Nonreciprocal XeCl laser-induced aggregation of β-crystallins in water solution

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Abstract: The aggregation of a β-crystallin water solution exposed to XeCl laser radiation demonstrates the dependence of scattering-exposure curve (scattering versus exposure) on laser intensity. The main features of this dependence can be understood by the relaxation of a partly denaturated state of a protein within some finite relaxation time. These photoactivated states originate from the absorption of UV photons. Two partly denaturated (photoactivated) monomers, as well as other aggregates, can aggregate, giving rise to sharply increasing probe light scattering after some lag time of irradiation.

OCIS codes: (140.2180) Excimer lasers; (350.3390) Laser materials processing; (290.5890) Scattering, stimulated; (350.1820) Damage; (000.1430) Biology and medicine.

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1. Introduction

Laser irradiation of materials can result in significant changes in their optical properties. In particular, nearly transparent samples can be transformed into opaque media due to light-induced scattering. The increase in scattering can sometimes be attributed to the aggregation of particles, resulting in the formation of larger objects, which scatter light more effectively than the initial, smaller particles.

One of the important examples is the aggregation of water-soluble proteins with UV laser light from a XeCl excimer laser (308 nm). This phenomenon is currently being investigated to elucidate cataract development.

The molecular mechanisms of the origin and development of certain forms of cataracts, including photocataractogenesis, are based on the degradation and aggregation of the main water-soluble proteins in the eye lens (α-, β-, and γ-crystallins) [1-3]. The light-induced degradation of these proteins, and thus the whole lens, stimulates processes associated with the development of the senile cataract, one of the most widespread forms of cataract [4]. Despite the fact that there are some comprehensive studies on the kinetics of crystallin aggregation produced by irradiation with UV lamps, very few papers are devoted to laser-induced crystallin aggregation [5-7]. The use of a laser provides the opportunity to control the irradiation conditions by fixing the wavelength and varying the pulse fluence and repetition rate. Nevertheless, as far as we have seen in the literature, the effects of UV-laser radiation were studied by the other groups at a constant energy density of light pulses (w) and a constant pulse repetition rate (F). It is thereby understood that the photo-aggregation kinetics obeys the reciprocity law, i. e. that the *scattering – exposure* curve is the same at different light intensities. Here, exposure is the product of the light intensity and exposure time. It is well established that after irradiation starts the solution of protein remains almost transparent during some period of time called the lag period. After the lag period, the scattering of the sample abruptly increases. Reciprocity suggests that this lag period is inversely proportional to the light intensity or that *lag exposure* in the scattering-exposure curve should not depend on the light intensity.

The particular goal of this work is to study the effects of UV radiation on a water solution of β-crystallin within a broad range of variation of the parameters w and F. We show in particular that the lag exposure depends on the light intensity in some way. In this sense, the '*non-reciprocity*' [8] of the response of the protein solution to UV laser light is observed. This *nonreciprocal* response comes neither from laser heating nor from optical nonlinearity, but rather is accounted for by the specific behavior of the irradiated protein molecules.

2. Material and methods

For this experiment, we used bovine lens β-crystallin from Sigma-Aldrich (USA). The protein solution was prepared as described elsewhere [9]. The optical setup for the experimental unit is presented in Fig. 1. The source of UV radiation was a XeCl laser LPX-200 (Lambda Physik) operating at 308 nm with a pulse energy of up to 450 mJ and a pulse repetition rate of up to 80 Hz. The experiments were conducted at a temperature of 22°C. To study the dynamics of protein aggregation under UV irradiation, we measured scattering-exposure curves, which show the intensity of the probe beam scattered in the cuvette with the protein solution versus the laser exposure D. The probe beam was generated by a single-mode (TEM_0) HeNe laser with a wavelength of 633 nm, a power of 10 mW, and a beam divergence of 1.1×10^{-3} rad. The intensity of the probe beam scattering was measured by the dark field method [10], which allowed measurement of the absolute value of the scattered power. We simultaneously measured the energy of 308 nm pulses that passed through the cuvette. A detector ("Gentec" ED-200) was placed immediately behind the cuvette. The detected signal is associated with both light absorption and scattering changes at 308 nm for the protein solution and enables us to follow the change in UV protein absorption at the initial stage of irradiation where the scattering is negligible. The signals from the energy meters and photodiode are processed and stored with an automated system. Before and after irradiation

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with various UV-laser exposures, we recorded the optical transmission spectra with a Specord M40 spectrophotometer. We observed some increase in the absorption of the protein solution at high exposures. The shape of the laser-induced transmission spectrum is analogous to that obtained earlier for a UV-lamp-irradiated water-soluble fraction of bovine lens [11].

Aggregation was confirmed independently by measuring the molecular weight distributions (MWD) chromatographically with a Superose 12HR column. The height of the quartz cuvette was 10 mm, and its lengths along the HeNe and XeCl laser beams were 10 mm and 5 mm, respectively.

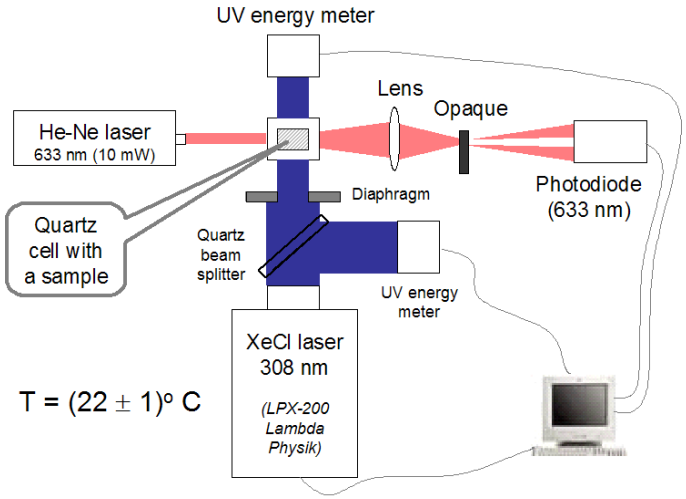

Fig. 1. Experimental setup.

In the preliminary experiments, we measured the absorption coefficient, α, of the β-crystallin solution with a concentration of 0.5 mg/ml in a cell 5 cm in length, and it was 0.05 cm^{-1} independent of the XeCl laser fluence in the range $w = (2 \div 300)$ mJ/cm^2. This indicates the lack of nonlinear absorption. With such an α and with a maximum experimental value of $w = 90$ mJ/cm^2, heating of the solution during irradiation did not exceed $\Delta T = 2°K$. Thus, the samples were irradiated at room temperature, and photothermal processes were absent. An air conditioner was used to keep the room temperature constant.

3. Results and discussion

The scattering-exposure curves obtained at different w and F are presented in Fig. 2 a, b. This demonstrates the existence of some lag exposure followed by a rapid increase in scattering. This lag exposure D^* is one of the major parameters to consider. For each laser pulse fluence w, there is a characteristic value for the repetition rate, F^*, below which D^* increases considerably (by several times), which suggests a sharp decrease in the probability of aggregation. At $w = 75$ mJ/cm^2 and $F = 4$ Hz, D^* is 100 J/cm^2, but at $w = 75$ mJ/cm^2 and $F = 0.7$ Hz, D^* is 300 J/cm^2. At low repetition rates for the laser pulses, the slope of the scattering-exposure curves decreases, whereas at high F the value of D^* and the slope of the scattering-exposure curves practically do not depend significantly on repetition rate.

The experimental curves in Fig. 2 a, b are analyzed to extract the dependence $D^*(I)$, $I = w \times F$, the result of the analysis is presented in Fig. 3.

The same shape was obtained for scattering-exposure curves, where the lag time had the same dependence on laser intensity, in our previous work devoted to the investigation of laser-induced aggregation of another water soluble protein, carbonic anhydrase [9], and also for investigations of the effect of laser radiation on the porcine lens substance [12]. However, the specific values of the characteristic exposures and intensities are different. These facts suggest that the observed intensity dependence of the laser-induced scattering kinetics is typical for water-soluble proteins irradiated at the wavelength of a XeCl laser.

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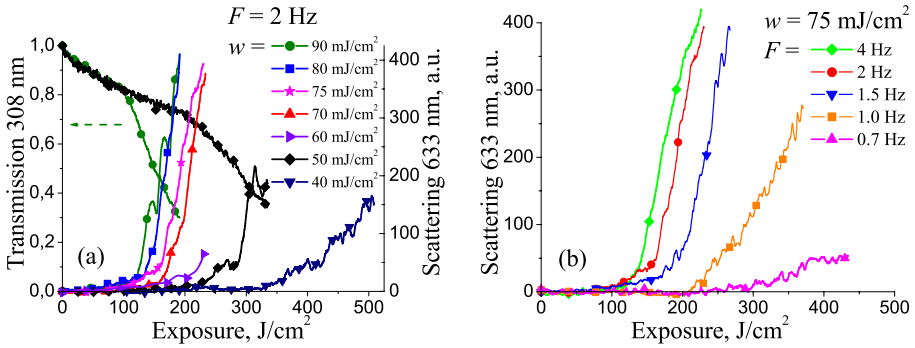

Fig. 2. The scattering-exposure curves for the irradiation of a solution of β-crystallin with a concentration of n = 0.5 mg/ml at a constant repetition rate (F), for different fluences (w) **(a)**; and at constant fluence with different repetitions rates **(b)**. In Fig. 2 a we also show the transmission curves at 308 nm. The abrupt change in the slope of the curves measured at different wavelengths takes place at approximately the same exposure. In Fig. 2 b the lag times for repetition rates 4, 2, 1.5, 1 and 0.7 Hz are 7.5, 18.5, 29, 49 and 95 min, correspondingly.

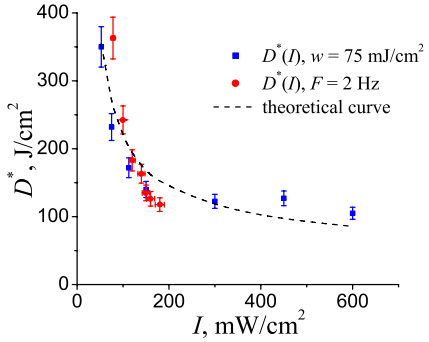

Fig. 3. The experimental (symbols) and theoretical (dotted) results for $D^*(I)$ with $I = F \times w$. Blue square symbols were obtained with w = const = 75 mJ/cm^2, red circles were obtained with F = const = 2 Hz. Fitting parameter values are I_0 = 90 mW/cm^2 and D_0 = 40 J/cm^2 (see the model in the text).

According to the modern understanding of the thermal aggregation of water-soluble proteins, temperature elevation results in the denaturation of protein molecules followed by their aggregation. *Photo-aggregation* is triggered by the absorption of photons. The tryptophan residue is responsible for photon absorption at the wavelength of irradiation here. The photon energy is converted to vibrational energy, and this energy propagates from the absorption site, increasing the number of excited degrees of freedom. At the initial moment, the local effective temperature is very high. It can provide some local thermal denaturation of the protein molecule regardless of the fact that the period of time during which the given site is heated is very short. Thus, the absorption of a photon can result with some probability in the creation of *a partly denaturated state* [13] for a protein molecule. We call the proteins in this partly denaturated state *photoactivated* proteins. Our explanation of the experimental data is based on the assumption that this *partly denaturated state of the protein relaxes within some relaxation time* τ. If, during this relaxation time, two photoactivated molecules meet each other, then there is some probability that they will aggregate. The aggregates are denaturated features, which can aggregate with other aggregates. The aggregated states are much more stable with respect to relaxation than the photoactivated states. In order to illustrate how these assumptions could explain the experimental data, let us consider the simplest model.

Let N_{10} be the number density of the initiate proteins (monomers) in solution, and let R be the number density of the photoactivated proteins. Let us consider the simplest case when the

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only relaxation process is the deactivation of the photoactivated monomers. The number density of irreversible aggregated monomers is *M*, and it increases whenever two photoactivated monomers combine with each other. The simple equations for *R* and *M* are:

$$\frac{dR}{dt} = \eta\sigma N_{10}\frac{I}{\hbar\omega} - \frac{R}{\tau} - k_2 R^2, \qquad \frac{dM}{dt} = k_2 R^2 \tag{1}$$

Here σ is the absorption cross-section, and η is the quantum efficiency of the photoactivation process. *I* is the laser intensity, and $\hbar\omega$ is the energy of photon. The second term on the right-hand side of the first equation accounts for the relaxation process, while the third term describes the aggregation.

Laser intensity in the set of Eqs. (1) is a periodic sequence of laser pulses. With typical values of parameters, it can easily be shown that *R(t)* tends to the limiting periodic sawtooth curve oscillating just near the constant average level for a time period t_{st} much smaller than the lag period in the scattering-exposure curve, that is $\int_0^{t_{st}} I(t)dt << D^*$. If the value of this level is much larger than the modulation depth, then the set of Eqs. (1) can be treated in continuous approximation where *I(t)* is changed for the constant averaged value *I*. In what follows we will consider this continuous approximation providing simple and clear formulas illustrating the main physical idea of the paper.

In this approximation, the stationary value of *R* reads:

$$R_{stat} = (2k_2\tau)^{-1} \times (\sqrt{1 + I/I_0} - 1) \tag{2}$$

where

$$I_0 = \frac{\hbar\omega}{\eta\sigma N_{10}} \cdot \frac{1}{4k_2\tau^2} \tag{3}$$

is some characteristic intensity. The number density *M* in this approximation can be estimated from the second Eq. (1) as $M = k_2 R_{stat}^2 t = k_2 R_{stat}^2 D I^{-1}$.

It is evident that the necessary condition for the scattering to be noticeable is that the total mass of the aggregates is high enough. If one assumes for simplicity that the change in scattering corresponds approximately to some almost fixed value of aggregated monomers:

$$M = M^* \tag{4}$$

then the lag exposure D^* at which condition Eq. (4) holds is:

$$D^* \approx \frac{D_0 I/I_0}{(\sqrt{1 + I/I_0} - 1)^2}, \tag{5}$$

where

$$D_0 = \frac{M^*}{N_{10}} \times \frac{\hbar\omega}{\eta\sigma} \tag{6}$$

is some characteristic exposure. It follows from Eq. (5) that at relatively high intensities $I >> I_0$:

$$D^* \approx D_0, \tag{7}$$

This indicates that the lag exposure at high intensities does not depend on intensity. In the other limit, $I << I_0$, because $\sqrt{1 + I/I_0} - 1 \approx I/2I_0$,

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$$D^* \approx 4D_0 \frac{I_0}{I}. \quad (8)$$

Thus, at low enough intensities the lag exposure is inversely proportional to intensity. All of these features correspond to the experimental findings in Figure 3. It should be noted that this is an oversimplified analysis only aiming to prove that the introduction of relaxation times for denaturation processes could explain the non-reciprocity of aggregation kinetics. Taking into consideration the value of the parameter I_0 = 90 mW/cm^2 (see Fig. 3) and the expression Eq. (3) allows estimating the value of the relaxation time τ from below. Using the known values $\hbar\omega = 6.62\times10^{-19}$ J, $\sigma = 5\times10^{-18}$ cm^2, $N_{10} = 10^{16}$ cm^{-3} and in consideration that $M^*/N_{10} < 1$ and $k_2 < 1.1\times10^{-11}$ cm^3/s provides estimation $\tau > 0.03$ s. The value of k_2 corresponds to diffusion limited aggregation of the activated monomers. It should be noted, however, that continuous quasi-stationary approximation for solution of the set of Eqs. (1) considered in this paper is valid if $\tau \geq 1$ s. A more comprehensive analysis based on considering the Smoluchovsky equations, which specifies condition (4) and clarifies the role of other relaxation times, will be published in a paper devoted to the mathematical aspects of the problem. In particular, in a more elaborated version, condition (4) rather means that the number density of dimers, i.e. aggregates possessing two monomers, should reach some critical value before the scattering abruptly increases. At the initial stage of aggregation, these conditions are very close to each other and provide the same dependence of the lag exposure on the laser intensity.

4. Conclusions

The photo-aggregation kinetics is characterized by the scattering-exposure curve (scattering of the probe beam vs. UV exposure). The scattering-exposure curve for XeCl laser-induced photo-aggregation of water soluble proteins, β-crystallins, demonstrate a dependence on laser intensity. This intensity varies with both laser fluence and repetition rate. At low enough intensities, the lag exposure and the slope of the kinetic curve depend strongly on intensity, whereas at high intensities, the dependence on intensity for both parameters practically vanishes. This feature can be explained with the model we present, which suggests that photon absorption by the protein results in photoactivation of a protein molecule and leads to aggregation. This photoactivation relaxes to the original state within some finite time, τ. The result of competition between relaxation and aggregation of the photoactivated proteins with each other depends on the laser intensity. At smaller intensities, relaxation is significant, and the intensity aggregation kinetics strongly depends on the laser intensity; at higher laser intensities, aggregation prevails over relaxation, and the intensity dependence vanishes such that only integrated exposure is important.

It should be pointed out that the relaxation time should strongly depend on the energy of the absorbing photon. The use of a monochromatic source, an excimer laser with relatively small photon energy belonging to the red tail of the tryptophan absorption band, but providing the nonthermal protein aggregation due to a sufficiently high intensity of laser radiation, allows one to observe the discussed phenomenon. Knowledge about the relaxation time can be important for treatment and prevention of cataract development in human eye. Of course, the role of other crystallins constituting the eye lens should also be taken into account.

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