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Low-frequency dynamics of DNA in Brillouin light scattering spectra[1)]

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Brillouin light scattering studies of deoxyribonucleic acid (DNA) in the temperature interval 297–375 K are presented. DNA fragment (119 bp) from the 1st intron of *D melanogaster limk1* gene with AT-rich insertion (28 bp, mutant agn^{ts3}) was used as an experimental sample. The temperature dependence of the hypersound velocity was found to exhibit anomalies in the vicinity of 347 and 335 K. Computer modeling of possible conformational states which might be attained by the DNA fragment under study has shown the existence of local structures that evolve with varying temperature. Combined analysis of experimental data and results of the modeling reveals a close relation between the anomalous behavior of Brillouin light scattering spectra and conformational DNA dynamics. The results are discussed in the framework of modern models of conformational DNA transformations.

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Since T.G. Morgan proposed the chromosome theory of heredity in 1914 and J. Watson and F. Crick discovered the double helix of deoxyribonucleic acid (DNA) in 1953, it was believed that the genetic material of chromosomes consists of genes harbored by DNA playing a role of only passive storage of genetic information. However, it turned out that through structural reconfigurations the double helix of DNA molecule can change its accessibility to the proteins that control gene expression. Local changes in the DNA conformation with deviations from the classical B-structure, accompany many cellular processes, such as transcription, replication, reparation, etc. Among nonconventional DNA conformations are hairpins, bubbles, triplexes, quadruplexes, and cruciform structures [1] (for more details see Supplementary materials). These local changes in DNA structure are promoted by alterations in thermodynamic parameters of a system (temperature, pressure, etc.).

An increase in temperature (from the room one) affects the local DNA structure and finally leads to such conformational transition as its melting, i.e., the distortion of the secondary ordered DNA structure due to strand separation in the double-stranded DNA. Thus, above the melting temperature DNA exists in form of single strands which can interact with each other solely on the basis of complementation between A and T, C and G, wherever they might encounter. According to the classical description of DNA melting, it is the process of transformation of a regular double helix of the DNA linear molecule into the coiled state [2]. The sugar-phosphate backbone together with the nitrogen bases acquires a higher freedom of rotation. A statistical segment includes 7 links, the distance between links in a single-stranded DNA being 6.8 A. Such a strand is referred to as a statistical coil. The helix-coil transition is reversible. As temperature decreases, two strands again unite thus forming a double helix, the distance between the nitrogen bases again being 6.8 A. However the Van der Waals interaction between pairs of bases is energetically favorable, and the distance reduces to 3.4 A. To come closer to each other, neighboring pairs rotate at an angle of 36°. In the helix state the double-stranded DNA is a rather rigid molecule with an extremely low freedom of rotation around individual bonds. A small flexibility of a strand is retained and the statistical segment increases 40-fold compared to a single-stranded DNA reaching approximately 300 base pairs. Melting of high-molecular DNA occurs near to 350 K depending on the distribution of AT and GC pairs along the molecule. The melting temperature grows proportionally to quantity of GC pairs. A simplest characteristic of the process is melting temperature T_m, defined as a temperature at which one half of DNA links are in a coiled state.

Phase (conformational) transformations of DNA are closely related to changes in its functioning. Phase transformations of biopolymers imply changes in the

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spatial (including local) structure of a macromolecule as a response to external factors (temperature, pressure, etc.). Recently, conformational transformations of DNA started to be intensively investigated by physical methods (see e.g. [3–5]), but the behavior of the low-frequency region of the vibrational spectrum of DNA is still poorly studied. At the same time, the frequency region from 1 to 1000 GHz reflects at best the local conformational changes, i.e., hops of atoms from one configuration to another and collective excitations. The biopolymer dynamics in this frequency region is similar to dynamics of glasses, overcooled liquids and other condensed media. For example, the vibrational spectra of proteins exhibit a boson peak, fast anharmonic motions similar to a fast picosecond relaxation in glasses, etc. [6]. Noteworthy, a soft Raman active mode was found in studies of phase transformations of DNA from A to B form under varying humidity [7]. These data were obtained with a high-contrast 6-pass Fabry-Perot interferometer enabling to trace mode softening from 30 to 15 cm^{-1}. High contribution of elastic and quasielastic light scattering in this frequency region makes Raman spectroscopy hardly amenable. On the contrary, the technique of Brillouin light spectroscopy covering this low-frequency region (1 to 1000 GHz) is more appropriate for studies of conformational DNA dynamics under varying external thermodynamic parameters [8].

Here we report the first results of Brillouin light scattering studies on low-frequency conformational dynamics of DNA from *Drosophila melanogaster* in the temperature interval 293–380 K covering the melting temperature.

The object of the studies was DNA fragment 119 bas pairs (bp) long from the 1st intron of *D melanogaster limk1* gene from mutant agn^{ts3} (thereafter DNA agn^{ts3}). *Drosophila* mutant agn^{ts3} is a mutation in the *agnostic locus*. The mutant manifests extremely high rate of amyloid-like inclusion formation in the brain and other tissues and drastically impaired learning and memory. Paradoxically, all these defects are abolished following 30 min exposure of an animal at 37 °C (310 K) [9, 10]. We have sequenced the *limk*1 gene (GeneBank: D*limk*1_*agnostic*ts3 – JX987487). Compared to the genome sequence, LIMK1 of the mutant carries the insertion of 28 bp in 1st intron partially homologous to dme-miR-1006.

The melting temperature (T_m) for this DNA fragment as calculated using the Oligo Calculator program [11] is 347 and 352 K. Therefore, we used an average value of $T_m = 349.5$ K. The procedure of DNA isolation and the nucleotide sequence of the isolated DNA fragment are described in Supplementary materials.

Solutions of agn^{ts3} DNA (496 mcg/ml) in sodium phosphate buffer were used for the Brillouin light scattering (BS) experiments. The light source was an argon laser with wavelength $\lambda = 488$ nm. A laser beam was directed and focused on the sample to be studied in the 180° scattering geometry by an optical system including mirrors and lenses. The light scattered from the sample was analyzed by a 3-pass piezoscanning Fabry-Perot interferometer and registered by a photoelectron multiplier. The device adjustment was controlled by a DAS-1 system (Burleygh). A quartz cuvette with the solution was placed into a home-made heater, which allowed us to change the sample temperature in the range 263–363 K with an accuracy of ±0.5 °C. The BS experiments on the DNA solution agn^{ts3} employed the 180° light scattering geometry. The distance between the interferometer plates was 7.5 mm, which corresponded to the free spectral interval of 20 GHz. The time of recording of one spectrum in the experiments was 300 s.

The scattering spectra were described by a sum of Gaussian corresponding to the Rayleigh scattering at the first and second orders and two Lorentzians that corresponded to the Stokes and anti-Stokes Brillouin doublet [8]. An example of spectrum fitting is given in Fig. 1.

Having determined the frequency shift of the BS components ($\Delta\nu$) as the position of the Lorentzian maximum with respect to the Gaussian maximum, we can calculate the hypersound velocity in the solution. The anti-Stokes (L_{AS}) and Stokes (L_{S}) shifts of the Brillouin doublet $\Delta\nu$ relative to the unshifted line (Rayleigh scattering) are related to the hypersound elastic wave velocity by

$$\pm\frac{\Delta\nu}{\nu} = \frac{2nV}{c}\sin\left(\frac{\theta}{2}\right), \qquad (1)$$

where ν is the incident light frequency, n is the refractive index, V is the elastic wave velocity, c is the velocity of light, and θ is the angle between the incident and scattered light. The experiments were conducted as follows: measurements were performed at first for the solution of sodium phosphate buffer ("buffer" in the text below) and then repeated for the solution of DNA agn^{ts3} in the buffer.

Fig. 1 presents experimental BS spectra for the DNA solution at different temperatures. It is evident that the positions (frequencies), intensities and half widths on half maximum of BS doublet change with increasing temperature.

Fitting of the spectrum yielded temperature dependences of the shifts of BS components for two solutions: DNA solution agn^{ts3} in the sodium phosphate buffer and sodium phosphate buffer. In order to carry out comparative analysis of the behaviors of the shift (hyper-

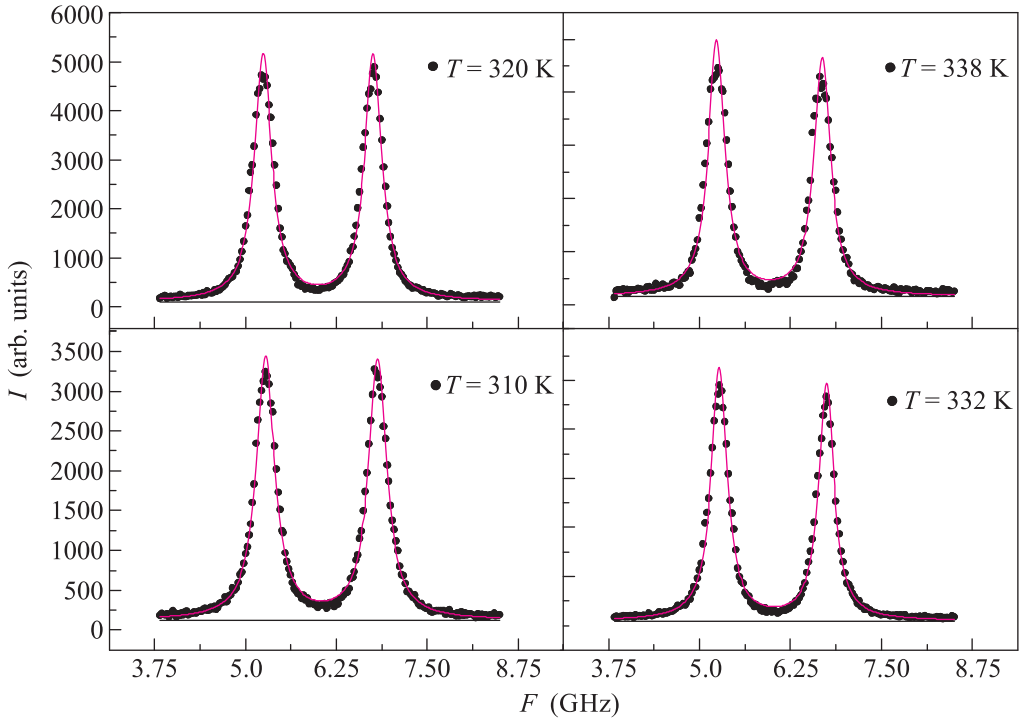

Fig. 1. Temperature evolution of Brillouin scattering doublet in the DNA solution agn^{ts3}

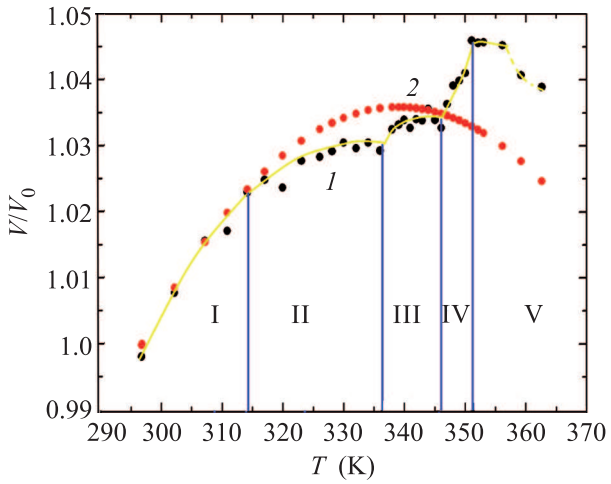

Fig. 2. Relative changes in hypersound velocity normalized to its magnitude at $T = 297$ K in the sodium phosphate buffer (curve *2*) and DNA agn^{ts3} solution (curve *1*) with temperature

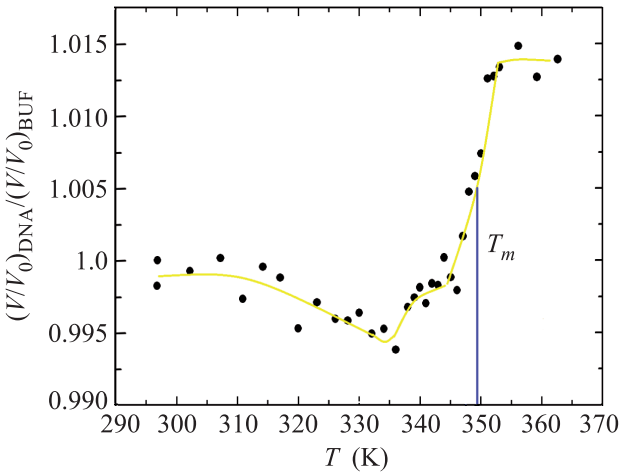

Fig. 3. Temperature dependence of the BS component shift normalized for the buffer for DNA agn^{ts3}

sound velocity) at varying temperature, two additional data processing procedures were used: (i) each dependence was normalized to its value at 297 K (Fig. 2) and, as a result, the relative change in the hypersound velocity was obtained; (ii) each dependence was also normalized to its value at 297 K and then the normalized dependences were additionally divided by the normalized dependence for the buffer (Fig. 3). If we neglect the temperature dependences of the refractive index and density because of their smallness in Eq. (1), we can regard the temperature dependence of the BS component shift as the dependence of the behavior of hypersound velocity on temperature (below we discuss just the behavior of hypersound velocity $\Delta\nu$). The sodium phosphate buffer is characterized by a monotonous increase in the hypersound velocity with increasing temperature to 340 K and then its decrease on further heating (Fig. 2, curve *2*). This temperature dependence of hypersound veloc-

ity in the buffer agrees with the behavior of elastic waves in water and water solutions [12] with varying temperature.

Let us consider now the temperature dependences of relative changes in hypersound velocity in the DNA agn^{ts3} solution (Fig. 2). We can divide the temperature dependences of relative value $\Delta\nu$ in DNA agn^{ts3} solution into three parts. The first is in the region of temperatures close to the room one: 294–315 K. As follows from Fig. 2, at these temperatures the behavior of the velocity in the DNA agn^{ts3} solution is similar to that in the buffer (part I, Fig. 2). At heating of the DNA agn^{ts3} solution the temperature dependence of hypersound velocity begins to deviate from the temperature dependence of velocity in the buffer in the vicinity of 315 K (see Fig. 2). It is well seen that, as temperature increases, the behavior of the hypersound velocity exhibits an anomaly with a minimum in the vicinity of 337 K. Thus, the second part of curve *1* in Fig. 2 can be limited by temperatures 315–347 K.

In the third part further heating of the DNA agn^{ts3} solution leads to a sharp change in the behavior of hypersound velocity: near 347 K there is a velocity upward jump extending for 5 degrees, and above it there are no peculiarities in curve *1*. Thus, the behavior of hypersound velocity in the DNA agn^{ts3} solution has two anomalies – in the vicinity of 337 and 347 K.

Analysis of the temperature dependences of half width on half maximum of BS components and their intensities do not have pronounced anomalies similar to those observed in the behavior of the frequency shift. However, the behavior of intensity of Rayleigh scattering with varying temperature (see Fig. 4) demonstrates an

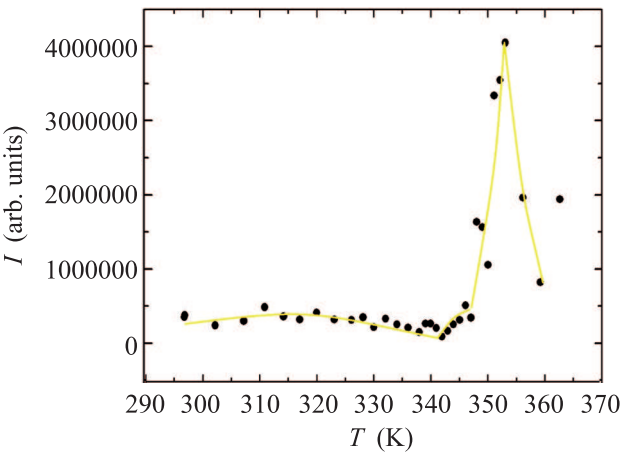

Fig. 4. Temperature dependence of the intensity of Rayleigh scattering in the DNA solution

anomalous dependence beginning from 347 K. Indeed, the intensity of Rayleigh scattering depends on temperature only slightly to about 330 K. An increase in temperature leads to a decrease in intensity which reaches a minimum at ∼342 K. Above 342 K the temperature dependence of intensity begins to grow and beginning from 347 K demonstrates a sharp anomaly with a maximum in the vicinity of 352 K. The intensity changes in 8 times!

Note that the intensity of Rayleigh light scattering is determined by extinction and its change with evolution of temperature [13]. Comparison of the behaviors of hypersound velocity and intensity of Rayleigh scattering shows that the "jump" in the hypersound velocity at 347 K agrees well with the anomaly in the intensity (see Figs. 2, and 4). The maximum in the behavior of the temperature dependence of the Rayleigh scattering intensity corresponds to the "end" of the jump in the hypersound velocity at 352 K. Thus, the calculated melting temperature of DNA agn^{ts3} in the vicinity of 349 K is consistent with the middle of the temperature region in which an anomalous behavior of Brillouin scattering spectrum is observed. Therefore, it can be stated that there is a connection between anomalies in the hypersound velocity and intensity of Rayleigh light scattering and melting and denaturation of DNA.

The anomalies observed in the behavior of Brillouin spectra stimulated bio-information analyses of the set of conformations for the DNA agn^{ts3} molecule by the *RNAstructure* program for two temperatures. The first was chosen to be the room temperature $T = 298$ K and the second was chosen to be the highest possible for the calculations $T = 310$ K. The sequence of nucleotides acids is shown in Table in Supplementary materials.

Figs. 5 and 6 show most possible calculated variants of the structure for corresponding DNA fragments at 298 and 310 K. The red line shows the 28 bp insertion, 5′-end of DNA is below (marked by a green line with indication of the number of nucleotides preceding the depicted structure), 3′ – end is above (marked by a blue line with indication of the number of nucleotides preceding the depicted structure), the left strand is the main one (see also Supplementary materials). Red figures in brackets indicate the starting and end sites of local strand separations according to agn^{ts3} sequence enumeration. Black figures show energies of structures at corresponding temperatures. The simulation was performed for a double-stranded DNA agn^{ts3}.

Under all conditions the structure of a double helix of DNA agn^{ts3} without hairpins is energetically favorable, but it is not the only possible. Calculations show that different local structures of DNA "ranging in energies" can exist along with the double helix (Figs. 5 and 6). Note that this picture is consistent with modern ideas about the necessary existence of local structures of

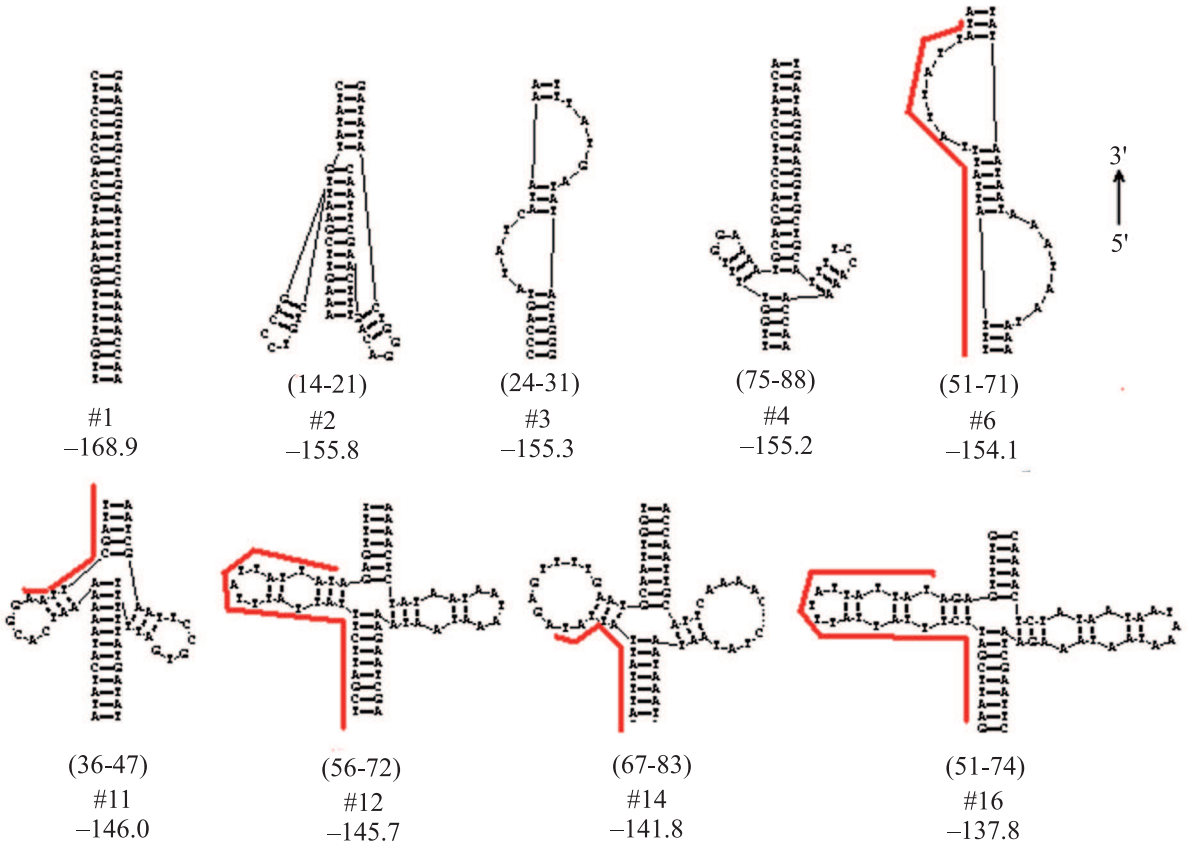

Fig. 5. (Color online) Set of most possible conformations of the DNA agn^{ts3} molecule at $T = 297\,\mathrm{K}$

DNA [1]. Let us consider the calculations in mode detail. At $T = 294\,\mathrm{K}$ DNA agn^{ts3} is seen to have two structures (bubbles) in the region of the insertion (Fig. 5). In the region of the insertion a number of small-size hairpin structures with participation of both the insertion and initial sequence are formed. The length of the hairpin structure is on the average 6–9 nucleotides, the maximum length of one bubble us 12 nucleotides.

At $T = 310\,\mathrm{K}$ the DNA agn^{ts3} reserves the structures with "bubbles" in the region of the insertion, and also some other structures (with a change in their sequence), while other structures transformed. The major change in agn^{ts3} DNA is an increase in the number of the least energetically favorable structures with a hairpin in the region of the insertion: in the most long hairpin strand separation due to inner base separation occurs leading to formation of bubbles instead of hairpins, strand separation also occurs in a number of other structures.

Thus, increasing temperature: (i) changes the sequence (by the bonding energy) of most energetically favorable structures, (ii) causes local changes in structures with the average bonding energy where separate hydrogen bonds rearrange. (iii) Changes in temperature lead to most considerable changes in energetically unfavorable structures – strand separation. (iv) Bonding energy reduces with increasing temperature.

As shown, the temperature dependence of the velocity of hypersound in the DNA solution sharply differed from the temperature dependence of hypersound velocity in the buffer (Fig. 2). Now we consider the temperature dependence of the behavior of relative changes in the hypersound velocity in the DNA agn^{ts3} solution normalized to the relative change in the hypersound velocity in the buffer which was used to prepare the DNA solution (Fig. 3). This temperature dependence reflects the "pure" dynamics of DNA "cleaning"from buffer dynamics. It can be seen that the temperature dependence is in the form of a step upwards at which additional anomalies in the vicinity of $T_1 = 335\,\mathrm{K}$ and $T_2 = 347\,\mathrm{K}$ occur (Fig. 3). It is worth here to turn to the behavior of hypersound velocity at structural phase transitions in crystals. According to the classical ideas about the behavior of acoustic phonons in the vicinity of structural phase transitions, anomalies in hypersound velocity are determined by the coupling between the order parameter (η) and deformation (u) [14]. If the coupling between the order parameter and deformation is of the $\eta^2 u$

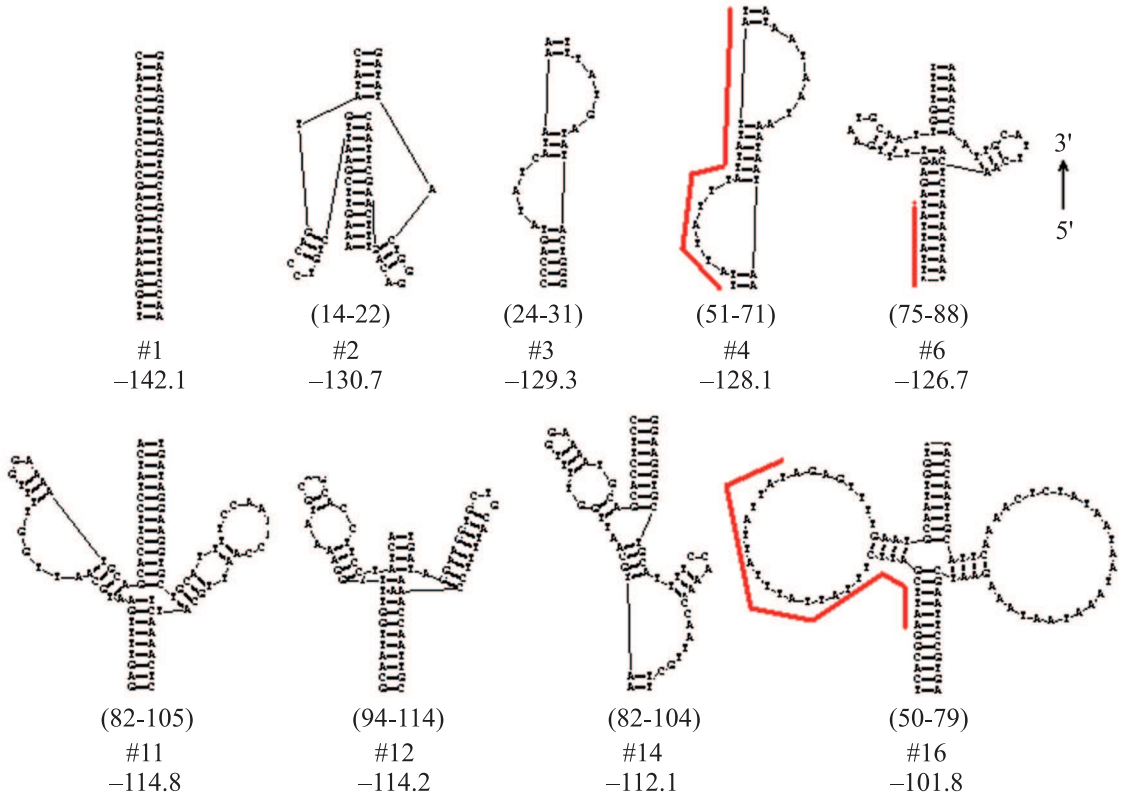

Fig. 6. (Color online) Set of most possible conformations of the DNA agn^{ts3} molecule at $T = 310$ K

type, the anomaly looks like a step upwards. Thus, the anomaly observed in the BS experiments for the DNA fragment resembles the anomaly in hypersound velocity at structural phase transition in a ferroelastic crystal if between the order parameter and deformation is of the $\eta^2 u$ type [15]. It is evident that it is impossible to use the same ideology for the description of the BS behavior for DNA and at classical structural phase transition in crystals, however it can be stated that the anomalies we observed points to structural changes occurring in DNA.

It follows from calculations of possible sets of structures formed at $T = 294$ and 310 K for the sequence of DNA studied not only classical double-stranded helix but also a modified helix with local structures of different types. The higher the temperature, the higher the probability of formation of such local structures with similar energies. This is confirmed by the behavior of hypersound velocity and intensity of Rayleigh scattering at heating. It can be seen from Figs. 5 and 6 that a change in temperature in the region 294–315 K does not lead to a change in the state of the system which follows from the absence of dependence of the low-frequency dynamics of DNA molecule on temperature (Figs. 2 and 3). This means that we mainly observe scattering from the double-stranded DNA structure.

At further heating, above 315 K, the conformational DNA dynamics is likely to begin to manifest itself in Brillouin scattering experiments, i.e., relative changes in velocity decrease near $T_1 = 335$ K. The minimum velocity corresponds to a minimum in free energy occurring at structural transformation DNA. Indeed, as follows from calculations, when temperature increases, all local structural changes shift into the region of the AT insertion. It is likely that when temperature increases from 315 to 335 K, the difference in free energies (ΔE) between different states of the DNA agn^{ts3} molecule monotonously decreases, and near 335 K $\Delta E \to 0$. Probably, in the vicinity of 335 K we observe a local minimum in the energy landscape of the system that occurs when free energies of the local structures of DNA formed become equal.

Further heating of DNA to 347 K leads to formation of the intermediate phase preceding DNA melting. As temperature grows from 347 to 352 K, the hypersound velocity expresienecs a "jump" of $\sim 1.5\%$. Studies of Brillouin light scattering at phase transitions in dielectrics crystals [15] and phase transformations in biopolymers,

such as denaturation of lysozyme [16–18], suggest that a jump in hypersound velocity of 1.5% points to a structural phase transition or change in conformation. In the case we consider the DNA helix-coil transition is implied. The critical behavior of intensity of Rayleigh scattering, i.e., the 8 fold increase in this region (see Fig. 4), agrees with this supposition [17, 18].

Another possible scenario of these phenomena is a stacking-interaction in the vicinity of DNA melting temperature [19]. Morozov et al. [19] suppose that scission of hydrogen bonds at melting leads to stacking-interaction of single-stranded DNA formed at melting. Then it can be supposed that in the vicinity of melting temperature structures consisting of stacking-interacting DNA strands are formed in some portions of DNA. Probably, these structures form networks at final denaturation of DNA (similar to denaturation of lysozyme [17]). The anomaly in light extinction that manifests itself as anomaly in Rayleigh scattering at denaturation in the vicinity of 352 K agrees well with this scenario.

Brillouin light scattering experiments with DNA agn^{ts3} revealed anomalies in the behavior of hypersound velocity and temperature dependence of Rayleigh light scattering intensity in the vicinity of 335 and 447 K. Calculations of possible conformational states at 297 and 310 K have shown that the classical double-strand structure of DNA agn^{ts3} is not the only possible and energetically favorable. Other local structures of DNA in the form of bubbles etc. which change with varying temperature can exist. Analysis of the behavior of hypersound velocity at varying temperature have shown that the anomalies can be attributed to the conformational DNA agn^{ts3} dynamics realized from 315 K. Anomalies in the behavior of hypersound velocity in the vicinity of 335 K are lkely to be due to the local energy minimum of the system in the region of 335 K, and the anomaly in the vicinity of 347 K is due to the intermediate phase that precedes melting of DNA agn^{ts3}. The behavior of hypersound also exhiits the 1.5% jump in the hypersound velocity attrinuted to melting of DNA agn^{ts3} and "helix-coil" transition. One more possible mechamism of the anomalies can be stacking-interaction and formation of a network of interacting strands of DNA. It is shown that at melting of DNA agn^{ts3} light extinction increases by a factor of 8 which is due to denaturation of DNA agn^{ts3} and structural transformations in this temperature region. No doubt, the phenomena observed in our study require further investigations, including small-angle neutron scattering and NMR.

Theoretical models that can describe the evolution of BS spectra in the temperature region considered are also necessary.

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