water-salt (~ 0.3 M NaCl) solution with water-salt (~ 0.3 M NaCl) ds NA solution. Al details of this technology can be found in [16].

2.3 *Measurements*

The absorption spectra were taken by Cary 100 Scan (Varian, USA) spectrophotometer. The circular dichroism (CD) spectra were recorded using a SCD-2 portable dichrometer (produced by the Institute of Spectroscopy of the Russian Academy of Sciences, New Moscow-Troizk) [31]. The CD spectra were represented as a dependence of the difference between the intensities of absorption of left- and right-handed polarized light (ΔA; $\Delta A = (A_L - A_R)$) on the wavelength (λ).

2.4 *Structure analysis of the DNA phases by SAXS*

Pellet (~ 3 mg) obtained by slow speed centrifugation (5,000 rev. $min.^{-1}$, 40 min., 4 °C; centrifuge K-23, Germany) of the DNA CLCD particles and the particles formed as a result condensation of DNA molecules pretreated with nano-Au (~ 15 nm) in PEG-containing water-salt solution was analyzed by small-angle X-ray scattering (SAXS). The measurements were carried out on a laboratory diffractometer Amur-K using a Kratky-type (infinitely long slit) geometry in the range of the momentum transfer $0.12 < s < 10.0$ nm^{-1}, where $s = (4\pi\sin\theta)/\lambda$, 2θ is the scattering angle, and λ = 0.1542 nm is the X-ray wavelength. The experimental scattering profiles were corrected for the background scattering from the solvent, and preliminary processed using standard procedures [32].

The repeating distances of the periodical motifs in the crystalline regions ($\bar{d} = 2\pi/s_{max}$), corresponding to the peak position (s_{max}) on the scattering patterns, were calculated using program PEAK [32].

The mean long-range order dimension, *L*, (the size of crystallites) and the degree of disorder in the system ($\Delta/\bar{d}$) were calculated from the standard equations as described in [33]. The low resolution shape of the nano-Au was reconstructed by *ab initio* method by protocol and the program GNOM [34] and DAMIN [35].

We have used three different approaches to clarify a problem of (ds NA-nano-Au) possible (if any) interaction:

- doping of linear ds NAs with nano-Au;
- condensation of (linear ds NA-nano-Au) complexes (if they are formed);
- doping of CLCDs formed by linear ds NAs with nano-Au.

3. Results

3.1 CD spectra of linear ds NAs doped with nano-Au

Here we have used a low concentration of ds NA molecules (~10^{-8} M) to prevent their nonspecific aggregation in solution.

To investigate if nano-Au can be bound to ds NAs, CD spectra for these biopolymers doped with nano-Au were measured first and compared with spectra for control samples without particles.

Figure 1, *a* shows that the amplitude of band at λ ~ 275 nm in the CD spectrum of linear ds DNA (B-form) in the presence of nano-Au (2 nm; curves 2–3) is somewhat diminished in comparison with the free ds DNA (curve 1).

However, in the case of linear ds poly(I)×poly(C) (A-form) doped with nano-Au the decrease in amplitudes of both bands in the CD spectrum of this polymer is more noticeable (Figure 1, *b*, compare curve 1 with curve 5).

The observed spectral changes in both cases detected at R values at about 2–8 (R is number of nano-Au per 1 NA molecule) are not consistent with denaturation of ds NA molecules [36].

Hence, under used conditions, the small alterations in the CD spectra of water-salt solutions containing linear ds DNA or ds poly(I)×poly(C) molecules doped with nano-Au can reflect a minor perturbation of the linear, ds structure of these molecules induced by nano-Au binding.

Because the concentrations of chemically synthesized nano-Au with diameters of about 5 and 15 nm were 10^{13} and 10^{12} particles ml^{-1}, respectively, we cannot experimentally obtain a high R value for these cases and, hence, we can not quantitatively definitely detect the changes in the CD spectra of linear ds NAs at their doping of with such nano-Au.

Comparison of data obtained using to the results of [37] suggests that the statement about binding of linear ds NAs with nano-Au, based only on modifications in their CD spectra, needs additional verification.

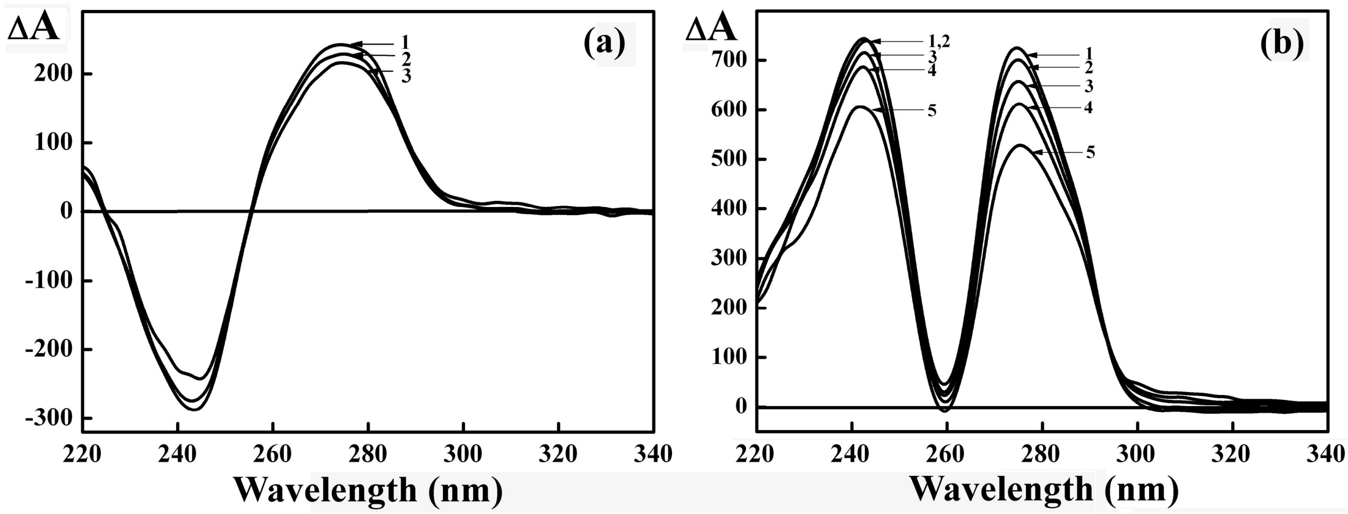

Figure 1. *a* – The CD spectra of ds DNA in absence (curve 1) and presence (curves 2–3) of different concentrations of nano-Au:
1 – $C_{nano\text{-}Au} = 0$ ($R = 0$); 2 – $C_{nano\text{-}Au} = 0.854\times10^{14}$ particle ml^{-1} (R = 3,91);
3 – $C_{nano\text{-}Au} = 1.667\times10^{12}$ particle ml^{-1} (R = 7,64). $C_{DNA} = 29$ μg ml^{-1}; 0.3 NaCl M + 0.002 M sodium phosphate buffer,

b – The CD spectra of synthetic ds poly(ribonucleotide) poly(I)×poly(*C*) in absence (curve 1) and presence (curves 2–5) of different concentrations of nano-Au:
1 – $C_{nano\text{-}Au} = 0$ ($R = 0$); 2 – $C_{nano\text{-}Au} = 0.174\times10^{14}$ particle ml^{-1} ($R = 0.285$);
3 – $C_{nano\text{-}Au}$ = 0,517×10^{14} particle ml^{-1} ($R = 0.848$); 4 – $C_{nano\text{-}Au} = 0.854\times10^{14}$ particle ml^{-1} ($R = 1.40$);
5 – $C_{nano\text{-}Au} = 1.667\times10^{14}$ particle ml^{-1} ($R = 2.732$). $C_{poly(I)\times poly(C)} = 30.4$ μg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer.
The average size of nano-Au is ~ 2 nm.
R is the ratio of the number of nano-Au to the number of DNA (or poly(I)×poly(*C*)) molecules in solution.
$\Delta A\times10^{-6}$ optical units; l = 1 cm

3.2 The phase exclusion from PEG-containing solutions of linear ds NAs pretreated with nano-Au

Here we utilize experimental approach: 'Solution of linear ds NAs was doped with nano-Au (of definite size) and was stored within 1 h at room temperature. Then the final composition was mixed under stirring with PEG solution. As a result, the phase exclusion of ds NA molecules (or (ds NA-nano-Au) complexes, if they are formed) takes place. After 1 h of additional exposition, the CD spectra of obtained mixture were measured'. (One can add that the process of phase exclusion of linear ds NAs is known since Lerman's experiments as «**ψ**-condensation» (**psi** is the acronym for ***p***olymer-***s***alt-***i***nduced) [38, 39].

In the case of linear, rigid, low molecular mass ds NAs it was accompanied by the formation of LC dispersion [16]. Particles of this dispersion have spatially twisted or a so-called «cholesteric» structure and the term «cholesteric liquid-crystalline dispersion» (CLCD) is used to signify dispersion formed by these particles [16].

The CD spectra of CLCDs formed by ds linear DNA and ds poly(I)×poly(C) pretreated with nano-Au (~ 2 nm) are compared in Figure 2, *a*.

First of all, one can see that the formation of CLCDs of both types of ds NA (compare curve I-1 and curve II-1) in PEG-containing water-salt solutions were accompanied by an appearance of abnormal bands in the CD spectra located in the region of absorption of nitrogen bases (λ ~ 270 nm). The appearance of these abnormal (negative or positive) bands univocally testifies the cholesteric twist of neighboring quasinematic layers in particles of NA dispersions [36].

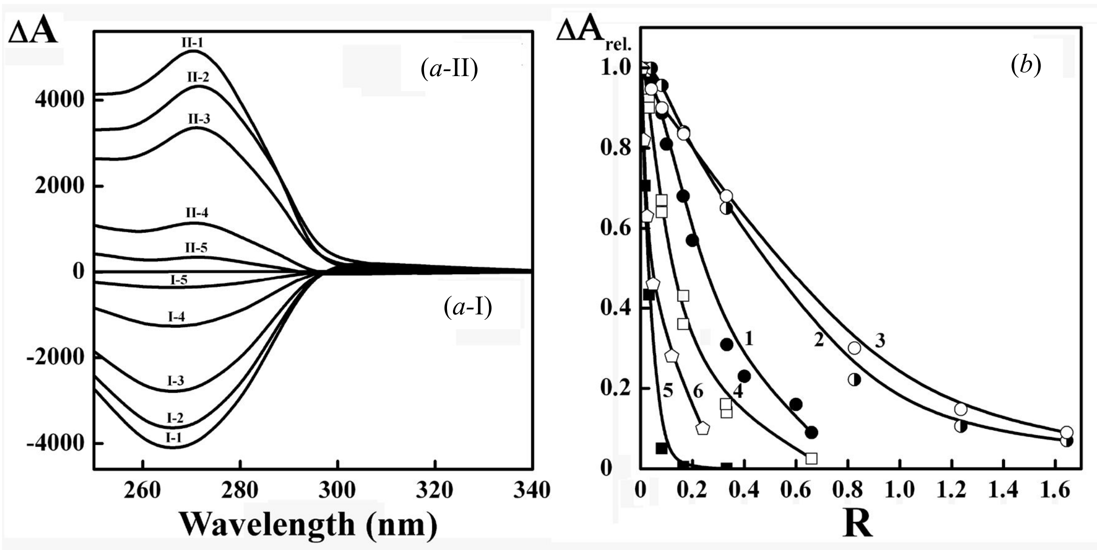

Figure 2. (a-I) – The CD spectra of LCDs formed by ds DNA molecules pretreated in solution of moderate (~0,3) ionic strength with different concentrations of nano-Au:
I-1 – *R* = 0; I-2 – *R* = 0.083; I-3 – *R* = 0.165; I-4 – *R* = 0.333; I-5 – *R* = 0.660.
C_{DNA} = 10 μg ml^{-1}; C_{PEG} = 170 mg ml^{-1}. 0.3 M NaCl + 0.002 M sodium phosphate buffer.
(a-II) – The CD spectra of LCDs formed by ds molecules of synthetic poly(ribonucleotide) poly(I)×poly(*C*) pretreated in solution of high (~0.3) ionic strength with different concentrations of nano-Au:
II-1 – *R* = 0; II-2 – *R* = 0.165; II-3 – *R* = 0.331; II-4 – *R* = 0.826; II-5 – *R* = 1.645.
$C_{poly(I)\times poly(C)}$ = 10.5 μg ml^{-1}; C_{PEG} = 190 mg ml^{-1}. 0.3 M NaCl + 0.002 M sodium phosphate buffer.
The average size of nano-Au is ~ 2 nm. *R* – see *Figure 1*. ΔA×10^{-6} optical units; l = 1 cm.
b – The dependence of the relative amplitude band (λ = 270 nm) in the CD spectra of the LCDs formed by linear ds DNA molecules and synthetic polynucleotides pretreated in solutions of high (~0.3) ionic strength with nano-Au of different sizes versus *R* value:
1 – DNA + 2 nm nano-Au; 2 – poly(I)×poly(*C*) + 2 nm nano-Au; 3 – poly(A)×poly(U) + 2 nm nano-Au;
4 – DNA + 5 nm nano-Au; 5 – DNA + 15 nm nano-Au;
6 – DNA + 10 nm nano-Pd (produced by pulsed laser ablation in water).
C_{DNA} = 10 μg ml^{-1}; C_{PEG} = 170 mg ml^{-1}. 0.3 M NaCl + 0.002 M sodium phosphate buffer.
$C_{poly(I)\times poly(C)}$ = 10.5 μg ml^{-1}; C_{PEG} = 190 mg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer.
$C_{poly(A)\times poly(U)}$ = 10 μg ml^{-1}; C_{PEG} = 190 mg ml^{-1}; 0.3 N NaCl + 0.002 M sodium phosphate buffer. $\Delta A_{отн.} = \Delta A/\Delta A_{max.}$

The negative sign of the band in the CD spectrum proves the left-handed twist of the right-handed DNA molecules (B-form, curve I-1), whereas the positive sign corresponds with the right-handed twist of the right-handed poly(I)×poly(C) molecules (A-form, curve II-1) in the formed particles [36].

Figure 2, *b* shows that in both cases the amplitudes of abnormal bands in the CD spectra drop. Hence, the decrease in the amplitudes of abnormal bands in the CD spectra (curves I-2 – I-5 and II-2 – II-5) do not depend on parameters of the secondary structure of ds NA molecules. The higher the concentration of nano-Au in solution, the greater the reduction in abnormal band in CD spectra of CLCDs for both types of NAs.

The CLCDs were formed as well by ds DNA molecules pretreated with 5, 15 nm nano-Au and with nano-Pd (the average size is about 10 nm). The CD spectra of all these CLCDs were measured and the dependences of abnormal band amplitudes on concentration of nanoparticles are compared in Figure 2, *b* (curves 4–6). One can see, first of all, that there is a strong dependence of spectral effect upon concentration of all nanoparticle types. The higher the concentration of nano-Au in solution, the greater the reduction in abnormal band in CD spectra of CLCDs of NAs. Secondly, spectral effect depends on the size of nano-Au, i.e. the greater the size of nano-Au, the smaller is the critical value of R for vanishing of abnormal bands in the CD spectra. The disappearance of abnormal bands for the CLCDs takes place at very low R values (below 1). For instance, in the case of big nano-Au (15 nm, curve 5) as was shown in R at about 0.2 (i.e., at 1 nano-Au per 5 ds DNA molecules). It is worth to note that the similar changes in the CD spectrum occurred also for linear ds DNA pretreated with nano-Pd (curve 6).

The CLCDs have been formed by ds poly(A)×poly(U) pretreated with 2 nm nano-Au. Figure 2, *b* (curve 3) shows disappearance of abnormal band in this case, as well.

The CD spectra shown in Figure 2 demonstrate clearly that the negatively charged nano-Au and nano-Pd must undoubtedly be bound to initial ds NA molecules to induce the changes in the abnormal optical activity of CLCD particles. The binding process depends definitely on the size of nanoparticles. Namely, the greater the size of nanoparticles, the more effective changes in the abnormal optical activity of the CLCD particles.

To confirm possible correlation between change in the abnormal optical activity of the CLCD particles and binding of nano-Au to ds DNA molecules the fluorescence images of the particles formed by DNA molecules, pretreated with the most ‘effective’ nano-Au (15 nm) (obtained DNA particles were also additionally doped with intercalating fluorescence dye – SG), were investigated. These images (Figure 3, *c*) were compared with the images in the CLCD formed by initial ds DNA (Figure 3, *a*). Also, both systems were diluted with water to diminish the osmotic pressure of PEG solution and to obtain its concentration below ‘critical’ value [16]. Figure 3, *b* shows that under these conditions (i.e. at low concentration of PEG) the spatial structure of ds DNA CLCD was disintegrated and ds DNA molecules were already existing in isotropic (noncondensed) state. This process results in full disappearance of fluorescence images of ds DNA CLCD. However, fluorescence images of CLCD formed by ds DNA molecules pretreated with nano-Au exist despite of its dilution (Figure 3, *d*). This is possible only if nano-Au connect neighboring ds DNA molecules and formed aggregate from DNA molecules can exist in the absence of high osmotic pressure of PEG-containing solution.

To get more information on the properties of condensed phase formed by ds DNA molecules pretreated with nano-Au (15 nm) we apply SAXS.

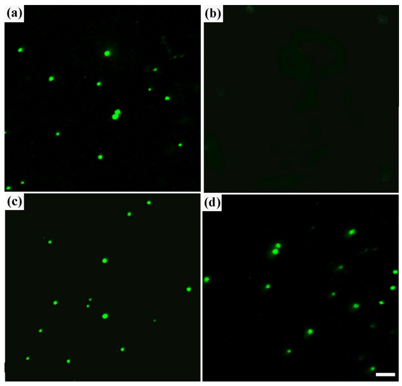

Figure 3. Fluorescent «images» of the free ds DNA CLCD particles (*a*) and the dispersion particles formed in the PEG-containing water-salt solution by the ds DNA molecules pretreated with nano-Au (*c*).
Both types of the DNA particles were doped with intercalating compound – cyanine dye SG.
(*b*) and (*d*) «images» obtained after dilution with water of initial PEG solutions containing particles shown in Figs. (*a*) and (*c*). The scale bar corresponds to 2 μm.
a – C_{DNA} = 15 μg ml^{-1}; C_{PEG} = 170 mg ml^{-1};
0.3 M NaCl + 0.002 M sodium phosphate buffer;
$C_{t\ SG} = 4.9\times10^{-6}$ M.
c – $C_{ДНК}$ = 15 μg ml^{-1}; C_{PEG} = 170 mg ml^{-1};
0.3 M NaCl + 0.002 M sodium phosphate buffer;
$C_{nano\text{-}Au} = 2.5\times10^{12}$ particle ml^{-1}; $C_{t\ SG} = 4.9\times10^{-6}$ M.
The average size of nano-Au is ~ 15 nm

Experimental scattering intensity profiles from the cholesteric phase of ds DNA (curve 1, control,) and from the phase formed by the ds DNA pretreated with nano-Au (curve 2) are presented in Figure 4. Scattering profile from the cholesteric phase formed by free linear ds DNA molecules demonstrates the characteristic Bragg peak typical for densely packed ds DNA molecules in CLCD particles with structural parameters shown in Table.

Figure 4 (curve 2) shows as well that pretreatment of linear ds DNA with nano-Au and phase exclusion of the formed complex (ds DNA-nano-Au) from PEG-containing solution results in: (i) the disappearance of the Bragg maximum, i.e. in a total, even nematic-like, disintegration of the orientational order of ds DNA molecules and spatial structure of the CLCD, and (ii) in the emerging of the characteristic scattering caused by big size nano-Au. The latter phenomenon allows us to determine a size of the nano-Au using ratio: $R = 4.49/s_n$, where s_n – position of the first minimum in the scattering pattern [40]. In our case $R = 4.49/0.64 \approx 7$ nm, that is, about 14 nm in diameter. The value, which fits results of 15 nm nano-Au TEM micrographs, should be considered as an average size due to polydispersity of the nano-Au sample.

Structural characteristics of the DNA cholesteric phase

Sample	s_{max}, nm^{-1} (± 0.1 nm^{-1})	$\bar{d}$, nm (± 0.1 nm)	L, nm (± 3.0 nm)	$\Delta/\bar{d}$ (± 0.01)
DNA liquid-crystalline phase	1.9	3.4	16.0	0.15

Note: s_{max} – the wave vector ($s = 4\pi\sin\theta/\lambda$; 2θ – the scattering angle; λ – the X-ray wavelength equal 0.1542 nm); $\bar{d}$ – the periodicity of the structure (the interhelical distance); L – the crystallite size; $\Delta/\bar{d}$ – the degree of disorder (Δ – the mean square deviation of distances between neighboring regularly packed structural elements)

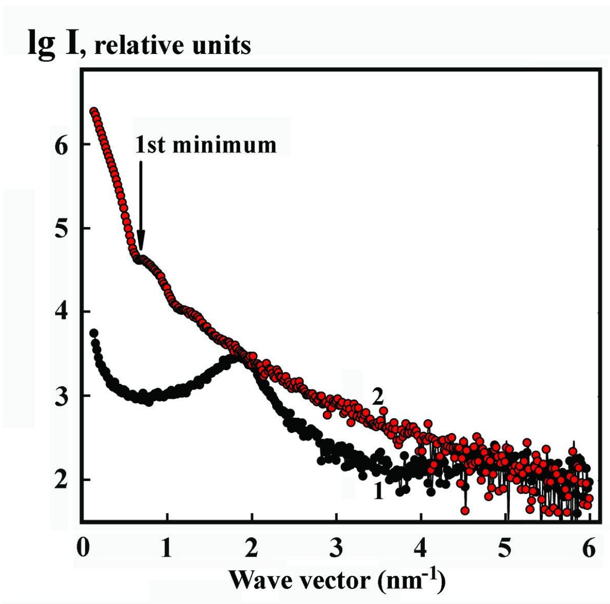

Figure 4. Experimental SAXS curves of phase obtained from the CLCD particles formed by free, linear, ds DNA molecules in PEG-containing water-salt solution (curve 1, control) and of phase formed in the PEG-containing water-salt solution by ds DNA molecules pretreated with nano-Au (curve 2). Arrow marks the first minimum on the scattering curve 2:
1 – C_{DNA} = 15 μg ml^{-1}; C_{PEG} = 170 mg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer;
2 – $C_{ДНК}$ = 15 μg ml^{-1}; C_{PEG} = 170 mg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer; $C_{nano\text{-}Au}$ = 2.5×10^{12} particle ml^{-1} (the average size of nano-Au is ~ 15 nm)

The results of SAXS demonstrate that 15 nm nano-Au affect the interaction between molecules of (ds DNA-nano-Au) complex. Instead of ordered spatial structure of ds DNA CLCD only random disordered aggregates are formed by molecules of these complexes at their condensation.

Hence, all results obtained in this section speak in favor of binding of nano-Au to the ds DNA molecules. However, such complexes of (ds DNA-nano-Au) complexes, in contrast to the free ds DNA molecules, lose an ability to form spatially twisted structure at their condensation. They are capable of forming only aggregates, which do not possess abnormal band in the CD spectrum.

3.3 Doping of CLCD particles formed by ds NA molecules with nano-Au

The CD spectra of CLCDs formed by ds NAs and doped with different concentrations of nano-Au (2 nm) were compared, after the formation of the dispersions, in Figure 5, *a*. In both cases the amplitudes of abnormal bands in the CD spectra drop. The higher the concentration of nano-Au in solution, the greater the reduction in abnormal band in CD spectra of CLCDs for both types of NAs. (Here a comment is in order. Any possible aggregation of independent nano-Au outside of the ds NA CLCD particles cannot induce any change in the value of the abnormal band in the CD spectra in the region of absorption of these molecules).

The dependences of abnormal band amplitudes on the concentration of nano-Au are compared in Figure 5, *b*. One can see, first of all, that there is dependence of spectral effect upon concentration of nano-Au, expressed as R value. Secondly, comparison of Figure 5, *b* to Figure 2, *b* clearly shows that the disappearance of abnormal bands in the case of the formed CLCDs takes place at R values, which are much (~ 25 times) higher in comparison to the case of CLCDs formed by ds NA molecules pretreated with nano-Au. Thirdly, the disappearance of abnormal bands in the CD spectra indicates diminishing in a twist in the spatial structure of CLCD particles.

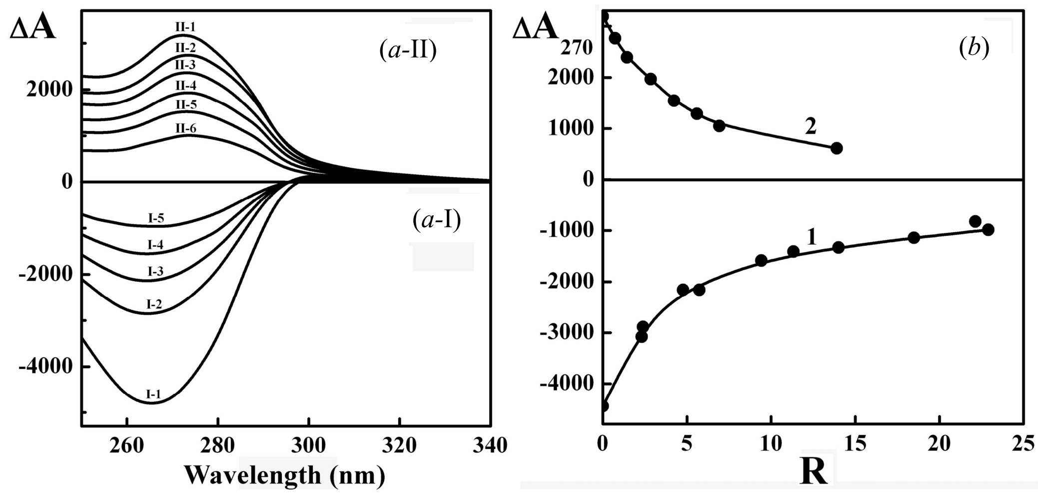

Figure 5. *a* – The CD spectra of DNA (*a-I*) and poly(I)×poly(*C*) CLCDs (*a-II*), doped with different concentrations of nano-Au.
(a-I): 1 – $R = 0$; 2 – $R = 2.39$; 3 – $R = 4.77$; 4 – $R = 9.42$; 5 – $R = 22.9$.
$C_{DNA} = 10$ μg ml^{-1}; $C_{PEG} = 190$ mg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer.
(a-II): 1 – $R = 0$; 2 – $R = 0.72$; 3 – $R = 1.44$; 4 – $R = 2.85$; 5 – $R = 4.23$; 6 – $R = 6.93$.
$C_{poly(I)\times poly(C)} = 12$ μg ml^{-1}; $C_{PEG} = 190$ mg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer.
The average size of nano-Au is ~ 2 nm. *R* – see *Figure 1.* ΔA $\times 10^{-6}$ optical units; l = 1 cm.
The CD spectra of NA CLCDs were measured 2 hrs after of their doping with nano-Au.
b – Dependences of the amplitude of band (λ = 270 nm) in the CD spectra of DNA CLCD (curve 1) and poly (I)×poly(C) CLCD (curve 2) doped with different concentrations of nano-Au on the R value:
$C_{DNA} = 10$ μg ml^{-1}; $C_{poly(I)\times poly(C)} = 12$ μg ml^{-1}; $C_{PEG} = 190$ mg ml^{-1}; 0.3 M NaCl + 0.002 M sodium phosphate buffer.
The average size of nano-Au is ~ 2 nm. *R* – see *Figure 1.* $\Delta A_{270} \times 10^{-6}$ optical units; l = 1 cm.
The CD spectra of NA CLCDs were measured 2 hrs after of their doping with nano-Au

Nevertheless, spatial structure of CLCD particles with ordered packing of 10^4 ds NA molecules [41] is not changed so easily at the nano-Au binding.

It is necessary to note, that doping of CLCDs formed by ds NAs with nano-Au sizes of 5 and 15 nm were accompanied only by a small (if any!) changes in the CD spectra [42].

Figure 5 demonstrates that nano-Au of small size (2 nm) was incorporated by this or that way into a spatial structure of CLCDs particles, i.e. such small nano-Au can interact even with ds NAs inside of CLCD particles, despite the very tight packing of these molecules.

The CD spectra of CLCDs formed by ds NAs and doped with nano-Au (2 nm) after their formation are compared in Figure 6, *a*. It can be seen that the doping of CLCDs with nano-Au reduces the amplitudes of the abnormal bands in the CD spectra for both types of formed dispersions. The curves characterizing the time dependences of the amplitude of the abnormal bands in the CD spectra of DNA and poly(I)×poly(C) CLCDs doped with nano-Au are compared in Figure 6, *b* (curves 1 and 2, respectively). It can be seen that the reduction of the band amplitude occurs in two stages. The duration of the first, fast stage is about 20–40 min (Figure 6, *b*). The slow stage does not lead, to any significant change in amplitudes of abnormal bands.

Hence, doping of NA CLCDs with nano-Au (2 nm; Figure 6) demonstrates that these negatively charged nanoparticles can be aggregated with tightly packed ds NA molecules. The interaction leads to a ‘disturbance’ of the spatial structure of CLCD particles causing a reduction in abnormal band in the CD spectra. Phenomenologically, this spectral change can be described as the unwinding of the cholesteric spiral, i.e., phase transition of cholesteric → nematic [43].

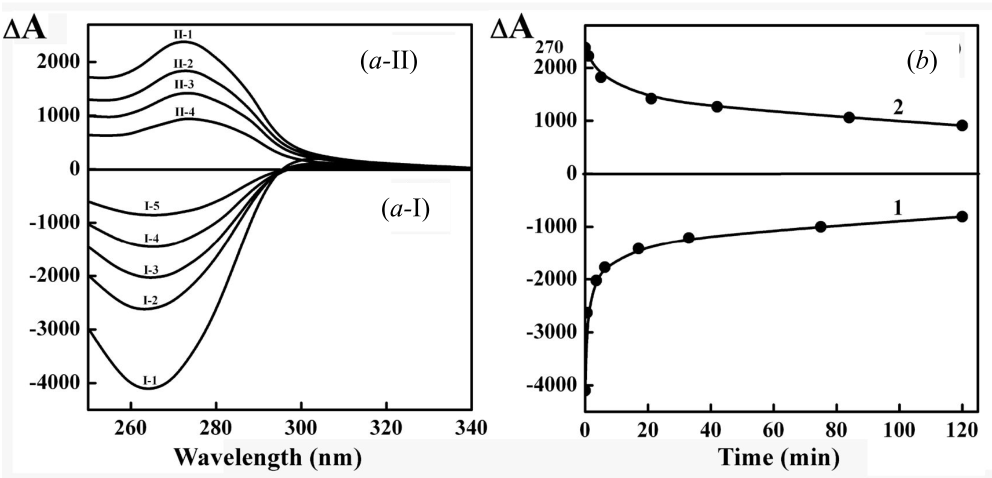

Figure 6. *a* – The CD spectra of DNA (*a-I*) and poly(I)×poly(C) CLCDs (*a-II*) measured after different time of their doping with nano-Au:
(a-I): I-1 – 0 min; I-2 – 0.6 min; I-3 – 3.5 min; I-4 – 17 min; I-5 – 120 min.
(a-II): II-1 – 0 min; II-2 – 5 min; II-3 – 21 min; II-4 – 120 min.
b – Time dependences of the amplitude of the abnormal bands (ΔA_{270}) in the CD spectra of DNA (curve 1) and poly(I)×poly(*C*) CLCDs (curve 2) doped with nano-Au:
$C_{DNA} = 10\ \mu g\ ml^{-1}$; $C_{poly(I)\times poly(C)} = 12\ \mu g\ ml^{-1}$;
$C_{PEG} = 190\ mg\ ml^{-1}$; 0.3 M NaCl + 0.002 M sodium phosphate buffer.
$C_{nano\text{-}Au} = 1.67\times10^{14}$ particle ml^{-1} (the average size of nano-Au is ~ 2 nm).
$\Delta A\times10^{-6}$ optical units; $\Delta A_{270}\times10^{-6}$ optical units; l = 1 cm

4. Discussion

The results presented above describe two different situations: i) doping of free, linear ds NA molecules in solution with negatively charged nano-Au, and ii) doping of CLCD particles formed by ds NA molecules with negatively charged nano-Au.

1. Considering minor spectral changes shown in Figure 1 we can suppose that negatively charged nano-Au are somehow attached to the free, linear ds NA molecules in solutions of high (~ 0.3) ionic strength. Note to the point that the authors of [21] showed earlier that the amplitude of band in the CD spectrum of synthetic ds DNA molecule (ds 25) of very low molecular mass (16.5×10^3 Da) treated with nano-Au (with an average diameter 13 nm and concentration of 9×10^{-9} M, i.e. $C_N \sim 5.43 \times 10^{12}$ particles ml^{-1}) is reduced compared to the free ds form.

Because we were not in position to repeat this result, due to pure experimental reasons, the statement concerning the interaction of negatively charged nano-Au with ds NA molecules, based on detection of minor changes in the CD spectra, needs additional verification.

2. The decrease in amplitudes of abnormal bands of CLCDs formed by linear ds DNA and poly(I)×poly(C) molecules pretreated with nano-Au (Figure 2) demonstrates that the negatively charged nano-Au are bound to linear ds NA molecules prior to their ψ-condensation to induce the shown spectral changes. The greater the size of nano-Au, the more effective the changes in the abnormal optical activity of the NA CLCD particles. To rationalize this observation two different mechanisms («chemical» and «physical») can be proposed.

4.1 The «chemical» mechanism of nano-Au-ds NA interaction

i) Having in mind the high negative charge of ds NA molecules and the anionic properties of nano-Au, the first question to answer is how these particles bind to these molecules [44].

ii) Indeed since both nano-Au and ds NAs are negatively charged, the long range electrostatic repulsion should suppress any aggregation. However, to obtain ds NA CLCDs we used the technology of intensive mixing for PEG-containing water-salt (~ 0.3 M NaCl) solutions with water-salt (~ 0.3 M NaCl) ds NA solutions was used. Under these conditions the electrostatic repulsion between nano-Au and ds biopolymer molecules is minimal. Hence, high salt concentration can facilitate the close approach of nano-Au to the ds NA molecule surface.

iii) If nano-Au are sufficiently close to ds NA molecule surface, the binding event will be accompanied by a partial substitution of the stabilizing organic anions on nano-Au for negatively charged phosphates of the DNA, which most likely bound the nano-Au due to the high electronegativity of the metal [44]. Besides, under these conditions, small-size nano-Au (~ 2 nm) can be sterically embedded into the major groves of ds DNA molecules, thereby causing a slight distortion in the structural geometry of the B-DNA [45, 46]. This mechanism requires a rather strong size limitation, because the nano-Au, larger than 2 nm, would be less likely to interact with a B-form of DNA or A-form of poly(I)×poly(C) in such a way due to sterical reasons [47].

Hence, the sterical embedment of nano-Au with sizes of 5 and 15 nm into the major groves, as a possible mechanism of (ds NA-nano-Au) interaction must be rejected.

iv) A single nano-Au of any size can, in principle, form complexes with nitrogen base pairs (namely, N7 atoms of purine and N3 atoms of pyrimidine) [17, 48] of ds NA. Taking into account the possibility of deformation of spatial structure of nano-Au [49, 50] chemical interaction between nitrogen bases and nano-Au appears to be a function of nano-Au size. In this case, an increase in the nano-Au size may lead to increase in the number of free electrons to share with nitrogen bases. But the efficiency of the formation of complexes between nitrogen bases of ds NAs and nano-Au is again limited due to the sterical location of nitrogen bases in the spatial structure of ds NAs, and thus cannot be considered as the main mechanism of (ds NA-nano-Au) interaction.

So, one has to look for some other explanation for why nano-Au bind to ds NA molecules. In our opinion, the «physical» mechanism (see below) is a good candidate.

4.2 The «physical» mechanism of nano-Au-ds NA interaction

Taking into account the fact that CLCDs of ds NAs were obtained at high salt concentration leading to a decrease in electrostatic repulsion between nano-Au and ds molecules, we consider other higher electric multipoles as a possible mechanism of nano-Au binding to ds NAs [21]. One can add here, that the theoretical simulations [51–53] which model the process for «metallization» of ds DNA fragment in an aqueous solution via nano-Au showed that nano-Au are polarized in the intrinsic electric field of the DNA molecule. In this case no special chemical functionalization of the surface of the nano-Au is supposed to be performed.

We advocate here the following mechanism of (nano-Au-ds NA) interaction. The charges of the phosphate groups of ds NAs induce a dipole in the highly polarizable nano-Au. This mechanism is quite short ranged ($\sim 1/d^4$), (where d is the distance between polarization center of nanoparticle and DNA base pair local charge). One might think that the Coulomb repulsion keeps the species apart at longer distances. Under our conditions, the high ionic strength (~0.3) of the water-salt solution screens the long-range repulsion so the nano-Au are allowed an approach to the DNA base pair charges. At a certain distance, the ion-induced dipole interaction takes over, resulting in a net attractive force. This is accompanied by fixation (immobilization) of nano-Au nearby the surface of ds NA molecules. Besides, the polarizability of a conducting sphere is proportional to the cube of the sphere radius, resulting in a large drop in polarizability when changing from 15 nm nano-Au to 2 nm nano-Au. This results in dropped efficiency of nano-Au-ds NA interaction with decrease in the size of nano-Au.

If this is the case, it means that 15 nm nano-Au can be bound with ds NAs stronger in comparison to 2 nm.

Figure 2 shows that pretreatment of ds NAs with 15 nm nano-Au is accompanied by much pronounced drop in an abnormal optical activity of the formed CLCDs in comparison to 2 or 5 nm nano-Au treatment.

If negatively charged nano-Au indeed bind to the ds NA molecules, the next question to answer is why the amplitude of abnormal bands in the CD spectra of CLCDs formed by ds NA pretreated with nano-Au, decreases?

Two points are important here.

1) It was shown earlier that the packing mode of ds DNA molecules in CLCD particles is determined at the moment of their «recognition» [54] during the spatial juxtaposition of neighboring DNA molecules. Spatially, this «recognition» process begins to be realized when the distance between neighboring DNA molecules corresponds to 5.0–10.0 nm [15, 55–59].

The peculiarities of the ds DNA secondary structure and the distribution pattern of «guest» molecules on their surfaces define not only their «recognition» but the mode of their ordering in quasinematic layers of spatial structure of dispersion particles. The theoretical considerations [36] reveal that the formation of the twisted structure of dispersion particles is determined by interaction between neighboring ds DNA molecules, which possess the «double» inherent anisotropy (related with helical structure and the presence of optically active carbon atoms in sugar residues) of DNA molecules. As a result, each subsequent quasinematic layer, formed by ds DNA molecules, is turned at a certain angle (approximately 0.5^o) with respect to the previous one. The rotation gives rise to the cholesteric LC structure of a dispersion particle. The ds DNA molecules (B-form) with a right-handed helical twist of the secondary structure form, as a rule, the particles of a left-handed helical packing of the quasinematic layers. This is demonstrated by the appearance of a negative CD band in the absorption region of DNA nitrogen bases. Hence, the anisotropic character of the free linear ds DNA molecules and sterical interaction between them specifies helically twisted packing of these molecules in particles formed.

However, the combination as shown in these two items can result in a distortion of the spatially twisted structure of the CLCD particles, and hence, in the decrease (down to full disappearance) of their abnormal optical activity (nematization of spatial structure). This means that abnormal band in the CD spectrum of the CLCD can be considered as a criteria, which reflects any mode of alteration of ds DNA structure induced by chemical or biological compounds.

2) The first experiments [13, 14] demonstrated that positively charged nano-Au chemically linked to ss DNA molecules do not prevent their hybridization. Obtained ds DNA molecules, after immobilization on a surface of the film for TEM, form planar superstructure, which consists of a few repetitive neighboring ds DNA molecules and nano-Au. In planar superstructure of type (DNA…Au…DNA…Au…DNA) spatial twist of neighboring DNA molecules is absent, despite anisotropy of these molecules [19, 60, 61].

Coming back to our case we can say the following. The formation of the spatial twisted structure of free ds DNA CLCD particles, as a result of the phase exclusion of these molecules, depends on relatively weak anisotropic contribution into electrostatic or Van-der-Waals interaction between neighboring ds DNA molecules. It can be very sensitive to the presence of metallic nanoparticles, i.e. nano-Au.

Taking into account that at the formation of CLCD particle with an ordered arrangement of ds NA molecules the «recognition» distance between these molecules is close to 5.0–10.0 nm, one can expect that if nano-Au are indeed fixed near the surfaces of these molecules, they must sterically prevent both «recognition» and proper spatial orientation of these molecules, which define mutual twist of ds molecules.

Besides, the facet spatial structure of nano-Au [11, 62] creates conditions under which neighboring ds DNA molecules in water-salt solutions of high ionic strength can be assembled on various facets of these nanoparticles [63]. This leads to disordered and uncorrelated arrangement of ds DNA molecules on surface of nano-Au, i.e., to the formation of ds DNA aggregates (Figure 7). In the framework of this model, the greater the size of nano-Au, the effective the formation of ds DNA aggregates.

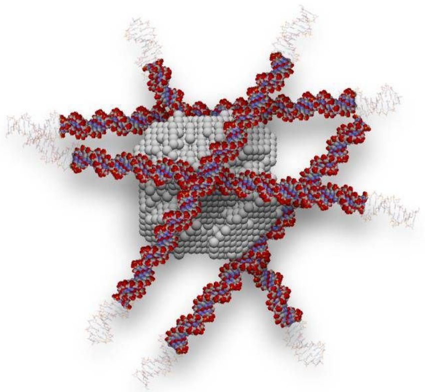

Figure 7. Proposed structure of aggregate formed by a few ds DNA molecules fixed on crystal facets of one gold nanoparticle (15 nm). Structure of gold nanoparticle that follows from the data of X-ray analysis was used

Results of SAXS support such consideration. They showed (Figure 4) that ds DNA molecules pretreated with 15 nm nano-Au and condensed in PEG-containing water-salt solution form aggregates with disordered arrangement of these molecules. If this is the case, the abnormal band in the CD spectrum typical for DNA CLCD with spatially twisted structure must be decreased with increase in nano-Au concentration and increase in nano-Au size. Figure 2 confirms this statement, i.e. the greater the size of nano-Au, the smaller is the R value of disappearance of abnormal band in the CD spectra.

Hence, negatively charged nano-Au are undoubtedly can be bound to initial ds NA molecules in water-salt solutions of high ionic strength and can form (ds DNA-nano-Au) complexes. Taking into account the possibility of uncorrelated positions of neighboring ds DNA molecules on surface of nano-Au, phase exclusion of these complexes results only in one process, i.e. in formation of disordered aggregates from ds DNA molecules linked via nano-Au (Figure 7). This allows one to suppose that the spatial topography of nano-Au may play a role of «random external field», which suppresses both ds DNA molecules recognition and formation of cholesteric structure from (ds DNA-nano-Au) complexes. Analogous transformation of lamellar phases under influence of nanoparticles, including nano-Au (2 nm), was considered in [8, 64].

Quite different process happens at doping of ds NA CLCDs with high concentration of nano-Au (2 nm) (Figures 5 and 6).

Taking into account that the changes in the CD spectra of ds NA CLCDs take place at high R values within 20–40 min (Figure 6, *b*), it was supposed that nano-Au with the size (~ 2 nm) comparable to the distance between ds DNA molecules in quasinematic layers (~ 3.4 nm) can quickly incorporate between these molecules ordered in the spatial structure of CLCD particles. (The nano-Au with the sizes equal to 5 nm and 15 nm are too «big» in comparison to the distance between ds NA molecules in quasinematic layers, and this prevents their incorporation into content of CLCD particles). Then, within a slow stage, which does not result in significant change in amplitude of abnormal band in the CD spectra, interaction between adjacent nano-Au is accompanied by formation of nano-Au clusters in the content of CLCD particles [43].

To check this scenario we made the SAXS measurements of the phases obtained as a result of slow centrifugation of ds DNA CLCD particles doped with different concentrations of nano-Au (2 nm) [42]. (The CLCD phase of free, initial ds DNA was used as a control sample). The results of SAXS measurements showed that all samples doped with nano-Au (2 nm) possess diffuse Bragg maxima. The doping is accompanied only by increasing from 0.4–0.5 nm of characteristic size (i.e., the distance between the ds DNA molecules arranged parallel in quasinematic layers. The mutual position of neighboring ds DNA molecules (and, correspondingly, quasinematic layers in ds DNA CLCD particles) changes albeit insignificantly [42, 65].

This means that doping of ds DNA CLCD with nano-Au (2 nm) does not lead to the modification in ordering of ds DNA molecules in the particles of CLCD.

However, the incorporation of nano-Au into quasinematic layers of ds DNA CLCD particles and their spatial ordering between these molecules in quasinematic layers must be «transformed» by this or that way into the changes in the total energy of interaction between neighboring ds DNA molecules. For instance, the «dipoles» of neighboring (DNA–nano-Au) complexes would tend to be arranged in parallel, which can induce a change in the helical twist of neighboring quasinematic layers. This means also that nano-Au may change the contributions determining the helical twist of neighboring quasinematic layers of NA molecules (in particular, the anisotropic contribution to the van der Waals interaction). In this case, the twist angle between the layers may turn to zero, which is equivalent to the untwisting of the cholesteric structure of dispersion particles at nano-Au doping. For this process the differences in the secondary structure of various ds NA molecules, are not important.

Figures 5 and 6 indeed demonstrate a quick decrease in amplitudes of abnormal bands in the CD spectra. However, in contrast to the cases shown in Figure 2, these spectral changes reflect the unwinding of the particle spatial structure (regardless of the origin of NAs) as a result of incorporation of nano-Au into CLCD particles formed by ds NA molecules [36]. This effect is unique as none of the other chemical or biological compounds can cause the complete unwinding of the spatial structure of CLCD particles as a result of their interaction with ds DNA molecules at the room temperature.

Therefore, the results presented above strongly suggest that the negatively charged nanoparticles of noble metals can interact with free ds NA molecules or with these molecules ordered in the spatial structure of the CLCD particles. This allows one to hypothesize that nano-Au can induce different damaging effects in mammalian cells which depend on the state of ds DNA molecules. The conclusions about the damaging effect of the small nano-Au are confirmed by experimental studies [66–69], which demonstrated their ability to reduce the locomotor activity of ejaculated human and bovine sperms by 25 and 22 %, respectively, as well as destroy the nuclear apparatus of mature gametes. Besides it was suggested that the small nano-Au are highly active with respect to the sperm cells of mammals and humans [66, 70, 71]. These results provide background to assume that the *in vitro* and *in vivo* action of nano-Au on spatially arranged ds DNA structures is similar to that of molecules that possess mutagenic activity.

5. Conclusion

Results presented above allow one to draw a few important conclusions.

1. The negatively charged nano-Au in solutions of high ionic strength can interact with free ds molecules of NAs or with these molecules ordered in the spatial structure of the CLCD particles. This interaction leads to decrease in amplitude of specific bands in the CD spectra.

2. The decrease in abnormal bands in the CD spectra of CLCDs can be induced by two very different reasons: i) formation of aggregates from ds NAs pretreated with nano-Au, or ii) disappearance of twist of spatial structure of ds NA CLCD particles doped with nano-Au.

3. The processes of dispersing nanoparticles and condensing ds DNA do not commute. If first one disperses nanoparticles in isotropic dilute solution of ds DNA, and then tries to condense the obtained mixture, orientationally ordered structures do not occur. If in other way around, one first condenses the ds DNA and only after formation of cholesteric structure, disperses the nanoparticles, the orientational order remains stable. This order evolves (from cholesteric to untwisted nematic-like) on increasing the nanoparticle concentration. Our working hypothesis is that the both procedures do not commute due to random anchoring field created by a complex topography of nanoparticle surfaces (facets). This random field disorients ds DNA long axis in a dilute solution (where ds DNA can more or less freely rotate individually). On the contrary in the condensed twisted structure the same random field cannot rotate cooperatively ordered ds DNA. Instead, the field disorients the nanoparticles.

4. The changes in the CD spectra of CLCDs do not depend on the methods of nano-Au synthesis, but depends on concentration and size of used nanoparticles.

5. According to preliminary measurements and evaluations, analogous spectral changes are typical also for nano-Pd.

6. It is not excluded, that difference in action of nano-Au on «free» and «condensed» ds DNA molecules can result in various damaging effects in living cells and be accompanied by various genetic consequences.

Acknowledgement

The authors are sincerely grateful to B. N. Khlebtsov and V. A. Khanadeev (Institute of Biochemistry and Physiology of Plants and Microorganisms, the Russian Academy of Sciences) for synthesizing high quality gold nanoparticle samples and for determining their parameters, as well as to P. G. Kuzmin, (Wave Research Center of Prokhorov General Physics Institute, the Russian Academy of Sciences) for laser ablation synthesis of palladium nanoparticles and for determining their parameters.

E. I. Kats participation in this work was supported by the Russian Science Foundation under Grant № 14-12-00475.

References

1. *Hegmann T., Qi H., Marx V. M.* Nanoparticles in liquid crystals: synthesis, self-assembly, defect formation and potential applications // J. Inorg. Organomet. Polym. Mater. 2007. Vol. 17. P. 483–508.
2. *Nealon G. L, Greget R., Dominguez C., Nagy Z. T., Guillon D., Gallani J. L., Donnio B.* Liquid-crystalline nanoparticles: hybrid design and mesophase structure // Beilstein. J. Org. Chem. 2012. Vol. 8. P. 349–370.
3. *Stamatoiu O., Mirzaei J., Feng X., Hegmann T.* Nanoparticles in liquid crystals and liquid crystalline nanoparticles // Top. Curr. Chem. 2012. Vol. 318. P. 331–393.
4. *Wang W., Efrima S., Regev O.* Directing silver nanoparticles into colloid-surfactant lyotropic lamellar systems // J. Phys. Chem. B. 1999. Vol. 103. P. 5613–5621.
5. *Firestone M. A., Williams D. E., Seifert S., Csencsits R.* Nanoparticle arrays formed by spatial compartmentalization in a complex fluid // Nano Letters. 2001. Vol. 1. № 3. P. 129–135.
6. *Eiser E., Bouchama F., Thathagar M. B., Rothenberg G.* Trapping metal nanoclusters in «soap and water» soft crystals // Chem. Phys. Chem. 2003. Vol. 4. P. 526–528.
7. *Kumar P. S., Pal S. K., Kumar S., Lakshminarayanan V.* Dispersion of thiol stabilized gold nanoparticles in lyotropic liquid crystalline systems // Langmuir. 2007. Vol. 23. P. 3445–3449.
8. *Pansu B., Lecchi A., Constantin D., Impéror-Clerc M., Veber M., Dozov I.* Insertion of gold nanoparticles in fluid mesophases: size filtering and control of interactions // J. Phys. Chem. C. 2011. Vol. 115. P. 17682–17687.
9. *Constantin D., Davidson P.* Lamellar mesophases doped with inorganic nanoparticles // Chem. Phys. Chem. 2014. Vol. 15. P. 1270–1282.
10. *Дыкман Л. А., Богатырев В. А., Щеголев С. Ю., Хлебцов Н. Г.* Золотые наночастицы: Синтез, свойства, биомедицинское применение. М. : Наука, 2008. 319 с. [*Dykman L. A., Bogatyrev V. A., Shchegolev S. Yu., Khlebtsov N. G.* Zolotye nanochastitsy: Sintez, svoystva, biomeditsinskoe primenenie (Golden nanoparticles: synthesis, properties and biomedical application). Moscow : Nauka Publ., 2008. 319 p. (in Russian)].
11. *Louis C., Pluchery O.* Gold nanoparticles for physics, chemistry and biology. London : Imperial College Press, 2012. 395 p.
12. *Skuridin S. G., Dubinskaya V. A., Rudoy V. M., Dement'eva O. V., Zakhidov S. T., Marshak T. L., Kuzmin V. A., Popenko V. I., Yevdokimov Yu. M.* Effect of gold nanoparticles on DNA packaging in model systems // Dokl. Biochem. Biophys. 2010. Vol. 432. P. 141–143.
13. *Mirkin C. A., Letsinger R. L., Mucic R. C., Storhoff J. J.* A DNA-based method for rationally assembling nanoparticles into macroscopic materials // Nature. 1996. Vol. 382. P. 607–609.
14. *Alivisatos A. P., Johnsson K. P., Peng X., Wilson T. E., Loweth C. J., Bruchez M. P., Scultz P.* Organization of nanocrystal molecules using DNA // Nature. 1996. Vol. 382. P. 609–611.
15. *Livolant F., Leforestier A.* Condensed phases of DNA: structures and phase transitions // Prog. Polym. Sci. 1996. Vol. 21. P. 1115–1164.
16. *Yevdokimov Yu. M., Salyanov V. I., Semenov S. V., Skuridin S. G.* DNA liquid-crystalline dispersions and nanostructures / ed. Yu. M. Yevdokimov. Boca Raton – London – New York: CRC Press, 2011. 320 p.
17. *Herne T. M., Tarlov M. J.* Characterization of DNA probes immobilized on gold surfaces // J. Am. Chem. Soc. 1997. Vol. 119. P. 8916–8920.
18. *Zanchet D., Micheel C. M., Parak W. J., Gerion D., Alivisatos A.P.* Electrophoretic isolation of discrete Au nanocrystal/DNA conjugates // Nano Letters. 2001. Vol. 1, № 1. P. 32–35.
19. *Kumar A., Pattarkine M., Bhadbhade M., Mandale A. B., Ganesh K. N., Datar S. S., Dharmadhikari C. V., Sastry M.* Linear superclusters of colloidal gold particles by electrostatic assembly on DNA templates // Adv. Mater. 2001. Vol. 13. P. 341–344.
20. *Storhoff J. J., Elghanian R., Mirkin C. A., Letsinger R. L.* Sequence-dependent stability of DNA-modified gold nanoparticles // Langmuir. 2002. Vol. 18. P. 6666–6670.
21. *Sandström P., Boncheva M., Åkerman B.* Nonspecific and thiol-specific binding of DNA to gold nanoparticles // Langmuir. 2003. Vol. 19. P. 7537–7543.
22. *Li H., Rothberg L.* Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles // Proc. Natl. Acad. Sci. USA. 2004. Vol. 101. P. 14036–14039.

23. *Nelson E. M., Rothberg L. J.* Kinetics and mechanism of single-stranded DNA adsorption onto citrate-stabilized gold nanoparticles in colloidal solution // Langmur. 2011. Vol. 27. P. 1770–1777.
24. *Liu J.* Adsorption of DNA onto gold nanoparticles and graphene oxide: surface science and applications // Phys. Chem. Chem. Phys. 2012. Vol. 14. P. 10485–10496.
25. *Duff D. G., Baiker A., Edwards P. P.* A new hydrosol of gold clusters. 1. Formation and particle size variation // Langmuir. 1993. Vol. 9. P. 2301–2309.
26. *Khlebtsov N. G., Bogatyrev V. A., Dykman L. A., Melnikov A. G.* Spectral extinction of colloidal gold and its biospecific conjugates // J. Colloid Interface Sci. 1996. Vol. 180. P. 436–445.
27. *Frens G.* Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions // Nature Phys. Sci. 1973. Vol. 241, Issue 105. P. 20–22.
28. *Shafeev G. A.* Laser-based formation of nanoparticles // Lasers in Chemistry / ed. M. Lackner. Wienheim: Wiley-VCH Verlag, 2008. Vol. 2. P. 713–741.
29. *Barcikowski S., Menéndez-Manjón A., Chichkov B., Brikas M., Račiukaitis G.* Generation of nanoparticle colloids by picosecond and femtosecond laser ablations in liquid flow // Appl. Phys. Lett. 2007. Vol. 91. P. 083113.
30. *Zipper H., Brunner H., Bernhagen J., Vitzthum F.* Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications // Nucleic Acids Research. 2004. Vol. 32, № 12. P. e103. DOI: 10.1093/nar/gnh101.
31. *Kompanets O. N.* Portable optical biosensors for detection of biologically active and toxic compounds // Physics-Uspekhi. 2004. Vol. 47, № 6. P. 630–633.
32. *Konarev P. V., Volkov V. V., Sokolova A. V., Koch M. H. J., Svergun D. I.* PRIMUS: A Windows PC-based system for small-angle scattering data analysis // J. of Appl. Crystallog. 2003. Vol. 36, № 5. P. 1277–1282.
33. *Vainshtein B. K.* Difraction of X-rays by chain molecules. Amsterdam-London-New York: Elsevier Publishing Company; 1966. 257 p.
34. *Svergun D. I.* Determination of the regularization parameter in indirect-transform methods using perceptual criteria // J. Appl. Cryst. 1992. Vol. 25. P. 495–503.
35. *Svergun D. I.* Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing // Biophys J. 1999. Vol. 76. P. 2879–2886.
36. *Yevdokimov Yu. M., Salyanov V. I., Skuridin S. G., Semenov S. V., Kompanets O. N.* The CD spectra of double-stranded DNA liquid-crystalline dispersions. New York: Nova Science Publishers, 2011. 103 p.
37. *Li H., Rothberg L.* Colorimetric detection of DNA sequences based unmodified gold nanoparticles // Proc. Natl. Acad. Sci. USA. 2004. Vol. 101. P. 14036–14039.
38. *Lerman L. S.* A transition to a compact form of DNA in polymer solutions // Proc. Natl. Acad. Sci. USA. 1971. Vol. 68. P. 1886–1890.
39. *Lerman L. S.* Intercalability, the ψ transition, and the state of DNA in Nature // Progress in molecular and subcellular biology / ed. F. E. Hahn. Berlin – Heidelberg – New York: Springer-Verlag, 1971. Vol. 2. P. 382–391.
40. *Feigin L. A., Svergun D. I.* Structure analysis by small-angle X-ray and neutron scattering. New York: Plenum Press, 1987. 335 p.
41. *Yevdokimov Yu. M.* From particles of liquid-crystalline dispersions to rigid deoxyribonucleic acid nanoconstructions // Liq. Cryst. Today. 2011. Vol. 20, № 1. P. 2–19.
42. *Yevdokimov Yu. M., Skuridin S. G., Salyanov V. I., Popenko V. I., Rudoy V. M., Dement'eva O. V., Shtykova E. V.* A dual effect of Au-nanoparticles on nucleic acid cholesteric liquid-crystalline particles // J. Biomater. Nanobiotechnol. 2011. Vol. 2. P. 461–471.
43. *Yevdokimov Yu. M., Salyanov V. I., Kats E. I., Skuridin S. G.* Gold nanoparticle clusters in quasinematic layers of liquid-crystalline dispersion particles of double-stranded nucleic acids // Acta Naturae. 2012. Vol. 4. P. 78–90.
44. *Zhang X., Servos M. R., Liu J.* Surface science of DNA adsorption onto citrate-capped gold nano-particles // Langmuir. 2012. Vol. 28. P. 3896−3902.
45. *Liu Y., Meyer-Zaika W., Franzka S., Schmid G., Tsoli M., Kuhn H.* Gold-cluster degradation by the transition of B-DNA into A-DNA and the formation of nanowires // Angew Chem. Int. Ed. 2003. Vol. 42. P. 2853–2857.
46. *Tsoli M., Kuhn H., Brandau W., Esche H., Schmid G.* Cellular uptake and toxicity of Au55 clusters // Small. 2005. Vol. 1, № 8–9. P. 841–844.
47. *Pan Y., Neuss S., Leifert A., Fischler M., Wen F., Simon U., Schmid G., Brandau W., Jahnen-Dechent W.* Size-dependent cytotoxicity of gold nanoparticles // Small. 2007. Vol. 3, № 11. P. 1941–1949.
48. *Jang N. H.* The coordination chemistry of DNA nucleosides on gold nanoparticles as a probe by SERS // Bull. Korean. Chem. Soc. 2002. Vol. 23. P. 1790–1800.
49. *Пичугина Д. А., Мажуга А. Г., Шестаков А. Ф.* Наночастицы золота: получение, строение, свойства и применение // Органические и гибридные наноматериалы: тенденции и перспективы / под ред. В. Ф. Разумова, М. В. Клюева. Иваново : Иван. гос. ун-т, 2013. С. 147–190

[*Pichugina D. A., Mazhuga F. G., Shestakov A. F.* Nanochastitsy zolota: poluchenie, stroenie, svoystva i primenenie // Organicheskie i gibridnye nanomaterialy: tendentsii i perspektivy / ed. V. F. Razumov, M. V. Klyuev. Ivanovo : Publisher Ivanovo State University; 2013. P. 147–190].

50. *Weitz D. A., Lin M. Y., Sandroff C. J.* Colloidal aggregation revisited: new insights based on fractal structure and surface-enhanced Raman scattering // Surf. Sci. 1985. Vol. 158. P. 147–164.
51. *Komarov P. V., Zherenkova L. V., Khalatur P. G.* Computer simulation of the assembly of gold nanoparticles on DNA fragments via electrostatic interaction // J. Chem. Phys. 2008. Vol. 128. P. 124909-1–124909-11.
52. *Zherenkova L. V., Komarov P. V., Khalatur P. G.* Simulation of the metallization of a fragment of a deoxyribonucleic acid molecule with gold nanoparticles // Colloid J. 2007. Vol. 69. P. 706–717.
53. *Komarov P. V., Zherenkova L. V., Khalatur P. G., Khokhlov A. R.* The self-assembly of a metalloorganic nanoaggregates based on the electrostatic interaction between DNA molecules and gold nanoparticles polarized in its field // Nanotechnologies in Russia. 2007. Vol. 2. P. 92–98.
54. *Yevdokimov Yu. M., Salyanov V. I., Golo V. L., Kats E. I., Spener F.* Double-stranded right-handed nucleic acid molecules of B-family do not "recognize" double-stranded right handed molecules of A-family at formation of liquid-crystalline dispersions in PEG-containing solutions // Sensory Systems. 2000. Vol. 14. P. 246–257.
55. *Livolant F.* Cholesteric organization of DNA *in vivo* and *in vitro* // Eur. J. Cell. Biol. 1984. Vol. 33. P. 300–311.
56. *Yevdokimov Yu. M., Skuridin S. G., Salyanov V. I.* Liquid-crystalline phases of double-stranded nucleic acids in *vitro* and *in vivo* // Liq. Cryst. 1988. Vol. 3. P. 1443–1459.
57. *Strey H. H., Parsegian V. A., Podgornik R.* Equation of state for DNA liquid crystals: fluctuation enhanced electrostatic double layer repulsion // Phys. Rev. Lett. 1997. Vol. 78. P. 895–898.
58. *Podgornik R., Parsegian V. A.* Charge-fluctuation forces between rodlike polyelectrolytes: pairwise summability reexamined // Phys. Rev. Lett. 1998. Vol. 80. P. 1560–1563.
59. *Kanduc M., Dobnikar J., Podgornik R.* Counterion-mediated electrostatic interactions between helical molecules // Soft Matter. 2009. Vol. 5. P. 868–877.
60. *Sastrya M., Kumar A., Datar S., Dharmadhikari C. V.* DNA-mediated electrostatic assembly of gold nanoparticles into linear arrays by a simple drop-coating procedure // Appl. Phys. Lett. 2001. Vol. 78. P. 2943–2945.
61. *Warner M. G., Hutchison J. E.* Linear assemblies of nanoparticles electrostatically organized on DNA scaffolds // Nature Mater. 2003. Vol. 2. P. 272–277.
62. *Kinge S., Crego-Calama M., Reinhoudt D. N.* Self-assembling nanoparticles at surfaces and iterfaces // Chem. Phys. Chem. 2008. Vol. 9. P. 20–42.
63. *Imura Y., Morita C., Endo H., Kondo T., Kawai T.* Reversible phase transfer and fractionation of Au nanoparticles by pH change // Chem. Commun. 2010. Vol. 46. P. 9206–9208.
64. *Constantin D., Davidson P.* Lamellar Lα mesophases doped with inorganic nanoparticles // Chem. Phys. Chem. 2014. Vol. 15. P. 1270–1282.
65. *Yevdokimov Yu. M., Skuridin S. G., Salyanov V. I., Popenko V. I., Shtykova E. V., Dadinova L. A., Volkov V. V., Khlebtsov N. G., Khlebtsov B. N., Kats E. I.* A new nanobiomaterial: particles of liquid_crystalline DNA dispersions with embedded clusters of gold nanoparticles // Nanotechnologies in Russia. 2014. Vol. 9. P. 194–202.
66. *Wiwanitkit V., Sereemaspun A., Rojanathanes R.* Effect of gold nanoparticles on spermatozoa: the first world report // Fertil Steril. 2009. Vol. 91. P. e7–e8.
67. *Kang B., Mackey M. A., El-Sayed M. A.* Nuclear targeting of gold nanoparticles in cancer cells induces DNA damage, causing cytokinesis arrest and apoptosis // J. Am. Chem. Soc. 2010. Vol. 132. P. 1517–1519.
68. *Khlebtsov N. G., Dykman L. A.* Biodistribution and toxicity of engineered gold nanoparticles: a review of *in vitro* and *in vivo* studies // Chem. Soc. Rev. 2011. Vol. 40. P. 1647–1671.
69. *Dykman L. A., Khlebtsov N. G.* Uptake of engineered gold nanoparticles into mammalian cells // Chem. Rev. 2014. Vol. 114. P. 1258–1288.
70. *Zakhidov S. T., Marshak T. L., Malolina E. A., Kulibin A. Yu., Zelenina I. A., Pavlyuchenkova S. M., Rudoy V. M., Dement'eva O. V., Skuridin S. G., Yevdokimov Yu. M.* Gold nanoparticles impair nuclear chromatin decondensation process in murine sperm cells in vitro // Biochemistry (Moscow) Suppl. Series A: Membrane and Cell Biology. 2010. Vol. 4. P. 285–288.
71. *Zakhidov S. T., Pavlyuchenkova S. M., Samoylov A. V., Mudzhiri N. M., Marshak T. L., Rudoy V. M., Dement'eva O. V., Zelenina I. A., Skuridin S. G., Yevdokimov Yu. M.* Bovine sperm chromatin is not protected from the effects of ultrasmall gold nanoparticles // Biology Bulletin. 2013. Vol. 40. P. 493–499.

Поступила в редакцию 27.10.2014 г.