INTERACTION OF DNA WITH SILVER NANOPARTICLES

G.M. GLIBITSKIY,[1] V.V. JELALI,[2] M.O. SEMENOV,[1] A.D. ROSHAL,[3] D.M. GLIBITSKIY,[1] O.YU. VOLYANSKIY,[2] G.G. ZEGRYA[4]

[1]A.Ya. Usikov Institute of Radiophysics and Electronics, Nat. Acad. of Sci. of Ukraine
(12, Academician Proskura Str., Kharkiv 61085; e-mail: glibit@ukr.net)

[2]I.I. Mechnikov Institute of Microbiology and Immunology, Acad. of Med. Sci. of Ukraine
(14, Pushkinska Str., Kharkiv 61057)

[3]V.N. Karazin Kharkiv National University, Institute for Chemistry
(4, Svoboda Sq., Kharkiv 61077, Ukraine)

[4]A.F. Ioffe Physical-Technical Institute, Russian Academy of Science
(26, Polytekhnicheskaya Str., St.-Petersburg 194021, Russian Federation)

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The dehydrational self-organization of DNA with Na^+ and Ag^+ ions and silver nanoparticles has been studied. It has been shown that the character of the formation of dendritic textures (the size of the area occupied on the film surface) is governed by the conformational state of DNA.

1. Introduction

Nowadays, the materials added with silver nanoparticles (SNs) find more and more applications. Silver nanoparticles are added to bandaging materials for medical purposes. They are used in surgery as an antibacterial means [1]. SNs are applied while designing nanocomposite molecular sensors, which are based on the binding of SNs with DNA at the molecular level [2,3]. In connection with the expansion of the SN application scope, there appeared the publications devoted to the researches of the toxicity of this material and its influence on the genetic material in cells [4–7]. The accumulation of SNs in the environment was shown to result in a deterioration of the ecological situation [8]. On the other hand, the data were obtained concerning a possibility to apply SNs to the treatment of oncological diseases [9]. In this connection, the study of the influence of SNs on the change of a tissue state at the cellular level is a challenging task. For today, it has been shown that the mechanism of biological action of silver ions is mainly determined by their interaction with DNA and consists in the creation of complexes with it [10].

Earlier, some researches have been carried out concerning the influence of the dehydration of protein solutions and suspensions of biological liquids (blood and blood plasma), as well as DNA solutions with various metal ions, on the formation of dendritic structures on the surface of films that are formed owing to the dehydration. For instance, in works [11–14], theoretical models for dehydration processes were developed, and the formation of dendritic structures was shown to be caused by inhomogeneities in the gel emerging at the dehydration, the inhomogeneities being formed by protein molecules and blood components.

In work [15], it was shown that the character of textures that are formed at the dehydration of DNA solutions with metal ions and the corresponding area covered by them on the film surface are connected with the mutagenicity of metal ions in the solution used in the film fabrication. In this work, we will carry out a comparative research of the dendritic textures and the occupied areas on the surface of films that are formed at the dehydration of DNA solutions with Na^+ and Ag^+ ions and their nanostructure.

2. Materials and Methods

In the preparation of solutions, we used Na-DNA from calf thymus (Serva and Sigma-Aldrich). The suspension of SNs 4 nm in dimension was prepared at the A.F. Ioffe Physical-Technical Institute of the Russian Academy of Science (St.-Petersburg). The DNA solution was prepared in two stages. First, the DNA specimen was maintained in a solution of the corresponding salt with a required concentration for 12 h at a temperature of 8–10 °C. Then the obtained DNA solution was stirred for 8 h with the help of a laboratory mixer.

Films from DNA solutions with silver nitrate and SNs were fabricated following the technique described in work [16].

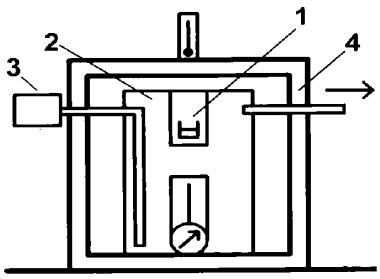

Fig. 1. Diagram of a setup for fabricating the DNA films: glass cuvette for solutions (*1*), vessel with inputs for air pumping (*2*), compressor for air supply (*3*), water thermostat (*4*)

The diagram of a setup used in the film fabrication from the solutions of Na-DNA from calf thymus with a concentration of 0.2 mg/ml and Ag^+ ions and the Na-DNA suspension with SNs is shown in Fig. 1. Cuvette *1* (20×20 mm^2 in size and filled with the solution) was arranged in hermetic chamber *2*, through which air was blown with the help of compressor *3*. The hermetic chamber was located in thermostat *4* (TKh-50). In the chamber, there were also a temperature sensor, a hygrometer, and vessels with silica gel to maintain the relative moisture (RM) of air at fixed levels. All metal salts were of chemically pure grade.

The monitoring of a structural state of DNA in the solutions with Ag^+ ions and silver nanoparticles was carried out with the use of ultra-violet (UV) spectroscopy by analyzing the absorption at the wavelength $\lambda = 260$ nm. At the same time, the structural state of DNA with SNs in films was monitored with the help of infrared (IR) spectroscopy in the interval 900–1800 cm^{-1}, where the reference absorption bands sensitive to the conformation of the sugar-phosphate backbone and the nitrogenous bases of DNA are located.

The spectral properties of SN suspensions with DNA and DNA solutions with metal salts were studied on a spectrophotometer Hitachi U2310. The IR spectra of moistened DNA films with SNs were registered on a double-beam spectrophotometer UR-20 in the spectral interval 1000–1850 cm^{-1}. A NaCl prism with a resolution of 6 cm^{-1} at 1600 cm^{-1} was used. The films were fabricated on fluorite substrates. The films were moistened following standard techniques in a hermetic cuvette with the use of saturated salt solutions [17, 18].

3. Results and Their Discussion

For the sake of comparative analysis at different zooms, the presented figures demonstrate the textures of films obtained at the dehydration from the following solutions:

- Figs. 2 and 3: 0.03 M $NaNO_3$ + Na-DNA;
- Figs. 4 and 5: 5.05×10^{-5} M $AgNO_3$ + 0.03 M $NaNO_3$ + DNA;
- Figs. 6 and 7: 15 μg/ml SNs + 0.03 M $NaNO_3$ + DNA.

The texture area on the surface of the film prepared from the solution with 5.05×10^{-5} M $AgNO_3$ + 0.03 M $NaNO_3$ (Fig. 4) amounts to $32 \pm 2\%$ of the total film area and decreases by 50% in comparison with the texture area on the surface of the film 0.03 M $NaNO_3$ + Na-DNA ($62 \pm 2\%$ of the total film area, Fig. 2). The texture area on the surface of the film obtained from the solution with 15 μg/ml SNs + 0.03 M $NaNO_3$ (Fig. 6) also decreases by 50% ($35 \pm 2\%$ of the total film area) in comparison with the texture area on the surfaces of the film 0.03 M $NaNO_3$ (Fig. 2). Those data bring us to a conclusion that the texture areas diminish owing to the interaction of DNA molecules with both silver ions and silver atoms on the surface of SNs. Since such an interaction has a mutagenic character [19, 20], i.e. it distorts the native DNA structure, we may suppose that the textures on the film are formed by salt composites with DNA molecules in a conformationally changed state.

Those data also testify that a reduction of the areas with silver ions and nanostructures is evidently associated with different characters of the interactions between DNA, on the one hand, and Na^+ and Ag^+ ions and SNs, on the other hand. To elucidate those assumptions, we carried out researches of the solutions used for the preparation of DNA films with silver ions and silver nanostructures in the UV range.

In our opinion, the intensity of the interactions of DNA with silver ions in $AgNO_3$ solutions and with Ag nanoparticles is determined by the number of particles (ions or atoms) that become associated with the DNA. Bearing in mind that metallic silver has a face-centered cubic lattice with a lattice constant of 4086 Å, we can evaluate the number of atoms in a nanoparticle. For instance, a nanoparticle 4 nm in diameter contains about 1500 atoms at the phase interface, i.e. only one third of the total number of atoms in the particle.

Taking into account that silver nitrate completely dissociates in aqueous solutions, i.e. Ag^+ ions completely transit into the aqueous phase at dissolving, we may suppose that, provided the mass concentrations with respect to silver are identical, the $AgNO_3$ solution contains three times as many chemically responsive silver ions as in the solution of nanoparticles.

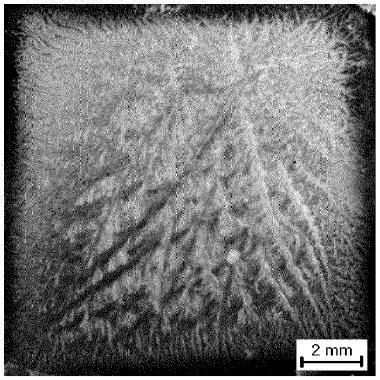

Fig. 2. Photo of the film obtained from a solution containing 0.03 M $NaNO_3$ + Na-DNA. $T = 40$ °C, RM $= 30 \pm 2\%$

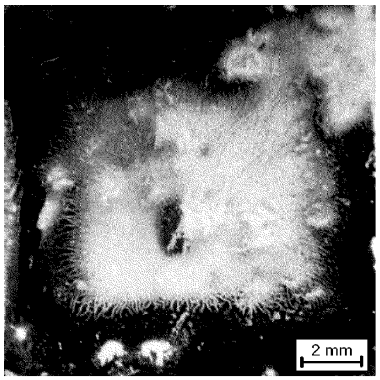

Fig. 3. Photo of the film obtained from a solution containing 5.05×10^{-5} M $AgNO_3$ + 0.03 M $NaNO_3$ + DNA. $T = 40$ °C, RM $= 30 \pm 2\%$

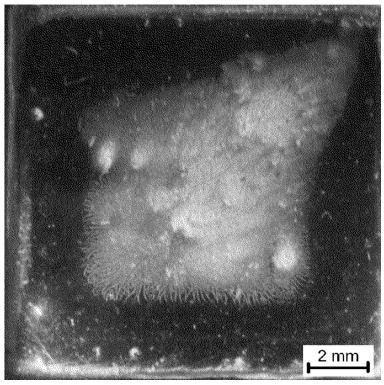

Fig. 4. Photo of the film obtained from a suspension containing 15 μg/ml SNs + 0.03 M $NaNO_3$ + DNA. $T = 40$ °C, RM $= 28\%$

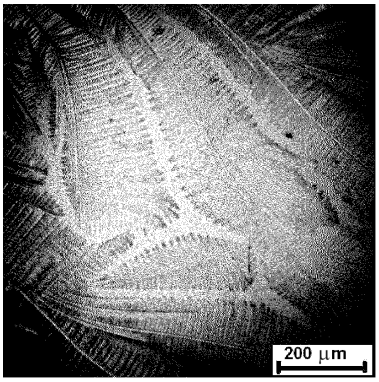

Fig. 5. Zoomed in part of the photo in Fig. 2

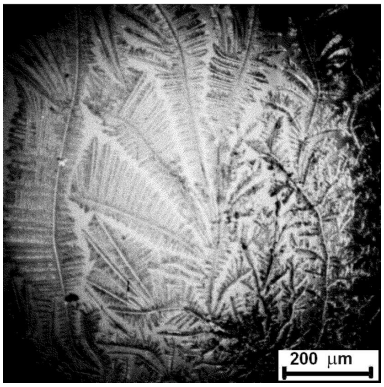

Fig. 6. Zoomed in part of the photo in Fig. 4

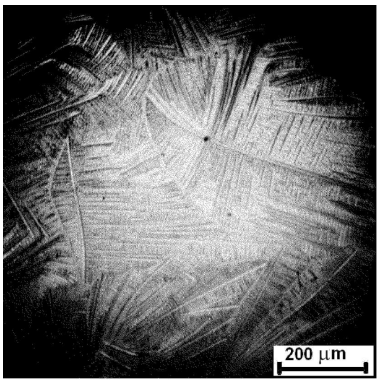

Fig. 7. Zoomed in part of the photo in Fig. 6

The interaction of silver ions in a solution and on the nanoparticle surface with DNA molecules may probably be of the same donor-acceptor character. Therefore, the band positions in UV absorption spectra can appreciably depend on the polarizing influence of ions on the chromophore DNA fragments. Evidently, the solvated Ag^+ ion in a solution exerts a much stronger polarizing action on the DNA molecule than the same ion that is built into the crystal lattice, and the charge of which is partially compensated by the electron gas.

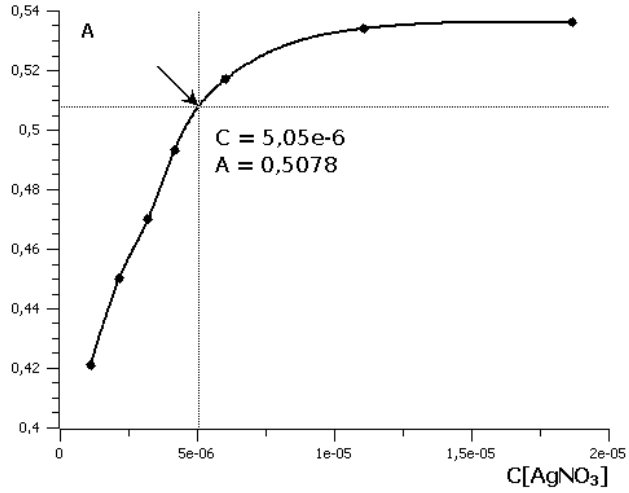

Fig. 8. Dependence of the optical density at the wavelength λ = 260 nm on the Ag^+ concentration

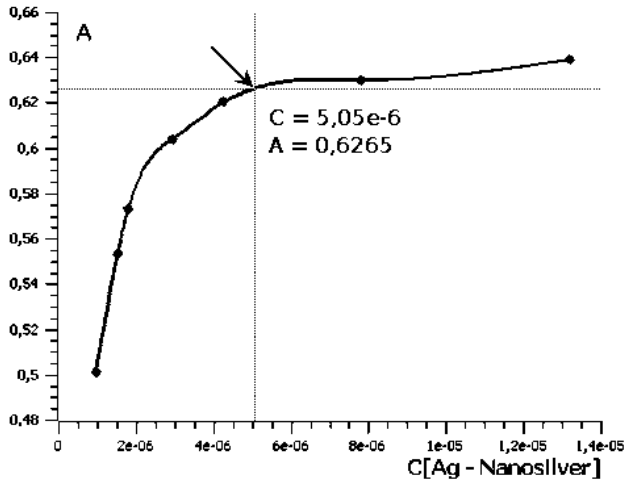

Fig. 9. Dependence of the optical density at the wavelength λ = 260 nm on the SN concentration

Na^+ ions are known to stabilize the DNA structure in a solution, with the hypochromism at the thermal denaturation amounting to 38–40%. To study the DNA structure, we determined the dependences of the optical density on the Ag^+ and SN concentrations at the wavelength λ = 260 nm. They are depicted in Figs. 8 and 9, respectively. One can see that, as the concentrations of silver ions and SNs increase, the intensity of the band grows, which testifies to the destruction of a DNA helical structure. The arrows in the figures point to those concentrations of DNA solution with Ag^+ ions and DNA suspension with SNs, at which the films were prepared. The calculation of the hypochromic effect in DNA at the corresponding Ag^+ and SN concentrations

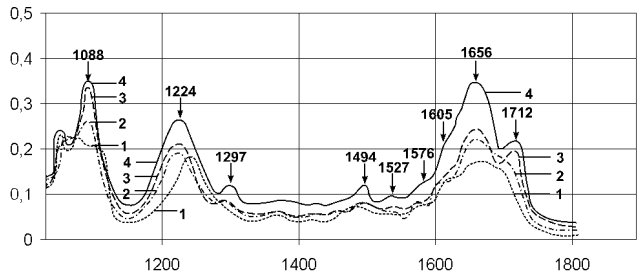

Fig. 10. IR spectra of native DNA at various relative moistures of 0 (*1*), 66 (*2*), 86 (*3*), and 92% (*4*)

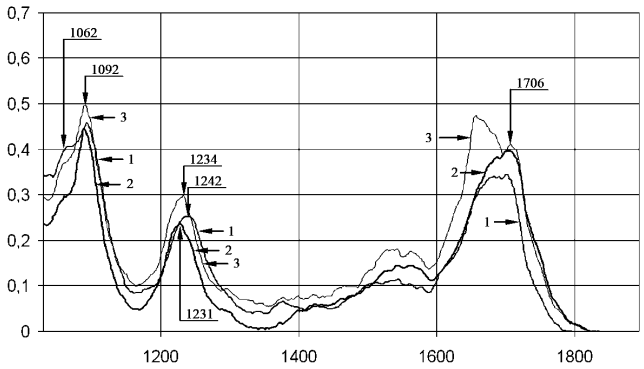

Fig. 11. IR spectra of DNA films with silver nanoparticles at various relative moistures of 40 (*1*), 76 (*2*), and 92% (*3*)

results in a value of 26–27%, which is equal to 70% of the total hypochromic effect in native DNA.

The data obtained testify that the secondary helical structure of DNA was violated, which probably brought about the formation of different texture areas. It is well known [17, 18] that the helical structure of Na-DNA becomes destroyed if the DNA is dehydrated. However, if those films are moistened to a RM of 92%, the helical structure becomes restored.

If the DNA films are formed from solutions with silver ions or suspensions with SNs, silver ions interact with dehydrated DNA groups and, under the influence of the interaction of DNA with Ag^+ ions and SNs, the secondary structure is destroyed further at the dehydration. To confirm this mechanism of destruction of the DNA helical structure by SNs, we studied the IR spectra of DNA films with SNs at various moistures. In Figs. 10 and 11, the IR spectra of native Na-DNA and Na-DNA with SNs, respectively, are depicted. One can see that, as the film moisture grows, Na-DNA transforms into the B-form of DNA at a relative moisture of 92%. This phenomenon is related with the formation of marker bands at RM=92%. Namely, the emergence of the band at ν = 1712 cm^{-1} testifies that nitrogenous bases have formed a regular

inter-pair stack structure of the B-form of DNA. The appearance of the band at $\nu = 1224$ cm^{-1} associated with the antisymmetric vibrations of phosphates and the deoxyribose band at $\nu = 1053$ cm^{-1} also testifies that a sugar-phosphate chain of the B-form of DNA has been formed.

However, as is seen from Fig. 11, the antisymmetric vibrations of phosphates in the DNA film with SNs at the same moisture (RM=92%) revealed themselves at the frequency $\nu = 1234$ cm^{-1}, which is not typical of the B-form of DNA. At the same time, the bands at $\nu = 1712$ and 1053 cm^{-1} are absent. Those data testifies that the helical structure of DNA is destroyed by nanosilver, and water molecules do not restore it. Probably, the interaction between water and DNA (nitrogenous bases and phosphate groups) is much weaker than that between water and SNs. For this hypothesis to be confirmed, further studies are needed.

Hence, the data of UV and IR spectroscopies testify that the character of formed dendritic structures (their area) is determined by the structural state of DNA.

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ВЗАЄМОДІЯ ДНК З НАНОЧАСТИНКАМИ СРІБЛА

Г.М. Глибицький, В.В. Джелалі, М.О. Семенов, О.Д. Рошаль, Д.М. Глибицький, О.Ю. Волянський, Г.Г. Зегря

Р е з ю м е

Проведено дослідження дегідратаційної самоорганізації ДНК з іонами Na^+, Ag^+ і наночастинками срібла. Показано, що характер формування дендритних текстур (розмір площі, що займає на поверхні плівки) визначається конформаційним станом ДНК.