Interaction of Nd:YAG Laser Radiation with Bovine Serum Albumin Solution

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Abstract. In this paper, the effect of Nd:YAG laser radiation on the properties of the BSA protein is investigated. A solution with a protein concentration of 5 mg/ml was irradiated for 30 minutes. After a 5-minute and 30-minute exposure, absorption spectra were taken, the particle size in the solution was determined by dynamic light scattering (DLS), the refractive index was determined, and fluorescent maps were taken. Raman spectroscopy of proteins was also performed. The results showed that after irradiation, the absorption of the protein solution decreases in the spectral range corresponding to amino acid residues. In DLS experiments, it was shown that the peak corresponding to protein molecules decreases, and the peaks corresponding to large aggregates (>100 nm) grow. Raman spectroscopy has shown that there is a decrease in intensity at a wavelength of 1570 cm^{-1}. There were no significant changes in the refractive indices and the shape of the fluorescent maps. The data suggest that partial denaturation of proteins took place.

1 Introduction

Proteins are one of the most important classes of organic compounds. The effect of various external factors on the properties of proteins is interesting both from a fundamental and applied point of view. Among the factors that can affect the structure and functioning of proteins, mechanical effects [1-4] (shaking, ultrasonic exposure, mixing, etc.) and exposure to external radiation have recently been of particular interest. This is primarily due to the active use of these effects in the pharmaceutical industry and surgery. Thus, in [5], the effect of shaking and external UV radiation on the properties of ipilimumab was compared. The results showed that after 45 days of shaking at 750 rpm, practically no changes in the fluorescence of solutions were observed. On the contrary, submicron particles were formed after prolonged irradiation, which indicated the formation of aggregates in solution.

In recent decades, the use of laser technologies in agriculture [6], medicine [7] and optogenetics [8] has been gaining popularity. Laser diagnostic technologies make it possible to analyze biological systems with high accuracy, determine the presence of diseases [9] and determine the qualitative and quantitative composition of the contents. In medical applications, lasers have also found their application in surgery [10]. This is due to

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the fact that laser radiation allows for minimally invasive operations with high accuracy. Nd:YaG laser with a wavelength of 1032 nm is interesting in medical applications due to the low absorption of its radiation in biological tissues [11].

In this paper, the effect of optical breakdown on the properties of the BSA protein is investigated. The phenomenon of optical breakdown in liquids has been well studied with respect to metals [12]. The breakdowns formed due to short laser pulses contribute to the formation of submicron and nanoparticles, as well as their further fragmentation [13]. The study of the effect of optical breakdown on the properties of proteins will help in the development of new therapeutic techniques that can cause minimal harm to the body.

2 Materials and methods

The irradiation of protein solutions was carried out at the facility described in detail in [14]. The samples were irradiated with Nd:YAG laser NL300 (Ekspla, Vilnius, Lithuania) with generation of the second harmonic at a wavelength of λ – 532 nm. The duration of one laser pulse was 4 ns, the pulse power was 2 MJ, the pulse repetition frequency was 1 kHz. Laser radiation was directed to a galvanomechanical system of mirrors, reflecting from which it penetrated into the test solution. The use of this system is necessary to ensure that optically-induced breakdowns are evenly distributed over the volume of the solution, as well as to avoid unnecessary nonlinear optical effects. The diameter of the laser beam was about 30 microns.

The Zetasizer ULTRA Red Label unit (Malvern Panalytical Ltd., Malvern, UK) was used to determine the particle sizes in the solution. The scattering of a He-Ne laser with a wavelength of 632.8 nm and a power of 12.5 MW was detected at angle of 174.7° at a temperature of 25 °C. The measurements were carried out in a polystyrene cuvette DTS0012, where 1 ml of the sample was poured. In each experiment, 5 measurements were carried out, the results were then averaged. For these experiments, a protein solution at a concentration of 0.4 mg/ml was used. The absorption spectra of the solutions were determined using a Cintra 4040 spectrophotometer (GBC Cintra 4040, Australia). Quartz cuvettes with an optical path length of 10 mm were used for measurements. 2 ml of a solution without protein was poured into the control cuvette, and 2 ml of a solution with protein at a concentration of 0.5 mg/ml was poured into the experimental cuvette. In each experiment, 6 measurements were carried out. A Jasco FP-8300 spectrometer (JASCO Applied Sciences, Canada) was used in fluorescence experiments. The experiments were carried out at room temperature (~22 °C) in a quartz cuvette with an optical path length of 10 mm, where 1.6 ml of a solution with a protein concentration of 5 mg/ml was poured. An Abbemat MW refractometer (Anton Paar, Graz, Austria) with three wavelengths: 435.8, 589.3 and 632.8 nm was used to measure the refractive index of solutions. 1 ml of the solution was poured into the cuvette, then the refractive indices at three wavelengths were examined. 5 measurements were carried out for each sample. The Senterra II Raman Microscope (Bruker Optik GmbH, Karlsruhe, Germany) was used to study the Raman scattering of samples.For this study, a BSA solution at a concentration of 5 mg/ml was dried on a CaF2 substrate. A laser with a wavelength of λ = 532 nm and a power of 12.5 MW was used for irradiation. The scattering was captured within 2 seconds using a 50x lens. 100 measurements were carried out for each sample. Measurements were carried out at three different points. The results were processed using the OPUS 8.2.28 program (Bruker Optik GmbH, Karlsruhe, Germany).

3 Results and discussion

After irradiation, experiments were carried out using optical techniques. Figure 1 shows the absorption spectra of BSA solutions. As can be seen from the spectral data, the absorption associated with amino acid residues decreased over time.

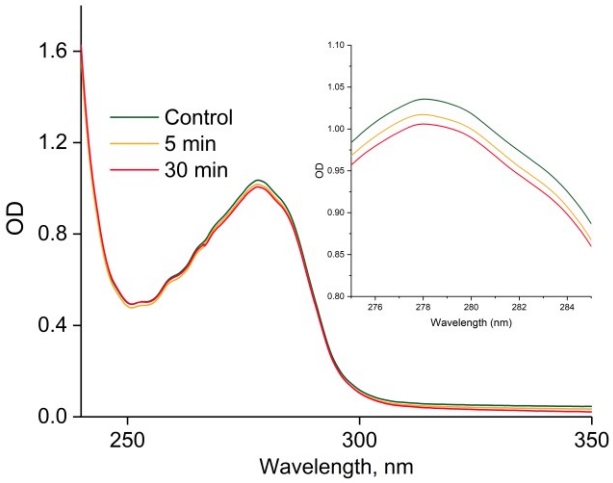

Fig. 1. Absorption spectra of control BSA solutions and solutions after laser exposure.

In Fig.2. fluorescence maps of solutions are presented. Based on the data obtained by fluorescence spectroscopy, it can be concluded that the excitation wavelength at which the maximum fluorescence value of the samples is recorded does not change and is 296 nm. Emission wavelengths corresponding to the maximum of fluorescence also practically do not change and are in the spectral range of 337.5-338 nm. The excitation wavelengths corresponding to the second fluorescence maximum are in the spectral range of 254-255 nm. At the same time, the emission wavelengths corresponding to the second peak are in the range of 334-336.5 nm. After Nd:YAG laser irradiation, the values of both peaks decrease.

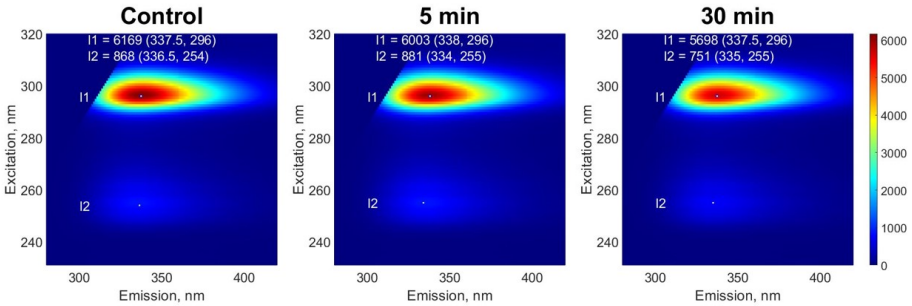

Fig. 2. Fluorescence maps of BSA solutions. Main peaks are shown as value in a.u. and emission and excitation wavelengths in brackets.

In Fig.3. The results of measuring the refractive coefficients for protein solutions are presented. As can be seen from the figure, measurements at all three wavelengths showed no significant differences in the refractive coefficients of the samples.

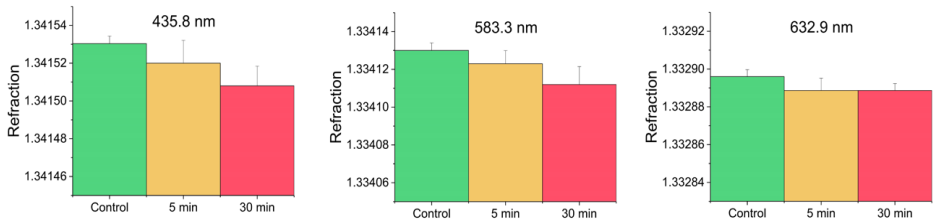

Fig. 3. Refractive indexes of BSA solutions, measured at 3 different wavelengths.

Figure 4 shows the intensity distributions of the radiation scattered at an angle of 174.7º along the diameters of the hydrodynamic shells of particles in solution. The figure shows that the initial solution contains both BSA molecules and aggregates. After 5 minutes of irradiation, there is a slight decrease in the first peak advising the BSA protein, and the value of the size corresponding to this peak increases. After a 30-minute exposure to Nd:YAG laser on a protein solution, a decrease in the first peak is observed, and the peaks corresponding to aggregates grow. In accordance with the Rayleigh relation, the intensity of the light scattered by the particle is I ~ d6, where d is the diameter of the corresponding hydrodynamic shell. Therefore, it can be argued that protein molecules, rather than their aggregates, predominate in solutions exposed to irradiation. At the same time, based on the fact that the intensity of subsequent peaks has increased, it can be argued that the number of aggregates in the solution has increased, which can be associated with partial denaturation of the protein and subsequent formation of corresponding clusters.

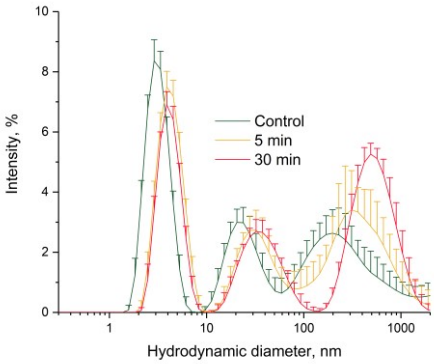

Fig. 4. Scattered light intensity distributions by hydrodynamic diameters of particles, obtained by DLS for control solution and solutions after laser exposure.

To find out whether protein denaturation took place, Raman spectroscopy of proteins was performed. The Raman spectra are shown in Fig.5. It can be seen that the spectra are generally similar, but there is a decrease in the scattering intensity at a wavelength of 1570 cm^{-1}. Therefore, it can be argued that there were conformational changes in proteins exposed to laser irradiation. Perhaps there was a partial denaturation of the molecules.

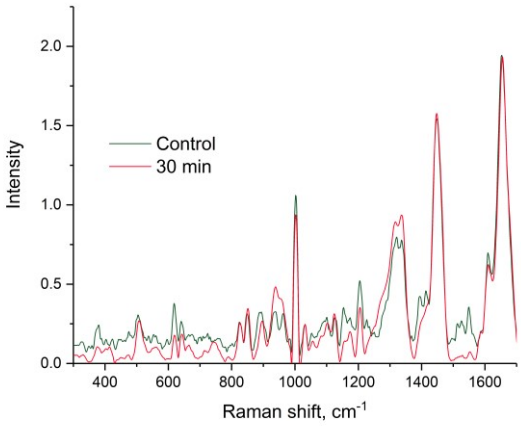

Fig. 5. Raman spectra of control sample and sample after 30-min laser exposure.

As is known, optically induced breakdown is a combination of different phenomena: generation of shock waves, generation of UV radiation, etc. [15]. As a result of these phenomena, partial denaturation and aggregation, destruction of the polypeptide chain and changes in the secondary and tertiary structure of molecules can occur. As a result of the analysis carried out by optical methods, it is shown that there are changes in the optical properties of the samples. A decrease in the optical density in the amino acid region was observed in the absorption spectra. Also, a decrease in optical density was observed in the longer wavelength region. With conformational changes in proteins, there is usually a change in the shape of the fluorescence map. In this case, no significant changes were found. At the same time, there was a decrease in fluorescence peaks, which may indicate partial denaturation of proteins in solution. Changes in the Raman spectra also indicate that structural changes of BSA molecules have taken place.

4 Conclusions

In this paper, the effect of optically induced Nd:YAG laser breakdown on the optical properties of bovine serum albumin (BSA) is investigated. It is shown that after 30-minute experiments, no significant differences were found in the refractive indices at wavelengths of 435.8 nm, 589.3 nm and 632.9 nm. The shape of the fluorescent maps also did not change significantly. At the same time, both fluorescence peaks located in the excitation spectral ranges of 254-255 nm and 296 nm decreased with time. The main peak decreased by 7.6%, the second peak decreased by 13.5%, which may indicate partial denaturation of proteins. At the same time, there was a decrease in absorption peaks at wavelengths corresponding to the absorption of amino acid residues. Raman spectroscopy showed a decrease in intensity at a wavelength of 1570 cm^{-1}, which can be associated with the destruction of the protein α-helix. Thus, it is shown that the laser-induced optical breakdown of the Nd:YaG laser is capable of causing conformational changes in the BSA solution. Measurements by the DLS method showed an insignificant shift of the first peak. The peaks corresponding to protein aggregates increased over time. Aggregates of about 400 nm were formed.

Acknowledgments

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References

1. N. F. Bunkin et al., ACS Omega, **5**, 24 (2020).
2. S. Movafaghi; H. Wu; F. Urdániz, I. M. Bull, D. S. Kelly, M. D. Randolph, T. W. Goodwin, Biotechnology journal, **15**, 9 (2020).
3. J. Liu, S. Tong, H. Sun, Y. Chang, H. Luo, H. Yu, Z. Shen, Biotechnology Progress, **37**, 1 (2021).
4. Y. Wang, C. Wu, International Journal of Biological Macromolecules, **212**, 465-473 (2022).
5. B. Fongaro et al., European Journal of Pharmaceutics and Biopharmaceutics, **176**, 54-74 (2022).
6. D. Burmistrov et al., MDPI Photonics, **8**, 12 (2021).
7. M. Badiceanu et al., Coatings, **12**, 1 (2022).
8. N. Keshmiri et al. Lasers in Surgery and Medicine, **54**, 2 (2022).
9. M. Moscovskiy et al., MDPI Photonics, **8**, 12 (2021).
10. B. Azadgoli, R. Baker, Annals of translational medicine, **4**, 23 (2016).
11. P. Bradley, British Journal of Oral and Maxillofacial Surgery, **35**, 1 (1997).
12. S. Dolgaev et al., Applied surface science, **186**, 1-4 (2002).
13. K. Ayyyzhy et al., Physics of Wave Phenomena, **27**, 2 (2019).
14. E. Nagaev, I. Baimler, A. Baryshev, M. Astashev, S. Gudkov, Molecules **27**, 19 (2022).
15. S. Kudryashov, D. Zvorykin Physical Review E, **78**, 3 (2008).