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Protein Dynamics in Brillouin Light Scattering: Termal Denaturation of Hen Egg White Lysozyme

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Thermal denaturation of hen egg white lysozyme has been investigated by Brillouin light scattering in the temperature range from 297 to 350 K. Anomalies in the temperature dependences of velocity and damping of hypersound and also in the behavior of the intensity of Brillouin components for the lysozyme solution at thermal denaturation have been revealed. These anomalies are attributable to phase transformations of the protein in the high-temperature region. It has been shown that Brillouin light scattering is a suitable tool for studying the structural evolution of proteins.

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Dynamics of globular proteins attracts considerable attention of researchers at present because of a necessity to understand how proteins work. The investigations performed by such techniques as elastic and inelastic light scattering [1, 2], X-rays scattering [3 – 5], NMR [6, 7], calorimetry [8 – 10], and others have resulted in the following sequence of phase transformations for small globular proteins at temperatures above the room temperature: native state of a protein – partly denatured state – fully denatured state – aggregation. The sequence varies from one protein to another and depends appreciably on pH of the protein solution, protein concentration, etc.

One of the most suitable model objects for studies of the protein dynamics is hen egg white lysozyme. It is known that an increase in the temperature of a lysozyme solution (from room temperature) leads to the transformation of a protein molecule from the native state to denatured state manifesting itself as an abrupt first-order phase transition [3 – 5, 11] in the vicinity of 333 K (the exact denaturation temperature depends on such parameters as pH of the protein solution and others [8]). A further increase in temperature gives rise to aggregates with a subsequent transition of the protein solution into a gel-like state at 343 K [11 – 14]. It is known that the intermolecular association or gelation of a protein solution upon heating is caused by intermolecular antiparallel β-sheet formation [11]. Recently, SAXS studies of a lysozyme solution have revealed that intramolecular changes in the protein structure are observed at temperatures well below the denaturation temperature [3 – 5]. It is likely that the instability of the intramolecular lysozyme structure at high temperatures triggers the transition from the native protein to a molten globular state.

Valuable information on structural transformations of protein solutions can be obtained in Brillouin light scattering experiments because this technique is sensitive to even minor structural changes and phase transformations in condensed media [15, 16]. The goal of our work was to study the structural transformations of hen egg white lysozyme by using Brillouin light scattering.

The objects of the investigation were a 0.1 M sodium acetate buffer (NaAc buffer, pH=4.55) and a solution of Hen Egg White Lysozyme (Fluka) with a concentration of 250 mg/ml in a NaAc buffer.

The light source was a neodymium YAG laser with a wavelength $\lambda = 532.15$ nm and power of 120 mW. The laser beam was directed by a set of lenses and mirrors to a microscope (Olympus BH-2) and was focused on the sample. The scattered light was collected and analyzed by a six-pass Sandercock tandem Fabry-Perot interferometer. A backscattering geometry was employed. The distance between parallel interferometer mirrors was adjusted to be 7.5 mm, which corresponded to a free spectral range of 20 GHz. The time of recording of one spectrum was 100 s. The solution to be studied was placed into a heated chamber (Lincom system, THMS600). The temperature of the solution was increased from 294 to 350 K with an accuracy of $\pm 0.1\,^{\circ}$C. The sample was kept for 30 minutes at each temperature prior to measurements.

Figure 1A shows the Brillouin scattering spectrum of the lysozyme solution measured at 342 K. The highest peak at the unshifted frequency should be the Rayleigh scattering caused by isobaric density fluctuations, and the lower peaks on each side of the Rayleigh peak are

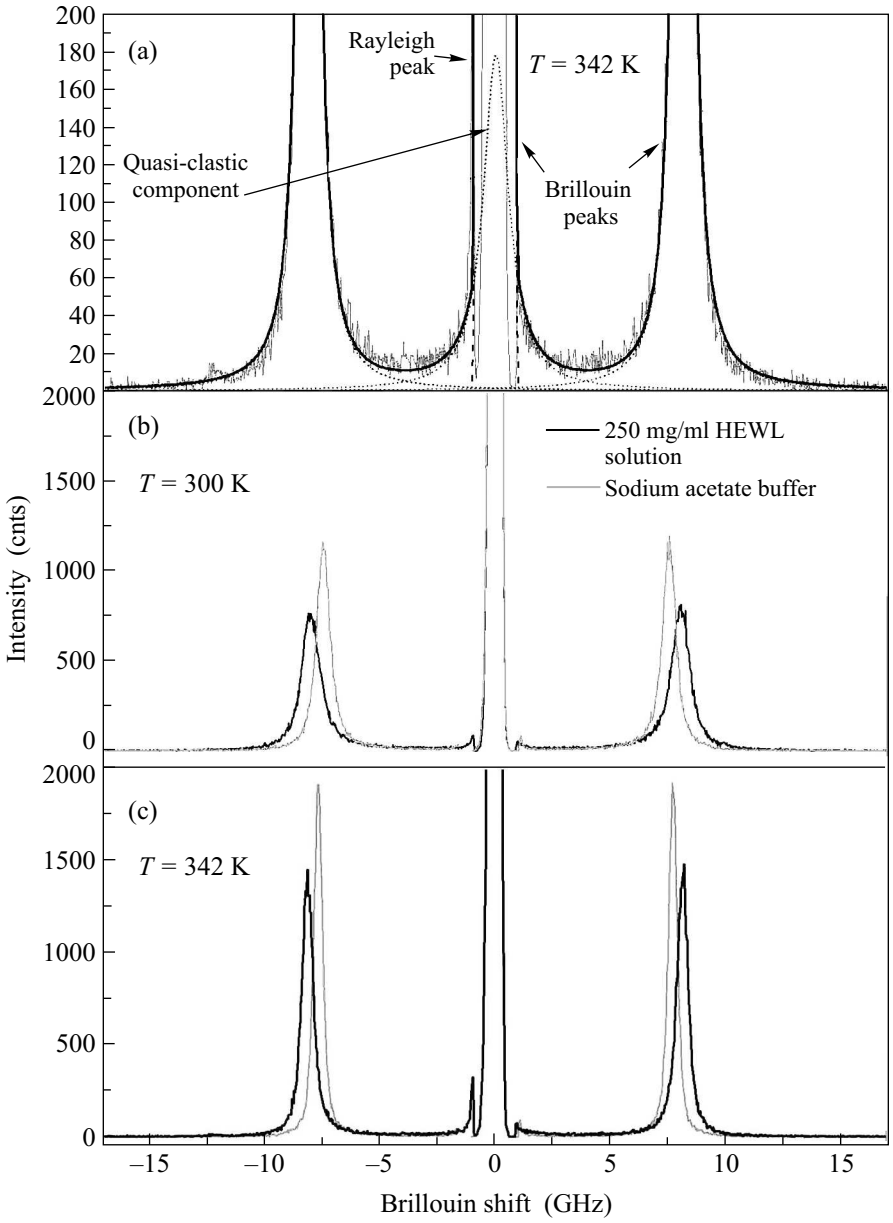

Fig.1. Experimental Brillouin light scattering spectra for the lysozyme solution and NaAc buffer at 342 and 300 K. Fitting was made as described in the text

Brillouin scattering peaks (components) which result from adiabatic density fluctuations. Unfortunately, the Rayleigh peak was extremely intensive for lysozyme solution. For this reason, a reference signal used by the control module of the interferometer was recorded in the experimental spectra of instead of the central component. There is an additional contribution into the Brillouin spectra at the unshifted frequency, i.e., quasielastic scattering due to relaxation processes in the liquid (it manifests itself on both sides of the Rayleigh line, see Fig.1). Fig.1A shows an example of fitting of the obtained experimental spectra. The scattering spectra were described by a sum of three Lorentzians and the Gaussian corresponding to the assumed Rayleigh scattering. Two of the Lorentzians corresponded to the Brillouin components and one corresponded to the quasielastic scattering. It is well seen (Fig 1B, C) that Brillouin spectra of the NaAc buffer and the lysozyme solution in the NaAc buffer change with increasing temperature.

The shift of the Brillouin peaks with respect to the unshifted line ($\Delta\nu$) defines the velocity v of a hypersonic elastic wave as

$$\pm\frac{\Delta\nu}{\nu} = 2n\frac{V}{C}\sin\frac{\theta}{2}, \qquad (1)$$

where $\Delta\nu$ is the Brillouin shift, ν is the incident light frequency, n is the refractive index, v is the velocity of elastic wave, c is the velocity of light, and θ is the angle between the incident and scattered light.

The Brillouin linewidth ($\delta\nu$) is related to the hypersound damping (Γ) by

$$\delta\nu = q^2\Gamma, \qquad (2)$$

where q is the wave vector.

By using the results of fitting of the spectra, temperature dependences of relative values of the shifts, linewidths, and intensities of the Brillouin peaks for these two solutions were plotted (Figs.2–4). The data

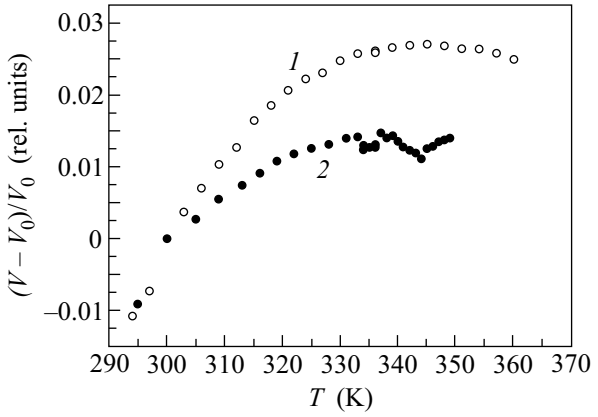

Fig.2. Relative changes in the Brillouin shift versus temperature for the NaAc buffer (curve *1*) and lysozyme solution (curve *2*). At $T = 300$ K the Brillouin shift $\Delta\nu_0 = 8.035$ GHz for scattering from the lysozyme solution, and $\Delta\nu_0 = 7.491$ GHz for light scattering from the sodium acetate buffer

were normalized to the magnitudes of the corresponding parameters obtained at $T = 300$ K because it can be believed that the solutions are in the equilibrium state at this temperature. A comparison of the temperature dependences of relative shifts, linewidths, and intensities of Brillouin peaks for the lysozyme solution and buffer gives information on specific features of the protein dynamics.

Fig.2 shows temperature dependences of the relative Brillouin shift for the NaAc buffer and the lysozyme solution in the NaAc buffer. According to Eq.(1), changes in the Brillouin shift correspond to changes in the velocity of hypersound (changes in the temperature dependences of refractive index can be neglected because of their smallness). Below we discuss the changes in the velocity of hypersound Δv. It is well seen (Fig.2, curve *1*) that in the case of scattering from the NaAc buffer Δv monotonically increases as temperature increases approximately to 343 K, and then it slightly decreases as the buffer is heated up to 360 K. The temperature dependences of the Brillouin shift and linewidth for the the NaAc buffer (Figs.2 and 3) are very similar to those of pure water [17].

Let us now consider the temperature dependences of relative changes in the velocity of hypersound in the lysozyme solution (Fig.2, curve *2*). The temperature dependences of Δv for the lysozyme solution can be conditionally divided into three parts. The first is 294–305 K. As can be seen from Fig.2, the behavior of the velocity of hypersound in the protein solution at these temperatures is similar to that in the buffer. As the lysozyme solution is heated to 333 K, the velocity of hypersound monotonically increases. Thus, the second part of curve *2* in Fig.2 can be taken to be the temperature region 305–333 K. Further heating of the lysozyme solution leads to a nontrivial behavior of the velocity of hypersound, i.e., in the vicinity of 333 K the velocity drops, then it becomes temperature-independent, and at 336 K the velocity abruptly increases. The next part of the temperature dependence (Fig.2, curve *2*) corresponding to the temperature region 336–350 K also exhibits an anomalous behavior with a characteristic minimum at $T = 343$ K. It can be supposed that the observed anomalies in the temperature behavior of the velocity of hypersound are associated with changes in the spatial organization of the lysozyme molecule.

Now we pass to consideration of the temperature dependences of the relative value of the Brillouin linewidth for the lysozyme solution (Fig.3, curve *2*) and the NaAc buffer (Fig.3, curve *1*). It is well seen (Fig.3) that,

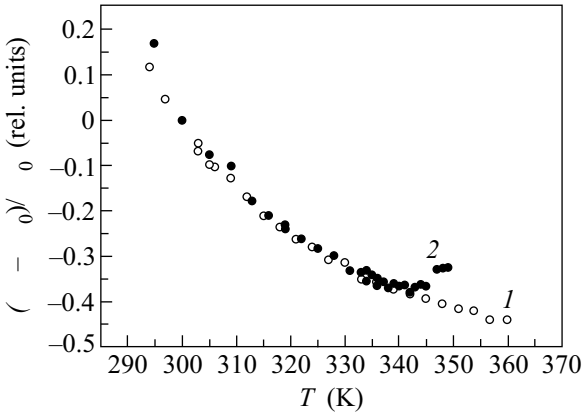

Fig.3. Relative changes in the Brillouin linewidth versus temperature for the NaAc buffer (curve *1*) and lysozyme solution (curve *2*). At 300 K the Brillouin linewidth (in the case of scattering from the lysozyme solution $\delta\nu_0 = 0.87384$ GHz, and for the case of light scattering from the NaAc buffer $\delta\nu_0 = 0.65512$ GHz

as temperature increases to $T \approx 342$ K, the hypersound damping exponentially decreases in both solutions. Above $T \approx 342$ K, the behaviors of hypersound damping in the buffer and protein solution differ, i.e., the hypersound damping in the buffer continues to decrease exponentially, while the damping of hypersound in the lysozyme solution increases. Note that at $T \approx 343$ K there is a minimum in the hypersound velocity (compare Figs.2 and 3).

A comparative analysis of the temperature dependences of relative changes in the scattering intensity for the lysozyme solution and the NaAc buffer also yields interesting results. It is well seen in Fig.4 that when

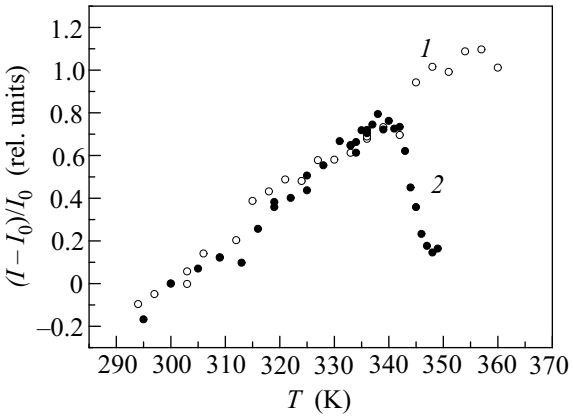

Fig.4. Temperature dependences of relative changes in the intensity of Brillouin components for the NaAc buffer (curve *1*) and lysozyme solution (curve *2*). I_0 is the intensity of the Brillouin components at 300 K. At $T = 300$ K the light scattering intensity of the Brillouin peak for the lysozyme solution was 840.5 a.u., and for the light scattering from the NaAc buffer it was 1167.7 a.u.

the sodium acetate buffer is heated, the intensity of light scattering from it monotonically increases in the entire temperature range considered. This is in good agreement with the behavior of the intensity of Brillouin components in low-viscosity liquids [15]. The intensity of light scattering from the lysozyme solution also monotonically increases with increasing temperature to $T \approx 340$ K (Fig.4, curve *2*), similar to the behavior of light scattering intensity for the buffer. Starting from the temperatures above $T \approx 340$ K, the dependences diverge, i.e., the intensity of the Brillouin components for the protein solution considerably decreases. This is due to an appreciable increase in light absorption and contribution of multiple scattering.

Thus, the temperature dependences of the hypersound velocity (Fig.2) and damping (Fig.3) and intensity of Brillouin components (Fig.4) for the protein solution exhibit anomalous behaviors at $T \approx 340$ K.

Analysis of the Brillouin scattering spectra has shown that the differences in the behaviors of the Brillouin components for the protein solution and buffer begin to manifest themselves at 305 K and are become pronounced at higher temperatures (see Fig.2). The behavior of the hypersound velocity in the lysozyme solution correlates well with small-angle X-ray scattering data [3–5]. Therefore, it can be concluded that the changes in the behavior of the velocity of hypersound in the lysozyme solution above 305 K can be caused by intramolecular structural changes in the interdomain correlation and in the polypeptide arrangement in the lysozyme molecule. It is supposed that the intramolecular structural change in lysozyme proceeds gradually above room temperature until a drastic collapse of the tertiary structure occurs and has the character of the second-order structural transition [3–5]. It is interesting to note that no noticeable differences in the behaviors of the hypersound damping and light scattering intensity for the lysozyme solution and buffer in the temperature interval 305–333 K were observed.

It can be seen in Fig.2 that the temperature dependence of the velocity of hypersound in the lysozyme solution has a step-like anomaly at $T \approx 333$ K. Such a behavior of the velocity of hypersound is typical of the systems experiencing the first-order phase transition [16]. The behavior of the hypersound velocity in the protein solution at $T \approx 333$ K points to sharp changes in the properties of the solution which can be associated only with the change in the spatial organization of the lysozyme molecule, i.e., with the collapse of its native structure and denaturation state. Possibly, there exists an intermediate state of lysozyme in this narrow temperate range which was also suggested by R.J. Green et al. [11].

The behavior of the relative hypersound velocity above 333 K continues to be abnormal: in the temperature interval 333–336 K the velocity is nearly independent of temperature. The IR spectroscopic and NMR studies of the thermal lysozyme denaturation have shown that in the "molten globular" state aggregates consisting of denatured lysozyme molecules can be formed through formation of intermolecular antiparallel β-sheets [11]. Possibly, start of formation of aggregates in the lysozyme solution leads to a step-like increase in the velocity of hypersound at 336 K.

It is interesting to note that lysozyme denaturation does not affect the temperature behaviors of hypersound damping and light scattering intensity up to 342 K: no differences between these parameters for the buffer and lysozyme solution were revealed in analysis of the Brillouin spectra. However, at $T = 342$ K the light scattering intensity for the lysozyme solution abruptly falls,

and damping begins to grow sharply (Figs.4 and 3, respectively). On the temperature dependence of the hypersound velocity, this temperature point is a minimum in the region of the high-temperature anomaly (Fig.2). Such an anomalous behavior of the light scattering can be due to phase transformations. Indeed, formation of aggregates in the protein solution is followed by formation of a protein network [13]. In other words, the solution passes into a gel-like state. As a result, the viscosity of the medium changes, multiple scattering appears, etc., which affect Brillouin scattering. It is likely that it is the formation of a protein network and transition into the gel-like state that are responsible for the critical behavior of light scattering in the vicinity of 342 K.

We have carried out Brillouin light scattering studies of the protein dynamics in the temperature range 297–350 K. By analyzing the obtained spectra, the evolution of the spatial organization of lysozyme has been traced.

We have shown that in the vicinity of 305 K the internal structure of the lysozyme molecule begins to undergo changes, which eventually lead to transformation of the solution into the gel-like state at 342 K. It has been found that all the phase transformations, i.e., the denaturation, formation of aggregates, and transition of the protein solution into a gel, manifest themselves in a pronounced way on the temperature dependence of the hypersound velocity, while the damping of hypersound and integral intensity of Brillouin components exhibit anomalies only at the protein solution – gel transition.

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