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Four-photon microwave laser spectroscopy of aqueous solutions of biopolymers

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***Abstract*. The four-photon laser radiation scattering spectra are obtained in the submillimetre range (75 – 95 cm^{-1}) for deionised water, aqueous solutions of DNA and α-chymotrypsin protein. Narrow resonances are recorded whose frequencies coincide (within the resolution power of a spectrometer) with rotational frequencies in the ground electronic state and vibrational state of ortho and para isomers of H_2O molecule in a gas phase and with the frequencies of the lines of H_2O_2 and OH^- molecules. It is shown that the resonance contribution of the rotational lines of ortho isomers of H_2O to the signal of four-photon scattering of native solutions of biopolymers increases by a factor of at least 8 compared to their contribution to the scattering signal in water, and becomes considerably larger than the contribution from the paraisomer lines. Denaturation of DNA after heating and cooling of the solution leads to the disappearance of such selectivity.**

Keywords: *nonlinear laser spectroscopy, four-photon scattering, low-frequency spectroscopy of biopolymers, hydration of macromolecules.*

1. Introduction

Analysis of the optical spectra of low-frequency molecular motion in liquids that form molecular complexes clarifies certain aspects of the intermolecular interaction, dynamics and topology of molecular associates, and peculiarities of the interaction of biological molecules with water which is their natural (native) medium. However, the use of classical methods of optical spectroscopy (IR absorption and spontaneous Raman scattering) in the spectral range from a few units to 100 cm^{-1} is hampered by a number of experimental difficulties caused by a strong absorption in samples and, hence, necessitates the use of thin films of the medium that can be heated considerably during measurements.

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Four-photon spectroscopy [1, 2] makes it possible to increase the signal-to-noise ratio significantly due to phasing of atomic and molecular motions in a macroscopic volume with the help of two laser waves with frequencies ω_1 and ω_2, whose difference $(\omega_1 - \omega_2)$ can be scanned over a broad spectral range – from near IR to the centimetre range. It is important that the frequencies of interacting waves lie in the transparency region of the medium and heating of the medium does not occur. The parameter being measured is the intensity of radiation at the frequency $\omega_s = \omega_1 - (\omega_1 - \omega_2)$ of the signal passing through a polarisation analyser crossed with the direction of polarisation of the wave $\boldsymbol{E}^{(2)}$. The source of this wave is the nonlinear polarisation [1, 2]

$$\boldsymbol{P}_i^{(3)} = 6\chi_{ijkl}^{(3)}(\omega_s;\omega_1;\omega_2;-\omega_1)\boldsymbol{E}_j^{(1)}\boldsymbol{E}_k^{(2)}\boldsymbol{E}_l^{(1)*}, \quad (1)$$

where, $\chi_{ijkl}^{(3)}$ is the cubic susceptibility tensor of the medium, and $\boldsymbol{E}^{(1)}$ and $\boldsymbol{E}^{(2)}$ are the amplitudes of interacting fields. The intensity I_s of the signal being recorded is $I_s \propto \left|\chi_{ijkl}^{(3)}\right|^2 I_1^2 I_2$.

By using this approach, the rotational spectrum of H_2O and D_2O molecules was observed in water [3, 4] in the range 0 – 100 cm^{-1}. The frequencies of the lines in this spectrum coincided to within 0.2 cm^{-1} with the rotational resonance frequencies of these molecules in the gas phase. In aqueous solutions of hydrogen peroxide [3] and of the α-chymotrypsin protein [5, 6], a considerable increase (by an order of magnitude) in the contribution of rotational resonances of water molecules to the four-photon scattering signal was observed, as in the case of an increase in the water temperature [7]. Such a behaviour of the rotational spectrum can be explained qualitatively by the presence of free H_2O molecules at the boundaries of water with microbubbles, solid microscopic impurities, and large biopolymer molecules

Note that an analysis of features of the formation of hydrogen bonds in media whose molecules have different nuclear spins of the hydrogen atoms is one of the fundamental problems in the physics of the liquid state and, in particular, the physics of water. Examples of such molecules are the ortho and para modifications of water molecules in which the total spin of hydrogen atoms is equal to unity (ortho molecules) or zero (para molecules). The ratio of the concentrations of ortho and para isomers of water molecules at room temperature is 3 : 1. Their rotational spectra are different [8] and can be identified quite well in the gas phase [9]. Earlier, it was found that passage of water vapour through a porous material with a developed surface enriches

the water vapour with ortho molecules [9]. Sorption of the spin isomers at the surface of dried organic films (DNA, lysozyme protein) and inorganic compounds also occurs at different rates [10].

The observed selective binding of spin isomers can be attributed to a higher mobility of continuously rotating ortho isomers of the water molecule, while para molecules that may not rotate are characterised by a higher ability to the formation of complexes. The existence and spectroscopic manifestations of ortho and para components of water in the liquid phase have not been substantiated so far. It is also not clear if any selectivity of spin isomers exists during intermolecular interactions in aqueous solutions.

The aim of this study is to analyse the rotational spectra of ortho and para isomers of H_2O molecules in distilled water, and in aqueous solutions of ordinary and denatured DNA and α-chymotrypsin. Measurements were made in the tuning range from -75 to $-95\ \mathrm{cm}^{-1}$ containing several intense separated rotational lines of ortho and para isomers of H_2O [8].

2. Experimental

Experiments were performed on the setup described in [11]. Two waves $\boldsymbol{E}^{(1)}$ and $\boldsymbol{E}^{(2)}$ with frequencies ω_1 and ω_2 propagated in the opposite directions in a cell with a sample. The input and output windows of the cell were made of fused quartz and had a low depolarisation level for transmitted laser radiation. The wave $\boldsymbol{E}^{(1)}$ (second harmonic radiation of a single-mode Nd : YAG laser) was circularly polarised. The tunable wave $\boldsymbol{E}^{(2)}$ (dye laser radiation) was linearly polarised. For such polarisations of the interacting waves [1, 11], the signal determined by the source (1) contains no contribution from nonresonant scattering.

The unit polarisation vectors of the signal wave at frequency ω_s and of the wave $\boldsymbol{E}^{(2)}$ are noncollinear, while the directions of their propagation coincide. The signal was separated by a Glan prism. The width of the instrumental function of the spectrometer ($\sim 0.2\ \mathrm{cm}^{-1}$) and the spectral range ($-1200 \ldots 300\ \mathrm{cm}^{-1}$) were determined by the output characteristics of the dye laser pumped by the third harmonic of the Nd : YAG laser and provided the computer-controlled frequency tuning of the wave $\boldsymbol{E}^{(2)}$. For each value of the frequency ω_2, the signal was averaged over 10–30 laser shots, and the laser frequency was then automatically tuned with a step of $\sim 0.119\ \mathrm{cm}^{-1}$. The zero frequency detuning was locked into the Brillouin resonances to within $0.02\ \mathrm{cm}^{-1}$; the subsequent wavelength tuning was monitored by the modes of a Fabry–Perot interferometer with a base of 7 mm. The error in four-photon scattering signal recording was computer controlled at the beginning of each measurement and usually did not exceed 10 %. The measurement error of resonance frequencies was determined by the instrumental function of the spectrometer ($\sim 0.2\ \mathrm{cm}^{-1}$). The residual slack of the sine mechanism of the rotating system for the diffraction grating in the dye laser resulted in an additional spread of the centre of the laser line frequency by $\sim 0.2\ \mathrm{cm}^{-1}$ for detunings exceeding $50\ \mathrm{cm}^{-1}$.

Measurements were made in milli-Q-water and aqueous solutions of α-chymotrypsin (with a concentration of $17\ \mathrm{mg\ mL}^{-1}$) and in native and denatured DNA ($15\ \mathrm{mg\ mL}^{-1}$) in the spectral range from -75 to $-95\ \mathrm{cm}^{-1}$. Denaturation of the DNA was caused by heating the solution up to 90 °C followed by cooling to room temperature. The samples were placed in a 100-mm-long thermally stabilised (at room temperature) cell having specially selected fused quartz windows that did not cause any additional depolarisation of the probe radiation. The four-photon mixing signal was formed in a region (~ 5 mm in length) of intersection of pump waves. No additional degassing of the liquids being analysed was performed.

The DNA from the milt of salmon fish was extracted in the presence of sodium dodecylsulphate followed by double precipitation using ethnol. To suppress light scattering, the DNA solutions were passed through a high-pressure homogeniser. The mean molecular mass of the DNA after such a treatment estimated from the characteristic viscosity of the preparation [12] was ~550 kDa (kilodaltons) for pH ~7.0 of the DNA solution. The DNA concentration was measured with a spectrophotometer [13].

3. Experimental results

Figure 1 shows the four-photon scattering spectrum in the range from -75 to $-90\ \mathrm{cm}^{-1}$ in water obtained by passing bidistilled water through filters with a pore diameter ~ 300 nm (Milli-Q-water). The spectra clearly display resonances assigned according to [8], to the most intense rotational transitions in the ground vibrational state of para and ortho isomers of H_2O molecules and to the rotational transitions of H_2O_2 and OH^- molecules that may be formed in water during laser flash photolysis [14, 15]. The line frequencies for all transitions differ from the tabulated values [8] within $0.5\ \mathrm{cm}^{-1}$, which is comparable with the instrumental function width and the spread in the dye laser frequency. Such a discrepancy appears quite acceptable because the frequencies of many lines of the rotational and vibrational–rotational spectra of water molecules differ by $\sim 0.1\ \mathrm{cm}^{-1}$ according to various estimates.

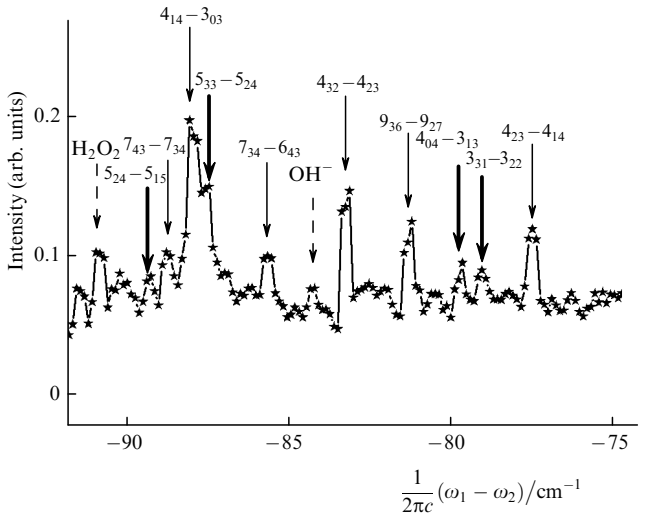

Figure 1. Four-photon scattering spectrum of milli-Q-water. Thin and heavy arrows show resonances of ortho and para isomers of H_2O, respectively. The rotational quantum numbers J_{K_a,K_c} of the initial and final levels of the corresponding transitions in the main isotope of H_2O molecules are indicated over the arrows; the dashed arrows indicate the resonances of H_2O_2 and OH^-.

Figure 2 shows the four-photon scattering spectra of aqueous solutions of the α-chymotrypsin (17 mg mL^{-1}), DNA (15 mg mL^{-1}) and the spectrum of milli-Q-water for comparison. One can see that the spectra of solutions of both biopolymers and milli-Q-water are similar and contain rotational lines of ortho and para molecules of water that were earlier observed in the aqueous solution of the α-chymotrypsin [4–6]. The spectra of both solutions display a substantial increase (by a factor of ~ 8) in the intensity of four-photon scattering rotational resonances compared to their intensity in the spectrum of milli-Q-water.

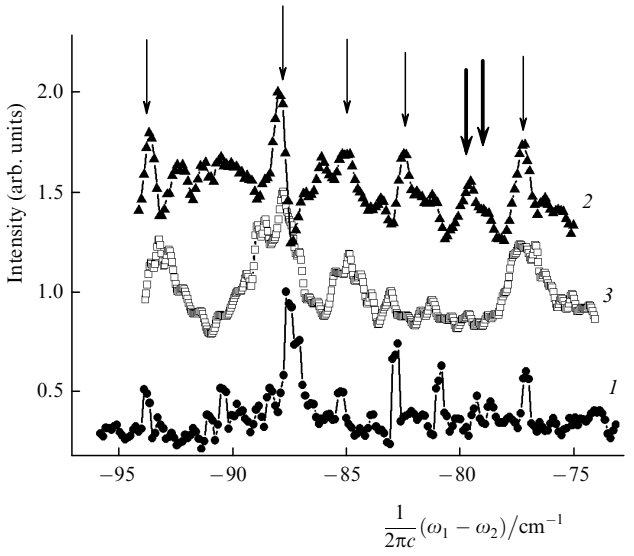

Figure 2. Four-photon scattering spectra of milli-Q-water (*1*) and aqueous solutions of the α-chymotrypsin protein (17 mg mL^{-1}) (*2*) and DNA (15 mg mL^{-1}) (*3*). The notation is the same as in Fig. 1. The intensity of spectrum (*1*) is increased by a factor of ~ 8.

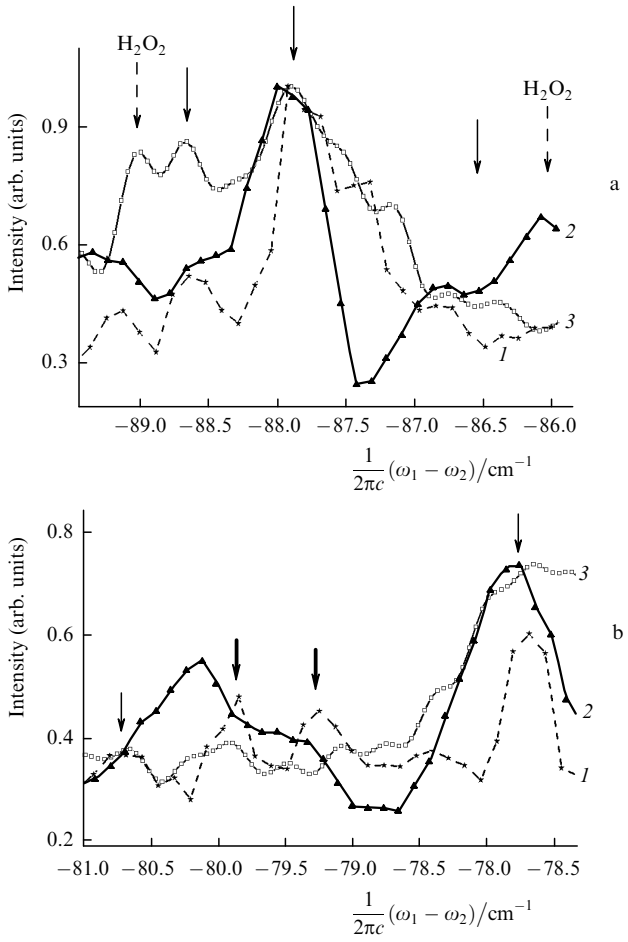

Figure 3. Fragments of the spectra of milli-Q-water (*1*) and aqueous solutions of α-chymotrypsin (17 mg mL^{-1}) (*2*) and native DNA (15 mg mL^{-1}) (*3*) presented in Fig. 2. The notation is the same as in Fig. 1.

The spectra of biopolymers were compared in several narrow spectral ranges. Figure 3 shows fragments of the spectra presented in Fig. 2 in the ranges from −86 to −90 cm^{-1} and from −77 to −81 cm^{-1}, which contain the characteristic lines of ortho and para isomers of water molecules. One can see (see Fig. 3a) that the resonances of ortho isomers of H_2O are present in the spectrum of milli-Q-water and in the spectra of the aqueous solutions of both biopolymers, while the lines of the para isomers of H_2O are suppressed in the spectra of the protein and DNA (Fig. 3b).

Figure 4 shows the four-photon scattering spectra for aqueous solutions of DNA (15 mg mL^{-1}) and denatured DNA two hours after the heating. The rotational resonances of ortho and para isomers of H_2O molecule and H_2O_2 molecules are observed in the spectra. One can see that denaturation of DNA in aqueous solution considerably modifies the rotational spectrum of H_2O molecules. In particular, the intensity of the rotational lines decreases to nearly one third of its initial value. In contrast to the aqueous solution of 'live' DNA, the rotational resonances of para isomers of H_2O are clearly distinguishable in the solution of denatured DNA. In the spectra of milli-Q-water and denatured DNA, the rotational lines of para isomers of H_2O are clearly seen in the vicinity of −80 and −79 cm^{-1}, while these lines are suppressed considerably in the spectrum of the live DNA. The spectrum of denatured DNA also displays a considerable increase in the intensity of rotational lines of H_2O_2.

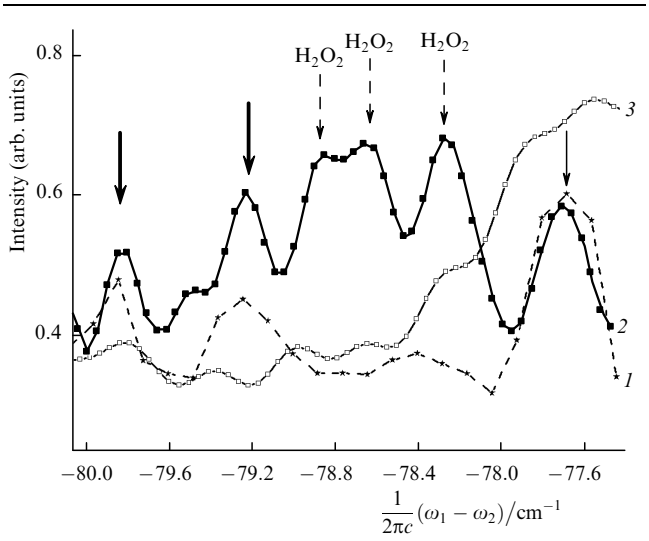

Figure 4. Four-photon scattering spectra of milli-Q-water (*1*) and aqueous solutions of denatured DNA (*2*) and DNA (*3*). The notation is the same as in Fig. 1. The intensity of spectrum (*2*) is increased by a factor of 2.5.

4. Discussion

Water is known to be a strongly associated liquid. Each molecule of water can form up to four hydrogen bonds with its neighbours. The average coordination number of hydrogen bonds at room temperature is 3.5 [16]. Our experiments show that four-photon laser scattering spectra of water and aqueous solutions exhibit narrow resonances at frequencies coinciding (within the instrumental function width) with rotational transition frequencies in the ground electronic and vibrational state of the H_2O molecule (Fig. 1). The spectral lines corresponding to the ortho and para isotopes of the H_2O molecule can be identified. Note that the vibrational–rotational spectra of the gas phase were observed for H_2O molecules and some other light molecules in nanodrops of liquid helium [17, 18] and in solid argon matrices [19] in which rotational and vibrational–rotational spectra of the OH, NH and NH_2 radicals coinciding with their spectra in gases were observed. In addition, the ortho–para conversion and variations in the concentration ratio of ortho and para molecules were observed in accordance with the Boltzmann factor upon cooling of an argon matrix from 27 to 4 K [19]. In some cases, the well-resolved vibrational–rotational spectrum was also observed in liquids at room temperature.The solution of HF in CCl_4 is an example of such a medium [20]. It was shown in [19, 20] that a spherical cavity of diameter ~ 4 Å, which is preserved during the orientational relaxation time (not exceeding 10^{-12} s for most of the liquids) is sufficient for free rotation of light molecules such as H_2O, NH_2 and HCl.

The appearance of the rotational lines of the H_2O molecule in aqueous solutions of biopolymers is probably explained by the physical properties of water hydrated at the surface of microscopic impurities [21–23]. It is well known [22, 23] that hydration leads to structurisation of water molecules in the solvate shell of the impurity. The spatial constraints emerging in this case prevent the formation of new hydrogen bonds between water molecules and lead to the formation of cavities at the surface of the microscopic impurity. As a result, the average number of hydrogen bonds per molecule is lowered considerably, thereby increasing the probability of the appearance of free molecules in the solvate shell. This is confirmed by molecular dynamic calculations [23] and X-ray diffraction experiments [21].

Spectra of the aqueous solutions of biological macromolecules (proteins, DNA) (Figs 2 and 3) exhibit rotational lines of intensity about an order of magnitude higher than the intensities of the corresponding lines in the spectrum of water. This suggests that biopolymer molecules are capable of increasing the effective concentration of free water molecules in the solvate shell of a macromolecule. This circumstance also indicates that the experimentally observed rotational lines of H_2O are not related to water molecules in air bubbles which may be present in the samples.

The increase in the intensity ratio of rotational lines of ortho and para isomers of water is not proportional to the increase in the concentration of DNA or protein in the solution. The intensity of the rotational spectrum of ortho isomers of water is considerably higher than the intensity of the rotational spectrum of para isomers in the solution of biopolymer molecules used in our experiments (Fig. 3). Note that selective sorption of the para isomers of water molecules from the gaseous phase was observed earlier [10] during their passage over the surface of films containing DNA, lysozyme and collagen.

The selectivity of interaction of ortho and para isomers of water with biological macromolecules is probably associated with a strong dependence of the nature of hydration (attraction or repulsion of H_2O molecules) on polarisability fluctuations of the H_2O impurity molecule complex, which was recently discovered in model calculations [22]. This characteristic may be different for ortho and para isomers of H_2O.

The selectivity of interaction decreases upon DNA denaturation (Fig. 4). Taking into account that the hydration energy determines the preference of a certain conformation of a macromolecule in the solution, it is obvious that the change in the concentration ratio of ortho and para molecules of water in a cell may considerably affect the equilibrium concentrations of functionally important states of macromolecules *in vivo*, and hence the functioning of cells.

5. Conclusions

Four-photon laser radiation scattering spectra have been obtained in aqueous solutions of biopolymers (proteins, DNA, denatured DNA), and deionised milli-Q-water in the submillimetre range ($-75 \ldots -95$ cm^{-1}). The rotational spectral lines of ortho and para isomers of the main isotope of water molecule, as well as H_2O_2 and OH^- molecules, were detected in the investigated spectra.

The resonance contribution from the rotational spectrum of H_2O molecules to the four-photon scattering signal increases considerably on passing from water to biopolymer solutions. The mechanism of this phenomenon is not quite clear, but it can be assumed that the presence of biopolymer molecules leads to a distortion of the initial topology of the network of hydrogen bonds in the hydrate shell, increasing the concentration of weakly bound and free H_2O molecules in microcavities at the biopolymer–water interface.

It was found that protein and DNA molecules in the native solution selectively interact with the para isomers of H_2O. No selective interaction was observed in the case of DNA denaturation.

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