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Raman spectra in range 75−1200 cm^{-1} of amino acids L-tryptophan, diphenyl-L-alanine and glycine in aqueous solution and in dehydrated films

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Raman-scattering spectroscopy was used for comparative study of characteristics of biomolecule oscillations in solutions and microcrystalline films. In range 75−1200 cm^{-1} we obtained Raman spectra of microcrystals of the indicated amino acids and their molecules in an aqueous solution. Attention was paid to accurate registration of frequency shifts of molecule oscillations in the solutions and in microcrystalline films formed from the solutions. The known publications do not contain such comparison. The mechanisms able to result in the identified shifts are discussed.

Keywords: Raman-scattering spectroscopy, crystals of amino acids, dehydration.

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1. Introduction

Amino acids (AA) are building blocks of living matter. From amino acids the more complicated molecules and structures are formed — for example, proteins, DNA. Unlike more complex biomolecules, the amino acids are relatively simple compounds, that are not subjected to significant conformational changes in solutions, which makes rather reliable comparison of their spectra in liquid and in crystal state.

AA glycine, diphenyl-L-alanine and L-tryptophan (hereinafter referred to as glycine, alanine, tryptophan) were selected for studies due to definite features of structure (Figure 1) and functional properties — they are representatives of various groups of AA (non-polar, polar, aromatic) [1], as well as due to the possibility to compare the obtained results with known study results.

Dynamics study of biomolecules has important information on force constants of interaction of their individual fragments, which is essential in terms of understanding the mechanisms of formation of more complex molecular structures [2,3], and provides information for computer simulation and directed synthesis of new compounds.

By now the amino acids and other biomolecules are considered as possible components of the hybrid microelectronic devices controlled by electrical and optical signals [4,5]. Use of biomolecules and their complexes as functional units of devices fundamentally and significantly increases the functionality of semiconductor microelectronic devices, for example, in nanophotonics and nanoplasmonics.

The present paper relates to study of Raman scattering by amino acids of three groups: alanine, glycine, tryptophan. The main attention was paid to line shifts in spectra of crystals relative to their position in aqueous solutions.

2. Experiment method and studied samples

Raman scattering was studied using modular micro-Raman spectrometer HORIBA-JOBIN-IVON 320, connected to optical microscope OLIMPUS BX41. Scattering was excited by helium-neon laser at wavelength 632.81 nm with power 2−5 mW.

Studies were conducted at a room temperature. The microcrystals were located on standard cover-glasses. For example, micrograph of alanine single crystals is shown in Figure 2.

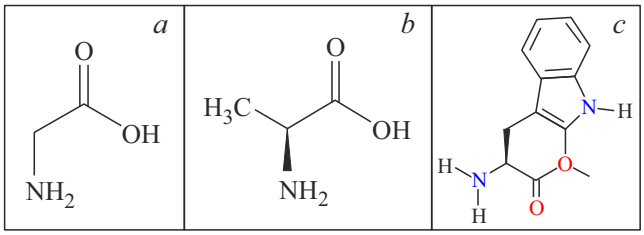

Figure 1. Molecular structure *a*) of glycine, *b*) alanine, *c*) tryptophan.

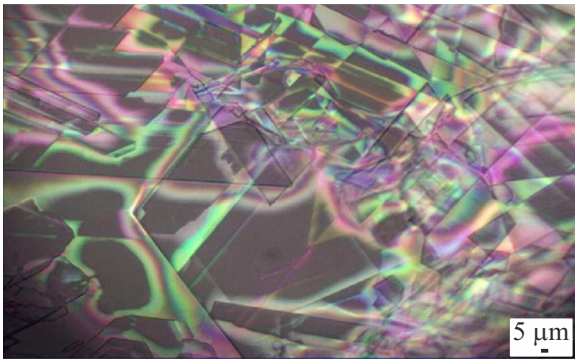

Figure 2. Dehydrated alanine microcrystals on substrate. Photo scale is shown in right bottom angle.

The absence of interference color bands on individual single crystals indicates that these are predominantly flat flakes of constant thickness. Also typical angles of microcrystals ($\sim 53°$; $105°$) shown in photo do not comply with angles of symmetric crystallographic syngonies, so we can suppose that crystals relate to monoclinic syngony.

For the microcrystaline samples preparation we used pre-developed technology of slow isothermal dehydration (lyophilization at atmospheric pressure in a thermostat at 25°C) to obtain the biomolecular films with polycrystalline domain structure on standard cover glasses [6].

Solutions during study were in quartz cuvettes. The cuvette wall thickness and solution layer thickness ensure reliable focusing of excitation inside the solution layer.

3. Results

In paper we obtained spectra of inelastic Raman spectra of microcrystals in films and of AA molecules in water solution In range $75-1200\,\mathrm{cm}^{-1}$.

Comparison of the obtained Raman spectra of microcrystals of glycine and its 5% solution in water is given in Figure 3.

Although available published studies of Raman scattering in AA do not cover the wavelength range studied in this paper, and thus is not copied by our measurements, we can say that on a whole the general appearance of spectra and positions of maximum generally correspond to the results of other authors, for example, [7]. Spectrum of crystals contains narrow and intense lines, as well as large number of less intense lines, slightly exceeding the noise level in intensity (not always identified in scale of Figures in magazine). The solution spectrum is less intensive, contains lower number of lines, at that the lines are noticeably wider relative to lines in spectrum of microcrystals

Relatively low intensity of Raman spectrum of the solution is easily explained by low concentration of glycine in solution. Such low concentration was selected to reduce the possible agglomeration of amino acid molecules.

The most interesting result is the noticeable relative difference in the positions of the corresponding lines of the two spectra. It is obvious that unlike statements in paper [8] the band shifts do not have common nature, and for different bands they differ in value and sign. For example, lines of microcrystals in range $1030-1045\,\mathrm{cm}^{-1}$ are located higher by energy than scattering lines in solution. But lines 500 and $890\,\mathrm{cm}^{-1}$ of microcrystals lies lower by energy than corresponding lines in spectrum of solution. Note also that part of lines in spectra of single-crystals do not present in spectra of solutions. Figure 3 also indicate such situations with double ended arrows. In some cases it is rather difficult to compare lines and their components. For example, during detail analysis we identified that for narrow and intense line $144.5\,\mathrm{cm}^{-1}$ in spectrum of microcrystals it is impossible to find correspondence in spectrum of solution, as near $150\,\mathrm{cm}^{-1}$ wide scattering peak occurs, it present in spectrum of solvent — water.

Spectra of microcrystals and solution of alanine (Figure 4) show the same trend. Spectra of microcrystals contain large number of lines. For major of lines in spectra the shift is insignificant. Significant shift towards lower energies was i.dentified in shortwave line of the range, near $1000-1017\,\mathrm{cm}^{-1}$.

It is interesting that, as in the case of glycine, the number of lines have no noticeable analogues in the spectrum of solutions. These are lines 279, 392, $658\,\mathrm{cm}^{-1}$. Disappearance of these lines (or their analogues) can not be explained by a simple change of scales or noise masking, because, for example, significantly less intense line $916\,\mathrm{cm}^{-1}$ has in spectrum of the solution the obvious analogue $918\,\mathrm{cm}^{-1}$.

Results obtained for tryptophan (Figure 5), are similar to those obtained for two other already shown amino acids. Maximum scattering peak of single-crystals, near $100\,\mathrm{cm}^{-1}$,

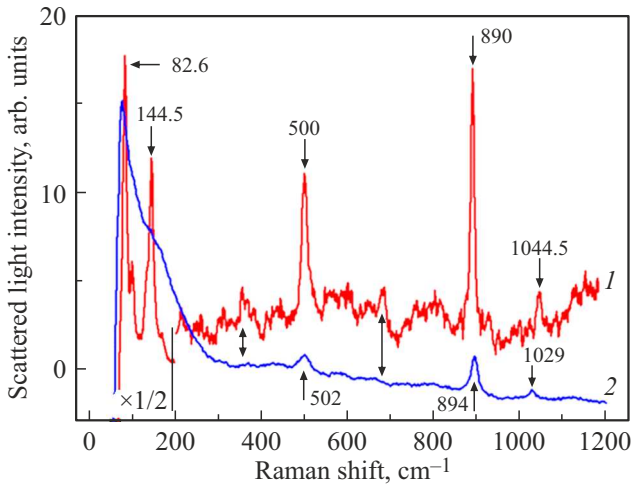

Figure 3. Raman spectra of microcrystals (curve *1*) and of 5% solution (*2*) of glycine amino acid in water. In range of energies below $200\,\mathrm{cm}^{-1}$ the intensity of Raman spectra of microcrystals was decreased by 2 times for scales comparison. The positions of the most intense lines are given. Detail explanations are given in the text.

Positions of lines and differences in positions in microcrystals and aqueous solution of amino acids of alanine, glycine, tryptophan (FWHM — full width half-maximum)

Glycine: Max, cm^{-1}//FWHM, cm^{-1}						
Type of oscillation	„Lattice“ modes		COO^-	Interpretation not determined	CN	
Crystal	82.6//11	144//12	500//12	890//5	1044.5//14	
Liquid	–	168//68 (?)	502//20	894//15	1028//12	
Δ (K–Zh)	–	–24	–2	–4	16.5	
Alanine: Max, cm^{-1}//FWHM, cm^{-1}						
Type of oscillation	„Lattice“ modes		COO^-		CC	CH_3 CH_2 CN
Crystal	106.5//6		527//13	847//10	916	1017//12	1109//6
Liquid	–		523//20	843//13	918	999//22	1108//14
Δ (K–Zh)	–		4	4	–2	18	1
Tryptophan: Max, cm^{-1}//FWHM, cm^{-1}						
Type of oscillation	„Lattice“ modes		C–C, ring breath CH_2^+	Interpretation not determined	C–C ring breath	
Crystal	96//24	154//20 {C}	752//7 {T}	844//10 {M}	1006//8	1115//7{c}
Liquid	–	171//45	772//10	870//10	998//6.5	110//13
Δ (K–Zh)	–	–18	–20	–26	8	5

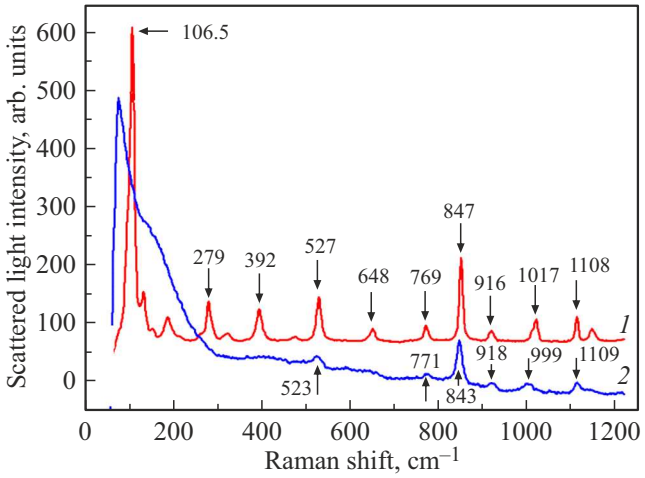

Figure 4. Raman spectra of microcrystals (curve *1*) and 5% solution (*2*) of alanine amino acid. The spectrum of solution is stretched vertically and shifted in graph for easy graphical comparison. For details see the text.

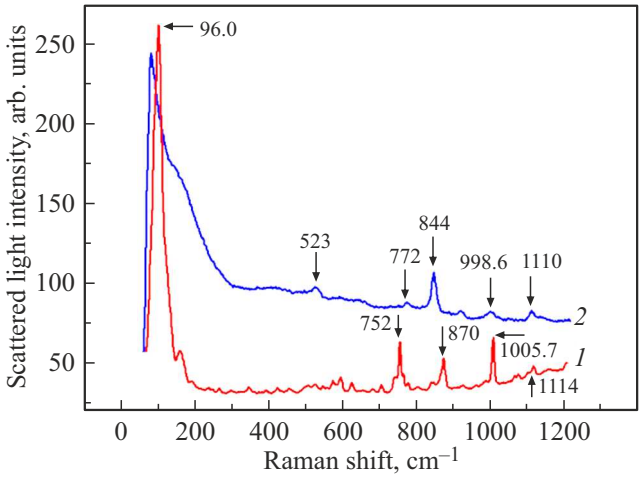

Figure 5. Raman spectra of microcrystals (curve *1*) and 5% solution (*2*) of tryptophan amino acid. The spectrum of solution is stretched vertically and shifted in graph for easy graphical comparison.

presumably, is shifted to range of low energies beyond the border of work range of the spectrometer. Like in previous cases at the low frequency end a weak wide band occurs near 150 cm^{-1}, which appears to be determined by scattering in the solvent (water). Due to relatively high difference in positions of lines for the given material (trytptophan) it is possible to compare the scattering peaks in microcrystals and solution with some doubt. We can

assume that line 772 cm^{-1} in solution corresponds to the line 752 cm^{-1} for microcrystals, i. e. the microcrystal line is shifted to lower energies relative to the line in solution. Line 998.6 cm^{-1} (similarly — presumably), corresponds to line 1005.7 cm^{-1}, and in this case the shift sign is reversed.

Sharp peak in most low frequencies for all solutions is due to spectral characteristic of step-filer of the spectrometer.

Table contains data on fragments of spectra at side of lower frequencies 75−1200 cm^{-1}, indicates (shift, cm^{-1})//(line width), difference of frequencies Δ(K−Zh), and correspondence of lines in spectra and oscillating modes of selected AA molecules.

As it was mentioned, the spectra of microcrystals contain a large number of low-intensity lines, which may be components of multiplets. Also the correspondence of lines in pairs of spectra is not always obvious. Due to these considerations the authors considered it appropriate in most cases to limit the position determination by three significant figures.

In general, spectra of molecules in the solution contain significantly less lines, and are less intensive then spectra of the same substance in cristalline state. Lines of molecules in spectra of solutions are wide as compared to spectra of molecules in microcrystals of dehydrated films. The most significant result is shift measurement between lines in spectra of films and solutions in reviewed part of spectrum.

4. Discussion of results

The obtained results once again emphasize the complexity of biomolecular objects compared to traditional objects of solid state physics.

The most noticeable fact is disappearance of some lines during transition from crystal (in our case, microcrystals) to solution. Such are, for example, lines 279; 392; 648 cm^{-1} in spectrum of alanine. Note first of all that these lines are at low frequency end of spectrum, and, hence, these are oscillations with lower energy of photons. We can suppose that such types of oscillations are more subjected to damping due to collisions with unordered moving molecules of the solvent, this is one of the reason of these lines absence in spectra of solutions. Another possible reason can be transverse nature of these oscillations, this is impossible in liquid and can be damped more strongly.

Similarly in solutions of all three amino acids narrow and strong lines are absent, while they are observed in spectra of crystals, and locate near 100 cm^{-1}. In terms of the position they can be related to acoustic modes. Their somewhat higher energy compared to acoustic modes in most inorganic crystals can be due to the fact that local oscillators in AA molecules are presumably made of light elements. If this assumption is true than such lines absence in the solution is explained by fundamental change of intermolecular interactions during transition from crystals to solution.

Some lines have one-to-one correspondence between the spectra of microcrystals and liquids. It is obvious that in this case the own oscillation of individual molecules is basic oscillatory system. In crystals such oscillation is most likely delocalized due to interaction between closely located identical molecules, and shall convert in one of oscillations of the crystal lattice.

The experiment results show that lines occurred in spectra of solutions are significantly broadened. According to existing ideas, the width increases, and resonant frequency of the oscillatory system shall increase upon damping increasing, this is universal principle [9]. Note that also decreasing of Coulomb interactions due to environment screening by polar molecules, and oscillating mass increasing shall be towards oscillation frequency decreasing. Separation of these contributions requires supercomputer calculations of the dynamic properties of such systems.

The mechanism of frequency increasing when moving from the spectra of microcrystals to the spectra of solutions is less obvious. Formally this shall be result of bond strength increasing of appropriate oscillator. Accidental compensation of interactions in the crystal is possible, but not likely, it disappears during transition to the solution. The first supposition is more probable. When substance containing hydrogen is diluted, new hydrogen bonds of molecules with environment can be formed [10]. In this case the elastic constant of the oscillatory system can increase.

Identification of contributions of individual suggested mechanisms will require further studies, including methods of supercomputer simulation.

Results relating to the high-frequency part of spectrum, would be presented in separate publication, as they are important for other physical and chemical properties of the biomolecules.

5. Conclusion

Spectra of Raman scattering of some amino acids in form of microcrystals and in solution are obtained. Shifts of spectral lines are traced during the transition from the spectra of microcrystals to the spectra of amino acid solutions. Such data in this range are absent in publications. It turned out that the indicated shift is not universal, as previously assumed, but is different for different molecules and different lines, up to change in the sign of the shift. Mechanisms responsible for the observed line shifts are suggested.

The made studies clarify the dynamic properties of glycine, alanine and tryptophan amino acids in solutions and dehydrated films of microcrystals, this in turn presents experiment data to clarify their physical and mathematical models.

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Conflict of interest

The authors of the paper declare that they have no conflict of interest.

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