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DNA Conformational Transformations under Hyperthermia

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Пропонується молекулярний механізм перетворень подвійної спіралі ДНК в умовах гіпертермії. Експериментально досліджуються затверділі трьохкомпонентні рідинні системи «NaCl-дистильована вода-сухий препарат ДНК». Показано, що плівки, сформовані при температурах 20˚С та 42˚С, відрізняються за своєю структурою. Встановлено, що імовірною причиною такої різниці є утворення уступів в спіралі ДНК, що виникають в результаті розриву водневих зв'язків під час динамічного фазового переходу при температурі 42˚С.

*Ключові слова: гіпертермія, конфірмаційні зміни подвійної спіралі ДНК***.**

A molecular mechanism of DNA double helix transformations under hyperthermia has been proposed. Evaporation of NaCl – distilled water – DNA system has been experimentally studied. To obtain the textures the aqueous solution with DNA molecules and sodium ions has been used. The textures formed after evaporation are showed to have different structure for the experiments at different temperature (20˚C and 42˚C). The experimental result shows that evaporation of water does not depend on concentration of DNA. The DNA helix bending as a result of rupture of hydrogen bonds in the course of dynamic phase transition in water at temperature of 42˚C is assumed to be a likely reason for this difference. The rupture of hydrogen bonds in water leads to a decrease in the degree of DNA dissociation, which in its turn changes the conformation of these molecules. At T = 42˚C the number of bended areas on the helix axes is much larger than at T=20 ˚C is showed.

Key Words: hyperthermia, DNA double helix transformation .

Статтю представив академік НАН України, д.ф.-м.н., проф. Булавін Л.А.

Introduction

Hyperthermia is a condition when temperature of the body or its areas increases over that which is required for normal metabolism and body functions as a result of external influence. It should be noted that in accordance with the generally accepted classification the states of hyperthermia are divided into three groups. For the first group the temperature is within the range 38-39 ºC, for the second group it ranges 39-40 ºC, for the third group it exceeds 41.1 ºC. The last group is the most dangerous condition as it is very close to the upper limit temperature which threatens the human life [1]. Currently, there is no accurate information about the statistical data on occurrence of pathological conditions in the human body under the influence of high temperature. The danger of this physiological state is related to the fact that the qualitative disturbances of biological processes occur in the human organism [2, 3].

Hyperthermia is widely used in oncology. To create a hyperthermia state there is a lot of methods (laser, electromagnetic, etc.) [4]. However, as it is noted in [5], today, the application of them does not solve a number of problems associated with the preservation of the functional state of tissues under external influence. In [6], the hypothesis about the properties of cancer cells to apoptosis and about DNA damage has been forwarded. Understanding the molecular processes occurring during metastasis paves the way for developing effective approaches to

treatment and prevention of cancer tumors. This paper deals with attempts to identify possible transformations in DNA conformations under conditions of hyperthermia.

Materials and methods

The object of study is a two-layer system in which the first layer is a glass plate and the second layer is a dried film of aqueous system distilled water-NaCl-DNA. The samples have been selected in such a way as to study the interaction of hydrogen ions from the solution with the ions of the substrate. For such interaction the result of molecular rearrangements is textures formed on the surface after evaporation of liquid [7]. The ions on the glass surface play an important role in the formation of textures [8].

To obtain the textures the aqueous solution with DNA molecules and sodium ions has been used. The solution has been prepared by dissolving dry DNA powder in distilled water. For this experiment the DNA molecules extracted from salmon testis with molecular mass of one molecule being equal to 10^7 a. u. m. have been used. This study has been carried out for the DNA solutions with concentrations 0% - 0.2%.

The aqueous solution with different concentrations of DNA is dropped on a glass plates manufactured by PJSC *Stekloprибor* (dimensions 25,4 × 76,2 mm and a thickness 1-1.2 mm). The drop mass has been determined through comparing the mass of the glass plate and the glass plate with solution, with the use of scales PS110/C/1. The drop is dried in the air tank at a constant temperature with connection to a digital thermostat TP2. The experiment has been carried out at temperatures of 20 ˚C and 42 ˚C. After formation of the texture on the glass substrate its mass has been measured by the scales PS110/C/1. With the help of an optical system comprising a microscope MBC-9 and a camera with photo eyepiece Canon PC1355 the texture images have been obtained.

Experiment results

Figure 1 shows the concentration dependences of change in mass of samples after drying (the difference between the mass of drop on glass m_1 and mass of texture m_2) at temperature 20˚C and 42˚C. These dependences are marked as Δm^{20} Δm^{42}, accordingly.

As one can see from Fig.1, at T = 20˚C, the mass of texture formed after evaporation for different concentrations of DNA remains practically the same. The random errors have been caused by the fact that in this experiment it is impossible to drop a liquid of precise mass on the glass surface.

The experimental result shows that evaporation of water does not depend on concentration of DNA. Fig. 2-3 show the textures formed at 20˚C and 42˚C.

The following experimental facts can be seen from Figure 2.3:

1) The salt aggregates obtained at T = 20˚C and T = 42˚C after evaporation of solutions without DNA appear as structures chaotically located on the surface of the substrate;

2) The salt aggregates obtained at T = 20˚C and T = 42˚C after evaporation of solutions containing DNA are structured as the textures characterized by cylindrical symmetry, the elements of which

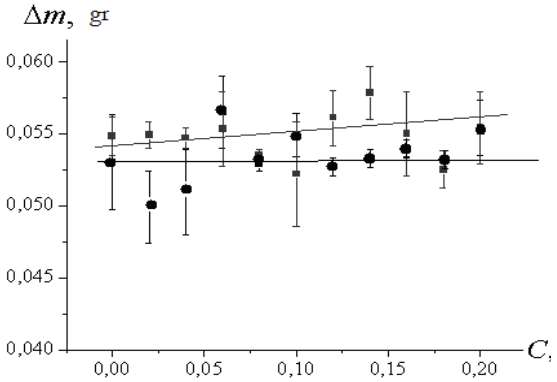

Fig. 1. Concentration dependence of change in mass of the samples at ●20˚C, and ■ 42 ˚C.

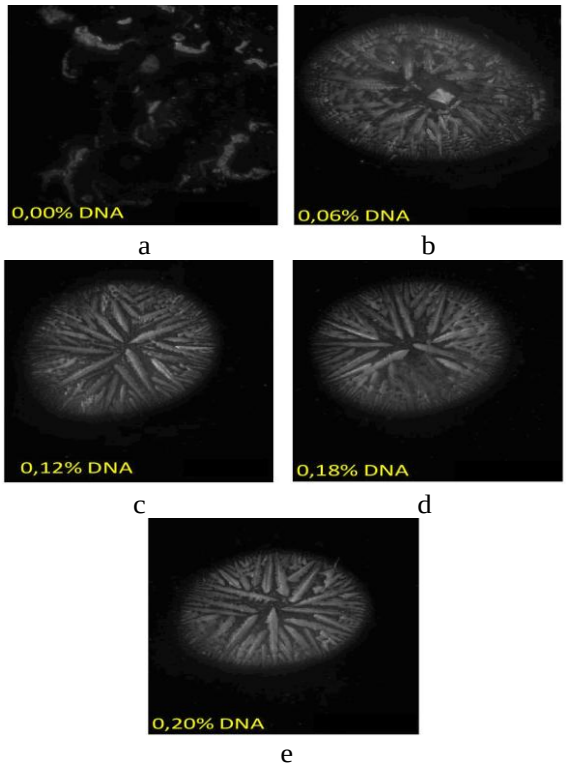

Fig. 2. Images of textures formed after evaporation of DNA water solution of different concentrations at T=20˚C (enlarged 9 times).

resemble rectangles with radially directed long axis; 3) At T = 20 ˚C, the number of much smaller texture fragments is larger than in the case of T = 42˚C.

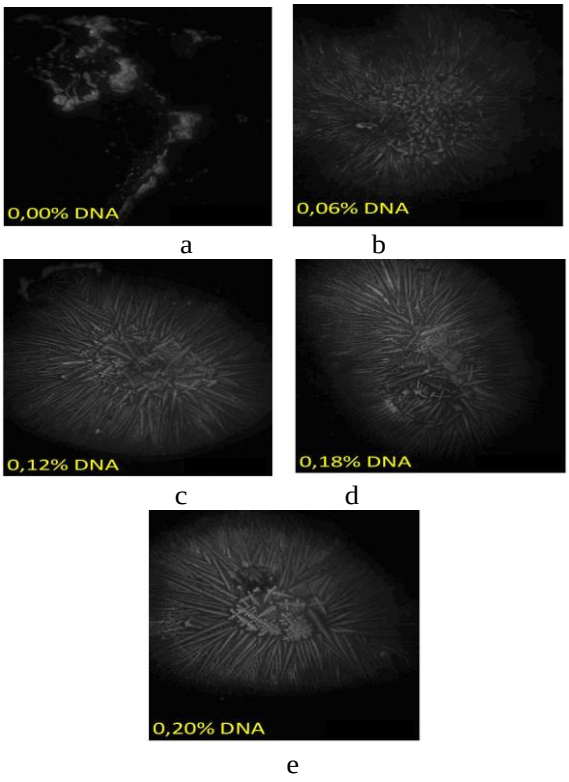

Fig. 3. Images of textures formed after evaporation of DNA water solution of different concentrations at T = 42˚C (enlarged 9 times).

Discussion

First of all, we try to explain why after evaporation of solutions with and without DNA the textures have different structures (Fig. 1 and Fig. 2).

In fact, in our experiment, we study crystallization of NaCl solution. It is known [9] that crystallization proceeds in two stages: formation of crystalline nucleus and its subsequent growth. Let us analyze the possible mechanisms of nucleus formation. NaCl solution contacts with the glass substrate. It is known [10] that the glass surface can have local defects (e.g. microscopic cracks, etc.). Broken structure in the defect area provokes the formation of so-called silanol groups SiOH. The defect area is an area where the crystalline nucleus is the most likely to appear. For this it is necessary that the particles of crystallizing substance can join a SiOH group. In the absence of DNA neither Na+ nor Cl- ions can do this. So, we can conclude that in the absence of DNA the NaCl crystallites are formed in the solution. After evaporation these crystallites precipitate on the substrate forming a chaotic structure observed in the experiment (see Fig.2a and Fig.3).

A different picture is observed for DNA water solution. Having oxygen in the phosphate groups this macromolecule can form hydrogen bonds with the silanol groups of substrate surface. Assuming the existence of a crystalline nucleus on the DNA molecules the growth of the nucleus occurs due to more and more ions joining Na + and Cl-. The main difference of this mechanism is that proliferation of crystallites takes place along the surface. In other words, we deal with epitaxy [10]. The fact which confirms the epitaxial mechanism of crystallization is cylindrical symmetry of the structure. It is known [10] that the process of crystallization is governed by temperature and concentration gradients, so that the direction of the crystallite growth turns out to be parallel to the direction of these gradients. In our case, the above gradients have radial direction. The long axes of rectangular texture fragments of salt aggregates have the same direction (Fig. 2c-2e, 3c-3e).

What are the nuclei of crystallization on DNA molecules? It is known [11] that in aqueous DNA solutions the DNA molecule dissociates splitting into the double helix with the negative ions O from phosphate groups and the positive ions Na+ surrounding the helix and forming its ionic atmosphere. On these double helices which are the decay products a certain number of Na atoms remains. The potential nuclei of crystallization arise when several sodium atoms are located close to each other. Clearly, such an arrangement can be achieved if: 1) the above mentioned atoms remaining on the double helix (Fig. 4) belong to the neighboring phosphate groups and 2) in this region the straightness of helix axis is broken to bring the sodium atoms closer to each other. More precisely, the second condition is not an independent requirement: it is automatically realized if the first condition is true.

It is well known [11] the DNA - molecule in the solutions has coil's configuration.

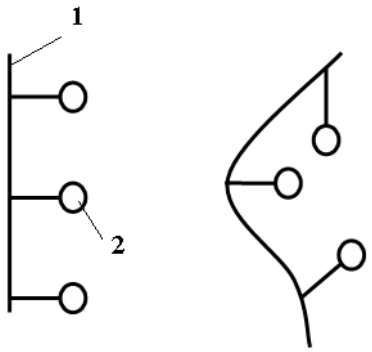

Fig. 4. Bending of the helix axis by residual sodium atoms (1 - helix axis, 2 - Na atoms)

This configuration consists of series-connected with each other segments. Each segment has the shape of a double helix, the axis of which is straightforward. The same is observed in solutions of DNA. This property is a result of the repulsive Coulomb forces acting between the oxygen ions of

phosphate groups. Neutralization of these ions by the positive sodium ions causes a decrease in these repulsive forces i.e. weakens the cause for the straightness of the helix axis. The residual Na atoms located one after the other (as shown on Fig. 4b) may weaken the force of repulsion in such a way as it can bend the helix axis at this point.

Each of the crystallites (rectangular texture fragment) on Fig. 2-3 grows from its nucleus. If so, in our experiment the amount of crystalline nuclei at T = 42˚C is much larger as compared with that at T=20 ˚C. According to the mechanism showed on Fig. 4 this means that in the first case the number of bended areas on the helix axes is much larger. Thus, at temperature T = 42 ˚C conformation of DNA molecules undergoes significant changes. What does cause these changes?

In [12, 13] it has been showed that in water at temperature T = 42 ˚C there is a dynamic phase transition. This shift significantly changes the structure of water, particularly, at T = 42˚C a lot of hydrogen bonds is broken. We guess that this is just that factor which causes conformational changes in DNA at the mentioned temperature. Indeed, the rupture of hydrogen bonds in water leads to a decrease in the degree of DNA dissociation, which in its turn changes the conformation of these molecules.

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