

Four-photon Rayleigh-wing spectroscopy of the aqueous solution of α -chymotrypsin protein

A.F. Bunkin, S.I. Lebedenko, A.A. Nurmatov, S.M. Pershin

Abstract. The spectra of coherent librations of H_2O molecules, coinciding in frequencies with the rotational spectrum of gaseous H_2O , are observed for the first time in aqueous solutions of α -chymotrypsin protein and hydrogen peroxide and in deionised Milli-Q water by the method of four-photon laser scattering in the frequency range 0–100 cm^{-1} . The resonance contribution of molecular librations to the four-photon scattering signal considerably increases in aqueous solutions compared to water. The resonances related to the lines of the ortho- and para-modifications of the natural isotope of the H_2O molecule in the gas phase are recorded in the four-photon scattering spectra. It is found that the protein molecule in aqueous solution selectively interacts with the para- H_2O , which makes it possible to interpret some features of the interaction of microwave radiation with biological objects and to develop a new class of laser biotechnologies.

Keywords: nonlinear laser spectroscopy, four-photon scattering, low-frequency spectroscopy of proteins.

1. Introduction

At present low-frequency resonances existing in molecules of proteins, amino acids, and polymers attract considerable interest because they contain information on the geometrical parameters of these macromolecules and processes of their interaction with solvent molecules during studies in native media. In most cases, this information can be obtained only by the methods of optical spectroscopy. However, the recording of Raman signals of these objects at frequencies below 100 cm^{-1} is complicated due to a high optical noise.

This problem can be solved by using four-photon Rayleigh-wing spectroscopy [1–3], which provides a considerable increase in the signal-to-noise ratio in low-frequency spectra due to phasing of the orientation and

translational motions of molecules with the help of two laser waves at frequencies ω_1 и ω_2 , whose difference is scanned within the Rayleigh wing. The parameter being measured is the polarisation state of radiation at frequency $\omega_s = \omega_1 - (\omega_1 - \omega_2)$, whose nonlinear source is [4, 5]

$$P_i^{(3)} = 6\chi_{ijkl}^{(3)}(\omega_s; \omega_1; \omega_2; -\omega_1)E_j^{(1)}E_k^{(2)}E_l^{(1)}. \quad (1)$$

Here, $\chi^{(3)}$ is the cubic susceptibility of a medium, which is proportional to the correlation fluctuation function of optical anisotropy; $E^{(1)}$ and $E^{(2)}$ are the amplitudes of the interacting fields; and $I_s \propto |\chi^{(3)}|^2 I_1^2 I_2$ is the detected signal intensity. Note that the laser field interacting with an ensemble of molecules specifies the phase of their vibrational and rotational modes in the entire volume of the medium irradiated simultaneously by light at frequencies ω_1 and ω_2 .

Recently [6], the spectrum of coherent rotations in liquid water, identical to the H_2O spectrum in a gaseous phase, was recorded by this method. Later, the rotational spectrum of water molecules was also observed in the aqueous solution of α -chymotrypsin protein in the region 0–5 cm^{-1} in the Rayleigh wing [7]. It was reasonable to assume that, if these lines are rotational lines of water molecules, the rotational resonances should be also observed at higher frequency detunings.

In this paper, we obtained the four-photon scattering spectra in the macroscopic volume of aqueous solutions of α -chymotrypsin protein in the region 0–100 cm^{-1} . This frequency range includes Brillouin resonances, the narrow and broad parts of the Rayleigh wing, the possible Raman resonances related to vibrational and orientation motions of the molecular fragments of the protein, as well as the intrinsic low-frequency spectra of water molecules.

2. Experimental

Experiments were performed on the setup described in [3]. The two counterpropagating waves $E^{(1)}$ and $E^{(2)}$ at frequencies ω_1 and ω_2 propagated in a sample cell. The entrance and exit silica windows of the cell provided a low depolarisation level of the transmitted laser radiation. The wave $E^{(1)}$ (the second harmonic of a single-mode Nd : YAG laser) was circularly polarised, while the tunable wave $E^{(2)}$ was linearly polarised. In the case of such polarisations of the interacting waves [5, 6], a signal determined by nonlinearity (1) does not contain the nonresonance contribution from the electronic subsystem of the medium.

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Because the polarisation unit vectors of the signal wave at frequency ω_s and of the wave $E^{(2)}$ are noncollinear and their propagation directions coincide, the signal was selected with a Glan prism. The width ($\sim 0.2 \text{ cm}^{-1}$) of the instrumental function of the spectrometer and its spectra range (from -1200 to 300 cm^{-1}) were determined by the output parameters of a dye laser, which was pumped by the third harmonic of a Nd:YAG laser and provided computer-controlled tuning of the $E^{(2)}$ wave. Signals for each value of frequency ω_2 were averaged over 10–30 counts and then the laser frequency was automatically tuned with a step of $\sim 0.05 \text{ cm}^{-1}$.

Measurements were performed in Milli-Q water (H_2O), the aqueous solution of α -chymotrypsin protein, and in the 5% solution of hydrogen peroxide (H_2O_2) in the spectral range from 0 to 100 cm^{-1} .

3. Experimental results

Figure 1 presents a fragment of the spectrum of Milli-Q water (H_2O) in the range $\pm 10 \text{ cm}^{-1}$ at an increased scale. The resonances at frequencies 2.26 ($4_{14} - 3_{21}$), 4.47 ($14_{69} - 15_{312}$), 7.76 ($5_{50} - 6_{43}$), 8.78 ($7_{70} - 8_{63}$), and 10.7 cm^{-1} ($10_{29} - 9_{36}$) (ortho-modification) and frequencies 3.21 ($5_{33} - 4_{40}$), 6.11 ($3_{13} - 2_{20}$) and 8.25 cm^{-1} ($14_{410} - 15_{313}$) (para-modification) are clearly observed, which can be assigned, with an accuracy to the instrumental function (0.2 cm^{-1}), to the rotational transitions in the ground vibrational state of the ortho- and para-spin-isomers of water molecules, respectively [8]. In parentheses are indicated the rotational quantum numbers J, K_a, K_c of the initial and final levels of the corresponding transitions in the natural isotope of the water molecule taken from the HITRAN data base [8]. In addition, the distinct resonances of hydrogen peroxide (H_2O_2) at ~ 1.8 and $\sim 2.8 \text{ cm}^{-1}$ were observed, which were also reported in [8]. These resonances were well reproduced in all measurements.

Figure 2 shows the spectrum of water in the frequency detuning range from -4 to -40 cm^{-1} . By using the

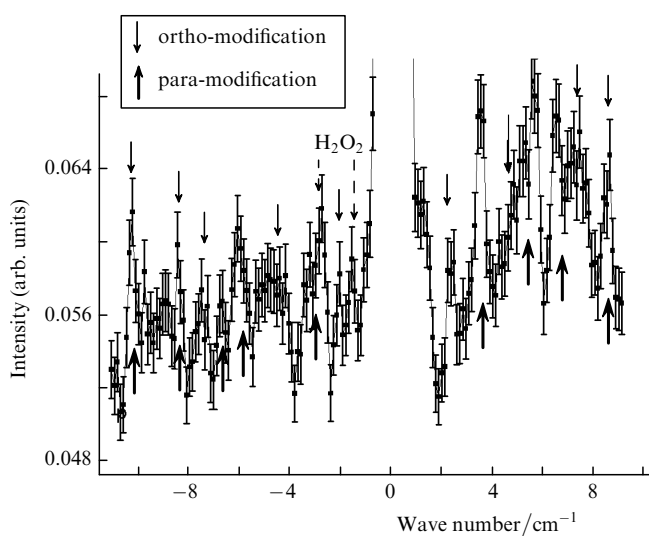


Figure 1. Fragment of the spectrum of Milli-Q water (H_2O) in the frequency range from -10 to $+10 \text{ cm}^{-1}$. The thin and thick arrows indicate rotational resonances assigned to the ortho- and para-modification of H_2O molecules, respectively.

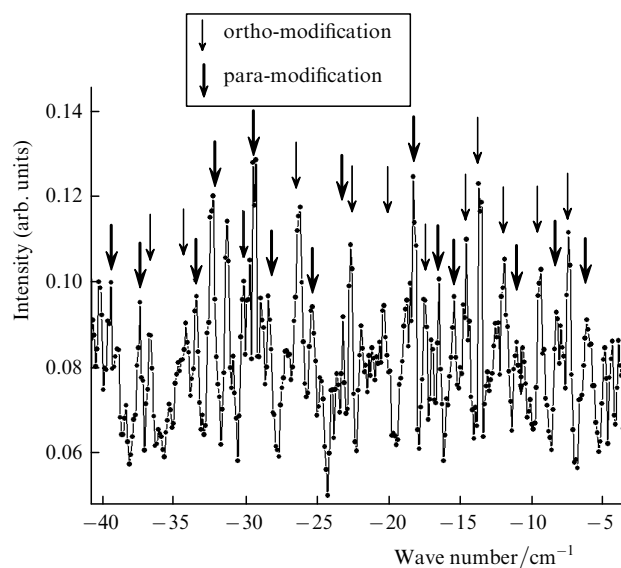


Figure 2. Four-photon scattering spectrum in water in the frequency detuning range from -4 to -40 cm^{-1} . The thin and thick arrows indicate rotational resonances assigned to the ortho- and para-modification of H_2O molecules, respectively.

HITRAN data base [8], all the resonances observed in the spectrum can be assigned to the different rotational transitions in the ground vibrational state of the ortho- and para-modifications of the natural H_2O isotope, similarly to resonances presented in Fig. 1. The resonances of the ortho- and para- H_2O are indicated by the arrows of different thickness (as in Fig. 1).

Figure 3 presents the spectra of water and the 5% solution of hydrogen peroxide in the frequency range from -35 to -65 cm^{-1} . Here, the resonances related to the ortho- and para-modifications of the water molecule are also observed, these resonances being present in the spectra of both liquids. Note that rotational resonances in the

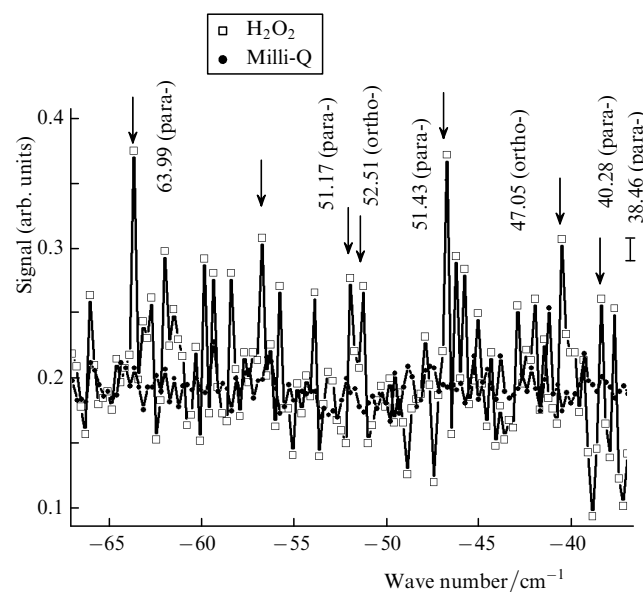


Figure 3. Fragments of the spectra of the 5% aqueous solution of H_2O_2 and Milli-Q water in the frequency range from -35 to -65 cm^{-1} .

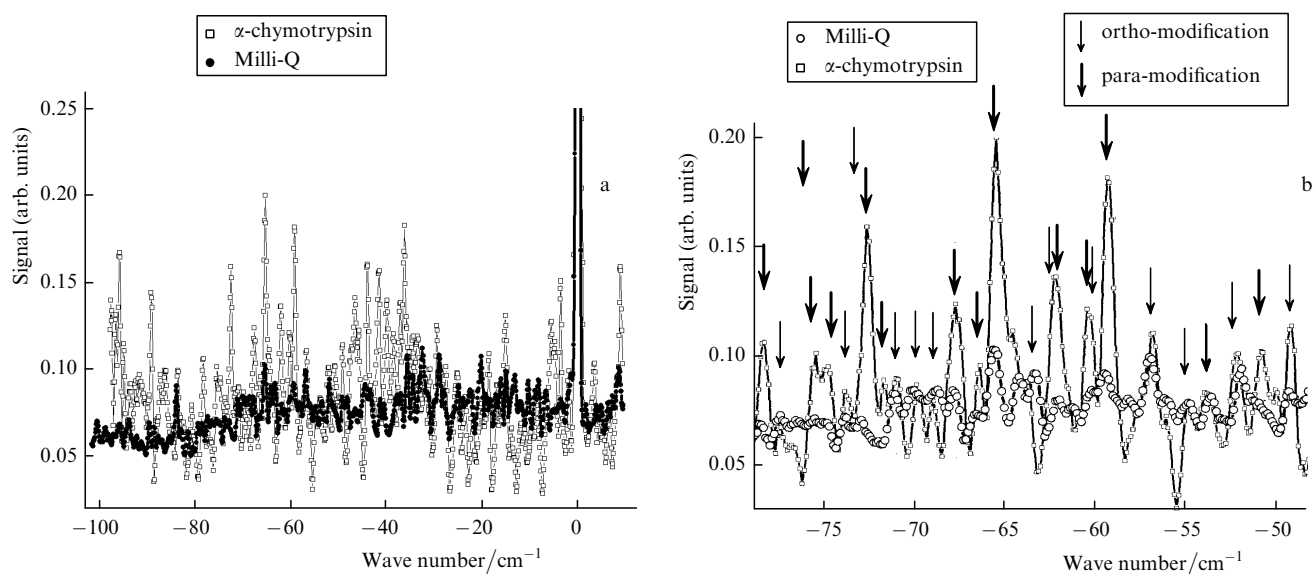


Figure 4. (a) Survey four-photon scattering spectra of Milli-Q water and the aqueous solution of α -chymotrypsin at the concentration 17 mg mL^{-1} in the frequency range from 10 to -100 cm^{-1} and (b) the spectra of these liquids in the frequency range from -48 to -78 cm^{-1} . The thin and thick arrows indicate rotational resonances assigned to the ortho- and para-modifications of H_2O molecules, respectively.

solutions of H_2O_2 and protein (Fig. 4) are more distinct than in water.

Figure 4a shows the survey spectra of Milli-Q water and aqueous solution of α -chymotrypsin at the concentration 17 mg mL^{-1} in the frequency range from 10 to -100 cm^{-1} . One can see that, as in the case of the H_2O_2 solution, the rotational resonances in the protein solution are more distinct than in the spectrum of water. Figure 4b shows a more detailed spectrum of the same liquids in the range from -48 to -78 cm^{-1} . The arrows indicate rotational resonances related to the ortho- and para-modifications of H_2O , which were identified by comparing them with data [8]. The resonances of ortho- H_2O molecules are observed at frequencies 77.3 ($9_{45} - 9_{36}$), 74.1 ($5_{14} - 5_{05}$), 70.6 ($3_{30} - 3_{21}$), 70.3 ($7_{34} - 7_{25}$), 68.4 ($10_{56} - 11_{29}$), 63.5 ($12_{310} - 13_{211}$), 62.3 ($5_{32} - 5_{23}$), 56.5 ($10_{47} - 11_{110}$), 54.8 ($3_{03} - 2_{12}$), 53.8 ($2_{21} - 2_{10}$) and 52.5 cm^{-1} ($6_{43} - 7_{16}$), while the resonances of para- H_2O molecules are observed at frequencies 78.3 ($10_{46} - 10_{37}$), 75.3 ($4_{04} - 3_{13}$), 74.5 ($3_{13} - 2_{02}$), 72.6 ($7_{35} - 8_{08}$), 71.4 ($6_{33} - 6_{24}$), 68.0 ($4_{31} - 4_{22}$), 66.5 ($10_{55} - 9_{64}$), 65.3 ($6_{24} - 5_{33}$), 61.9 ($2_{20} - 2_{11}$), 59.9 ($6_{24} - 6_{15}$), 58.9 ($7_{35} - 6_{42}$) and 51.0 cm^{-1} ($4_{13} - 3_{22}$). In parentheses are indicated the rotational quantum numbers J , K_a and K_c of the initial and final levels of the corresponding transitions in the natural water isotope taken from the HITRAN data base [8]. Note that in the protein solution the rotational resonances related to the para- H_2O molecules dominate.

4. Discussion

Our experiments have shown that the spectra of four-photon scattering of laser radiation in water exhibit narrow resonances at frequencies coinciding, with an accuracy to the instrumental function width of the spectrometer, with the frequencies of rotational transitions in the ground electronic and rotational states of the H_2O molecule. We have identified the resonances related to the ortho- and para-modifications of the natural water isotope. The same

resonances were observed in the spectra of aqueous solutions of hydrogen peroxide ($5\% \text{ H}_2\text{O}_2$) and α -chymotrypsin protein at the concentration 17 mg mL^{-1} . The latter circumstance suggests that the fraction of free water molecules in the protein and hydrogen peroxide solutions increases.

The increase in the contribution of the rotational spectrum to the four-photon scattering signal in the H_2O_2 solution compared to H_2O can be explained by a weakening of the intermolecular interaction in hydrogen peroxide compared to water because H_2O_2 molecules form two hydrogen bonds with H_2O , whereas water molecules form four hydrogen bonds [9, 10]. We can assume that the hydrogen bond between water molecules and α -chymotrypsin protein is weaker than with other water molecules. The dominating role of the rotational resonances of para- H_2O molecules observed in the protein solution suggests that these molecules interact selectively with biological macromolecules. The physical nature of this selectivity is not clear and requires further studies.

5. Conclusions

By using four-photon coherent scattering of laser radiation in aqueous solutions of α -chymotrypsin and hydrogen peroxide and in deionised Milli-Q water in the range $0.2 - 100 \text{ cm}^{-1}$, we have recorded for the first time the spectrum of coherent librations of H_2O molecules coinciding in frequency with the lines in the vibrational spectrum of H_2O in the gas phase. The resonance contribution of coherent librations of H_2O molecules to the four-photon scattering signal considerably increases in aqueous solutions compared to water and rises upon dilution. The four-photon scattering spectra exhibit resonances related to the spectral lines of the ortho- and para-modifications of the natural H_2O isotope observed in the gas phase. It has been found that the protein molecule in solution interacts selectively with para- H_2O molecules. This effect makes it possible to interpret the selective action of microwave radiation on

biological objects and to develop in the future a new class of laser biotechnologies.

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