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Quantum-Mechanical Analysis of the Intensity Distribution in Spectra of Resonant Raman Scattering Spectra of Aqueous Solutions of Tyrosine

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Abstract—Quantum-mechanical calculations of the intensity distribution in the resonant Raman scattering spectra of aqueous solutions of tyrosine excited by laser radiation with wavelengths of 244, 229, 218, 200, and 193 nm, as well as in the nonresonant Raman scattering spectrum excited at a wavelength of 488 nm, are performed. Satisfactory agreement is achieved between the calculation results and the experimental data. It is shown that the changes in the intensity distribution observed in the spectra with a change in the excitation wavelength from 244 to 193 nm correlate with the determined changes in the contribution made by excited electronic states into the scattering tensor components. It is noted that it is necessary to take into account the Herzberg–Teller effect and that the number of excited electronic states taken into account considerably affects the calculated relative intensities of lines. The possibility of existence of several tyrosine conformers in aqueous solution at room temperature is shown.

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Raman scattering (RS) spectroscopy is an efficient tool for studying biomolecules, including peptides and proteins, based on the knowledge of the structure, properties, and conformational and chemical transformations of amino acids entering their composition. Tyrosine is one of the most important aromatic amino acids used to synthesize many biologically active compounds, in particular, thyroid hormones, melanin, and adrenaline. It plays an important role in regulation of blood pressure and urination, possesses strong stimulating properties, helps organisms to withstand stresses, and stabilizes nervous system.

Taking into account the important role played by tyrosine in functioning of living organisms, it is not surprising that its structure, properties, and spectra have been extensively studied by many authors [1–16]. The fluorescence spectra of tyrosine in a supersonic jet were studied in [1], and the two-photon absorption and fluorescence spectra were investigated in [2, 3], but these work contain no detailed analysis of the vibrational structure of the spectra. The electronic-vibrational absorption and spontaneous RS spectra of tyrosine, tryptophan, and their mixtures were theoretically and experimentally studied in [4]. The calculation was performed by the TDDFT method with the PBEO functional and 6-311++G(d,p) basis set. The wavelengths corresponding to electronic transitions in the range of 210–280 nm and the dipole moments were determined, and the lines characteristic of tyrosine and tryptophan in peptide spectra were separated.

In characterizing the state of the art in the study of the vibrational spectra of tyrosine, it should be noted that not all the lines of the vibrational structure have yet been unambiguously identified. The vibrational spectrum of isolated tyrosine was studied in detail in [5], in which the frequencies of vibrations were calculated using a Gaussian 92 complex and the lines were identified taking into account the data available in the literature by the time that the article was written. Due to the absence of experimental data on tyrosine spectra in a gas phase or in an insulating matrix, the authors used the spectra of tyrosine powder from [6].

An important place is taken by the studies of tyrosine spectra in solutions, first of all in aqueous solutions. In [7], the RS spectra of tyrosine in an aqueous solution and in D_2O were measured and analyzed upon excitation at a wavelength of 488 nm at room temperature. In addition, the vibrational frequencies of complexes “tyrosine + $7H_2O$ molecules” and “tyrosine + $7D_2O$ molecules” were calculated by the quantum-mechanical DFT/B3LYP/6-31++G method, which allowed the authors to identify the spectral lines. Based on the analysis of the effect of water molecules, the possibility of formation of tyrosine conformers due to a flexible side chain was suggested and four most probable conformers were specified.

The authors of [8] studied the effect of solvent on the vibrational spectra of tyrosine and para-cresol in the range of 1150–1300 cm^{-1}. It was noted that the oxygen bond strongly affects the positions of lines corresponding to the excitation of vibrations 7a and 9a. Thus, the frequency of vibrational line 7a in neutral tyrosine is 1255 cm^{-1}, in the case of formation of a hydrogen bond by the oxygen atom in the OH group (tyrosine acts as an acceptor), this frequency is 1240–1230 cm^{-1}, at the same time, for a strong hydrogen bond of the phenol group (tyrosine acts as a donor), this frequency is 1275–1265 cm^{-1}. The 9a vibration frequency is very sensitive to the position of the OH group with respect to the benzene ring, being 1180 cm^{-1} if the OH group lies in the ring plane and decreasing to 1170 cm^{-1} if the OH group falls out of this plane.

One of the first works devoted to the investigation of resonant Raman scattering (RRS) in an aqueous solution of tyrosine is study [12], in which the RRS spectrum excited at a wavelength of 200 nm was measured and described. The most intense lines correspond to the excitation of vibrations 8a (1617 cm^{-1}), 8c (1601 cm^{-1}), 1215 cm^{-1}, 1 (858 cm^{-1}), 2 × 16a (838 cm^{-1}), 7a (1268 cm^{-1}), and 9a (1184 cm^{-1}).

An RRS spectrum of an aqueous solution of tyrosine excited by laser radiation with a wavelength of 235 nm is presented in [13]. In the range studied (500–2000 cm^{-1}), the most intense lines were observed at 1617, 1180, 1210, and 853 cm^{-1}.

In [14], the RRS spectra of tyrosine were measured upon excitation by laser radiation at wavelengths of 240 and 192 nm. It was noted that the intensities of lines corresponding to the excitation of vibrations 8a, 8c, and 9a are higher in the case of excitation at 192 nm than at 240 nm. Work [15] discusses the actual achievements in studying the RRS spectra of amino acids, presents the RRS spectrum of tyrosine excited by radiation with a wavelength of 193 nm, and identifies the main spectral lines.

The contribution of excited electronic states (EESs) to the scattering tensor components was discussed in [16] based on calculation of the RRS spectra of tyrosine excited by radiation with wavelengths of 229 and 244 nm. The change in the calculated intensities of lines corresponding to the excitation of four most typical vibrations (ν_{9a} (1204 cm^{-1}), ν_{8a} (1655 cm^{-1}), ν_{8b} (1630 cm^{-1}), and ring stretching vibrations (1226 cm^{-1})) with a change in the number of EESs taken into account was studied in detail; it was shown that an increase in the number of EESs taken into account to 20 allows one to considerably correct the line intensities. In addition, it was found than an important role is played by the electronic-vibrational mixing of a considered electronic state with other EESs. The frequencies of vibrations were calculated using a harmonic approximation, which explains the overestimations of the calculated vibrational frequencies compared to the corresponding experimental values by 30–40 cm^{-1}. The theoretical data were compared with the experimental results for the RRS spectra of an aqueous solution of tyrosine with a concentration of 0.02 mg/mL, as well as with the data of [13, 14]. Note that the necessity of taking into account the contribution of highly excited electronic states to the scattering tensor components was previously shown by analyzing the spectra of other polyatomic molecules in [17, 18], in which attention was paid to an important role of the Herzberg–Teller effect played in the analysis of the intensity distribution.

Overall, the study of the vibrational and electronic-vibrational spectra of tyrosine in different phase states is not completed. The presence of rather comprehensive experimental data on the RS and RRS spectra of aqueous solutions of tyrosine requires calculations of the intensity distribution in the spectra excited at different wavelengths, as well as determination of the regularities and mechanisms of the formation of observed distribution patterns. In this connection, it is very important to perform quantum-mechanical calculations of the RRS and RS spectra of tyrosine excited by radiation with a large set of wavelengths (488, 244, 229, 218, 200, and 193 nm). This study is not only of interest itself, but also serves as a basis for subsequent analysis of the conformational composition of tyrosine in different phase states and for studying the structure of peptides, because the characteristic lines of tyrosine are promising for application as markers.

Let us turn to the RS spectra of tyrosine in an aqueous solution. In the present work, we calculate the RS activity using the B3LYP hybrid functional and its variations with the 6-311++G(d,p) basis set with the use of the Gaussian-03 [19] and ORCA [20] software packages. Next, according to [21], we find the intensity distribution in the RS spectra of the aqueous solution of tyrosine excited at a wavelength of 488 nm. The geometric parameters of the tyrosine molecule correspond to the parameters taken in [7].

The calculation results are presented in Table 1 together with the experimental data from [7] for a tyrosine aqueous solution in the range of 600–1650 cm^{-1}; lines with a relative intensity lower than 0.1 are omitted. In [7], the authors also calculated the RS spectra of tyrosine in a complex with seven water molecules by the DFT/B3LYP/6-31++G(d,p) method, which is of undoubted interest, but the difference between the calculated and experimental vibrational frequencies turned out to be rather large and, in some cases, reached 100 cm^{-1}. In the present work, the effect of solvent was taken into account using a reactive field model (dielectric constant $\varepsilon = 78.39$, SCRF method [19]); in this case, the difference between the calculated and experimental vibrational frequencies does not exceed 50 cm^{-1} and, on average, is 20 cm^{-1}. For comparison, we also performed calculations with the use of the B3LYP hybrid potential based on the

Table 1. Frequencies and relative intensities of the RS lines of the aqueous solution of tyrosine excited at a wavelength of 488 nm

Frequency, cm^{-1}			Relative intensity		
calculation 1*	calculation 2*	experiment [7]	calculation 1*	calculation 2*	experiment [7]
555	554	582	0.01	0.03	0.1
636	652	643	0.02	0.08	0.2
654	705	703	0.09	0.06	0.1
791	828	806	0.15	0.08	0.4
843	854	830	0.14	0.47	0.5
856	887	850	0.57	0.11	0.5
893	896	865	0.13	0.02	0.2
948	937	907	0.08	0.06	0.1
969	957	948	0.04	0.01	0.1
978	978	968	0.01	0.01	0.1
1010	987	1040	0.13	0.03	0.2
1089	1102	1068	0.04	0.10	0.2
1179	1180	1178	0.28	0.30	0.3
1226	1220	1210	0.73	0.57	0.5
1240	1259	1250	0.05	0.41	0.2
1265	1275	1268	0.41	0.01	0.3
1329	1335	1327	0.28	0.21	0.2
1341	1358	1336	0.01	0.07	0.2
1357	1368	1361	0.03	0.04	0.2
1367	1380	1415	0.25	0.24	0.1
1433	1389	1445	0.04	0.14	0.2
1462	1461	1516	0.01	0.01	0.15
1484	1477	1504	0.11	0.07	0.1
1538	1535	1537	0.01	0.01	0.1
1627	1623	1604	0.2	0.14	0.5
1647	1631	1616	1.00	0.03	1.0
1657	1643	–	0.04	1.0	
1773	1755	–	0.2	0.23	–

*Calculations 1 and 2 are performed using the software complexes [19] and [20], respectively.

spin-restricted Kohn—Sham (RKS) method [22, 23] in the RIJCOSX approximation [24] with the 6-311G++(d,p) basis set using the ORCA 3.0.3 software package [20]. In this case, the average discrepancy between the calculated and experimental results was 21 cm^{-1}, the maximum discrepancy being 56 cm^{-1}. This fact testifies that the ORCA programs can be successfully used for calculating RS spectra together with the well-known Gaussian-03 software.

Comparison of the calculation results with the experimental data shows that, in general, the character of intensity distributions is satisfactorily reproduced by our calculations. For example, the most intense lines (in decreasing order) lie at 1616 (8a), 1604 (8b), 1210 (totally symmetric benzene ring stretching), 830 (2 × 16a), and 850 (1) cm^{-1}. However, the calculated relative intensities of some lines, for example, of lines at 555, 636, 791, 978, 1341, 1357, 1462, and 1538 cm^{-1} (according to calculation 1* from Table 1), are underestimated compared to the experimental values. The most probable reason for this discrepancy is the existence of several conformers of tyrosine in the aqueous solution.

Note that the existence of two highly intense lines in the range of 830–850 cm^{-1}, which are assigned to the fundamental ring vibrations υ_1 and to the out-of-plane vibration mode $2\nu_{16a}$, between which the Fermi resonance is observed, is one of the distinguishing features of the RS spectrum of tyrosine. These very char-

Table 2. Calculated and experimental relative intensities of the RRS lines of the aqueous solution of tyrosine excited by laser radiation with different wavelengths

Frequency, cm^{-1} [7]	Relative intensity									
	$\lambda = 244$ nm		$\lambda = 229$ nm		$\lambda = 218$ nm		$\lambda = 200$ nm		$\lambda = 193$ nm	
	calculation	experiment [16]	calculation	experiment [16]	calculation	experiment [14]	calculation	experiment [12]	calculation	experiment [15]
643	0.10	0.2	0.06		0.03		0.02		0.02	
703	0.03		0.05		0.03		0.07	0.1	0.02	
806	0.03		0.04		0.03		0.04	0.1	0.04	
830	0.10	0.3	0.09	0.1	0.08	0.1	0.19	0.25	0.19	0.3
850	0.22		0.12		0.12	0.1	0.31	0.4	0.24	
948	0.05		0.04		0.05		0.07	0.1	0.06	
1040	0.03		0.02		0.03		0.04	0.1	0.03	
1068	0.03		0.03		0.04		0.07	0.1	0.06	
1178	0.41	0.5	0.30	0.4	0.32	0.4	0.31	0.35	0.21	0.25
1210	0.32	0.4	0.14	0.2	0.15	0.2	0.87	1.0	0.79	1.0
1250	0.05	0.1	0.04		0.13	0.2	0.26	0.4	0.21	0.3
1268	0.06	0.1	0.09		0.07		0.31	0.4	0.33	0.35
1336	0.03		0.02		0.02		0.05	0.1	0.06	0.1
1445	0.05		0.04		0.04		0.07	0.1	0.08	0.1
1516	0.01		0.01		0.02		0.02	0.1	0.01	
1604	0.34	1.0	0.59	1.0	0.66	0.8	0.63	0.8	0.57	0.6
1616	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0

acteristic spectral lines can be used as markers when investigating the structure of proteins [4, 9–11].

Data on the vibrational characteristics and the electronic structure of the molecule obtained by calculating the RS spectra of aqueous solutions of tyrosine were used to calculate the RRS spectra excited at wavelengths of 193, 200, 218, 229, and 244 nm. The intensity distributions in the spectra were analyzed by the quantum-mechanical method [17] in the Herzberg–Teller approximation taking into account the frequency effect, the Dushinskii effect, and the contribution of highly excited electronic states to the scattering tensor components.

The calculated relative intensities of lines together with the experimental data of [12, 14–16] are presented in Table 2; lines with a relative intensity below 0.1 are omitted. In the process of calculations, we revealed a considerable variation in the intensity distributions with increasing number of excited electronic states taken into account. In our case, it is reasonable to take into account all electronic states for which the difference between the energy of the 0–0 transition and the energy of the transition to the resonance electronic state does not exceed 10 eV. In addition, a considerable role is played by the electronic-vibrational mixing of electronic states, which also leads to a change in the relative intensities of spectral lines.

With decreasing wavelength of exciting radiation, i.e., with passing from the RRS spectrum excited at a wavelength of 244 nm to the spectrum excited at 193 nm, one observes a change in the character of the intensity distribution in the spectrum. In particular, the intensities of lines in the range of 1150–1300 cm^{-1} increase and the dominant role of the lines in the range of 1600–1620 cm^{-1} is retained. The intensities of lines 1 and 2 × 16a (850 and 830 cm^{-1}) are characterized as intermediate in the spectra excited at wavelengths of 244, 200, and 193 nm and as weak in the spectra excited at 229 and 218 nm. These changes are easily seen when analyzing the contributions of different excited electronic states to the scattering tensor components.

The spectra excited by radiation with wavelengths of 200 and 193 nm have similar characters of intensity distributions. This is not surprising because, in fact, the resonance occurs with one and the same excited electronic state and with only slightly different contributions of the electronic states close to the resonance one due to a difference between the excitation photon energy and the energy of transition to the considered electronic state. The similarity of the spectra excited at wavelengths of 218 and 229 nm is explained in a similar way.

Comparison of the intensity distributions in the RS and RRS spectra of the aqueous solution of tyrosine shows a relative decrease in the intensities of lines at 850 and 830 cm^{-1} and an increase in the intensity of lines in the range of 1150–1300 cm^{-1} in the RRS spectra compared to the RS ones; this behavior is well reproduced by our calculations.

Thus, the presented results of quantum-mechanical calculations of the RS and RRS spectra of the aqueous solution of tyrosine allowed us to reproduce the main regularities in the intensity distributions in these spectra and to explain the difference between the spectra excited at wavelengths of 244 and 193 nm by different contributions of excited electronic states to the scattering tensor components. It is found that the number of excited electronic states taken into account considerably affects the relative intensities and that one must take into account the electronic states with the difference between the energies of the 0–0 transition and the transition to the resonance electronic state lower than 10 eV. The Herzberg–Teller effect, the frequency effect, and the Dushinskii effect should also be taken into account.

A satisfactory qualitative agreement between the calculated and experimental RS and RRS spectra excited at wavelengths of 488, 244, 229, 218, 200, and 193 nm is achieved. Some discrepancies between the calculated and experimental data indicate the existence of several conformers of tyrosine in aqueous solutions, which confirms the conclusions of work [7].

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