

## Study of low-frequency dynamics of short peptides by Brillouin light scattering and Monte-Carlo global energy minimization

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**Abstract.** Brillouin light scattering studies of the behavior of hypersound velocity in solutions of short peptides Asp-Ser, Glu-Asp-Arg, Ala-Glu-Asp-Leu with widely varying concentrations at temperatures ranging from 295 to 360K are presented. As shown by analysis of experimental data and molecular simulation, the dipeptide Asp-Ser is characterized by formation of dimers which decompose at high temperatures. High-concentration solutions of tri- and tetrapeptides form aggregates at high temperatures which are preserved on cooling.

### 1. Introduction

The structure and dynamics of proteins and other biopolymers attract considerable attention. It has been shown that changes in structures and dynamics of proteins, DNA, and polysaccharides can lead to changes in their activity and function, which can result in different diseases. For example, it has been shown that one of the mechanisms responsible for neurodegenerative diseases is the conformational dynamics of proteins and/or its violation [1].

Phase transformations in biopolymers are intensively studied by Brillouin light scattering that gives information about dynamic processes at frequencies from 1 to 1000 GHz [2]. Brillouin scattering was successfully used for studies of thermal denaturation of lysozyme and DNA melting. On heating, the phase termed as the molten globula state, was found in lysozyme, and the subsequent temperature increase up to denaturation lead to formation of aggregates, organized in a gel-like structure [3, 4]. The conformational dynamics leading to melting of the DNA double helix was observed in Brillouin studies of DNA melting [5]. Thus, Brillouin light scattering is a highly informative technique for the investigations of phase transformations in biopolymers.

This paper deals with Brillouin light scattering studies of the low-frequency dynamics of short peptides. Peptides control the majority of processes in human organism, for instance, diverse functions in the central nervous system. The deficiency of peptides can accelerate ageing.

Peptides are natural or synthetic compounds build from amino acid residues connected by peptide (amide) bonds C(O)-NH. According to the number of amino acid residues they are classified as dipeptides, tripeptides, tetrapeptides, etc. The peptides containing up to 10 amino acids are called oligopeptides, more than 10 are called polypeptides. Though the short

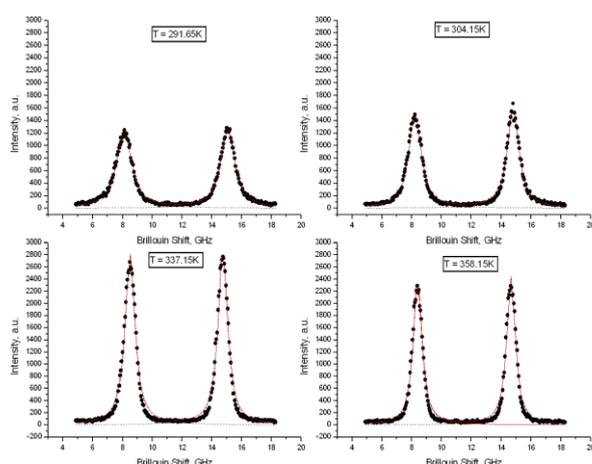


peptides used in our studies are very effective in clinics [6, 7], the mechanisms of their functioning, the structure and dynamics of peptides in solutions are still poorly studied.

## 2. Materials and Methods

Three peptides, i.e., dipeptide AD7 (Asp-Ser), tripeptide pinealon (Glu-Asp-Arg) and tetrapeptide bronchogen (Ala-Glu-Asp-Leu), were used. The peptides were synthesized at the Institute of Bioregulation and Gerontology (St.Petersburg, Russia) Peptide concentrations were analysed in the range from 0.05 mg/l to 10 mg/l. Peptides were dissolved in the sodium-phosphate buffer (pH = 7.0 for AD7, pH = 7.45 for bronchogen and pinealon).

Brillouin light scattering experiments were performed using a 3-pass Fabry-Perrot interferometer. The light source was an argon laser with wavelength  $\lambda=488$  nm and power 100 mW. The laser beam was transmitted through the optical scheme and focused in the sample. The  $180^\circ$  light scattering geometry was employed. The distance between the interferometer plates was 7.5 mm corresponding to a free spectral interval (FSR) of 23 GHz. The time for spectrum recording was 300 s.



**Figure 1.** Brillouin spectra obtained for the solution of bronchogen ( $C = 5$  mg/l) at different temperatures

Temperature of solutions was increased from 293 to 364 K with an accuracy of  $\pm 0.1^\circ\text{K}$ . Examples of experimental Brillouin spectra obtained for the solution of bronchogen at different temperatures are presented in Fig.1. The dashed line is fitting. To describe the Brillouin doublet, the Lorentz function was used.

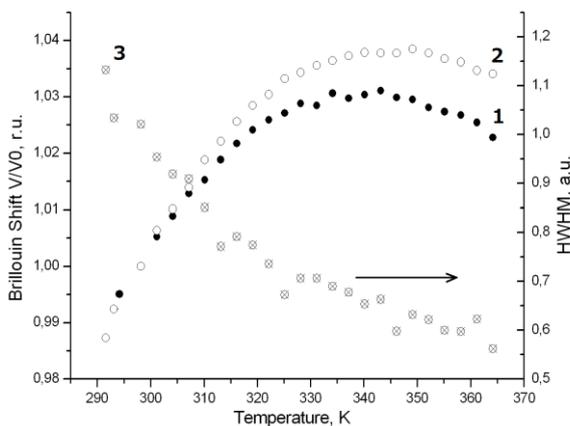
Models of spatial structures of DS dipeptide complexes were obtained by the Monte-Carlo global energy minimization as implemented in the ICM-Pro software package for molecular modeling of proteins and their complexes with ligands (Molsoft LLC, USA) [8]. All calculations were done in the ECEPP/3 force field with the all atom approximation [9]. All standard torsion angles ( $\varphi$ ,  $\psi$ ,  $\omega$  and  $\chi_i$ ) of amino acids were allowed to vary during the Monte-Carlo energy minimization. The ICM default energy parameters including van-der-Waals, electrostatic, torsion energy interactions, hydrogen bonding and solvation energy term were used in the calculations. Free energy of peptide hydration was considered basing on calculations of peptide solvent accessible surface areas and continuum approximation model for protein solvent interactions [10].

### 3. Results and discussion

As follows from Fig.1, the position, i.e., frequency  $\Delta\nu$ , and the halfwidth at half-maximum (HWHM) of the Brillouin components change with increasing temperature. By using expression

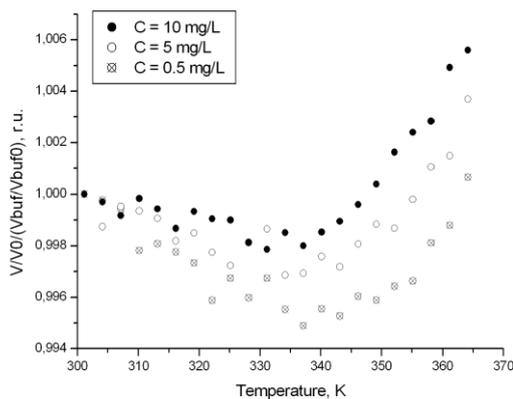
$$\pm \Delta\omega/\omega = \pm \Omega/\omega = \pm \Delta\nu/\nu = 2n(\nu/c)\sin(\theta/2)$$

where  $\nu = \omega/2\pi c$ ,  $\theta$  is the scattering angle, and  $n$  is the refractive index, the hypersound velocity in the solution is found. We discuss the behavior of the Brillouin shift assuming that changes in the refractive index with varying temperature are small. The behavior of the Brillouin shift is proportional to the hypersound velocity.



**Figure 2.** Temperature dependence of attenuation (3) and relative changes in the Brillouin shifts for bronchogen (2) and buffer (1)

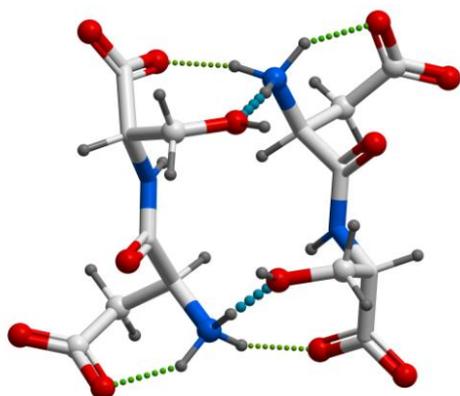
The experiments and calculations yielded temperature dependences of the shift and HWHM of the Brillouin components for the buffer solution and for solutions of peptides with different concentrations. Fig.2 shows relative changes in the Brillouin shift for the sodium-phosphate buffer and for the solution of bronchogen with  $C=5$  mg/l. As a basic for normalization the velocity at  $T=298.15$  K was used.



**Figure 3.** Temperature dependence of hypersound velocity for the solution of dipeptide AD7 normalized to the buffer

It is evident from the Fig.2 that the hypersound velocity in the sodium-phosphate buffer monotonically grows as temperature increases to 340 K. On further heating the hypersound velocity decreases (Fig.2). Interestingly, the relative change in the hypersound velocity in bronchogen (curve 2, Fig. 2) is similar to that of the buffer up to 310 K. Further temperature

increase leads to a deviation of the curve 2 from the curve 1 which points to a peptide dynamics *per se* contribution to the temperature dependence of velocity. Similar behavior was observed for lysozyme solutions [3]. The temperature dependence of hypersound velocity in bronchogen has no anomalies, indicating that this peptide does not show phase transformations. A similar behavior – without anomalies and with a monotonic decrease with increasing temperature is demonstrated by the HWHM.



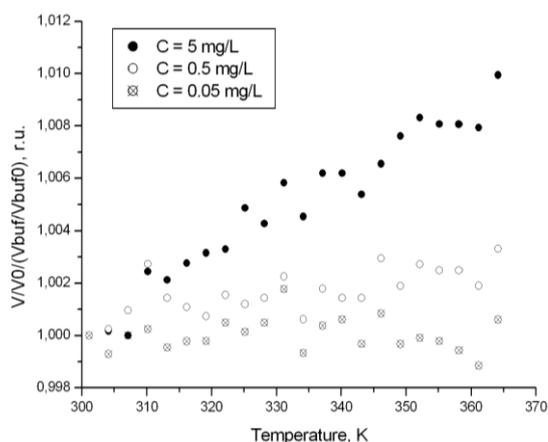
**Figure 4.** Dipeptide dimer.

To compare the behavior of hypersound velocity upon temperature variations in solutions with different peptide concentrations, the data were additionally processed. Each dependence was normalized to its magnitude at 305 K and then the normalized dependences were divided by the normalized dependence for the buffer (Fig.3, 5). As a result, we obtained the plot that showed the dynamics of given peptide. The normalized temperature dependence of hypersound velocity for the solution of dipeptide AD7 differs from that for the buffer (Fig.3): near 335-345 K there is a minimum in the velocity observed for all the concentrations studied. A further increase in temperature leads to a monotonic increase in the normalized hypersound velocity. Numerical analysis of possible configurations of dipeptides has shown that the energetically favorable situation is when AD7 exists in the solution in the form of dimers. Indeed, the lowest energy conformation obtained in the global energy minimization is a densely packed symmetrical complex with a head-to-legs arrangement of peptide main and side chains. In this conformation peptide alpha-amino-group forms multiple hydrogen bonds with C-terminal COO- group, as well as with hydroxyl group of Ser side chain. Hydrogen bond between the side chain of Asp- with peptide alpha-amino-group provides additional stabilization for the conformation of dipeptide complex. Nevertheless, other polar groups in the peptide main chain not involved in the intermolecular hydrogen bonding remain fully accessible for solvent hydration. All these observations make the lowest energy conformation a likely candidate for the DS dipeptide complex.

It should be noted that dimers are formed in the initial solution. It can be supposed that the anomalies observed in the hypersound velocity in the vicinity of 340 K are attributable to the decomposition of dimers.

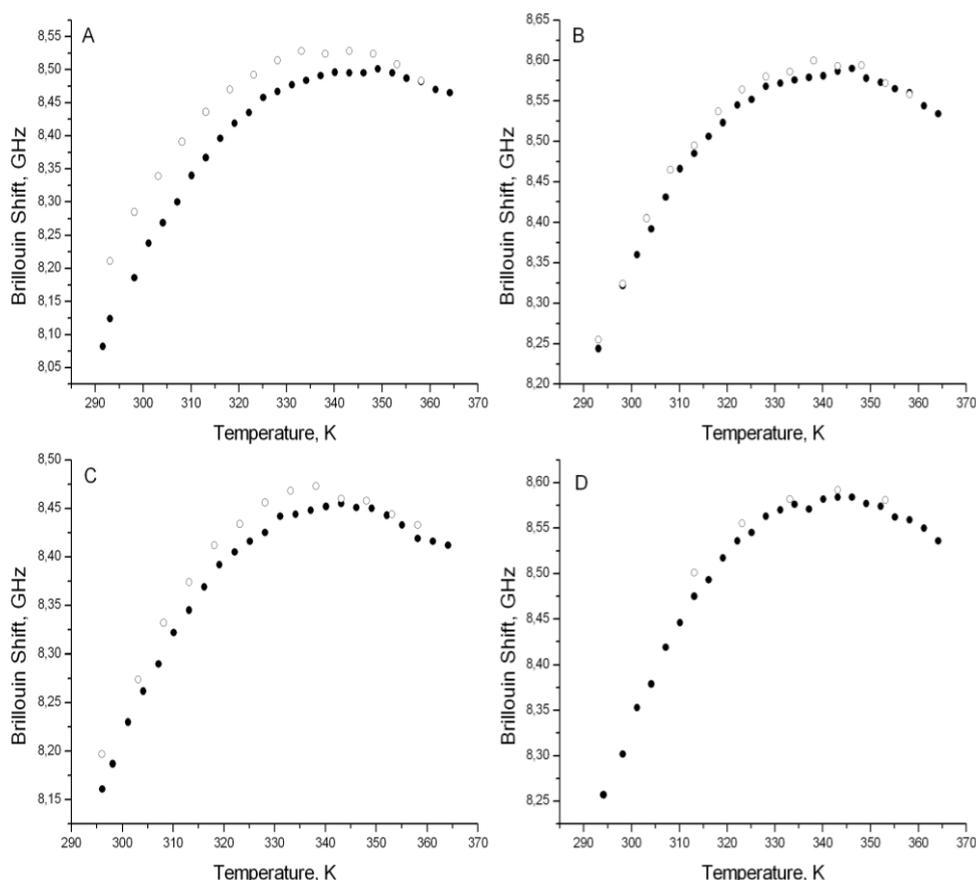
The temperature dependences of the hypersound velocity normalized to the buffer for bronchogen and pinealon are similar. However, they markedly differ from that of AD7: the hypersound velocity for these peptides monotonically increases with increasing temperature (Fig. 5) at all concentrations. Calculations show that formation of the structures consisting of two and more molecules in these compounds is energetically unfavorable. The absence of anomalies on heating is in agreement with the calculations.

Let us consider the behavior of hypersound velocity on heating and cooling for different concentrations of bronchogen and pinealon. At high concentrations, the hypersound velocity behaves differently on heating and cooling (Fig. 6 A, C) below 350 K (Fig. 6).



**Figure 5.** Temperature dependence of hypersound velocity for the solution of bronchogen normalized to the buffer

Suggestively, a structural change in the spatial organization of peptides occurs at 350 K. The differences in the behavior of hypersound velocity on heating and cooling for high concentrations of peptides can arise due to formation of aggregates between peptide molecules at decreasing temperature. For lower concentrations of pinealon and bronchogen, no differences in the behavior of hypersound velocity on heating and cooling are observed (Fig.6 B, D). This indicates a reversible character of conformational rearrangements of these peptides when no aggregates are formed.



**Figure 6.** Temperature dependence of Brillouin shift for bronchogen in concentrations 5 mg/l (A) and 0.5 mg/l (B) and for pinealon in concentrations 5 mg/l (C) and 0.5 mg/l (D) at cooling.

#### 4. Conclusions

Studies of the hypersound velocity behavior upon varying temperature in solutions of short peptides with different concentrations demonstrated that the peptide dynamics depended both on their concentration and structure. Dipeptide AD7 in the solution at concentrations studied was capable of self- organization into dimeric structures which decomposed in the vicinity of 345 K. Tri- and tetrapeptides did not form sufficiently stable complexes on heating and their behavior was governed by their equilibrium dynamics. The molecules of pinealon and bronchogen in high-concentration solutions were likely to form aggregates on cooling down from the high-temperature region. This was not observed in low-concentration solutions of pinealon and bronchogen.

#### Acknowledgments

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