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ЕРЕВАНСКИЙ ФИЗИЧЕСКИЙ ИНСТИТУТ
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MECHANICAL PROPERTIES OF DNA FILMS

ЦНИИатоминформ
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ԴՆԹ-ի ԹԱՂԱՆԹՆԵՐԻ ՄԵԽԱՆԻԿԱԿԱՆ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԸ

Ցույց է տրվել, որ ԴՆԹ-ի թաղանթի ցունգի մոդուլը ($E = 0,02 - 0,025$ գն/մ^2) հիդրատացիոն ջրի քանակի նվազման հետ, աճում է մինչև $E = 0,5 \div 0,7$ գն/մ^2 արժեքը: E -ի կախումը հիդրատացիայից ունի քարդ, ոչ միանվագ բնույթ: Դենատուրացված ԴՆԹ-ի թաղանթների ցունգի մոդուլը մեծ է նատիվից: E և Θ փոփոխման բոլոր առանձնահատկությունները, որոնք նկատվում են նատիվ ԴՆԹ-ի թաղանթների մոտ՝ դենատուրացվածների մոտ անհետանում են: Նատիվ և դենատուրացված ԴՆԹ-ների հիդրատացիայի իզոթերմերը տարբերվում են, և կախված են դենատուրացման ձևից: Հայտնաբերվել է, որ ջերմաստիճանի աճի հետ՝ ավելի քան $\frac{1 \text{գ } H_2O}{\text{գ չոր ԴՆԹ}}$ պարունակող թաղանթների ցունգի մոդուլը կրում է՝ հիդրատացիայի փոփոխությամբ ուղեկցվող մի շարք թռիչքաձև փոփոխություններ: Ջրի փոքր քանակության դեպքում $< 0,3 \frac{\text{գ } H_2O}{\text{գ չոր ԴՆԹ}}$ E -ի փոփոխությունը՝ կախված ջերմաստիճանից, դառնում է սահուն: Հալման ջերմաստիճանը կախված է ջրի քանակից:

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INTRODUCTION

The DNA rigidity problem is an important one in the physics of biopolimers. This parameter is of great importance for quantative understanding of the microscopic properties of DNA, its packing in chromosomes and virus particles, properties of circular closed and superhelical DNAs.[1,2,3] A number of experimental and theoretical works[4-13] are devoted to the study of this problem. In DNA rigidity calculations based on experimental data, the rigid stick model is used and, consequently, the problem of persistent length arises. In the experiments on the study of mechanical properties of DNA in solution, there is mainly observed one of the conformations of DNA, the B-form. It is also known, that in the solid phase (films, oriented fibres), depending on the environment (hydration, temperature), DNA may be in various conformational states. The works using the DNA microwave absorption are of special importance in the experiments on the study of DNA rigidity.[14,15] In one of the recent works of this series[15], they spoke of a frequency dependence of the mechanical properties and the author said it was "paradoxical" that at high frequencies water acquired properties of solids. But, is it water solely that changes its properties at high frequences? We think it is not. Frequency dependence of the mechanical properties of many polymers is studied in detail.[16] This phenomenon is also studied in case of biopolymers, e.g., proteins.[17] One could suppose there was such dependence for DNA.

The purpose of this paper is to study the DNA viscoelastic properties in the frequency range from 50Hz to 20kHz. At such frequencies free water can have no "paradoxical" mechanical

properties. As the DNA films are compounds consisting of DNA and water, we have also investigated their hydration isotherms.

MATERIALS AND METHODS

To measure the Young's dynamical modulus E and the logarithmic decrement ϑ of DNA films, V.N. Morozov's micromethod was used.[18,19] The method is based on the analysis of resonance transverse vibrations of plates supported as cantilevers. A method and an experimental chamber for measuring E and ϑ as functions of temperature are described in Ref.[17] A hydration isotherm measuring method allowing to study microobjects weighing up to 0.02mg, is described in Ref.[20]

Amorphous films of cattle spleen and calf thymus DNAs were investigated. The films were prepared by slow drying of DNA saturated water solution on a teflon sheet, at 10°C. There were obtained from 5 to 20 μm-thick films which were cut to form 0.5-2.0mm long and 0.05-0.1mm wide rectangular plates. Samples of that size were supported as cantilevers, and the resonance frequencies of their transverse vibrations were in the range from 50Hz to 20kHz. One can change the resonance frequency by changing the sample's length. These procedures are comprehensively described in Ref.[17] The relative humidity from 97 to 32% was supported by $CaCl_2$ water solution of different concentrations. The relative humidities of 15 and 10% were obtained with the help of LiCl and $ZnCl_2$ saturated water solutions, respectively. For heating up hot air was blown into the chamber radiator. The heating rate was 1 deg/min. The chamber temperature was measured by a copper-constantan thermocouple. The measurement accuracy was 0.1°C.

FREQUENCY DEPENDENCE OF E AND ϑ

Dependences of E and ϑ on frequency were measured. The frequency was changed by fractional shortening of the samples. Thus we succeeded in changing the transverse vibrations resonance frequency within 50Hz and 20kHz.

The experiments have shown that it is only at high humidities ($A \geqslant 85\%$) that E increases with the frequency. The lower the humidity, the weaker the frequency dependence. At frequencies lower than 200Hz, at $A \geqslant 85\%$, there is observed a strong decrease of E and an increase in ϑ. All the further investigations were carried out at frequencies $>$ 200Hz, where E depends on the frequency weakly.

DEPENDENCE OF E AND ϑ ON HYDRATION

In Fig.2 the Young's modulus and the logarithmic decrement of an amorphous film of calf thymus DNA are shown as functions of relative humidity at 25°C, A=95%, $E=0.02 \cdot GN \cdot m^{-2}$. For comparison note, that for protein amorphous films $E \approx 1$ GN m^{-2} (at the same relative humidity)[19], i.e. two orders of magnitude higher than it is for DNA. As it is seen from Fig.2, the Young's modulus essentially increases with decreasing relative humidity. This increase takes place through a number of transitions followed by plateaus. According to literature, in solid state, at $A \geqslant 95\%$, the B-form of DNA is observed. The B-A transition region is not a monotonic one and, probably, this transition takes place not smoothly. The region of $A < 60\%$ is also characterized by a monotonic behaviour of E. This is the case with all the samples (8 experiments).

The logarithmic decrement, which characterizes the internal friction in samples[17,18] at the sites of these transitions, has marked extrema.

E and ϑ of cattle spleen DNA films were also investigated (Fig.3). It is seen that here too the rigidity strongly increases with decreasing hydration. Despite the fact that at high humidity (A=90%) these films are somewhat softer than the preceding ones, at low humidity (A=30%) they become more rigid. The monotonic behaviour of these samples is somewhat different from the preceding ones.

The reversibility of dehydration was also checked (Fig.4). In the counter process (hydration) a hysterisis is observed - E is larger than it is at dehydration, but the peculiarities of a direct process recur.

EFFECT OF THERMAL DENATURATION

As it is known, the DNA helicity stability is specified by different intra- and intermolecular interactions. These interactions are affected by hydration, temperature.[21] We have also studied the effect of denaturation on the viscoelastic properties of DNA films. As hydration of DNA films depends on the temperature, salt content, molecular ratio of anions to cations, we have also investigated the DNA films hydration isotherms.

We ahve earlier[20] developed a micromethod allowing to study the hydration of samples weighing from 0.1 to 0.01mg with an accuracy of 0.1%. The method is based on the analysis of the transverse resonance vibrations of a microrod supported as a cantilever, to the free end of which the sample is fastened.

Bronze rods with rectangular cross section were used in this work. Such rods of 10 μm constant thickness, 200 μm width and 1÷2mm length, have 1÷2kHz intrinsic resonance frequency of transverse vibrations. Test DNA films were prepared directly on the free end of the rod by drying the DNA saturated solution on it.

Isotherms of hydration of native DNAs denatured in solution and in solid phase were studied. For denaturation in solution the DNA water solution was boiled for 30 minutes. For denaturation in solid phase, the native DNA film was kept at t=150°C for 30 minutes. All the experiments were started at high humidity, A=95%, which then was gradually lowered. For complete removal of water (A=0%) the sample was heated up to 150°C and kept at that temperature for one hour. The water content estimate was made relative to the point of A=0. The counter process (from A=0% to A=95%) was not studied, because at that temperature the sample was denatured. Fig.5 shows the isotherms of hydration of cattle spleen DNA films. As it is seen, the isotherm of hydration of DNA denatured in solution strongly differs from the other two ones. It can be explained by the fact, that in solution DNAs are completely denatured rolling up into coils and this, in its turn, leads to their tighter packing up in the solid phase. Native films and those denatured in solution differ from each other slightly, but these differences are experimentally proved. Probably, the intermolecular contacts do not allow the molecules to roll up into a coil. It is known that the first water surface layers are formed at low humidity. The definite difference in water content of the three isotherms testifies to the fact, that the intermolecular contacts in these films are different too.

Fig.6 shows the dependence of E on the humidity of different cattle spleen DNA films. These curves have different behaviours. Native films display nonmonotonic behaviour with changing humidity. These peculiarities vanish in case of DNAs denatured in solution, while in case of those denatured in the solid state they are partially conserved. The behaviour of the films denatured in solution reminds one the analogous dependence for globular proteins.[19] The Young's modulus of a denatured DNA film and that of native globular proteins are of the same order: at A=95%, E=0.2 and 0.5 GN m^{-2}, respectively. Globular biopolymers are probably characterized by much larger Young's moduli than the helical ones are. The loss of the peculiarities of E(A) at denaturation indicates to the fact, that it is a manifestation of intramolecular conformation changes. To this indicates also the smoothing of the logarithmic decrement curves at denaturation (Fig.7). The solid-phase denaturation at t=150°C does not take place completely. The similarity of curves 1 and 2 in Figs. 6,7 is an indication of that. The intermolecular contacts in dry films do not let the molecules to completely roll up into a coil and be packed up tighter as it takes place during denaturation in solution.

PREDENATURATION EFFECTS IN DNA FILMS

The study of the helix-coil transition in nucleic acids may provide information about intramolecular processes characterizing the structure of molecules. We have also studied the relation of DNA films viscoelastic properties with intramolecular structural transformations taking place at thermal denaturation. The experiment was carried out as follows. A rectangular plate cut

off from a DNA film and clamped in micropincers was placed in a chamber where the required humidity was created. During 10-12 hours the chamber was kept at t=25°C. Then it was sealed and the experiment began. Hot air was blown into the radiator to heat up the chamber. The temperature change rate made 0.5 deg/min. The temperature was measured by a copper-constantan thermocouple, the end of which was put into a Dewar vessel full of a water+ crushed ice mixture with t=0°C. The measurement accuracy was 0.1deg.

In Fig.8 the Young's modulus is presented as a function of temperature. E is normalized to the value at t=25°C, at which A=95% humidity was created, the water content being h=1.3g H_2O/ g dry DNA. It is seen that for humid samples the module undergoes a number of step-like changes. In the top right corner of Fig.8 a curve is presented, which characterizes the reversability of these jumps. If the films are not heated up to the denaturation temperature (when the sample is heated up to 75°C), then in the counter process (cooling) these jumps are not repeated. It should be noted that, when cooling, the behaviour of already heated samples depends on the degree of hydration. Recurrence of experiments is a very good one at $A \leqslant 65\%$. The higher the humidity, the worse the recurrence. But even at preliminary humidity of A=95%, when the melting curve has the most distinct jump-like form, there is some recurrence. The results of three different experiments are presented in Fig.9 . It is seen, that they roughly coincide (in Fig.9 these sites are marked by arrows). In Ref.[21] by means of IR-spectroscopy, at much the same temperatures, they observed changes in the saccharophosphate skeleton. It is probable that the DNA films mechanical properties changing too is an indication of intramolecular changes.

Curves for DNA films melting at A=55% and 75% are presented in Fig.10 . It is seen that for a dry sample the Young's modulus smoothly changes and at the moment of DNA denaturation E begins to increase. It is probably due to intermolecular cross-links formation under such conditions. The vanishing of step-like transitions at low humidities suggests, that they are an indication of intramolecular changes, for it is known that at low humidities the DNA molecules are partially "broken".

To intramolecular changes also indicates the dependence of the logarithmic decrement on temperature (Fig.11). Fig.11 presents the values of ϑ for different water contents at A=95%, h=1.3g H_2O/g DNA and A=55%, h=0.1g H_2O/g DNA. As it is seen, at high humidities ϑ undergoes pronounced breaks which vanish at low humidities. The logarithmic decrement is proportional to the internal friction, and the conformation changes in molecules affect the internal friction. It could be affected by intramolecular interactions. But, probably, their contribution is low, A=55% . When heating at A=55%, intramolecular cross-links are formed which lead to an increase in E (see Fig.10), but the logarithmic decrement remains unchanged (see Fig.11).

The viscoelastic properties of DNA films depend on hydration (see Figs.2,3). That is why the DNA film hydration changing was measured during the heating up. The measurements were carried out by the method described in Refs.[17,20]

In Fig.12 data for the wettest samples, A=95% and A=75%, are presented. As it is seen, when heating up films with water content of h=1.3g H_2O/g DNA (A=95%), hydration changes jumpwise and up to t=90°C is throughout lower than at the initial point of t=25°C. It should be noted that in literature there are data

on the DNA films hydration changing at heating,[21] but the curves presented there had been obtained when the chamber's airtightness was upset. We put a test and obtained similar curves. Thus, under conditions of our experiment, even at water content of 1.3g H_2O/g DNA, there is observed a 5-6% desiccation. At humidities lower than 55% this change is even smaller (1%). But, as it is seen from Figs.2,3 , the decrease of hydration leads to an increase in the Young's modulus. And the changes observed in E at heating up can be explained only by changes in the properties and interactions of molecules.

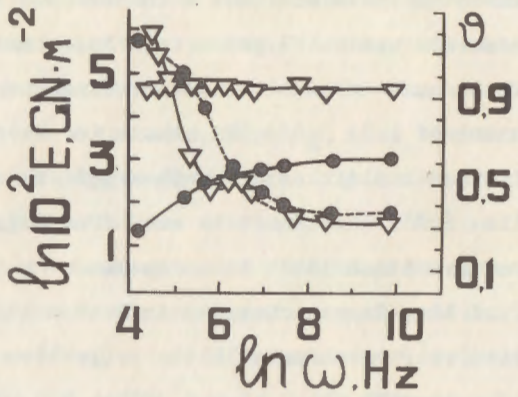

Fig.1

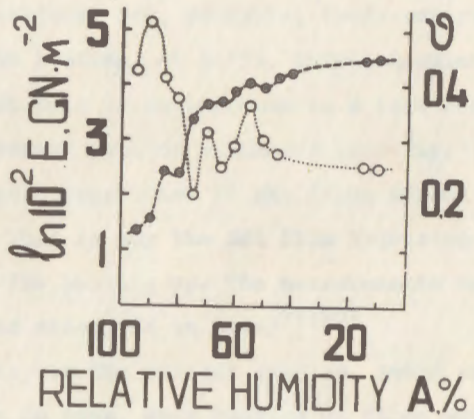

Fig.2

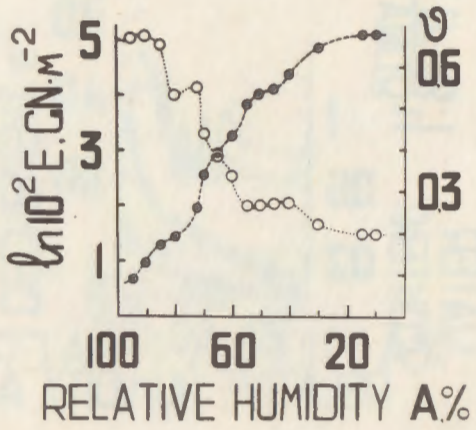

Fig.3

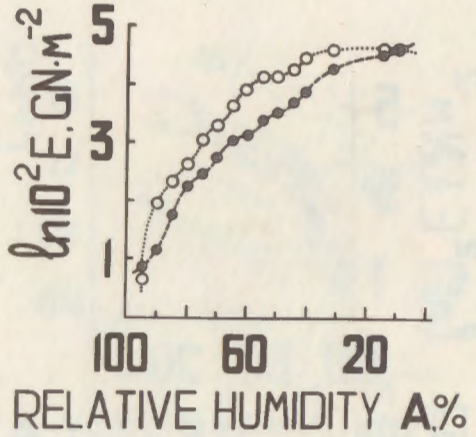

Fig.4

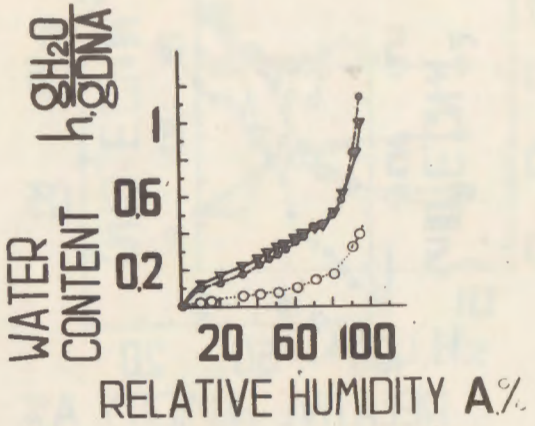

Fig.5

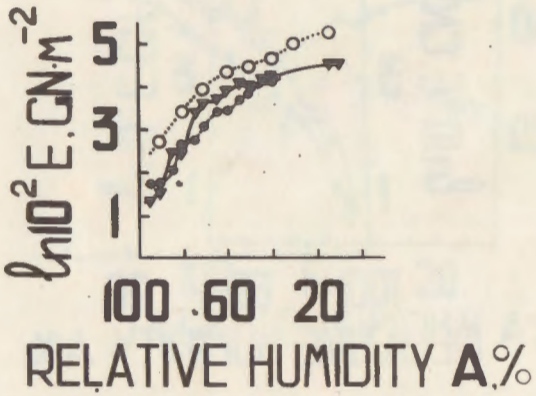

Fig.6

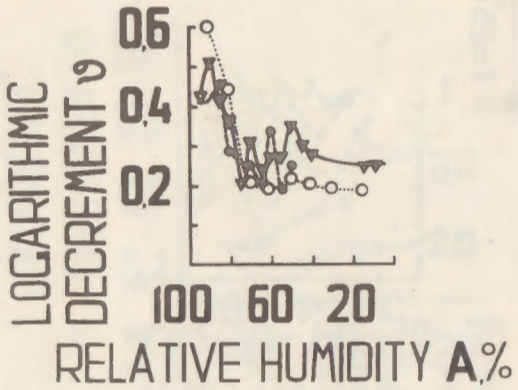

Fig.7

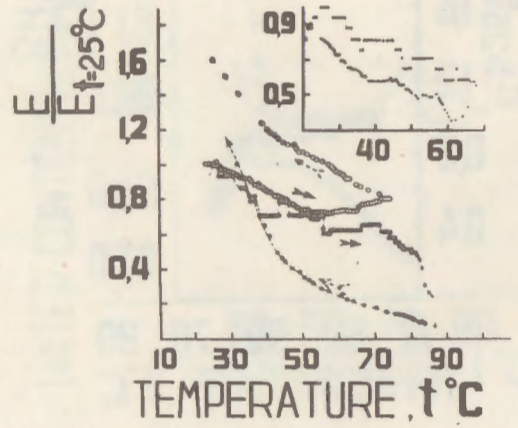

Fig.8

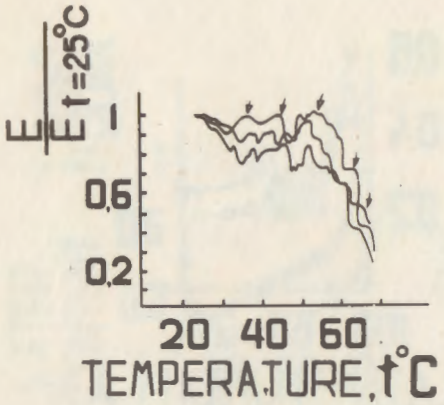

Fig.9

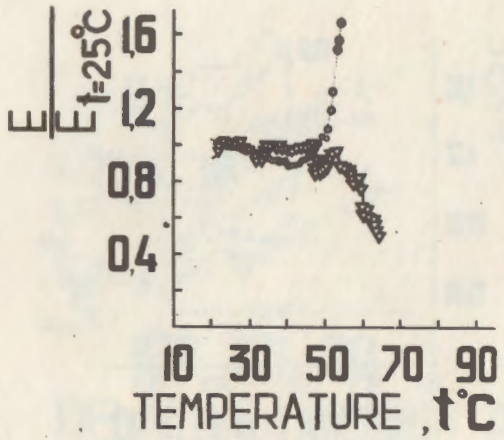

Fig.10

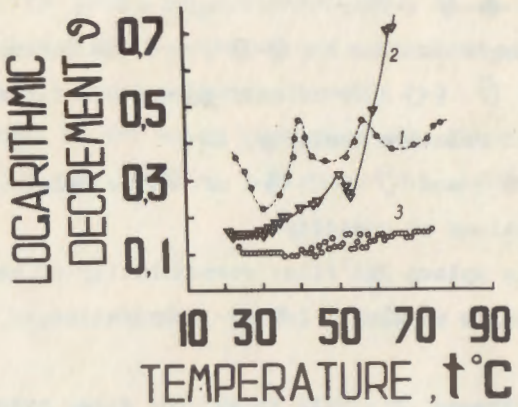

Fig.11

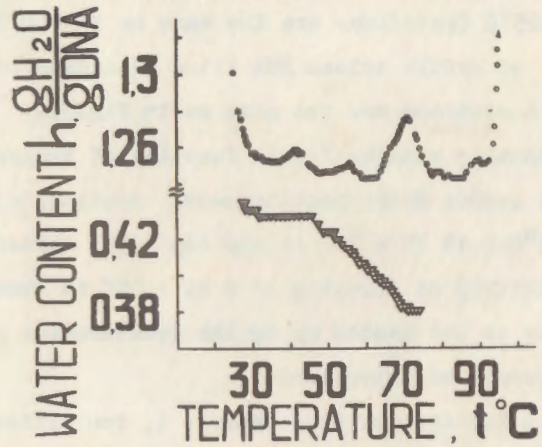

Fig.12

FIGURE CAPTIONS

Fig.1 E and ϑ as functions of frequency at different humidities: -●—●- A=95%; -▽—▽- A=50% .

Fig.2 The Young's modulus E (-●—●-) and the logarithmic decrement ϑ (··○··○··) of calf thymus DNA films as functions of relative humidity, A% .

Fig.3 E (-●··●-) and ϑ (··○··○··) of cattle spleen DNA films as functions of humidity.

Fig.4 A cattle spleen DNA film: reversibility of changes in the Young's modulus E (-●—●-)dehydration, ··○··○·· hydration).

Fig.5.The isotherms of cattle spleen DNA films hydration at t=25°C: 1)-△—△- native, 2)-●··●- denatured in film, 3)··○··○·· denatured in solution.

Fig.6 E of cattle spleen DNA films as a function of humidity at t=25°C (notations are the same as in Fig.5).

Fig.7 ϑ of cattle spleen DNA films as a function of humidity (notations are the same as in Fig.5).

Fig.8 The Young's modulus E as a function of temperature:
1) at A=95%; -●—●-; heating; ➔ cooling; ⇢
E_t=25°C=2.18 GN m^{-2} . In the top right corner the reversibility of changing of E at A=95% is shown: the sample is not heated up to the denaturation point.

Fig.9 Recurrence of experiments:
E as a function of temperature, t, for different samples. The preliminary humidity A=75% .

Fig.10 The Young's modulus E as a function of temperature:
1) -▽—▽- A=75%, E_t=25°C=0.28 GN m^{-2} ;
2)-○—○- A=55%, E_t=25°C=0.76 GN m^{-2} .

Fig.11 The logarithmic decrement as a function of temperature:
1) A=95% ; 2) A=75% ; 3) A=45% .

Fig.12 Changes in the water content of DNA films at heating:
1)-○—○- at A=95% ; 2) -▽—▽- at A=75% .

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