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Resistance of α-Crystallin Quaternary Structure to UV Irradiation

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Abstract—The damaging effect of UV radiation (λ > 260 nm) on bovine α-crystallin in solution was studied by small-angle X-ray scattering, gel permeation chromatography, electrophoresis, absorption and fluorescence spectroscopy, and differential scanning calorimetry. The results obtained show that damage to even a large number of subunits within an α-crystallin oligomer does not cause significant rearrangement of its quaternary structure, aggregation of oligomers, or the loss of their solubility. Due to the high resistance of its quaternary structure, α-crystallin is able to prevent aggregation of destabilized proteins (especially of γ- and β-crystallins) and so to maintain lens transparency throughout the life of an animal (the chaperone-like function of α-crystallin).

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Key words: α-crystallin, quaternary structure, UV radiation, small-angle X-ray scattering

α-Crystallin, along with β- and γ-crystallins, is one of main structural and functional proteins of the vertebrate eye lens. It is an oligomeric polydisperse protein consisting of noncovalently bound ~20 kDa polypeptide subunits of two types [1].

According to different authors, native α-crystallin isolated from the external part (cortex) of the lens has mean molecular mass ~700-800 kDa [1-4]. The tertiary structure of α-crystallin subunits and their arrangement within α-crystallin oligomers (quaternary structure) remain unknown. There are different structural models of α-crystallin oligomers [1, 3-6]. We believe that the micelle-like model of the quaternary structure of this protein is quite probable [6]. The number of subunits in α-crystallin oligomers and thus the size and molecular mass of these oligomers can change depending on temperature, pH, ionic strength, and some other physicochemical factors [2, 4, 5, 7].

Like small heat shock proteins, α-crystallin is characterized by chaperone-like activity [8] and has amino acid sequences homologous to these proteins [5]. For this reason, α-crystallin is considered a member of this protein family. The chaperone-like activity of α-crystallin is revealed in efficient prevention of aggregation in solution of β- and γ-crystallins and some other proteins when their structure is destabilized by different denaturing factors (heating [7-10], UV-irradiation [11, 12], dithiothreitol treatment [13]). It was shown that under conditions of heat denaturation of proteins, the mechanism of α-crystallin chaperone-like activity involves formation of α-crystallin complexes with destabilized proteins [7, 9, 10]. It is supposed that hydrophobic interactions play an important role in formation of such complexes [14].

Lens turbidity or a cataract can be explained by aggregation of crystallins with destabilized structure. The chaperone-like activity of α-crystallin is considered an important protective mechanism that prevents aggregation of destabilized proteins in the lens and thus contributes to maintenance of lens transparency during the entire life of an animal [1]. This aspect is supported by detection in lens of α-crystallin complexes with β- and γ-crystallins [10, 15].

UV radiation is a factor leading to development of cataract [16]. UV irradiation causes oxidation of SH groups in crystallins [17], changes in native conformation of the latter [18], and formation of covalent cross-links between polypeptides of these proteins [17, 19, 20]. Such destructive transformations of proteins upon UV irradiation result in formation of large protein aggregates and

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protein solution turbidity [11, 12, 20, 21]. Probably similar molecular mechanisms of UV-induced cataract development are active *in vivo*. Apparently in this case UV radiation also affects α-crystallin in the lens. Taking into account that, most likely, α-crystallin in a lens prevents aggregation of destabilized proteins and just owing to this it carries out protective function, the investigation of the effect of UV radiation on α-crystallin structure and properties seems an important task.

There are many works dealing the effect of UV radiation on the structure and properties of α-crystallin [19, 20, 22-31]. UV irradiation of α-crystallin solutions as in the case of other crystallins results in photooxidation of polypeptide residues, accumulation of yellow chromophores, change in native conformation of α-crystallin polypeptides (subunits), and formation of covalent cross-links between subunits [19, 22, 23, 25-28]. The chaperone-like activity of α-crystallin decreases in response to UV irradiation [23, 24], and during aging the resistance of α-crystallin in lenses to UV radiation decreases [29].

Comparison of the effect of UV irradiation (λ = 308 nm) on α-, β-, and γ-crystallin solutions has shown that α-crystallin solutions remain transparent, whereas solutions of β- and γ-crystallins become very turbid [20, 21]. It follows from these results that in the case of α-crystallin formation of large aggregates causing turbidity occurs at much higher (5-20 times) doses of UV irradiation than in the case of β- and γ-crystallins. What happens to the quaternary structure of this protein during UV irradiation before the beginning of formation of such aggregates and what causes such high resistance of α-crystallin to UV radiation? We could not find answers to these questions in the literature. Therefore, in this work we have studied the effect of UV radiation on the quaternary structure of α-crystallin and used small-angle X-ray scattering [32] to study the size and shape of the protein macromolecules in solution.

MATERIALS AND METHODS

α-Crystallin was isolated from the bovine eye lenses of ~1-year-old animals. The peripheral part (cortex) of the lens was homogenized in buffer (20 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, 3 mM NaN_3, 1 mM EDTA, pH 6.8) at the volume ratio 1 : 15, and water-insoluble components were removed by centrifugation (30 min, 30,000*g*). The resulting crystallin solution was fractionated by gel permeation chromatography on a column (2.5 × 90 cm) with Toyopearl HW55 gel (Toyo Soda, Japan) at the solvent flow rate 1.5 ml/min. The eluate optical density was registered at 280 or 206 nm using an Uvicord S 2138 UV detector (LKB, Sweden) joined to a computer by means of an analog-to-digital converter (ADC) E-24 (L-card, Russia). The column was calibrated using a set of standard proteins (Pharmacia AB, Sweden). Collected α-crystallin fractions were concentrated by ultrafiltration on Biomax 30 membranes with exclusion limit of 30 kDa (Millipore, France) and stored at 5°C. α-Crystallin concentration in solutions was determined on a UV-1700 spectrophotometer (Shimadzu, Japan) by absorption at 280 nm using specific absorption coefficient A_{280} (1%, 1 cm) = 8.45 [4].

α-Crystallin solutions were UV irradiated at 37°C by filtered radiation of a DRSh-1000 mercury lamp (λ > 260 nm, ~9 W/m^2) as described in [12]. In different experiments α-crystallin concentration in solution upon UV irradiation varied from 1 to 8 mg/ml, irradiation time from 1 to 7 h. The effect of UV radiation on the state of the α-crystallin was followed as described in [12] by changes in I_{405} intensity of measuring light beam from a photodiode (λ = 405 nm), which passed through a cell with the α-crystallin solution at right angle to the beam from the DRSh-1000 lamp. The signal from the detector measuring I_{405} was passed via a logarithmic amplifier to the ADC connected to the personal computer to register the dependence of the extent of the measuring beam extinction $Q_{405} = -k\cdot\log(I_{405})$ on the time of UV irradiation (k is coefficient of proportionality). It should be noted that Q_{405} value differs from absorbance $A = -\log(I_{405}/I^0_{405})$ only with a constant item and a constant multiplier (I^0_{405} is intensity of the measuring light beam falling on the cell with α-crystallin solution).

In addition to UV-irradiated α-crystallin solutions, those of α-crystallin incubated at 37°C without UV irradiation were studied along with control α-crystallin solutions that did not undergo UV irradiation and incubation at 37°C.

α-Crystallin solutions after UV irradiation were analyzed by gel permeation chromatography as described above. The polypeptide composition of α-crystallin was analyzed by electrophoresis in 12.5% polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate as described in [33]. Molecular mass of polypeptides was determined using a set of standard proteins (Sigma-Aldrich, USA). Gels were scanned on an Epson Perfection 4180 photoscanner (Epson, Japan). Profiles of protein distribution along gel lanes were obtained using the Image J 1.34s program (NIH, USA) for scanned images.

Absorption spectra of α-crystallin solutions in the UV and visible regions were measured on a Shimadzu UV-1700 spectrophotometer. Intrinsic fluorescence emission spectra of α-crystallin were measured on a Shimadzu RF-5301PC spectrofluorimeter with excitation at 280 nm.

α-Crystallin solutions were subjected to microcalorimetric analysis using a DASM-4 high-sensitivity differential scanning microcalorimeter (Institute of Biological Instrument Engineering, Russia) at the scanning rate 1°C/min and constant pressure 2.2 atm. The concentration of α-crystallin in solutions was 1.5 mg/ml.

α-Crystallin solutions were investigated by small-angle X-ray scattering as described in [7] using an automatically controlled small-angle diffractometer with a linear coordinate detector [34] (CuK_α X-rays, λ = 0.154 nm).

Curves of small-angle X-ray scattering were processed using the Primus [35] and Gnom [36] programs, which made it possible to obtain for α-crystallin intensity of scattering at zero angle $I(0)$, radius of gyration R_g and distance distribution function $P(r)$. Collimation correction as described in [37] was done before the Primus program was used.

The $I(0)$ and R_g values are approximation parameters of the initial part of the intensity curve according to Guinier formula:

$$I(S) = I(0)\exp(-(SR_g)^2/3), \quad (1)$$

where $S = 4\pi\sin\theta/\lambda$, λ is X-ray wavelength, and 2θ is scattering angle.

The distance distribution function $P(r)$ is connected with scattering intensity $I(S)$ by spherical Fourier transformation [32]:

$$P(r) = \frac{r^2}{2\pi^2}\int_0^{S\max} I(S)\frac{\sin(Sr)}{Sr}S^2 dS \quad (2)$$

and is a convenient presentation of structural information contained in the curves of small-angle X-ray scattering. The view of the $P(r)$ function provides information about the shape of protein macromolecules. The r value, at which $P(r)$ goes to zero, can be used to estimate maximal size of these macromolecules.

The molecular mass M of α-crystallin was determined using ratio:

$$M = \frac{I(0)}{Kc}, \quad (3)$$

where $I(0)$ is the intensity of scattering at zero angle, c is the mass protein concentration in solution, and K is a coefficient of proportionality which depends on the particular experimental conditions of intensity measurements. The coefficient K was determined on the basis of ratio (3) by intensity of small-angle X-ray scattering at zero angle $I(0)$ by a solution of bovine serum albumin (Sigma-Aldrich) purified from contaminants of oligomers of this protein by gel permeation chromatography. Albumin concentration in solution was measured with a spectrophotometer using specific absorption coefficient $A_{280}(1\%, 1\text{ cm}) = 6.66$ [38].

It should be noted that R_g, M values, and distance distribution function $P(r)$ determined by small-angle X-ray scattering are the statistical mean for α-crystallin in all oligomers of this polydisperse protein.

RESULTS

UV irradiation of α-crystallin solutions resulted in slight monotonous lowering of light transmission (λ = 405 nm) by these solutions (Fig. 1). After UV irradiation α-crystallin solutions acquired a yellow shade in transmitted light but remained transparent. Precipitation of α-crystallin was not observed during UV irradiation and subsequent continuous (over two months) storage of irradiated solutions at 5°C.

Figure 2 shows absorption spectra of α-crystallin solution in its initial state and 2, 4, and 7 h (curves *1-4*) after UV irradiation. As seen in Fig. 2, the absorption spectra are significantly changed after UV irradiation. The absorbance increases in the UV and visible spectral

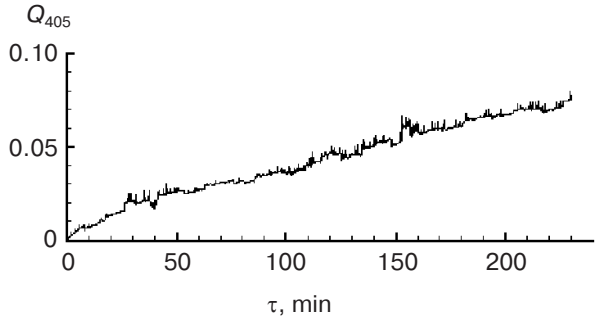

Fig. 1. Dependence of the extent of measuring light beam extinction Q_{405} by α-crystallin solution (8 mg/ml) on the time of UV irradiation τ at 37°C.

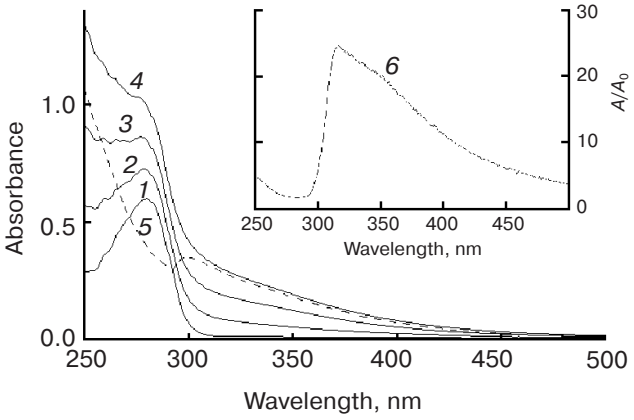

Fig. 2. Absorption spectra of initial α-crystallin solution (*1*) and that after UV irradiation at 37°C for 2, 4, and 7 h (*2-4*, respectively). Dotted line (*5*) shows the differential absorption spectrum of α-crystallin after UV irradiation for 7 h (the difference between spectra *4* and *1*). The insert (curve *6*) shows the ratio of absorbance A of α-crystallin solution irradiated by UV light for 7 h (spectrum *4*) to absorbance A_0 of unirradiated α-crystallin solution (spectrum *1*). The concentration of α-crystallin during UV irradiation was 8 mg/ml. For measuring absorption spectra, α-crystallin solutions were tenfold diluted by buffer.

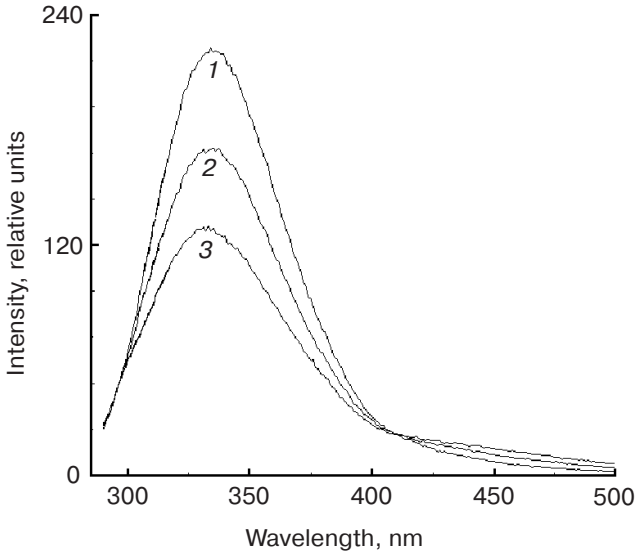

Fig. 3. Fluorescence spectra of initial α-crystallin solution (*1*) and after UV irradiation at 37°C for 2 and 4 h (*2* and *3*, respectively). α-Crystallin concentration during UV irradiation was 8 mg/ml.

regions and the longer the irradiation time the more pronounced is this increase. These spectral changes are indicative of significant damage to α-crystallin under our conditions of UV irradiation. Similar changes in absorption spectra after UV irradiation of mixtures of all crystallins of the bovine eye lens and α-crystallin alone were reported elsewhere [17, 22, 27, 30].

Differential absorption spectra obtained by subtraction of the spectrum of the initial unirradiated α-crystallin from that of UV-irradiated α-crystallin are not marked; there is only a single small maximum at ~300 nm. One such differential spectrum obtained after α-crystallin irradiation for 7 h is shown by the dotted line in Fig. 2 (curve *5*).

The increase in absorbance of α-crystallin solution upon UV irradiation is most pronounced in the near UV region ($\lambda > 315$ nm). It is especially clear on curves showing ratio of absorbance of irradiated α-crystallin solutions to absorbance of initial unirradiated solution. One such curve for α-crystallin irradiated for 7 h is shown in the inset in Fig. 2. Relative increase in absorbance after UV irradiation grows with decreasing wavelength to 315 nm and reaches 25-fold level at $\lambda = 315$ nm after irradiation for 7 h (inset in Fig. 2). Such relative absorption increase upon decreasing wavelength in visible region can explain the emergence of yellow shade in α-crystallin solutions after UV irradiation.

UV irradiation is known to result in photooxidation of tryptophan residues in proteins [22, 25]. Among products of tryptophan oxidation are N-formyl-kinurenine, kinurenine, and 3-hydroxykinurenine. They are characterized by strong absorption near 260 nm and broad less intensive absorption peaks at 300-400 nm: N-formyl-kinurenine has a peak centered near 320 nm, while kinurenine and 3-hydroxykinurenine have peaks centered near 360 nm [27, 39]. Spectral changes in α-crystallin solutions caused by UV irradiation (Fig. 2) can be explained by accumulation of such products of photooxidation of tryptophan residues. It can also be supposed that these changes, at least in part, are caused by increased light scattering due to aggregation of α-crystallin oligomers. However, results of small-angle X-ray scattering and chromatography do not support this supposition.

Fluorescence spectroscopy and electrophoresis are also indicative of significant changes in α-crystallin state after UV irradiation under our experimental conditions (Figs. 3 and 4).

As seen in Fig. 3, the intensity of α-crystallin intrinsic fluorescence in response to UV irradiation strongly decreases (approximately by half after irradiation for 4 h), which is probably caused by destruction of aromatic amino acid residues in α-crystallin. The decrease in α-crystallin fluorescence intensity in response to UV irradi-

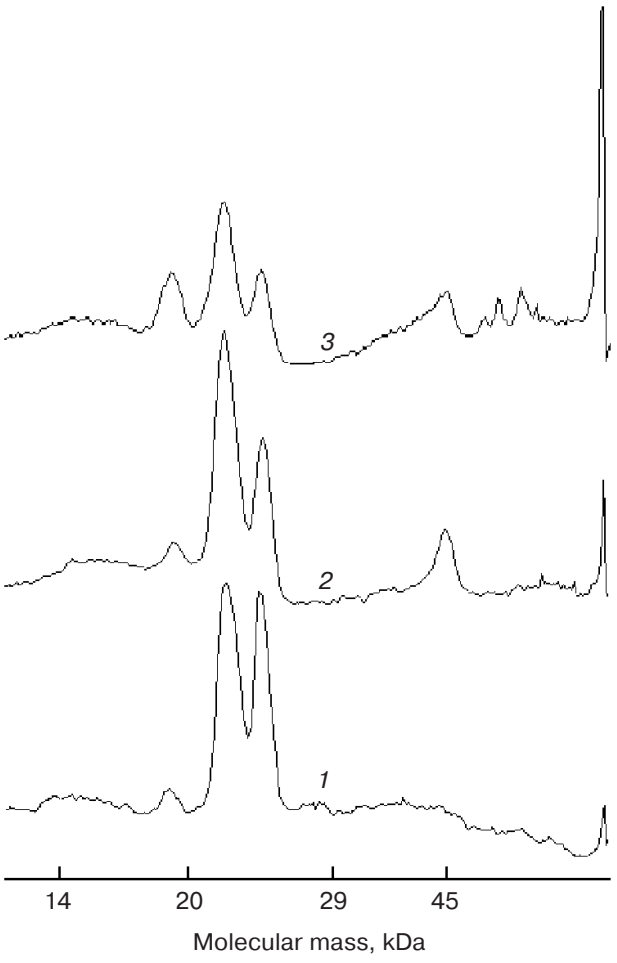

Fig. 4. Results of gel electrophoresis of initial α-crystallin (*1*) and after UV irradiation at 37°C for 2 and 4 h (*2* and *3*, respectively). α-Crystallin concentration during UV irradiation was 8 mg/ml. The molecular mass scale was obtained using a set of standard proteins.

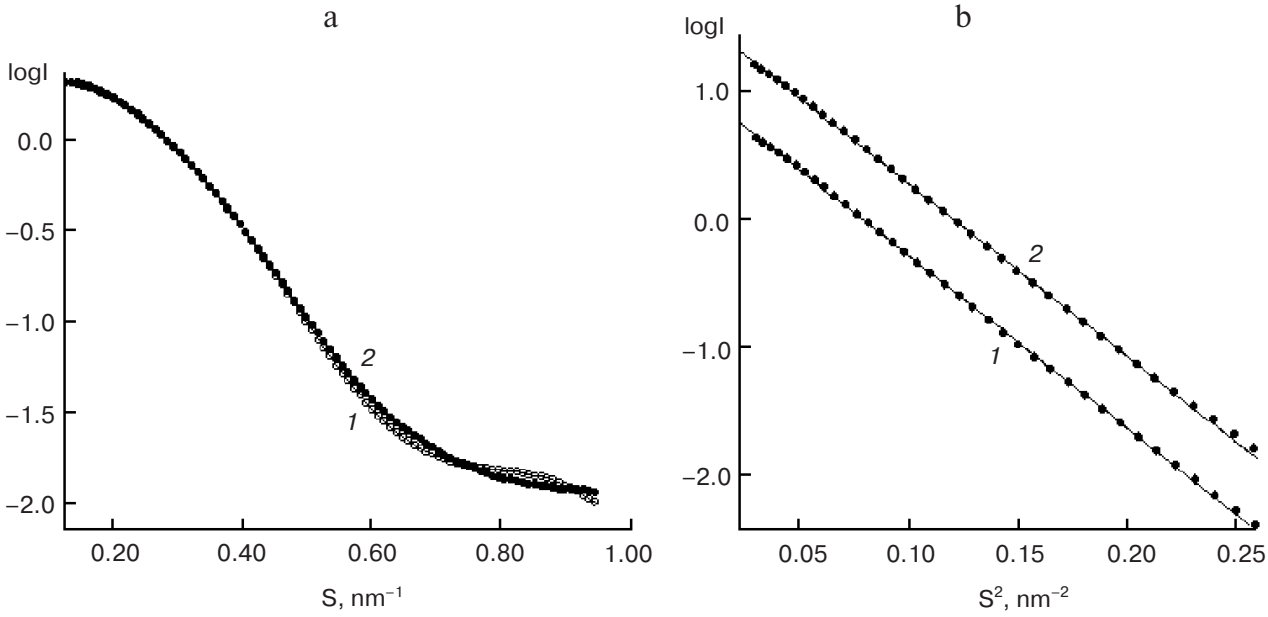

Fig. 5. Curves of α-crystallin small-angle X-ray scattering in initial state and after UV irradiation at 37°C for 4 h (a, curves *1* and *2*) and the same curves in Guinier coordinates, shifted relative each other in vertical direction (b). α-Crystallin concentration during UV irradiation was 8 mg/ml. The data were smoothed and collimation corrected.

ation (Fig. 3) corresponds to data in the literature [22, 25, 30, 31]. A slight shift of fluorescence maximum towards the blue region after UV irradiation (by approximately 2-3 nm after UV irradiation for 4 h) can be caused by somewhat increased extent of hydrophobicity of the chromophore microenvironment in α-crystallin.

Results of gel electrophoresis (Fig. 4) show that UV irradiation results in formation of numerous covalently cross-linked aggregates of α-crystallin polypeptides, dimers (M = 45 kDa) and multimers. The molecular mass of some such aggregates can reach 200 kDa or more because some of them do not penetrate the gel (the last narrow peak to the right in Fig. 4). Besides, UV irradiation results in increase in relative content of polypeptides with molecular mass below 20 kDa (Fig. 4), probably formed after photolysis of α-crystallin subunits. Estimation of the content of aggregates of covalently bound α-crystallin polypeptides by protein band areas on gel electrophoresis profiles (Fig. 4) has shown that UV irradiation for 4 h caused covalent cross-links in ~50% of total α-crystallin polypeptides, ~10% of them underwent photolysis without cross-links (M = 18 kDa), and ~40% remained in the initial state.

To study the main problem of our work concerning the effect of UV irradiation on quaternary structure of α-crystallin and revealing initial stages of its possible aggregation, we used small-angle X-ray scattering.

The curve of small-angle X-ray scattering by initial (control) α-crystallin solution, that did not undergo UV irradiation and incubation at 37°C, is shown in Fig. 5a (curve *1*). The initial part of this curve is approximated by Guinier formula (1) and has in Guinier coordinates the form of a straight line (Fig. 5b, curve *1*). The mean radius of gyration (R_g) of α-crystallin, obtained using the Primus program based on nine measurements of α-crystallin solutions from different isolations, was 6.33 ± 0.05 nm (0.05 nm is the standard deviation). The distance distribution function $P(r)$, calculated using the Gnom program (Fig. 6, curve *1*), has shape characteristic of globular particles [32]. The maximal size of α-crystallin oligomers

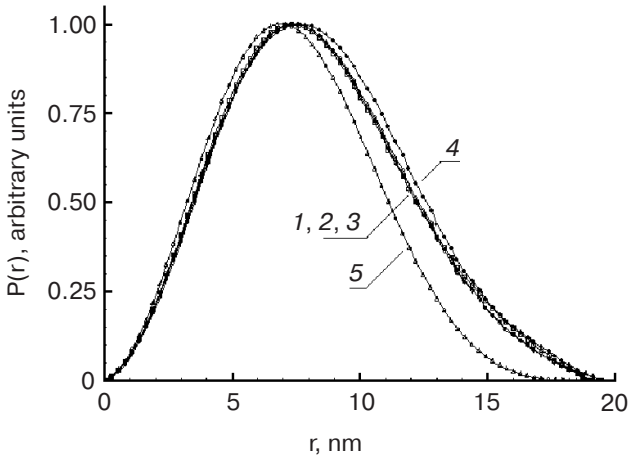

Fig. 6. Distance distribution functions for initial α-crystallin (curve *1*), after UV irradiation for 2, 4, and 7 h at 37°C (curves *2-4*, respectively), and after incubation for 8 h at 37°C without UV irradiation (curve *5*), calculated from curves of small-angle X-ray scattering using the Gnom program. α-Crystallin concentration during UV irradiation and incubation at 37°C was 8 mg/ml.

determined from the condition that $P(r) = 0$ was $L_{max} \approx$ 19 nm. Molecular mass value $M = 780$ kDa was obtained for α-crystallin (mean of two measurements in which values 755 and 810 kDa were obtained). The values for R_g, M, L_{max}, and $P(r)$ function are in agreement with the results of previous small-angle X-ray investigations of α-crystallin [3, 4, 7, 12].

The long linear part on the Guinier plot should be noted (Fig. 5b). The $S \cdot R_g$ product from 0.6 to 3 corresponds to this part. Such character of scattering intensity shows that α-crystallin oligomers probably have an extended shape with axial ratio of about 1.8. Our model calculations have shown that the extended Guinier region up to $S \cdot R_g \approx 3$ should be also observed for a polydisperse system of such asymmetrical particles with the size distribution characteristic of α-crystallin.

UV irradiation of α-crystallin solutions did not result in significant changes in the small-angle X-ray scattering curves. An example is the curve of small-angle X-ray scattering for α-crystallin solution irradiated for 4 h (Fig. 5a, curve *2*). The initial parts of the small-angle scattering curves for UV-irradiated α-crystallin solutions as well as for control solutions are approximated well using the Guinier formula (1) (Fig. 5b, curve *2*). Values of radius of gyration R_g and molecular mass M for α-crystallin after UV irradiation are given in the table.

It is seen in the table that R_g and M values for α-crystallin solutions irradiated by UV radiation at concentration 8 mg/ml for 2 and 4 h practically did not change compared to control samples. The distance distribution function $P(r)$ and maximal size L_{max} for these UV-irradiated α-crystallin samples also did not change within the limits of experiment accuracy (Fig. 6, curves *2* and *3*).

Very slight increase in R_g and M values for α-crystallin solution that had been irradiated for 7 h at the concentration 8 mg/ml, more pronounced after sample storage (see table), was observed along with a small shift to the right of the distance distribution function (Fig. 6, curve *4*). Analysis of the shape of the small-angle scattering curves showed that these small changes in R_g, M, and $P(r)$ are probably caused by appearance in solution of a small amount of aggregated α-crystallin oligomers (no more than ~3% of the total protein mass).

Somewhat lower R_g and M values were obtained for UV-irradiated α-crystallin solution at lower concentration (3 mg/ml), which may be indicative of very slight reduction in the mean size of α-crystallin oligomers in this experiment (see table).

Thus, as follows from the results of small-angle X-ray scattering, no major changes are observed under our conditions of UV irradiation in quaternary structure of α-crystallin oligomers or their aggregation, although the

Effect of UV irradiation at 37°C and incubation at 37°C without UV irradiation on radius of gyration R_g and molecular mass M of α-crystallin (data of small-angle X-ray scattering)

Number of experiment	Conditions of incubation of α-crystallin at 37°C			R_g, nm	M, kDa
	UV irradiation	Incubation time, h	α-Crystallin concentration during incubation, mg/ml		
	without incubation (control)	–	–	6.33 ± 0.05*	780
1	UV	2	8	6.34 6.24**	780 –
2	UV	4	8	6.37	790
3	UV	4	8	6.32	780
4	UV	7	8	6.46 6.53***	790 820
5	UV	2	3	6.17	720
6	without UV	2	3	6.14	680
7	without UV	4	8	6.15	710
8	without UV	8	8	5.94	640****

* Mean value and standard deviation for nine control samples.
** After storage for 80 days at 5°C.
*** After storage for 17 days at 5°C.
**** Calculated from radii of gyration with assumption that M is proportional to R_g^3.

data of spectroscopy and electrophoresis show (Figs. 2-4) that significant damage to the native structure of α-crystallin subunits occurs.

It should be stressed that according to the results of small-angle X-ray scattering, UV irradiation does not cause the appearance in solution of a substantial number of protein particles differing by size from native α-crystallin oligomers. The small-angle scattering also does not identify in solution covalently bound aggregates of α-crystallin subunits whose molecular mass can reach (according to electrophoresis) 200 kDa and more (Fig. 4). This means that all covalently bound aggregates of α-crystallin subunits revealed by electrophoresis (Fig. 4) are located inside α-crystallin oligomers.

Size invariability of α-crystallin oligomers upon UV irradiation under our experimental conditions was confirmed by gel permeation chromatography. Chromatograms of solutions of initial α-crystallin and that irradiated at 37°C for 1 and 2 h at the concentration 1 mg/ml are shown in Fig. 7. All chromatograms (Fig. 7) contain a single peak corresponding to the release from the column of a protein with mean molecular mass 700-800 kDa. This peak has practically identical position and width for both initial and UV-irradiated α-crystallin. No appearance of additional peaks was observed after UV irradiation. The inset (Fig. 7) shows the results of electrophoresis for the same α-crystallin solutions that were studied by gel permeation chromatography. The results of electrophoresis and chromatography in Fig. 7 show that UV irradiation is accompanied by appearance of a significant number of covalently bound aggregates of α-crystallin subunits with molecular mass over 40 kDa, and all these aggregates are inside α-crystallin oligomers.

Thus, although electrophoresis clearly shows that covalent cross-links between α-crystallin subunits have appeared after UV irradiation, neither small-angle X-ray scattering nor gel permeation chromatography reveal structural changes in α-crystallin oligomers, aggregation of the latter, or appearance in solution of protein structures differing in size from native α-crystallin oligomers.

In our work, UV irradiation was carried out at 37°C. It is known that the quaternary structure of α-crystallin is temperature-dependent. At temperatures near 37-40°C a gradual rearrangement of α-crystallin quaternary structure occurs which lowers the number of subunits within oligomers and hence the oligomers acquire smaller size and lower molecular mass [2]. On increasing the temperature above ~60°C rapid rearrangement of α-crystallin quaternary structure takes place, which results in irreversible doubling of the number of subunits in each oligomer and therefore its molecular mass (for example, see [7]).

To check the effect of elevated temperature on α-crystallin oligomeric form in our experiments on UV irradiation, we incubated α-crystallin at 37°C without UV irradiation. The results of small-angle X-ray scattering

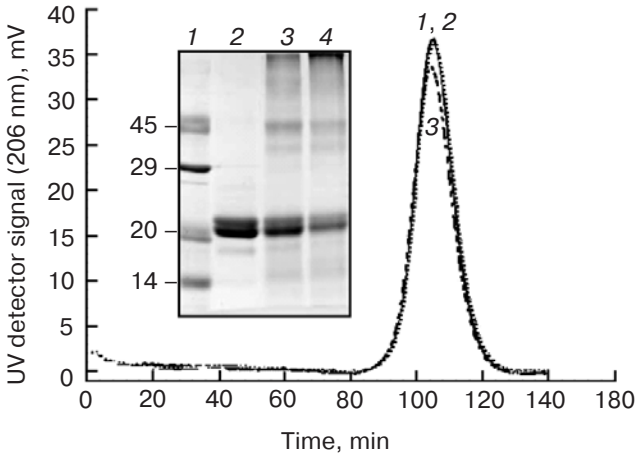

Fig. 7. Chromatograms of control and UV-irradiated at 37°C α-crystallin: continuous line (*1*) shows unirradiated α-crystallin, dotted line (*2*) corresponds to α-crystallin irradiated for 60 min, broken line (*3*) shows α-crystallin irradiated for 120 min. The inset shows the results of electrophoresis: *1*) a set of standard proteins; *2*) control; *3*) UV irradiation, 60 min; *4*) UV irradiation, 120 min. α-Crystallin concentration during UV irradiation was 1 mg/ml.

showed (see table) that such incubation at 37°C actually results in a slight reduction in mean radius of gyration and mean molecular mass of α-crystallin. The change of the distance distribution function points to the reduction of α-crystallin oligomer maximal size to ~17 nm after 8 h long incubation at 37°C (Fig. 6, curve *5*).

Thus, our results (table) show that the decrease in α-crystallin oligomer size, happening at 37°C without UV irradiation, is not observed after UV irradiation at 37°C. This is probably due to cross-linking of α-crystallin subunits by UV radiation. Such cross-linking can lower the mobility of α-crystallin subunits and thus hinder rearrangement of α-crystallin oligomers at 37°C.

The question how structural changes in α-crystallin and formation of inter-subunit cross-links in its oligomers upon UV irradiation influence structural transition of this protein at a higher temperature, namely at ~60°C, seems interesting.

To examine this question, we comparatively analyzed UV-irradiated and control α-crystallin solutions by differential scanning calorimetry. Heat absorption curves with the peak at temperatures near 64 and 62°C were obtained for the initial α-crystallin and that after 4 h long irradiation, respectively (Fig. 8). This peak, like those in other works on differential scanning calorimetry (for example, [40]), is indicative of structural transformation of α-crystallin upon heating. As seen in Fig. 8, UV irradiation results in approximately 30% decrease in the amount of heat absorbed during the transformation, and the transformation temperature decreases by approximately 2°C. This probably shows that UV irradiation reduces temperature resistance of α-crystallin and/or a

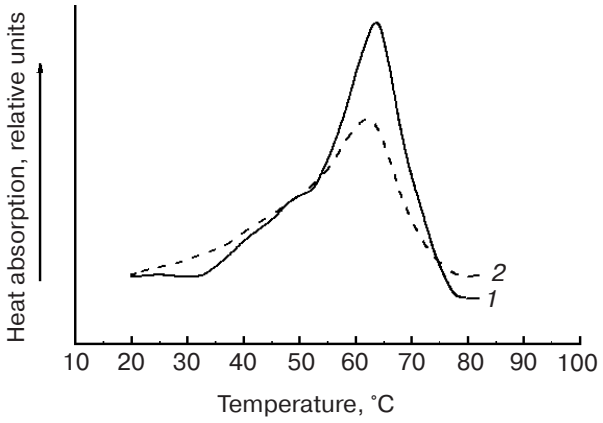

Fig. 8. Results of differential scanning calorimetry of initial α-crystallin solution (*1*) and after UV irradiation at 37°C for 4 h (*2*). α-Crystallin concentration during UV irradiation was 8 mg/ml.

part of α-crystallin resulting in structural transformation at ~60°C.

DISCUSSION

Investigation of the effect of UV radiation on structure of eye lens proteins (crystallins) is important for understanding molecular mechanisms of cataract formation.

No doubt, as follows from our data and much in the literature, UV irradiation results in structural changes in polypeptide subunits of α-crystallin. These changes are revealed in destruction of a significant fraction of tryptophan residues and/or in conformational rearrangements near these residues, in accumulation of products of photooxidation of α-crystallin polypeptide residues, and in formation of aggregates of covalently bound polypeptide subunits of this protein.

However, no effect of UV irradiation on α-crystallin quaternary structure was found in this work by small-angle X-ray scattering (see table and Figs. 5 and 6). This conclusion was supported by gel permeation chromatography (Fig. 7). Small-angle X-ray scattering and gel permeation chromatography in this case are mutually complementary. Small-angle X-ray scattering can determine a number of α-crystallin structural parameters (radius of gyration, maximal size, molecular mass) and reveals possible changes in external geometric shape of α-crystallin oligomers and the character of subunit arrangement in these oligomers. On the other hand, gel permeation chromatography reveals very large α-crystallin aggregates in solution whose dimensions are beyond the resolving power of small-angle X-ray scattering.

Our results show that formation in response to UV radiation of covalent cross-links between α-crystallin subunits happens first within the oligomers of this protein. Probably cross-links between α-crystallin oligomers can emerge only under very high doses of UV irradiation, when the majority of α-crystallin subunits are already damaged. This should result in aggregation of α-crystallin oligomers and solution turbidity.

Most likely, just such aggregation of α-crystallin oligomers caused the sharp increase in absorbance of α-crystallin solution upon UV irradiation by an excimer laser (λ = 308 nm) which began at energy exposure ~ 1600 J/cm^2 [21]. At exposure below ~1600 J/cm^2 a latent period without increase in absorbance of the α-crystallin solution was observed [21].

However, in this work we have observed monotonous increase in absorbance of α-crystallin solution during the whole period of UV irradiation (Fig. 1). This can be explained by the fact that at λ = 405 nm, as used in this work, the sensitivity of recording is much higher than at λ = 670 nm as was used in [21]. Actually, sensitivity of recording changes in the α-crystallin solution absorbance upon UV irradiation decreases as wavelength at which the recording is carried out increases (Figs. 2 and 9). As seen in Fig. 9, when the recording wavelength was increased to 600 nm (and moreover to 670 nm) we did not succeed in recording change in absorbance of α-crystallin solution upon UV irradiation.

The absence in this work of sharp increase in absorbance of α-crystallin solution upon UV irradiation, which was observed in [21], is most likely explained by lower doses of UV irradiation compared to those in [21]. Slight increase in mean values of radius of gyration, molecular mass, and a shift of the distance distribution function obtained in this work after α-crystallin UV irradiation for 7 h (see table and Fig. 6) suggest that under

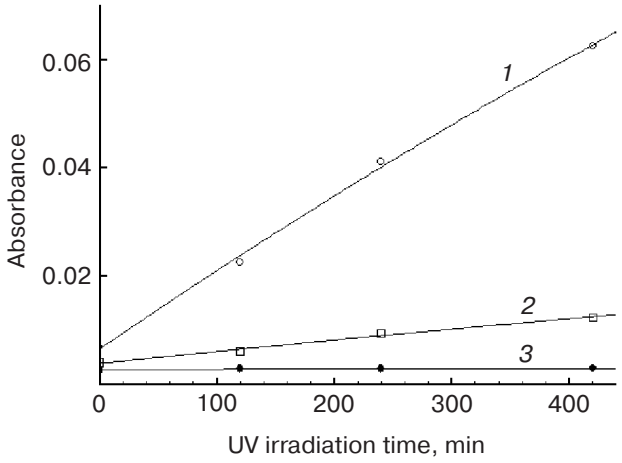

Fig. 9. Absorbance of α-crystallin solution before and after UV irradiation at 37°C for 2, 4, and 7 h measured on a spectrophotometer: *1*) absorbance for λ = 405 nm; *2*) for λ = 500 nm; *3*) for λ = 600 nm. α-Crystallin concentration during UV irradiation was 8 mg/ml. For measuring absorbance the α-crystallin solutions were tenfold diluted by buffer.

our experimental conditions we have approximated the beginning of α-crystallin oligomer aggregation observed in [21] at energy exposure above ~1600 J/cm^2.

It was reported in [29] that by gel permeation chromatography it was registered the increase in size of α-crystallin oligomers after UV irradiation (λ = 365 nm) of bovine eye lenses (from 2-4-year-old animals) in organ culture. Results of our work are indicative of equal size of α-crystallin oligomers before and after UV irradiation. Differences in results can be caused by different experimental conditions. First, irradiation at 365 nm used in [29] damages proteins by means of a succession of photochemical reactions, whereas irradiation in the range of 260-315 nm, used in this our work, directly interacts with aromatic amino acid residues of α-crystallin. Second, the experiment in [29] was carried out *in situ*, while in this work it was done *in vitro*. Perhaps in [29] α-crystallin interacted with γ- and β-crystallins or other macromolecular lens components.

Results of works [20, 21] show that α-crystallin is characterized by a significantly higher resistance to aggregation caused by UV irradiation compared to β- and γ-crystallins. The same conclusion follows from comparison of the effect of UV radiation on solutions of α- and β_L-crystallins under our experimental conditions. Thus, UV irradiation of β_L-crystallin solution under the same conditions resulted in strong solution turbidity caused by β_L-crystallin aggregation with subsequent precipitation of ~30% of this protein [12].

Most likely, high resistance of α-crystallin quaternary structure to UV radiation (and possibly to other factors that destabilize protein structure) is due to the dynamic oligomeric structure of this protein. Being in close contact within the oligomer, in the case of structural damage α-crystallin subunits first of all bind other subunits within the same oligomer. In this case, damage even of numerous subunits within an α-crystallin oligomer does not result in major rearrangement of its quaternary structure, aggregation of oligomers, and the loss of their solubility. This property of α-crystallin should be of exclusive physiological significance for preservation of its chaperone-like function *in vivo*. Probably just owing to this, α-crystallin is able to prevent aggregation of γ- and β-crystallins and maintain lens transparency throughout the life of the animal. It can be supposed that α-crystallin, as a chaperone-like protein, protects itself against aggregation in the lens.

Probably aggregation of α-crystallin oligomers is possible only at very high doses of UV irradiation when a major fraction of protein subunits is damaged. *In vitro* this results in intensive turbidity of α-crystallin solution as in [21] and α-crystallin precipitation. It can be expected that in this case chaperone-like activity of α-crystallin should sharply decrease. However, such high doses of UV irradiation for α-crystallin in the lens seem highly improbable and evidently without physiological significance.

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