LASER OPTICAL BREAKDOWN MODIFIED PHYSICAL PROPERTIES OF LYSOZYME IN AQUEOUS SOLUTION

T.A. Matveeva[1], *I.V. Baimler*[1], *K.V. Artemiev*[1], *I.V. Gorudko*[2], *R.M. Sarimov*[1*]

[1] Prokhorov General Physics Institute of the Russian Academy of Sciences, 38 Vavilova St., Moscow, 119991, Russia;
[2] Belarusian State University, 4 Nezavisimosti Av., Minsk, 220030, Belarus.

* Corresponding author: rusa@kapella.gpi.ru

Abstract. Physico-chemical characteristics of optical-induced break-down depend weakly on the protein concentration. This factor distinguishes lysozyme from other aqueous solutions which contain objects with nano-size. The laser radiation of the lysozyme aqueous solution during different time periods shows patterns that are quite similar to the classical patterns. In this particular paper, it has been observed that the catalytic activity of lysozyme is decreasing after the laser radiation. The optical density of the protein solutions is increasing. The fluorescence intensity of the lysozyme solution is determined by the amino-acid residues. It decreases before and after the laser light influence. The position of the maximum of the excitation and emission did not change. The shape of the fluorescence signal on the 3D maps also does not change significantly. This observation suggests the degradation of the amino-acid residues. The viscosity and the pseudo plasticity of the lysozyme aqueous solutions increased. However, there is no massive damage of the polypeptide chain. On the contrary, the optical break-down shows the intensive forming of the lysozyme aggregates.

Keywords: lysozyme, HEWL, lazer irradiation, reactive oxygen species, catalytic activity, protein aggregation, protein fluorescence.

List of Abbreviations

HEWL – hen egg white lysozyme
OD – optical density
ZS – zetasizer
IR – Infra-Red
DLS – dynamic light scattering
ROS – reactive oxygen species

Introduction

The unique capability of a laser is to concentrate the energy in the volume, in time and in the spectral range. These features make this instrument an irreplaceable application in many areas of human activity: in medicine, for instance (Letokhov, 1985). A major intervention in the pathological process happens during the disease treatment. Such cases occur in surgery (Khalkhal *et al.*, 2019). The progress in science and technology leads to the approach when the mechanical instruments in surgery are replaced with laser-based instruments (Zhang *et al.*, 2022). The pulse laser radiation impacts the body tissues. This impact is defined by the combination of the wavelength, energy density, and the emission pulse duration. With the variation of these parameters it is feasible to vary the effects of the laser radiation on the body tissues. By changing the radiation pulse it is possible to separate the thermal and non-thermal influence (Vaghasiya *et al.*, 2022).

Nowadays the pulse lasers with a wide range of pulse duration (from milli- to femtoseconds) are in use. Mostly in use are nanosecond lasers. The use of nanosecond lasers and lasers with shorter pulse duration leads to non-linear processes. Those are: optical break-down on the target surface, multi-photon absorption, forming and development of the plasma, generation of the shock waves (Kanitz *et al.*, 2019). Evidently it is a complicated task to create a united algorithm of choosing a laser for a specific case with such a big amount of non-linear processes. A specific approach is applicable in every particular case (Nanni & Alster, 1998). From the first point of view it made the task much more complicated. On the other hand, it is feasible to vary the methods of influence on the body tissues.

One of the most widespread surgical lasers is the laser with active medium Nd:YAG (Safir *et al.*, 2022). Such laser is being used in endoscopic access operations in pulmonology, gastroenterology, and urology; in esthetic cosmetology for hair removal; in oncology for laser coagulation of tumors (Gibson &

Kernohant, 1993). In the modulated regimen with the pulse duration of 10 ns it was applied in ophthalmology, for the glaucoma treatment, for instance (Slagle *et al.*, 2022). The majority of the body tissues on the Nd:YAG wavelength (1064 nm) have a low absorption coefficient (Lanka *et al.*, 2021). Such radiation has several millimetres of effective penetration depth into the tissues and also it provides a good haemostasis and coagulation (Katta *et al.*, 2022). A great advantage of Nd:YAG laser is an opportunity to deliver the radiation in the target zone with the fiber-optical waveguides. The use of the endoscopic and fiber instruments provides the spread of the laser radiation into the lower and upper parts of the gastro-intestinal tract with almost non-invasive method (Wang & Chocat, 2010).

The medical use of the laser radiation is connected with the development of the non-linear processes in body tissues during the laser absorption. One of the major non-linear processes is optical break-down. Optical break-down is a fast-developing irreversible process of the environment conversion from transparent to a very absorptive. This process is happening under the influence of the intensive radiation (Shafeev, 2012). Optical break-down can be obtained by the excess of the threshold value of the energy density of the laser radiation (Bunkin & Bunkin, 1993). The process of optical break-down development has a probabilistic character at the energies close to threshold values (Kovalchuk-Kogan *et al.*, 2015). It is known that the optical break-down happens less often when the medium for radiation is free from the impurities (Kononenko *et al.*, 2022). The research on the optical break-down in liquids shows that the nano-sized impurities in the medium lead to the increase of the optical break-down probability and the decrease of the threshold values of the laser radiation energies (Frias Batista *et al.*, 2021). The process of optical break-down in liquids happens few times more intensive when nanoparticles are present in the medium (Al-Kattan *et al.*, 2021). It is not clear whether protein molecules, being nano-sized objects, could lead to the increase of the optical break-down probability in aqueous solutions. It is also not evident what is happening with the protein sample after the impact of the optical break-down. In order to find answers for these two questions the standard Nd:YAG laser with pulse duration of 10 ns has been used in this study. The hen egg white lysozyme (HEWL) was used as a model protein. HEWL is one of the most investigated proteins; moreover it has a very low cost. It is worth mentioning, that HEWL became the second protein and the first enzyme which was investigated with the x-ray crystallography (Blake *et al.*, 1965). Also it became the first enzyme that contains the full sequence of all twenty standard amino acids (Canfield, 1963).

Materials and Methods

Laser exposure

A photograph of the optical break-down in the sample with HEWL during laser exposure is represented in Figure 1.

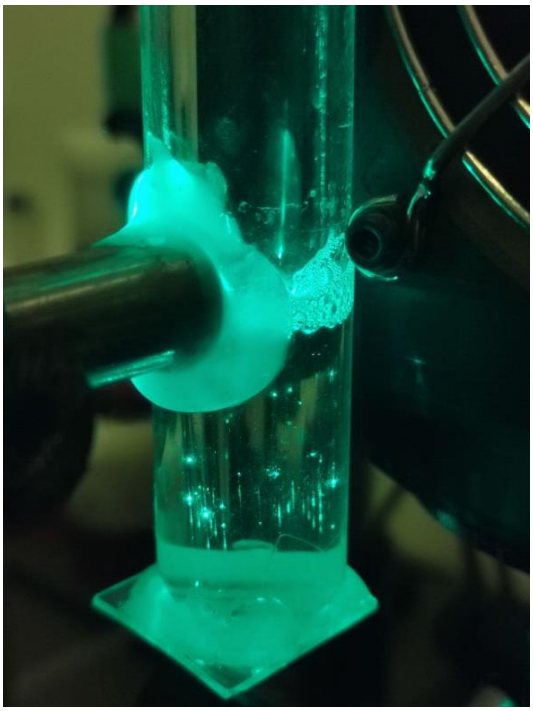


Fig. 1. Optical breakdown in the sample with HEWL during laser exposure

Nd:YAG laser (Ekspla NL300) is used as a source of laser radiation. The parameters were the following: pulse duration $\tau = 4$ ns, frequency $\upsilon = 1$ kHz, wavelength $\lambda = 532$ nm, impulse en-

ergy ε = 2 mJ. The laser light was focused in the center of the cuvette and was displaced among the straight line of 1 cm length with the velocity of 500 m/s. All that was performed using the galvano-mechanical system of mirrors. It is necessary to initiate the optical break-down in the unperturbed environment as well as to eliminate the thermal defocusing and additional scattering on the vesicles of the generated gas. These can be achieved with the transition of the laser light in the cuvette. Part of the laser radiation was redirected to the pin-photodiode by the mirror (reflection coefficient is 5%). This was done in order to start the time sweep of oscilloscope. The prepared protein solution was put into the glass cuvette with the 25 ml volume. Inside the cuvette, on one of the sides, the piezo-film acoustic sensor was attached in parallel to the scan line. The pin-photodiode for plasma flash registration was set at a distance of 3-4 cm from the cuvette. The signals from the sensor and photodiode were registered with the use of the digital oscilloscope GW Instek GDS-72204E. The photo shoot of the plasma flashes was performed with the use of the digital photo camera Canon EOS 450D (exposure time 10 ms, ISO 800). For each experimental point there were at least 50 photos in one series. The analysis of the acoustic signals from the sensor and the plasma signals from the pin-photodiode was done with the use of the specially created software LaserCav (https://drive.google.com/drive/folders/1WQmaSCA4mx2HyRSCxtSiik5MWNku9piR). The analysis of the plasma images from the photo camera was performed with the use of the software LaserImage (https://drive.google.com/drive/folders/1YRNF2p7qpejlGP55QBiqM108LSGAseaE).

Materials

Hen egg white lysozyme (>20000 U/mg, A-3711, Applichem), *Micrococcus lysodeikticus* (lyophilized cells, ATCC No. Aldrich, St. Louis, MI, USA). The water used for the experiments was obtained by distillation and deionization to a resistivity of ~18 MΩ/cm.

Absorption spectra

Absorption spectra were measured on a Cintra 4040 (GBC Cintra 4040, Australia) in quartz cuvettes with an optical path length of 10 mm at room temperature (~22 °C). The HEWL concentration was 0.4 mg/ml. The absorption spectra were measured with six-eight samples for each group.

Enzyme Assays of the HEWL

The activity of HEWL was examined using the lysis of *M. lysodeikticus* cells, at room temperature, as described in (Shugar, 1952). Here, 4 μL of HEWL (0.4 mg/ml) was collected from the initial solution diluted 10 times in water, and added, at 100 μL and a concentration of 40 μg/ml, to 2.5 ml of the micrococcus diluted in 20 mM of K_2HPO_4 (pH = 7.0) to an OD of about *0.7–0.8* (λ = 450 nm). The activity was measured by decreases in OD at the same wavelength with a spectrometer (GBC Cintra 4040, Australia) for the first two minutes after the addition of lysozyme. Measurements of lysozyme activity were carried out in four samples for each group. The measurements were carried out at a room temperature (~22 °C).

Dynamic Light Scattering

Zetasizer ULTRA Red Label (Malvern Panalytical Ltd., Malvern, UK) was used to obtain information on hydrodynamic particle diameters. A 1 ml solution of lysozyme with a concentration of 0.4 mg/ml was measured in a plastic cuvette at 25°C. Five independent experiments were carried out for the control and for each point of influence. The intensity distributions of the hydrodynamic diameters were calculated using the ZS Xplorer program and algoritm (Penkov & Penkova, 2021).

Fluorescence spectroscopy

Fluorescence of HEWL in water was studied on a Jasco FP-8300 spectrometer (JASCO Applied Sciences, Canada). Measurements of a 1.8 ml solution of lysozyme with a protein concentration of 10 mg/ml were carried out in quartz cuvettes with an optical path length of 10 mm at room temperature (~22 °C). Each sample was measured three times. The figures show typical spectra; with repeated measurements, the intensity maxima change by several percent.

Viscosity measurement

SmartPave 102 rheometer (Anton Paar GmbH, Austria) was used to obtain viscosity data of protein solutions. The measuring set was DG26.7 with C-PTD200 cell with 3.8 ml of each sample. All measurements were made at the temperature 25 °C, reducing shear rate from 1000 to 100 s^{-1}, using RheoCompass™ software (Anton Paar GmbH, Austria). The concentration of proteins in solution was 10 mg/ml.

Fourier Transform Infra-Red Spectrometry

Protein samples were studied on an FSM-2202 IR-Fourier spectrometer in the range of 400–4000 cm^{-1}. One beam of IR radiation passes through the cuvette compartment of this spectrometer, so it was necessary to sequentially obtain the comparison spectrum and the spectrum of the sample under study. The diameter of the IR beam in the constriction (in the middle of the cell compartment) was 11 mm. The comparison spectrum was the transmission spectrum of a clean germanium plate with dimensions of 19×49 mm. The plate was placed vertically in the beam constriction so that the beam passed through its middle. Protein solutions with a volume of 90 μl were applied with a calibrated Portlab 14205799 dispenser to the middle of the germanium plate in the form of a film with dimensions of ~19 × 25 mm. The films were dried for 20 minutes at a temperature of 24 °C and a relative humidity of 26%.

The spectra were recorded with a resolution of 2 cm^{-1} and 50 averaging.

Results

Catalytic activity

The impact of the laser radiation time of the lysozyme aqueous solution on its catalytic activity was investigated (Fig. 2).

It is observed that the catalytic activity of the lysozyme has decreased after the impact of the laser radiation on the lysozyme aqueous solution. In particular under the laser radiation for 30 minutes the lysozyme activity statistically significant decreased for almost 30%. In order to study the reason of this activity decrease, spectral research of the lysozyme aqueous solution has been performed.

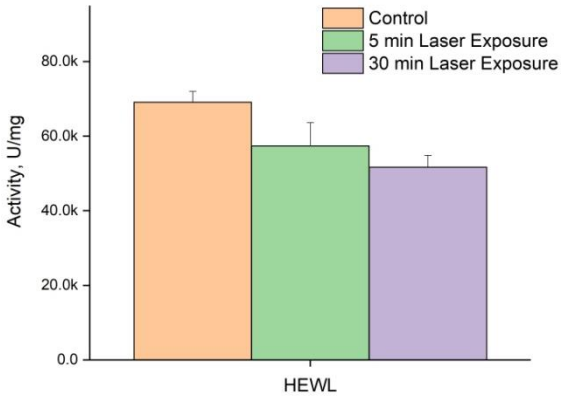


Fig. 2. The impact of the laser radiation time of the lysozyme aqueous solution on its catalytic activity (n = 4, Mean ± SD). *** – p = 0.001, significance levels for one-way ANOVA

Optical density

In the Figure 3 the impact of the laser radiation time on the optical density of the lysozyme aqueous solution is presented. It has been shown that the optical density of the lysozyme solution increases under the influence of the laser radiation. Moreover, the optical density in the 250–280 nm wavelength shows a linear increase for 30 min of the laser radiation. The absorption of the protein molecules in aqueous solutions increases for almost 0.05 points both in the local minimum (252 nm) and in the local maximum (280 nm). It is essential to mention that such changes happen in the longer wavelength part of the spectrum (the right side of the peak, 310–320 nm). Therefore the absorption intensity increases in the spectral band of the absorption of the aromatic amino acids residues, and in the range longer than 310 nm.

So it is possible to propose few choices in such case. First - the chemical modification of aromatic amino acid residues when the optical density increases. Also the change in absorption may be possible due to the partial protein denaturation. Moreover, the optical density can increase because of the non-specific scattering of the minor protein aggregation.

Fluorescence

The impact of the laser radiation time on the fluorescence of the lysozyme protein solution is represented in Figure 4.

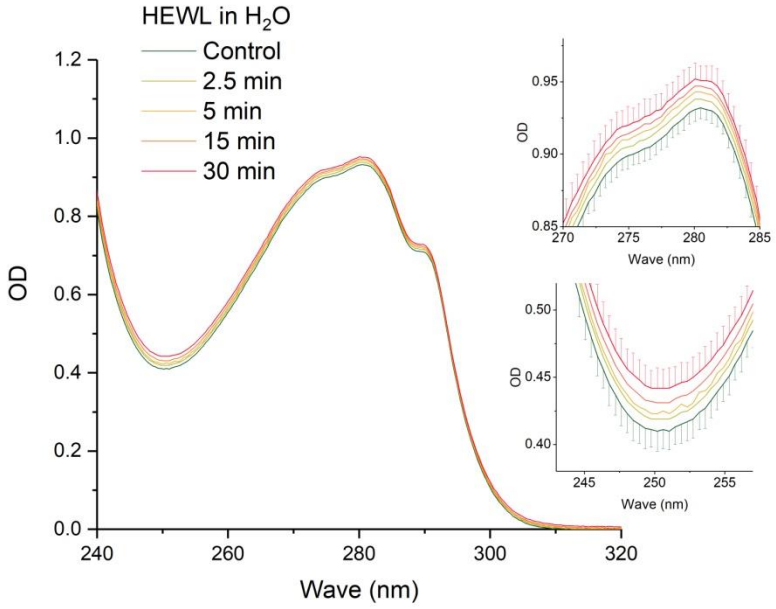


Fig. 3. The impact of the laser radiation time of the lysozyme aqueous solution on its optical density (n = = 6-8, Mean ± SD). On the upper right spectrum the change of the optical density in the area of the local maximum of 280 nm is presented. On the lower right spectrum the change of the optical density in the area of the local minimum of 250 nm is presented. The data was obtained with the use of differential double-beam spectroscopy in the sub-nanosized resolution

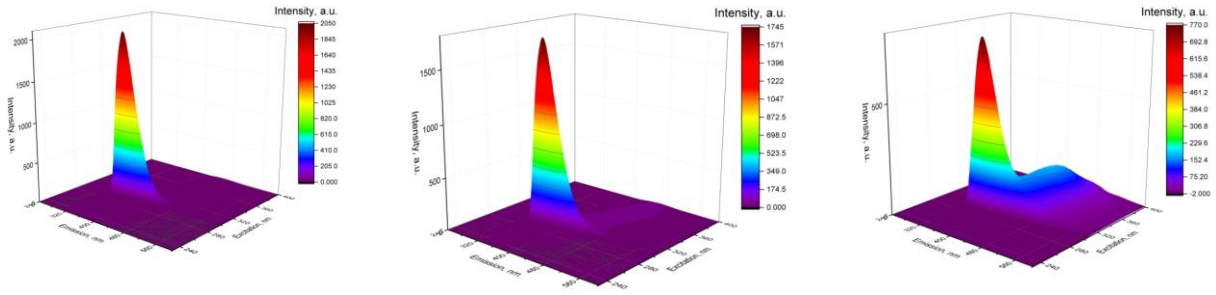


Fig. 4. The impact of the laser radiation time on the fluorescence of the lysozyme aqueous solution 10 mg/ml (typical spectrum). The 3D fluorescence maps are represented. On the abscissa axis is the emission wavelength range in nm (λ_{em}). On the ordinate axis is the extinction wavelength range in nm (λ_{ex}). The fluorescence intensity is represented in the arbitrary units by the color scale, which is unified for all three spectra

It is shown that the fluorescence excitation maximum is observed at 304 nm and does not change after the laser radiation influence both for the first 5 minutes and for the rest half hour. The fluorescence intensity decreases for 15% during 5 minutes of laser radiation, also it decreases for more than 60% during 30 minutes of laser radiation. Thus, the emission maximum for the intact protein solution and for the protein solution after 5 minutes of laser radiation is equal to 337–338 nm. The emission maximum shifts to the longer wavelength for few nanometers (343 nm) after the impact of laser radiation on the protein solution during 30 minutes.

The shape of the fluorescence spot on the 3D maps does not change substantially. It is important to mention that a slight fluorescence spot with the maximum of 434 nm appeared when the protein solution is influenced with laser radiation for 30 minutes at excitation

of 350 nm. The fluorescence in this area might signify the formation of the protein aggregates. Thus it is shown that the fluorescence intensity of aromatic amino acid residues is decreased under the laser radiation influence on the protein solutions. Such a change could be connected both with the degradation of the aromatic amino acids and with the change of the secondary structure.

Therefore, it is more likely that the protein underwent structural changes; however, it is not evident what the reason is for that. To make it clearly the light refraction coefficient was registered and rheological research has been performed.

Refraction coefficient

The impact of the laser radiation time on the light refraction coefficient of the lysozyme protein solution at the wavelengths 438 nm, 589.3 nm, 632.8 nm (Fig. 5). The refraction coefficient of lysozyme does not change significantly after the impact of laser radiation.

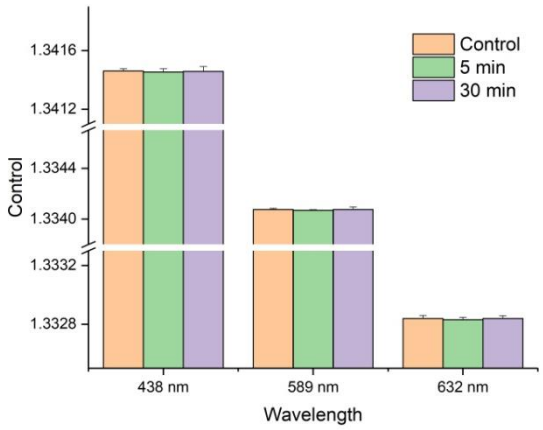


Fig. 5. The impact of the laser radiation time on the refraction coefficient of the lysozyme protein solution at the wavelengths 438 nm, 589.3 nm, 632.8 nm. The data recorded with the use of the precision refractometry (n = 3, Mean ± SD)

Viscosity

The impact of the laser radiation time on the viscosity of the lysozyme protein solution was investigated at different mixing velocities (Fig. 6). It is shown that the lysozyme aqueous solution has a pseudo plasticity feature. Pseudo plasticity is a feature of a liquid, which states that the viscosity of the liquid decreases when the shear stress increases. The impact of the laser radiation on the lysozyme aqueous solution leads to the increase of the viscosity.

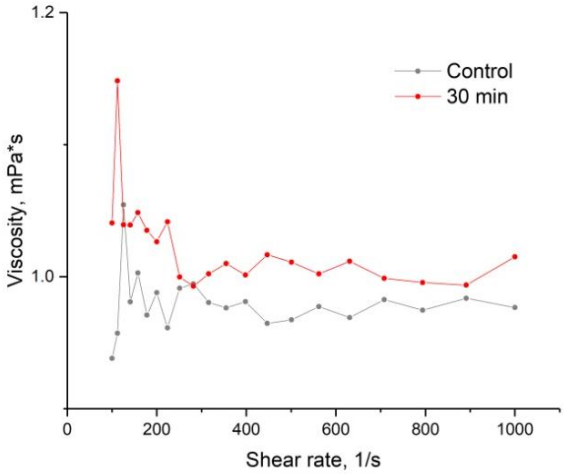


Fig. 6. The influence of the laser radiation time on the viscosity of the lysozyme protein solution with different mixing velocities (n = 3, Mean)

Moreover at the higher mixing velocities, the viscosity of the control solution and the solution irradiated during 30 minutes differs less than 10%. At the lower mixing velocities the viscosity of the control solution and the solution irradiated during 30 minutes differs for 60%. Meaning that the laser radiation lead to the increase of the lysozyme solution pseudo plasticity and its viscosity. So there is an increase of resistance of the movement of one part of the liquid among the other. Thus protein molecules more intensively interact with each other. Usually in protein solutions it is called aggregation. To verify this assumption the evolution of the sizes of the light-scattering particles in lysozyme aqueous solution was studied.

Size distribution

The impact of the laser radiation time on the evolution of the size distribution of the lysozyme and its aggregates in aqueous solution is represented in Figure 7. It is shown that the intact sample consist of the individual lysozyme molecules and also the aggregates with average hydrodynamic diameter 15 and 80 nm. For one aggregate with size 80 nm in the solution there is ~$4{,}6\times10^{9}$ individual lysozyme molecules. For

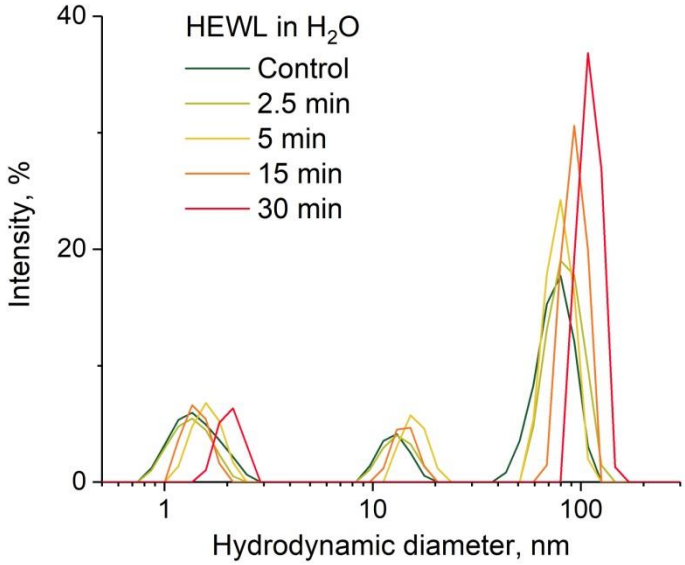
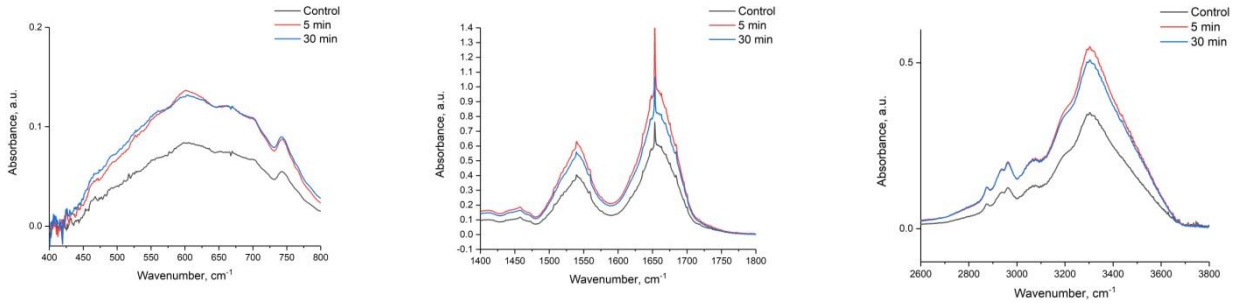


Fig. 7. The impact of the laser radiation time on the evolution of the size distribution of the lysozyme and its aggregates in aqueous solution (n = 5, Mean). The data was recorded with the use of the dynamic light scattering method



400-800 nm 1400-1800 nm 2600-3800 nm

Fig. 8. FTIR spectra (absorbance, −log(T)) of films of samples 0, 5, and 30

one aggregate with size 15 nm in the solution there is ~1,3×10^6 individual lysozyme molecules. There is an increase of the average hydrodynamic diameter of the individual molecules fraction for almost 30–40% under the laser radiation. It is necessary to mention that the hydrodynamic diameter of the lysozyme aggregates and the quantity of the aggregates increased. Thus, for one aggregate with 80 nm size is about 3,1×10^9 individual lysozyme molecules in the solution after 30 minutes of laser radiation. In other words the quantity of the big aggregates increased for one third. The dose-dependent character of changes is observed. The changes in the evolution of the size distribution became more and more significant with the extension of the laser radiation time.

Absorption spectra

The absorption spectra (absorbance, −log(T)) of the control, 5 minutes, 30 minutes samples are presented in the Figure 8. The absorption spectra are presented with the adjustment for the squares of the sample films, from which the thickness of the film depends on. The thickness of the films is not known therefore the

"absorbance" axis is presented in arbitrary units. The square of the film of the control sample is fewer for 10% compared to the samples "5 minutes" and "30 minutes" (both are the same).

Discussion

It is observed that after the impact of laser radiation on the lysozyme aqueous solution the catalytic activity of lysozyme is decreased (Fig. 2). The complex analysis of the lysozyme protein molecules have been performed with the use of the optical methods and rheometry. It is revealed that under laser radiation the optical density of the protein solution is increased (Fig. 3). Moreover, the optical density increase is observed in the bandwidth of absorption of the aromatic amino acid residues. This observation probably defines the damaged state of those amino acid residues. Also the increase of optical density is observed in the longer wavelength (310 nm), which confirms the protein denaturation and aggregation. Therefore it is possible to make several assumption of the development. During the optical break-down a big amount of reductive and oxidative elements are formed (Barmina *et al.*, 2017). The generation of UV radiation, shock acoustic waves, microvolumes with substantial temperature increase is observed (Kudryashov & Zvorykin, 2008). In such selection of stimuli the following can be observed: 1) chemical modification of amino acid residues; 2) polypeptide chain fragmentation; 3) change of tertiary and secondary structure of lysozyme molecules; 4) partial denaturation and 5) aggregation of molecules. All the scenarios described above can lead to the protein damage and the loss of catalytic activity. It is necessary to note that the similar list of events happens with proteins during the oxidative stress (Davies, 2005). It is developed in the living systems during the impact of different abiotic factors (Bruskov *et al.*, 2012), during inflammation (Sharapov *et al.*, 2021), at the state of hypoxia (Sokolov *et al.*, 2022), during the development of the range of diseases (Shaparov *et al.*, 2021), and so on (Zakharova *et al.*, 2018).

The fluorescence of the lysozyme protein solution after the impact of the laser radiation is investigated (Fig. 4). Usually the excitation maximum is located in the 275-290 nm bandwidth (Teale & Weber, 1957). In this study the maximum was registered at the 304 nm. Such case is normally observed when there is a high protein concentration. The fluorescence intensity is substantially decreased under the laser radiation impact. In such case the emission maximum does not change significantly. The chemical modification of the fluorophore normally impacts on the shape of the emission spot on the 3D fluorescence maps. However, it has been noted that the shape of the fluorescence spot on the 3D maps does not change crucially. Therefore it is possible to say that there is no substantial chemical modification of aromatic amino acid residues under the impact of optical break-down. It is more likely that its degradation or significant change of the secondary structure of the molecule is happening.

The major increase of water molecules in the hydrated shell of the protein happens during the partial denaturation; in this case the change of the refraction coefficient is observed (Sarimov *et al.*, 2018). In this study with a high accuracy the refraction coefficient was registered on the different wavelengths (Fig. 5), there were no crucial differences registered. The protein aggregation, as well as the soft one, always leads to the change of the rheological features (Purwanti *et al.*, 2011). It is established that the optical break-down leads to the increase of the viscosity of the lysozyme aqueous solution, as well as to the increase of the pseudo plasticity of the colloidal solution (Fig. 6). These kinds of changes in the solution correspond to the more intensive interaction of protein molecules to each other (aggregation). The development of aggregation is confirmed with the evolution by the size of the light-scattered particles in lysozyme aqueous solution (Fig. 7). It is interesting to note, that after the laser radiation impact there was no shift of the peak of the individual molecules in the area of smaller sizes. Evidently this correspond the absence of the major damage of the polypeptide chain and the presence of the parts of the protein molecule in the solution. On the contrary, the peak of the individual molecules shifts to the area of bigger sizes

(from 1.5 nm to 2 nm at approximate intensities). The spherical increase of the size for 30-40%, which is utilized by the DLS method, is compliant with the formation of the macromolecules aggregates. It is easy to count, with the use of the formula 4 from the study (Bobylev *et al.*, 2015), that for one dimer-molecule there are 2 monomers in the initial state. Therefore, it is possible to state that the major amount of lysozyme is in dimeric molecule state after 30 minutes of laser radiation impact.

Conclusion

Therefore it is shown that optical break-down in the protein solutions flows with high efficacy, the formation of plasma and acoustic oscillations is observed. The physico-chemical features of optical break-down very little depend on the protein concentration. This fact distinguishes lysozyme solution from other aqueous solutions that contain nano-sized objects. The principles that observed during laser radiation of lysozyme aqueous solution are quite similar to those observed in the colloidal solutions of other nano-sized objects.

The decrease of the lysozyme catalytic activity after the impact of laser radiation on the lysozyme aqueous solution is noted. The increase of optical density of the protein solutions is observed both in the absorption maximums of aromatic amino acid residues and in the longer wavelength bandwidth. Moreover, the fluorescence intensity of lysozyme solution, which is defined by the aromatic amino acid residues, is substantially decreased after the impact of laser radiation. Although there is no change of the positions of excitation and emission maxima. The shape of the fluorescence spot on the 3D maps does not change significantly. It is possible to say that there is a degradation of the aromatic amino acid residues under the optical break-down.

However, there were no major differences of the refraction coefficient recorded at the different wavelengths. In other words the total area of protein molecules does not change.

It is confirmed that the viscosity of the lysozyme aqueous solution increases under optical break-down, the pseudo plasticity of the colloidal solution increases as well. The measurements performed with the DLS method confirm that there is no major damage of the polypeptide chain. On the contrary, under the optical break-down the intensive formation of dimer molecules is observed.

Contribution: concept and research preparation – S.R.M; research planning – S.R.M., G.I.V.; research conducting – M.T.A., B.I.V., A.K.V.; writing and drafting of article – M.T.A., B.I.V., A.K.V, S.R.M.; project administration – S.R.M., G.I.V. All authors have read and agreed with the published version of the manuscript.

Acknowledgements

This work was supported by a grant of the Ministry of Science and Higher Education of the Russian Federation (075-15-2022-315) for the organization and development of a Worldclass research center «Photonics».

References

AL-KATTAN A., GROJO D., DROUET C., MOUSKEFTARAS A., DELAPORTE P., CASANOVA A., ROBIN J.D., MAGDINIER F., ALLONCLE P., CONSTANTINESCU C., MOTTO-ROS V. & HERMANN J. (2021): Short-pulse lasers: A versatile tool in creating novel nano-/micro-structures and compositional analysis for healthcare and wellbeing challenges. *Nanomaterials* **11**(3), 1-34 https://doi.org/10.3390/nano11030712.

BARMINA E.V., GUDKOV S.V., SIMAKIN A.V. & SHAFEEV G.A. (2017): Stable products of laser-induced breakdown of aqueous colloidal solutions of nanoparticles. *Journal of Laser Micro Nanoengineering* **12**(3), 254-257 https://doi.org/10.2961/jlmn.2017.03.0014.

BLAKE C.C.F., KOENIG D.F., MAIR G.A., NORTH A.C.T., PHILLIPS D.C. & SARMA V.R. (1965): Structure of hen egg-white lysozyme: A three-dimensional Fourier synthesis at 2 Å resolution. *Nature* **206**(4986), 757-761 https://doi.org/10.1038/206757a0.

BOBYLEV A.G., PENKOV N.V., TROSHIN P.A. & GUDKOV S.V. (2015): The effect of dilution on the aggregation of polycarboxylated C60 fullerene nanoparticles. *Biophysics (Russian Federation)* **60**(1), 30-34 https://doi.org/10.1134/S0006350915010078.

BRUSKOV V.I., KARP O.E., GARMASH S.A., SHTARKMAN I.N., CHERNIKOV A.V. & GUDKOV S.V. (2012): Prolongation of oxidative stress by long-lived reactive protein species induced by X-ray radiation and their genotoxic action. *Free Radical Research* **46**(10), 1280-1290 https://doi.org/10.3109/10715762.2012.709316.

BUNKIN N.F. & BUNKIN F.V. (1993): The new concepts in the optical breakdown of transparent liquids. *Laser Phys* **3**(1), 63.

CANFIELD R.E. (1963): The amino acid sequence of egg white lysozyme. *Journal of Biological Chemistry* **238**(8), 2698-2707.

DAVIES M.J. (2005): The oxidative environment and protein damage. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1703**(2), 93–109 https://doi.org/https://doi.org/10.1016/j.bbapap.2004.08.007.

FRIAS BATISTA L.M., KUNZLER K., JOHN M.G., CLARK B., BULLOCK A., FERRI J., GUPTON B.F. & TIBBETTS K.M. (2021): Laser synthesis of uncapped palladium nanocatalysts. *Applied Surface Science* **557** 149811 https://doi.org/10.1016/j.apsusc.2021.149811.

GIBSON K.F. & KERNOHANT W.G. (1993): Lasers in medicine-A review. *Journal of Medical Engineering and Technology* **17**(2), 51-57 https://doi.org/10.3109/03091909309008351.

KANITZ A., KALUS M.R., GUREVICH E.L., OSTENDORF A., BARCIKOWSKI S. & AMANS D. (2019): Review on experimental and theoretical investigations of the early stage, femtoseconds to microseconds processes during laser ablation in liquid-phase for the synthesis of colloidal nanoparticles. *Plasma Sources Science and Technology* **28**(10), 103001 https://doi.org/10.1088/1361-6595/ab3dbe.

KATTA N., SANTOS D., MCELROY A.B., ESTRADA A.D., DAS G., MOHSIN M., DONOVAN M. & MILNER T.E. (2022): Laser coagulation and hemostasis of large diameter blood vessels: effect of shear stress and flow velocity. *Scientific Reports* **12**(1), 8375 https://doi.org/10.1038/s41598-022-12128-1.

KHALKHAL E., REZAEI-TAVIRANI M., ZALI M.R. & AKBARI Z. (2019): The evaluation of laser application in surgery: A review article. *Journal of Lasers in Medical Sciences* **10**(4), S104-S111 https://doi.org/10.15171/jlms.2019.S18.

KONONENKO V.V., ASHIKKALIEVA K.K., ARUTYUNYAN N.R., ROMSHIN A.M., KONONENKO T.V. & KONOV V.I. (2022): Femtosecond laser-produced plasma driven nanoparticle formation in gold aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry* **426**, 113709 https://doi.org/10.1016/j.jphotochem.2021.113709.

KOVALCHUK-KOGAN T., BULATOV V. & SCHECHTER I. (2015): Optical breakdown in liquid suspensions and its analytical applications. *Advances in Chemistry* **2015**https://doi.org/10.1155/2015/463874.

KUDRYASHOV S.I. & ZVORYKIN V.D. (2008): Microscale nanosecond laser-induced optical breakdown in water. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics* **78**(3), 036404 https://doi.org/10.1103/PhysRevE.78.036404.

LANKA P., FRANCIS K.J., KRUIT H., FARINA A., CUBEDDU R., SEKAR S.K.V., MANOHAR S. & PIFFERI A. (2021): Optical signatures of radiofrequency ablation in biological tissues. *Scientific Reports* **11**(1), https://doi.org/10.1038/s41598-021-85653-0.

LETOKHOV V.S. (1985): Laser biology and medicine. *Nature* **316**(6026), 325-330 https://doi.org/10.1038/316325a0.

NANNI C.A. & ALSTER T.S. (1998): Complications of cutaneous laser surgery: A review. *Dermatologic Surgery* **24**(2), 209-219 https://doi.org/10.1111/j.1524-4725.1998.tb04139.x.

PENKOV N.V. & PENKOVA N. (2021): Key differences of the hydrate shell structures of atp and mg·atp revealed by terahertz time-domain spectroscopy and dynamic light scattering. *Journal of Physical Chemistry B* **125**(17), 4375-4382 https://doi.org/10.1021/acs.jpcb.1c02276.

PURWANTI N., SMIDDY M., JAN VAN DER GOOT A., DE VRIES R., ALTING A. & BOOM R. (2011): Modulation of rheological properties by heat-induced aggregation of whey protein solution. *Food Hydrocolloids* **25**(6), 1482-1489 https://doi.org/10.1016/j.foodhyd.2011.02.027.

SAFIR M., HECHT I., SHARON T., EINAN-LIFSHITZ A. & BELKIN A. (2022): Application of Nd:YAG laser to the anterior vitreous in malignant glaucoma – a systemic review and meta-analysis. *Graefe's*

Archive for Clinical and Experimental Ophthalmology **260** 2981–2990 https://doi.org/10.1007/s00417-022-05640-7.

SARIMOV R.M., MATVEYEVA T.A. & BINHI V.N. (2018): Laser interferometry of the hydrolytic changes in protein solutions: the refractive index and hydration shells. *Journal of Biological Physics* **44**(3), 345–360 https://doi.org/10.1007/s10867-018-9494-7.

SHAFEEV G.A. (2012): *Laser Ablation in Liquids*.</i>In Principles and Applications in the Preparation of Nanomaterials. Ed G. YANG. New York, Jenny Stanford Publishing. p 1192 https://doi.org/10.1201/b11623.

SHAPAROV M.G., GUDKOV S.V., LANKIN V.Z. & NOVOSELOV V.I. (2021): Role of Glutathione Peroxidases and Peroxiredoxins in Free Radical-Induced Pathologies. *Biochemistry (Moscow)* **86**(11), 1418–1433 https://doi.org/10.1134/S0006297921110067.

SHARAPOV M.G., GUDKOV S.V. & LANKIN V.Z. (2021): Hydroperoxide-Reducing Enzymes in the Regulation of Free-Radical Processes. *Biochemistry (Moscow)* **86**(10), 1256–1274 https://doi.org/10.1134/S0006297921100084.

SHUGAR D. (1952): The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. *Biochimica et biophysica acta* **8**302-309 https://doi.org/10.1016/0006-3002(52)90045-0.

SLAGLE G., BAHR T., WELBURN K., KATURI J., QUITTNER-STROM E. & SPONSEL W. (2022): Safety, efficacy, and timing of Nd:YAG laser goniopuncture after nonpenetrating deep sclerectomy for glaucoma: A retrospective cohort study. *European Journal of Ophthalmology* **32**(5), 2870–2879 https://doi.org/10.1177/11206721211056585.

SOKOLOV A.V., DUBROVSKAYA N.M., KOSTEVICH V.A., VASILEV D.S., VOYNOVA I.V., ZAKHAROVA E.T., RUNOVA O.L., SEMAK I.V., BUDEVICH A.I., NALIVAEVA N.N. & VASILYEV V.B. (2022): Lactoferrin Induces Erythropoietin Synthesis and Rescues Cognitive Functions in the Offspring of Rats Subjected to Prenatal Hypoxia. *Nutrients* **14**(7), https://doi.org/10.3390/nu14071399.

TEALE F.W. & WEBER G. (1957): Ultraviolet fluorescence of the aromatic amino acids. *The Biochemical journal* **65**(3), 476-482 https://doi.org/10.1042/bj0650476.

VAGHASIYA H., KRAUSE S. & MICLEA P.T. (2022): Thermal and non-thermal ablation mechanisms in crystalline silicon by femtosecond laser pulses: Classical approach of the carrier density two temperature model. *Journal of Physics D: Applied Physics* **55**(17), https://doi.org/10.1088/1361-6463/ac4dca.

WANG Z. & CHOCAT N. (2010): Fiber-optic technologies in laser-based therapeutics: Threads for a cure. *Current Pharmaceutical Biotechnology* **11**(4), 384-397 https://doi.org/10.2174/138920110791233271.

ZAKHAROVA E.T., SOKOLOV A.V., PAVLICHENKO N.N., KOSTEVICH V.A., ABDURASULOVA I.N., CHECHUSHKOV A.V., VOYNOVA I.V., ELIZAROVA A.Y., KOLMAKOV N.N., BASS M.G., SEMAK I.V., BUDEVICH A.I., KOZHIN P.M., ZENKOV N.K., KLIMENKO V.M., KIRIK O.V., KORZHEVSKII D.E., MENSHCHIKOVA E.B. & VASILYEV V.B. (2018): Erythropoietin and Nrf2: key factors in the neuroprotection provided by apo-lactoferrin. *BioMetals* **31**(3), 425–443 https://doi.org/10.1007/s10534-018-0111-9.

ZHANG H., LI M. & CEN Z. (2022): Excimer Laser Corneal Refractive Surgery in the Clinic: A Systematic Review and Meta-analysis. *Computational and Mathematical Methods in Medicine* **2022**, 7130422 https://doi.org/10.1155/2022/7130422.