

Photoaggregation of crystallins (main proteins of eye lens) under the effect of XeCl laser radiation.

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ABSTRACT

UV light is one of primary factors associated with cataract formation in the eye lens. α -, β -, γ -Crystallins maintain lens transparency, and damage to these proteins plays a major role in cataract formation. The effect of XeCl laser radiation (308 nm) on β_L -crystallin solution is studied. The strong dependence of protein aggregation kinetics on both laser fluence (w) and repetition rate (F) is investigated. The kinetics features are similar to those of carbonic anhydrase photoaggregation studied previously [1, 2].

Keywords: α -, β -, γ -crystallin, carbonic anhydrase, aggregation, UV laser, fluence, repetition rate, theoretical model.

1. INTRODUCTION

The effect of UV radiation on eye lens is the principal natural factor of cataract emergence and development. It is based on aggregation of main eye lens proteins such as α -, β - and γ -crystallins. Photoaggregation is accompanied by formation of high-molecular compounds that make lens opaque. Molecular mechanism of UV-induced crystallin aggregation is still a poorly studied problem. We carried out experimental research on kinetics of aggregation of model protein (carbonic anhydrase) [1-2], alpha-crystallin and diverse forms of normal beta-crystallin under the effect of XeCl laser radiation. In the course of irradiation we measured the dependence of scattering intensity of a test beam (633 nm) versus UV dose D and plotted kinetic curves [2]. Simultaneously, an increase in absorption and scattering of radiation at 308 nm was measured. For analysis of the aggregate state of proteins, molecular-weight distributions and optical spectra were measured. It was revealed that the photoaggregation rate strongly depends on laser fluence w and pulse repetition rate F .

A comprehensive study of the effect of XeCl laser radiation on carbonic anhydrase solution was carried out [1,2]. A strong dependence of the probability of high molecular weight aggregate formation on laser fluence and pulse repetition rate was found. Using the high-efficient liquid chromatography method, the formation of dimers, trimers, oligomers up to polymers with apparent $M_m > 2000$ kDa with increasing UV irradiation dose was demonstrated. Changes in absorption of radiation at 308 nm and scattering of radiation at 308 nm and 633 nm depending on dose were studied. A technique for comprehensive study of the effect of XeCl laser radiation on protein solutions was suggested and used.

A theoretical model was developed, which describes photoaggregation processes occurring in a carbonic anhydrase solution under continuous and pulsed UV irradiation. The simplified model which considered only dimer formation not only ensured qualitative coincidence of the theoretical dependence of dose D^* , starting from which scattering of a test beam begins, vs. fluence and repetition rate of UV pulses with experimental curves, but also provided some quantitative estimates [1, 2]. It was found that for each value of w there exists a characteristic value F^* below which aggregation velocity decreases substantially (by several times). An increase in absorption and scattering of radiation at 308 nm was shown to start at doses one order of magnitude less than D^* that is typical of the beginning of scattering of radiation at 633 nm.

In the study we report here, we compared UV photoaggregation of different forms of β -crystallins. The main result consists in determining an analogy in the behavior of kinetic curves of carbonic anhydrase and human eye lens protein (β_L -crystallin).

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2. MATERIALS AND METHODS

A physiological phosphate-buffered β_L -crystallin from a bovine eye lens («Sigma» C5163) solution at pH = 7,2 and different protein concentrations was transmitted through a membrane filter with pore sizes of 0,45 μm (Sartorius). Prior to UV irradiation, a vial with protein was defrosted from -40°C to a room temperature under a water flow and then was centrifuged during 15 minutes at acceleration of $3.5 \cdot 10^3\text{ g}$. A source of UV radiation was a XeCl laser LPX-200 (Lambda Physik) with energy per pulse up to 450 mJ and pulse repetition rate up to 80 Hz. A test beam was from a single-mode (TEM_0) HeNe laser ($\lambda = 633\text{ nm}$) with power of 10 mW and beam divergence of $1.1 \times 10^{-3}\text{ rad}$. The optical scheme differed from those used in Refs. [3, 4] and is illustrated in Fig. 1.

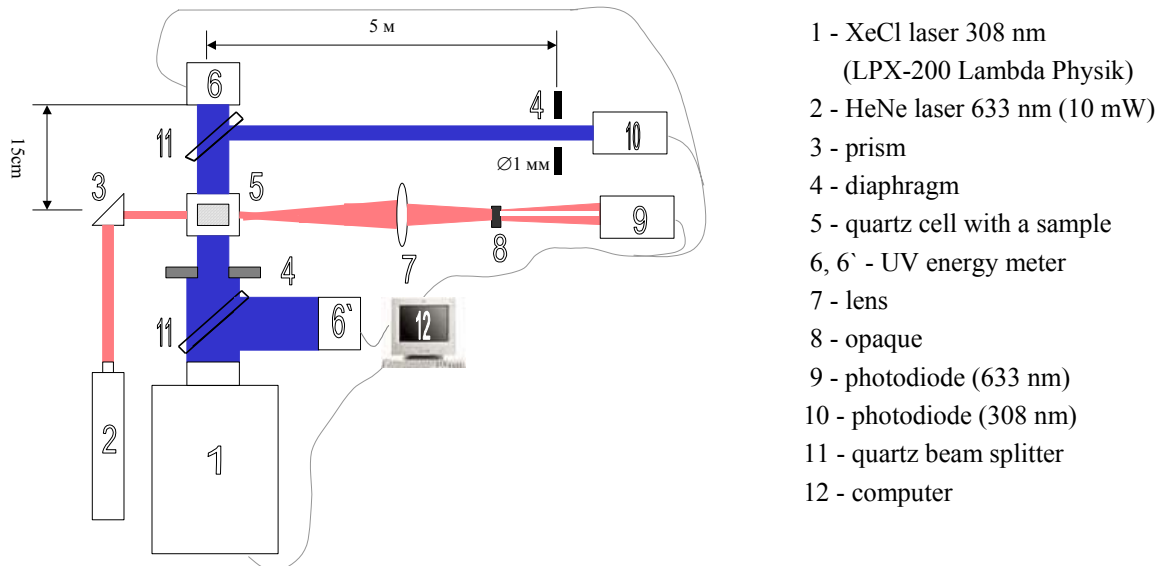


Fig. 1. Experimental scheme for crystallin solution irradiation.

The principal difference of the experimental scheme with β_L -crystallin from a scheme employed earlier for irradiation of carbonic anhydrase [1, 2] consists in the use of an automated measurement system designed for acquiring information from all photodetectors and storing it in the electronic form. The automated system averages all signals over a given number of pulses. At the output of the excimer laser a quartz plate 11 is added to divert part of laser beam for energy measurements. This diverted radiation comes onto an energy meter 6', designed by one of our co-authors using piezoeffect in a LiNbO_3 crystal [7]. Then the signal from 6' is processed by the automated information acquisition system 12. The signal acquired in such a way is used to determine UV laser fluence in the cell with protein and to sum the dose accumulated by a sample. Signals of energy meter 6 and photodiodes 9 – 10 are also processed and stored by the automated system. This makes it easier to conduct the experiment and reduces the time needed for processing of the results. In other aspects, geometry and elements used are the same as in the scheme described in detail in Ref. [2].

3. RESULTS AND DISCUSSION

The absorption coefficient α of β_L -crystallin solution with concentration of 0,5 mg/ml in a cell 5 cm in length in the range of XeCl laser radiation fluences $w = (40-90)\text{ mJ/cm}^2$ was $0,1\text{ cm}^{-1}$. At such α and at maximal value of $w = 90\text{ mJ/cm}^2$ heating of the solution during irradiation did not exceed $\Delta T = 2^\circ\text{C}$. Thus, the samples were irradiated at a room temperature. An air conditioner was used to keep the room temperature constant.

The behavior of kinetics curves obtained at different w and F is presented in Fig. 2 and Fig 3. Both for carbonic anhydrase [2] and for β_L -crystallin solution, for each w there is a characteristic value of F^* , below which D^* considerably increases (by several times), evidencing a sharp decrease in the probability of aggregation. At

$w = 40 \text{ mJ/cm}^2$ $F^* \sim 9 \text{ Hz}$, and at $w = 75 \text{ mJ/cm}^2$ $F^* \sim 4 \text{ Hz}$. But for the β_L -crystallin solution and carbonic anhydrase these values are different.

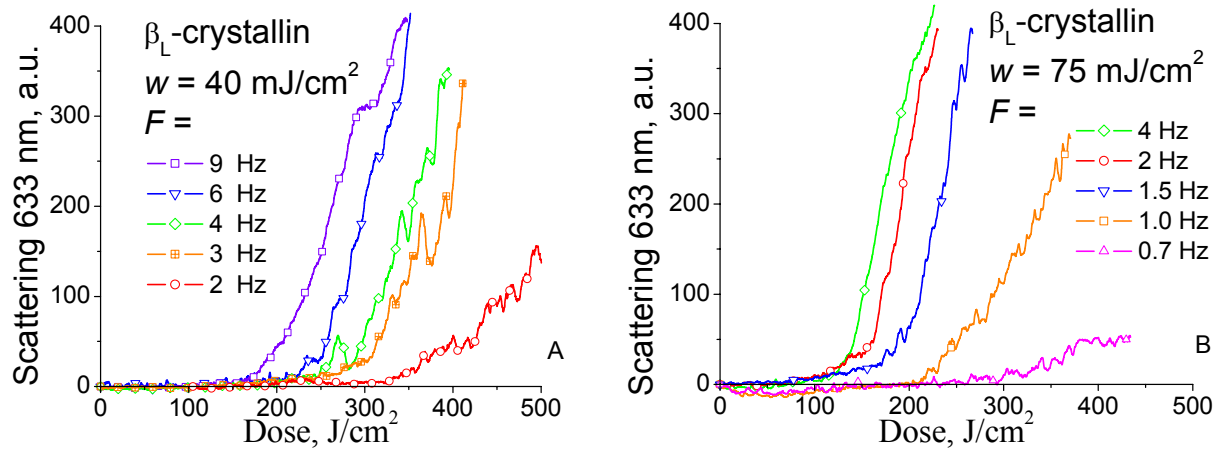


Fig. 2. A, B. The kinetic curves for solution of β_L -crystallin with $n = 0.5 \text{ mg/ml}$ for constant fluence (w) and different repetitions rates (F).

Sets of kinetics curves for constant w and varying F are presented in Fig. 2. It can be seen that when frequency increases, not only dose of the beginning of scattering of a trial He-Ne laser beam decreases, but also the slope of the kinetics curves increases. This indicates that there is a dependence of the probability and speed of the formation of aggregates on the regime of irradiation. There are regimes at which signal scattering either was not observed in the experiment ($w = \text{const}$, $F - \text{small}$), or did not become more efficient ($w = \text{const}$, $F > F^*$) – the dose at which scattering of 633 nm radiation starts and the slope of the kinetics curve are constant. The experimental curves 2 a,b were analyzed to determine the character of the dependences $D^*(F)$, and the result is shown in Fig. 4.

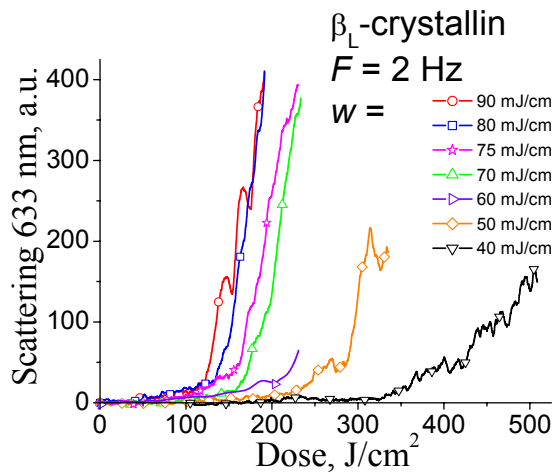


Fig. 3. The kinetic curves for solution of β_L -crystallin for constant repetition rate (F) and different fluences (w).

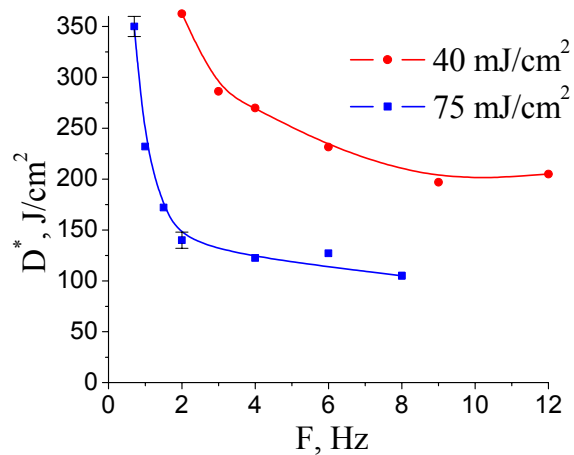


Fig. 4. The dependence of D^* on F .

A set of kinetic curves plotted for a fixed value of $F = 2 \text{ Hz}$ and different fluences w is presented in Fig. 3. Figure 5 shows a descending dependence of the irradiation dose needed for scattering to start on density of pulse energy.

The behavior of kinetic curves in Figs. 2, 3 qualitatively coincides with that of relevant dependences obtained earlier for carbonic anhydrase. The results presented in Figs. 4, 5 qualitatively coincide with the theoretical solution of

the problem of dimer formation that causes an increase of scattering of 633 nm radiation in a carbonic anhydrase solution irradiated by an excimer laser. This allows us to use the earlier developed theoretical model for the description of processes occurring in the β_L -crystallin solution irradiated by pulse laser radiation at a wavelength of 308 nm.

In protein solutions irradiated by different UV doses at fixed values of $w = 75 \text{ mJ/cm}^2$ and $F = 2 \text{ Hz}$, the molecular weight distributions (MWD) (see Fig. 6) and optical spectra (Fig. 7) were measured. When measuring MWD we evaluated the appearance of different aggregated forms of β_L -crystallin during irradiation, but not in the way we did it for the carbonic anhydrase solution [2]. In the chromatogram of the initial sample of carbonic anhydrase, the main fraction of monomer molecules and dimer forms were seen. At $D = 0,1D^*$ the dimer fraction considerably increased and oligomer forms appeared. At $D = D^*$ the dimer and oligomer forms further increased and a fraction of higher molecular weight protein aggregates appeared in the region of exclusion limit of the column (from $M_m = 2000 \text{ kDa}$ and more). A dose of $1,5D^*$ led to the growth of the content of the higher molecular weight protein aggregates, including forms with apparent molecular weight more than 2000 kDa (to obtain proportional chromatography profiles different susceptibility was used in measurements of optical density of solution at a wavelength of 280 nm). Nevertheless, not all the results of the experiment repeat the earlier results for carbonic anhydrase. For β_L -crystallin the behavior of MWD vs irradiation dose is different. At an increasing dose, the fraction with doubled molecular weight (dimers) is not clearly observed, while a peak corresponding to initial material (monomer) broadens toward both higher and lower molecular weights. This MWD behavior may serve as an argument for the hypothesis that under irradiation not only reactions of photoaggregation but also reactions of photolysis take place in a protein solution. These processes lead to the formation of aggregates with a molecular weight that is somewhat more or somewhat less than in the initial material (including fractional), up to very high weights. The latter statement is confirmed by the existence of a peak in the region of the exclusion limit of the column ($M_m = 2000 \text{ kDa}$) in Fig. 6. As for the carbonic anhydrase, increasing the number of components with different weights inevitably leads to a decrease in the amount of initial material.

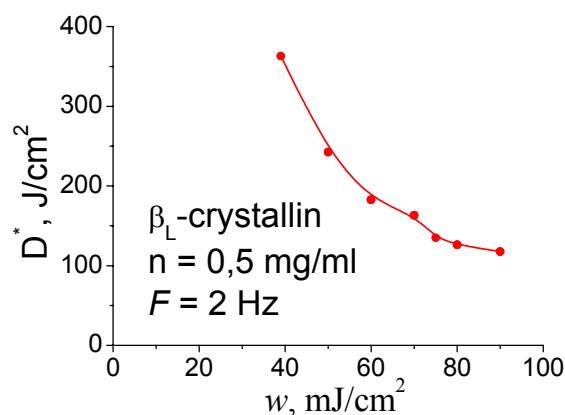


Fig. 5. The dependences of $D^*(w)$.

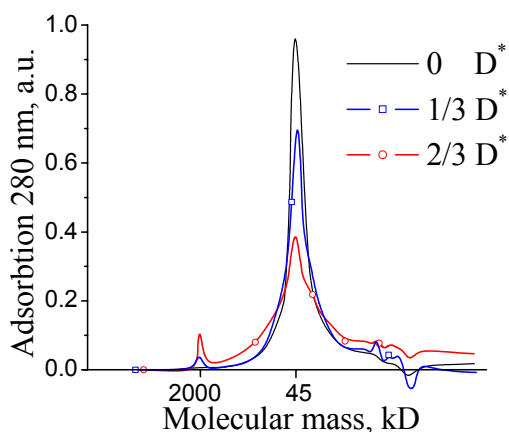


Fig. 6. Molecular weight distributions before (0) and after irradiation at different doses.

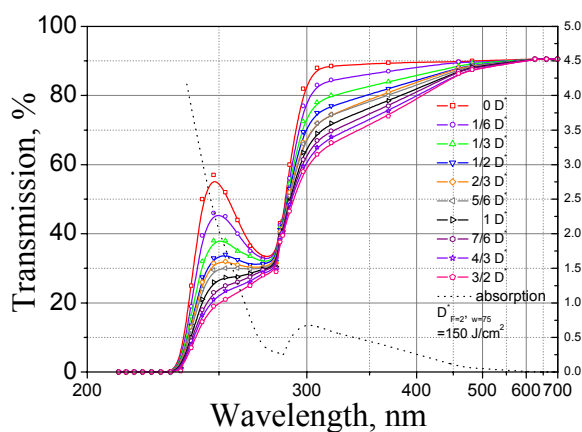


Fig. 7. Optical transmission spectra after irradiation by different doses.

Figure 7 shows changes in the optical transmission spectrum of protein at a growing UV irradiation dose. It can be seen that with the increasing dose the transmission monotonically decreases over the whole spectral range. The same figure demonstrates the spectrum of induced absorption at $D = 1,5D^*$. Its form is analogous to that obtained in Ref. [6]

for a UV-irradiated water-soluble fraction of bovine lens. In Fig. 8 changes in the transmission of r β A3-crystallin solution in the process of UV irradiation at wavelengths of 308 nm are presented. Determining the dependence $\alpha(D)$ for radiation at $\lambda = 308$ nm using a set of spectra (Fig. 7) and by transmission curve like in Fig. 8 yielded similar results [2].

In contrast to the experiments conducted for carbonic anhydrase, for β -crystallin a series of additional experiments was performed to determine the efficiency of aggregation versus solution concentration (Fig. 8). As a result, it was found out that a decrease in concentration by several times may lead to a decrease in photoaggregation speed by several orders of magnitude. This result is in good agreement with the theory developed for carbonic anhydrase and applicable to describe processes occurring at β -crystallin aggregation. According to this theory, two photomodified molecules take part in the formation of aggregate, which explains the nonlinear dependence on initial concentration of solution.

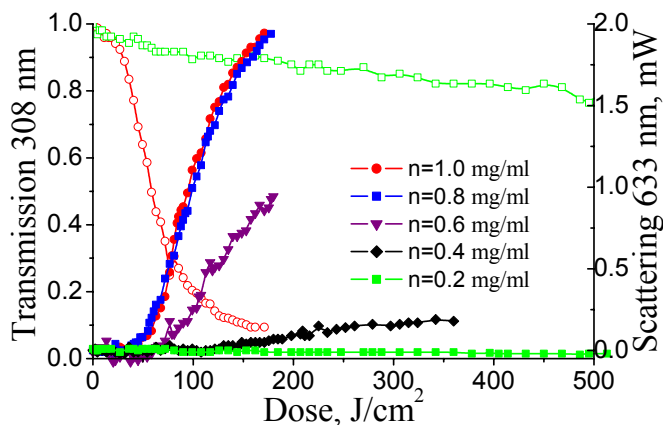


Fig. 8. The dependences of scattering and transmission of solutions with different concentrations r β A3-crystalline vs. dose of UV-irradiation.

Information acquired in this additional study can be used for developing an improved model of photoaggregation of eye lens protein.

4. CONCLUSION

A comprehensive study of the effect of XeCl laser radiation on β -crystallin solution is carried out. A strong dependence of the probability of high molecular weight aggregate formation on laser fluence and pulse repetition rate is found.

A technique for comprehensive study of the effect of XeCl laser radiation on protein solutions has been suggested in [1,2] and is used here. It is shown that the dependence of dose, starting from which scattering of a test beam begins, vs. fluence and repetition rate of UV pulses, $D^*(F,w)$, for β -crystallins qualitatively coincides with the same dependences for carbonic anhydrase (CA).

The main result of this work consists in determining an analogy of kinetic curves behavior of model protein of carbonic anhydrase and human eye lens protein (β -crystallin).

Results presented in Figs. 4,5 qualitatively coincide with the theoretical solution of the problem of dimer formation that causes an increase of 633 nm radiation scattering in a carbonic anhydrase solution irradiated by an excimer laser. This conclusion allows us to use the earlier developed theoretical model for description of processes taking place in a β -crystallin solution under irradiation by pulse laser radiation at 308 nm. This model attributes dimer formation to aggregation of two photoactivated monomers. It explains qualitatively the dependence of $D^*(F,w)$ [1-2].

A sharp nonlinear dependence of the efficiency of photoaggregation on protein solution concentration is found.

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