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Thermal denaturation of egg protein under nanosecond pulsed laser heating of gold nanoparticles

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Abstract. **Thermal denaturation of egg protein in the presence of gold nanoparticles via their heating at the plasmon resonance wavelength by the pulsed radiation of the second harmonic of an Nd : YAG laser (532 nm) is investigated. The experimental dependence of the protein denaturation time on the mean laser power is obtained. The heating temperature of the medium with gold nanoparticles is calculated. The numerical estimates of the temperature of the heated medium containing protein and gold nanoparticles (45.3 °C at the moment of protein denaturation) are in good agreement with the literature data on its thermal denaturation and with the data of pyrometric measurements (42.0± 1.5 °C). The egg protein may be successfully used to investigate the specific features of laser heating of proteins in the presence of metal nanoparticles under their excitation at the plasmon resonance wavelength.**

Keywords: *egg protein, gold nanoparticles, second harmonic radiation of an Nd : YAG laser, plasmon resonance, thermal protein denaturation.*

1. Introduction

Thermal destruction of cells by heating metal nanoparticles with laser radiation at the plasmon resonance wavelength was first proposed in 2003 by Pitsillides et al. [1] as a new promising method of therapy, first of all, targeted at oncological diseases. Since that time in numerous papers, devoted to this topic, different particular problems have been considered, from investigation of distribution of nanoparticles in cell structures [2] and their targeted delivery to the pathological cells [3, 4] to manufacturing rod nanoparticles with prescribed aspect ratio to provide the shift of plasmon resonance into the IR region [5, 6].

Photoinduced death of cells in the presence of nanoparticles under the action of laser radiation was demonstrated in cell cultures by counting the cells that died after irradiation [7, 8], as well as in pathogenic bacteria [9]. In the *in vivo* experiments the dynamics of hampering the growth of animal tumours in the process of treatment was studied.

Direct heating of metal nanoparticles in tissues by laser radiation is less investigated. Radiation of the second harmonic of the Nd : YAG laser, most often used to heat spherical gold nanoparticles at the plasmon resonance wavelength (520 – 550 nm), practically does not penetrate into biological tissues. On the other hand, the technology of manufacturing metal nanorods that may be heated using the radiation with greater wavelength remains rather expensive and complex; moreover, nanorods penetrate into the cells worse than spherical nanoparticles. It is accepted that the nanoparticle size of 30 – 50 nm is the best for penetration into cells [10], while nanorods typically have greater dimensions. Moreover, under laser heating the nanorods are subjected to fast transformation into nanospheres (reshaping), loosing the band of longitudinal plasmon resonance in their spectrum [11], so that they can no more serve as efficient convertors of laser IR radiation into heat.

Experimenting with tissues implies histological methods, which in the case of studying the heat effect on the samples do not provide unambiguous answer, because the process of sample preparation includes its treatment with hot paraffin. Moreover, it is necessary to solve the problem of marking the irradiated area in the process of histological section preparation to provide its proper imaging.

At present two basic mechanisms of pathological cell damage under laser heating of nanoparticles are discussed:

(i) thermal denaturation of intracellular proteins caused by the heat acquired by nanoparticles in the course of laser excitation;

(ii) evaporation of a thin layer of liquid around the heated nanoparticle, producing micro-bubbles that damage the cell membrane [12].

In cell experiments these two mechanisms cannot be separated. Therefore, it is desirable to develop a simple experimental technique that will makes it possible to study the denaturation mechanism solely, eliminating the effect of micro-bubbles. Moreover, the cell structures are rather complex and their study requires a complex of optical and electron microscopy methods. In such experiments it is rather difficult to determine the optimal characteristics of laser radiation for efficient photo-thermo-conversion.

It is known that efficient conversion of laser radiation into heat requires the power density of laser radiation $10^5 - 10^{10}$ W m^{-2} [13]. At the same time, most frequently

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in the experiments the heating of nanorods or nanoshells is performed using a 808-nm, cw semiconductor laser with the mean power 450 – 600 mW, which is not enough to obtain the required power density.

Using pulsed lasers and choosing properly the energy and temporal characteristics, it is possible to achieve the maximal efficiency of radiation-to-heat conversion for a given type of nanoparticles with their environment taken into account.

In the present paper an experimental approach is proposed to investigate the features of thermal decomposition of some biological objects depending on the characteristics of the laser radiation with the use of a conventional model biological medium – the protein of a chicken egg. The object of study was the white of a fresh chicken egg that consists of 12 % protein and 86 % water [14] and is optically transparent in the visible range in the natural state. Under denaturation of the protein the sample becomes optically non-transparent in the visible range, which is easily detected using optical methods. Moreover, the laser heating of the egg white can be easily detected remotely using a pyrometer.

2. Samples and research methods

The source egg white was extracted from a fresh raw chicken egg mechanically, without mixing with the egg yolk and without dilution. The gold nanoparticles were obtained using the method of laser ablation of a solid target in a liquid environment. This method is widely used to obtain nanoparticles of different composition, morphology and size and has a number of advantages over other methods [15]. It is efficient when small amounts of nanoparticles are required, e.g., in medical and biological studies, since it allows the production of aqueous solutions of gold nanoparticles without external impurities, inherent, e.g., in chemical synthesis methods. Detailed description of the synthesis setup, at which the colloidal solutions of gold nanoparticles were obtained, is given in [16]. Ablation was caused by the focused radiation of the second harmonic of the LS-2134UTF Nd : YAG laser (LOTIS TII, Minsk) with the wavelength 523 nm, the pulse duration 7 ms and the pulse repetition rate 15 Hz. The target of bulk gold (99.99 %) in distilled water was exposed to the laser radiation with the initial peak power density at the target surface 2 GW cm^{-2}, which exceeds the ablation threshold by more than an order of magnitude under the given conditions. With growing the number of particles in the solution, the power density of radiation on the target surface decreased. The time of exposure was 20 min, the volume of liquid was 10 mL, and the total radiation energy on the sample was 1.6 kJ. The concentration of nanoparticles in the probe was determined by measuring the mass loss of the bulk target and dividing it by the liquid volume. In the basic sample it amounted to 600 mg L^{-1}.

According to the data, obtained with the CM12 transmission electron microscope (TEM) (Philips), the shape of a gold nanoparticle is close to spherical (Fig. 1). The mean diameters of the particles, determined from the Gaussian bimodal approximation of the size distribution, were 9.9 and 20.3 nm with the distribution half-width 5.8 and 6.5 nm, respectively (Fig. 2). The size distribution was constructed using 230 measurements of nanoparticle diameters in three TEM images.

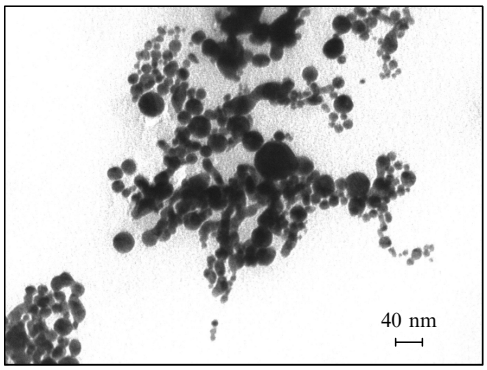

Figure 1. TEM image of gold nanoparticles obtained using the method of laser ablation of a solid target in distilled water.

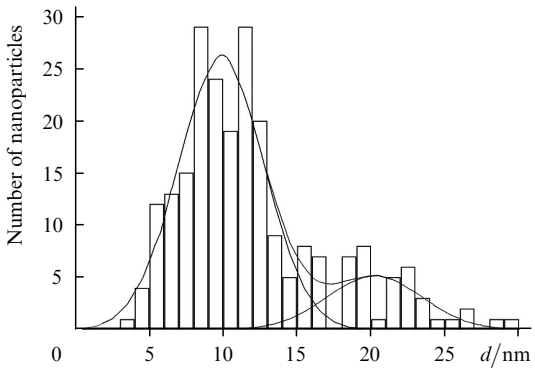

Figure 2. Diagram of the diameter distribution of gold spherical nanoparticles.

The linear absorption and fluorescence spectra of the samples were recorded using the CM 2203 spectrofluorimeter (SOLAR, Minsk). Figure 3 shows the absorption spectrum of the egg white with gold nanoparticles at the concentration 120 mg L^{-1} [optical path length 1 cm, curve (*1*)] and the absorption spectrum of the egg white without nanoparticles [optical path length 0.12 mm, curve (*2*).] The band of intense protein absorption lies in the UV region, while the plasmon resonance band of gold nanoparticles with the maximum at 533 nm is located in the visible part of the spectrum. The large background absorption in spectrum (*1*) over the entire range is associated with sufficiently strong scattering. The fluorescence spectrum of the egg white sample [curve (*3*)] is typical for tryptophan fluorescence and has a maximum at 330 nm; the addition of gold

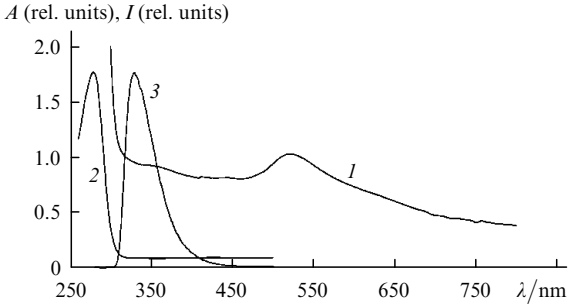

Figure 3. Absorption spectra of egg white with gold nanoparticles [optical path length 1 cm, (*1*)] and without them [optical path length 0.12 mm, (*2*)] and the spectrum of protein fluorescence *I* (3).

nanoparticles does not change the fluorescence spectrum. No fluorescence inherent in gold nanoparticles was detected.

The optical scheme for measuring the protein denaturation under the laser heating of gold nanoparticles was rather simple. The second harmonic of the Nd : YAG laser with the parameters indicated above was directed into the sample cuvette as a non-focused beam with the diameter 5 mm. In the course of experiments the mean laser power at the input and output of the cuvette was monitored using the NOVA II energy and mean power meter with a 12A-P calorimetric detector (Ophir Optronics Ltd, Israel). The input mean power varied from 0.49 to 1.64 W, the pulse power density being 15 – 50 mW cm^{-2}.

The samples for laser excitation studies consisted of the egg white with addition of aqueous solution of gold nanoparticles without stabilisation at a volume ratio of 4 : 1. Therefore, the mass concentration of gold nanoparticles in the probe was 120 mg L^{-1}. For comparison we used the protein solution, diluted with distilled water at a volume ratio of 4 : 1. To prepare the samples the components were thoroughly mixed during 10 – 15 min in the cylindrical measuring glass manually with a glass rod until a homogeneous uniform medium was obtained, after which the samples were kept still during 60 min to remove air bubbles that appear in the process of mixing. To stimulate the bubble exit from the solution, the glass was periodically carefully shaken.

In the course of the experiments the linear absorption and fluorescence spectra of the samples under study were monitored with the periodicity of 30 min to assure the stability of the optical properties of the medium.

The probe volume under laser irradiation was 0.5 cm^3; quartz spectrometric cuvettes were used with the optical path length 0.5 cm. The temperature of the sample was measured remotely using the AR 1300 pyrometer, the measurement error did not exceed 1.5 °C at a temperature of about 40 °C.

3. Results and discussion

A sample of the egg white without nanoparticles was subjected to laser irradiation for 15 min. The intensity of transmitted radiation did not change in the entire range of the used powers of exciting radiation. Figure 4 presents the time variations of the transmission for protein samples at different powers of the incident radiation. Note that the introduction of the colloidal solution of gold nanoparticles into the sample reduces the transmitted radiation intensity by nearly 10 times because of the absorption and scattering of radiation by nanoparticles. The normalised transmission curve (*2*) has two regions, namely, the region of a smooth decrease and the slump of transmission as a result of formation of an optically dense bulk clot of denaturated protein in the sample (Fig. 5). The measurement of the sample temperature with the pyrometer has shown that the temperature of the sample without nanoparticles remains close to room temperature (26 ± 1.5 °C), while in the sample with nanoparticles during the clot formation the temperature increases up to 43 ± 1.5 °C. Therefore, the nature of the observed clot is associated with the denaturation of the egg protein in the excited volume.

When the mean power of laser radiation is changed, the time of the clot formation (defined as the time interval between the beginning of exposure and the middle of the transmission slump) is also changed. The corresponding dependence is presented in Fig. 6. At the mean power 0.77 W and lower no protein denaturation with clot formation was observed for 10 min of irradiation. This may be an evidence of the threshold character of the effect, which, however does not follow directly from the obtained dependence.

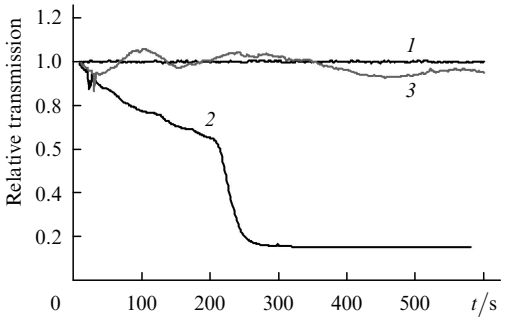

Figure 4. Time dependences of relative transmission of laser radiation, passing through a cuvette with the studied solution (optical path length 0.5 cm) for protein (mean excitation power 1.15 W) (*1*) and protein with nanoparticles (mean excitation power 1.15 and 0.49 W, respectively) (*2*, *3*). The transmission is normalised to its value at the initial moment of irradiation.

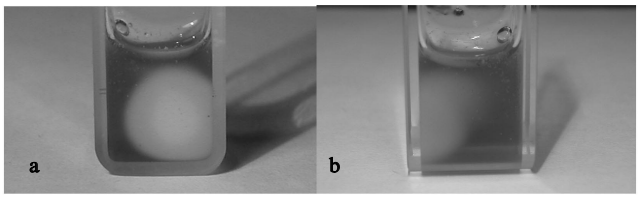

Figure 5. Photograph of the clot in the cuvette with the egg white that appears after denaturation caused by the pulsed laser irradiation: front view (a) and lateral view (b).

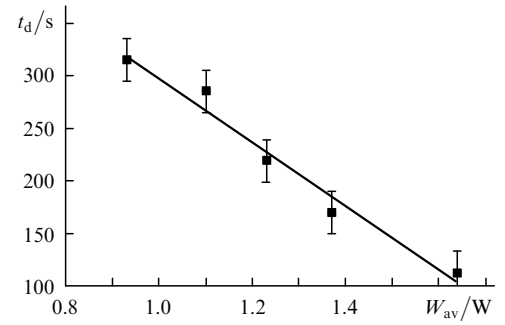

Figure 6. The time of 'bulk' denaturation of protein t_d versus the mean power of laser radiation W_{av}.

This experimental approach may be developed to estimate the efficiency of laser heating of nanoparticles.

For the laser beam diameter 5 mm and the optical path length 5 mm the irradiated volume of the sample amounts to 0.16 cm^3. The concentration of nanoparticles equals 120 mg L^{-1}, the total mass of nanoparticles in the irradiated volume of the sample $m = 19 \times 10^{-9}$ kg. The specific heat of bulk gold $c \approx 0.13$ kJ kg^{-1} K^{-1} [17]. There is no data about the specific heat of gold nanoparticles, and so we assume the

specific heat of the used nanoparticles having the diameter 10 nm and greater to be comparable with that of bulk gold in our calculations.

Based on the analysis of the absorption spectra, we find the optical density of the sample to be $D = 0.115$ at the irradiating wavelength. One can conclude that in the course of laser excitation with the characteristics, specified above, 23 % of the incident radiation is absorbed, 67 % is scattered and 10% passes through the sample and is registered by the calorimeter.

At the maximal mean radiation power of 1.64 W the pulse energy is $Q = 0.11$ J, and if 23 % of the energy is absorbed, then $Q = 0.0253$ J.

Thus, the mean heating of gold nanoparticles (without taking into account the heating of the surrounding medium) per single pulse is

$$T - T_0 = \frac{Q}{cm} \approx 10^4 \text{ K}. \quad (1)$$

Under real conditions the energy transfer from nanoparticles to the solvent molecules occurs during the nanosecond pulse action. Additional nonlinear scattering by the local inhomogeneities of the refractive index around the nanoparticles absorbing the radiation also takes place [18, 19], which shields pump radiation. As a result the maximal temperature of the particle heating amounts to $\sim 10^3$ °C.

Considering the laser heating of the entire medium, we replace its physical parameters (specific heat and density) with those of pure water, since the concentrations of nanoparticles and protein are sufficiently small. In this case the specific heat of the medium is $c \approx 4.2$ kJ kg^{-1}K^{-1} [17]. All the energy, absorbed during a single pulse, is dissipated in the irradiated medium, therefore

$$T - T_0 = \frac{Q}{cm} = 0.038 \text{ °C}. \quad (2)$$

The repetition rate of the laser pulses is $f = 15$ Hz. Let us assume that during the time between the adjacent pulses the heat from the heated nanoparticles has time to dissipate over the entire sample volume (0.5 cm^3) and to rise its temperature per pulse by

$$T - T_0 = 0.012 \text{ °C}. \quad (3)$$

We also assume that in the first approximation the system is heat-insulated, i.e., it is not cooled between the pulses. Then, taking into account the fact that the protein denaturation is observed after $t = 112.7$ s, and $f = 15$ Hz, we get the number of laser pulses, causing protein denaturation, equal to 1.7×10^3.

The net heating of the sample by all laser pulses yields 20.3 °C.

At room temperature 25 °C the sample is heated up to 45.3 °C, which agrees well with the temperature measured with the pyrometer. Analogous estimates at the mean exciting power 1.1 – 1.64 W yield 44 – 48 °C.

Thus, the preliminary estimates, carried out using constant thermophysical parameters and without taking into account the heat transfer to air and the heat capacity of the cuvette, show that the considered physical model is quite real and explains well the mechanism of thermal denaturation of protein under the pulsed laser irradiation of gold nanoparticles at the plasmon resonance wavelength.

In our opinion, the specific feature of heating of gold nanoparticles in the protein environment with low-frequency laser radiation is the presence of two processes, a fast and a slow one. The fast process is based on the heating of the particle during a single pulse, after which it has time to dissipate the heat into the medium. In this case the protein molecules in the immediate environment of the particle are denaturated in the first place. They can be visually observed as white turbid microparticles in the medium. These particles cause monotonic lowering of transmission during the initial period of time [see Fig. 4, curve (*2*) in the range up to 200 s].

The slow mechanism manifests itself as the bulk coagulation of protein in the entire region of laser action, heated due to multiple-pulse excitation to the temperature of denaturation.

In the case of cw excitation the fast mechanism is absent, and when the threshold excitation power is achieved, only the bulk coagulation of protein occurs, since the heat transfer has no time to compensate for the temperature rise due to laser heating.

Pulsed excitation due to the fast mechanism requires smaller mean laser powers, and the protein denaturation is observed before the heating of all irradiated volume up to the critical temperature. Therefore, increasing the concentration of nanoparticles in the region of pathology and raising the pulse power by using shorter, e.g., femtosecond, pulses it is possible to induce selective thermal damage of cells not only on the surface of an organ, but also inside it, which is important in the treatment of oncologic diseases.

4. Conclusions

As a result of the studies of thermal protein denaturation in the presence of gold nanoparticles excited by nanosecond laser radiation in the plasmon absorption band (second harmonic of Nd : YAG laser, 532 nm), and using the dependences of the medium transmission on the exposure time at different power densities, we have proposed two mechanisms of thermal denaturation: local fast denaturation during the pulse and bulk slow denaturation due to gradual increase in the temperature of the entire medium under multi-pulse irradiation. Quantitative estimates of the possible medium heating under such irradiation are performed, thereby demonstrating good agreement with the experimental results.

The proposed experimental approach to the study of laser heating of biological media using the protein from a chicken egg as a model medium is easily implementable and may serve as a convenient research tool.

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