Age-Related Absorption of the Human Lens in the Near-Ultraviolet Range

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ABSTRACT

The purpose of the present study was to determine the age dependence of the ultraviolet (UV) absorption of the different parts of the human crystalline lens. Cryostat sections of human cadaveric lenses (60 μm) were cut. The UV absorbance of nine samples, derived from different parts of the lens, was determined using a Shimadzu scanning spectrophotometer. The absorbance of the anterior and posterior lens capsules was measured separately. The absorption coefficients were calculated from the measured absorbance and values taken at 280 as well as at 360 nm were compared statistically. ANCOVA analysis of the values taken at 280 and at 360 nm wavelengths shows that correlation between the absorption coefficients and age can be found only in the case of the posterior layers. These results suggest a differential age-dependent increase of the UV absorption of the posterior layers compared to the anterior ones and can be related to the differential protein expression in the anterior and posterior parts. Posterior crystalline lens capsules have higher absorption coefficients than the anterior ones regardless of age.

INTRODUCTION

The human lens has an effective capacity to absorb UV radiation (1–3) and along with the other parts of the eye, plays an important role in preventing the retina from ocular light damage. Several studies have provided evidence that higher UV exposure, as well as the age, increase the prevalence of certain eye diseases (4–7), especially that of different types of cataract (8–13). The age dependence of light absorption by the human crystalline lens has been the center of interest for a long time. Weale (1) found a systematic age-dependent increase in the absorbance both in the visible light and near-UV range. In accordance with this, transmission spectra of human lenses (3,14) of different ages show that the older lenses transmit less amount of light of wavelengths longer than 300 nm. Since the lens grows continuously, higher absorption can be caused by the longer light path, but physiological yellowing of the older lens can also contribute to the increased absorbance (15).

It is known that the various parts of the lens absorb UV light differentially, that is, absorbance increases in anteroposterior direction (2,16,17). On the other hand, studies on age-related changes do not provide an explanation how this absorption gradient varies with age. Moreover, very little is known about the absorption capacity of the lens capsules and their age-related changes (18).

Therefore, the aim of the present study was to determine the age dependence of the UV absorption of the different parts of the human crystalline lens.

MATERIALS AND METHODS

Cryostat sectioning. A total of 38 human lenses, from the age of 7 to 81 years, were used for measuring the age-related changes of the UV absorbance of the human lens. The human lenses were used with the approval of the Human Ethics Committee of University of Szeged, Albert Szent-Györgyi Clinical Centre and the study was conducted in accordance with the provisions of Declaration of Helsinki for experimentation involving human tissue. Lenses were obtained with a postmortem delay of 6 h and were stored at −70°C until used.

Only those lenses which showed no cataract formation upon postmortem examinations were used for measurements. To determine the absorption of the various parts of the lens, the same procedure was used as reported in our earlier paper (17). Briefly, 60-μm thick consecutive sections were cut in a cryostat (Leica 1850 CM) at −18°C. Before sectioning, the anterior and posterior lens capsules were removed and stored in physiological saline separately. During this process, some of the capsules were damaged and could not be used for measurements, thus altogether 19 anterior and 27 posterior capsules were examined. During cryostat sectioning a total of nine sections were kept for measurements in an anteroposterior order: two samples from the anterior cortex (sample 1 and 2), five samples from the nucleus (samples 3–7) and two samples from the posterior cortex (samples 8 and 9), for further details see Fig. 1 and our earlier work (17).

As a result of the sectioning method, samples 4–7 always derived from the juvenile nucleus (19–21), thus the age-related changes of the sections taken from the nucleus are comparable, despite the fact that the thickness of the full lens increases with age.

Following UV measurements, cryostat cross sections were cut from the anterior and posterior capsules to determine the thickness of the capsules. Thickness of the anterior capsules varied from 21 to 26 μm, whereas that of the posterior ones were relatively constant in the range of 8 and 11 μm.

Absorption measurements. The dependence of the UV absorbance of the samples as function of wavelength was measured by a scanning spectrophotometer (model UV-2101PC UV- VIS; Shimadzu,
Kyoto, Japan) at room temperature. The absorbances and the absorption coefficients of the samples were determined as reported in our earlier work (17). The round 2 mm beam used for spectrophotometric measurements made it possible to measure only the central regions of the samples (Fig. 1). The investigated wavelength range was 240–400 nm, as in the range below 240 nm, the absorbance values often approached or reached the upper limit of the device.

Statistical analysis. Descriptive statistics were calculated for each layer separately. Continuous variables were expressed as mean ± SD. The tendency between age and absorption coefficients at 280 nm as well as at 360 nm was characterized with Pearson correlation coefficients and significance tests for correlation for each layer separately. Analysis of covariance (ANCOVA) model with factor layer and covariate age was used for further statistical analysis. A P-value < 0.05 was considered to be statistically significant. The calculations were computed through the use of IBM SPSS version 24.

RESULTS

Absorption spectrum of lens sections

The UV absorption of the lenses was examined in seven different age groups: 7–19 (n = 3), 20–29 (n = 3), 30–39 (n = 6), 40–49 (n = 10), 50–59 (n = 9), 60–69 (n = 4) and 70–81 (n = 3) years. There was only one lens younger than 10 years old available, so the age range of the first group was extended to include this sample. Due to the lack of samples, a similar expansion of the 70–81 years age group was performed. For statistical analysis, the age was considered as a continuous value and no age groups were formed. Figure 2 shows the averaged absorption coefficients of the nine sample layers as function of wavelength in three different age groups. The absorption spectrum slightly increases toward the shorter wavelengths and has a maximum at the wavelength of 280 nm. It can be seen that value of the maximum absorption increases in the anteroposterior direction, except in the case of the youngest group (7–19 years), in which the increasing tendency is not explicit and the samples 4–7 have almost equal absorption capacity.

The reported absorbance spectra of the human lens show another absorption peak around 360 nm (1,2,22). Figure 3 presents the averaged absorption coefficients of three age groups in the 300–400 nm wavelength range. For clarity, data related to Samples 1, 3, 5, 7 and 9 only are shown. The posterior layers have higher absorption capacity, but the increasing tendency is not as explicit as in the case of the peak at 280 nm. It is also noticeable that only the spectra of the youngest lenses show a small peak around 360 nm meanwhile the absorption is slightly decreased at 320 nm. As the detection limit of the spectrophotometer was calibrated in a way to measure the peak at 280 nm, the absorbance in the UV-A range presumably could have been detected with lower precision. Therefore, the fine absorption changes are visualized to a lesser extent compared with the previously published results.

Age-dependent changes of absorption

Although UV radiation at wavelengths shorter than 300 nm is presumably absorbed by the cornea, the 280-nm wavelength, where the absorption spectrum has a maximum, offers a plausible way to visualize accurately the age-related changes of the UV absorption of the lens. On the other hand, as the cornea is not able to fully absorb the UV radiation longer than 320 nm, it is physiologically also relevant to investigate the age-related changes in the UV-A range. The averaged absorption coefficients at 280 nm as well as at 360 nm of the anterior and posterior cortex and that of the nucleus were calculated in the following way: average of the values of samples 1 and 2 provided the absorption coefficient of the anterior cortex, that of the nucleus was calculated from the data of layers 3–7, and the average of the spectra
of samples 8 and 9 resulted in the absorption spectra of the posterior cortex. These calculations were performed on every single lenses. The averaged values of the absorption coefficient at 280 nm as function of age are shown in Fig. 4. The second order polynomial fit clearly shows that the age-related changes of the posterior cortex are the most pronounced compared to the other two parts. The absorption increases until the age of 60 where it reaches a plateau and slightly decreases after the age of 70.

Averaged absorption coefficients taken at 360 nm are presented in Fig. 5. Both the nucleus and posterior cortex show increasing absorption capacity with age but changes of the nucleus are the most noticeable.

To determine exactly how the age affects the absorption properties of the different layers and whether this effect is significant, statistical analysis was performed. Values of the absorption coefficients taken at 280 nm of the nine sample layers of every single lens were collected. The ANCOVA model revealed a borderline significant interaction between age and layer ($P = 0.057$). This result is significant at 10% level, with respect to the moderate sample size, this suggests that the effect of age depends on the layer. Changes of coefficient values as function of age were examined for each layer. Table 1 shows the correlation coefficients between age and the absorption coefficients at 280 nm and the results of significance tests for each layer. It can be seen that the correlation was significant in layers 6 ($R = 0.371, P = 0.022$), 7 ($R = 0.49, P = 0.002$) and 9 ($R = 0.363, P = 0.041$).

Figure 6 shows the values of the absorption coefficients taken at 280 nm of samples 6, 7 and 9 as function of age. Indeed, these results demonstrate a weak, but significantly increasing tendency of absorption.

In the case of absorption coefficients taken at 360 nm, the ANCOVA statistical model revealed a significant interaction between age and layer ($P < 0.01$), showing that the effect of age depends on the layer position in the UV-A range. Table 2 displays the correlation coefficient values between age and the absorption coefficient. The correlation proved to be significant in layers 5 ($R = 0.379, P = 0.047$), 6 ($R = 0.614, P = 0.000$) and 7 ($R = 0.676, P = 0.000$). In Fig. 7, the absorption coefficients taken at 360 nm of samples 5, 6 and 7 as function of age are presented. Indeed, the values show the increased absorption capacity of the nuclear region of the aged lenses.
Figure 5. Averaged values of absorption coefficients taken at 360 nm as function of age. Solid lines show the second order polynomial fit. Age-related changes of the nucleus are the most pronounced.

Table 1. Test P-values of the ANOVA analysis related to the absorption coefficients taken at 280 nm.

| Sample layers | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R             | 0.056 | 0.16 | 0.158 | 0.136 | 0.215 | 0.371 | 0.49 | 0.096 | 0.363 |
| P-values      | 0.737 | 0.337 | 0.343 | 0.414 | 0.207 | **0.022** | **0.002** | 0.582 | 0.041 |

Values <0.05 were considered to be statistically significant (bold-faced values).

Figure 6. Absorption coefficients at 280 nm of the three age-dependent sample layers are shown as function of age. Significant R and P-values are indicated.

Table 2. Test P-values of the ANOVA analysis related to the absorption coefficients taken at 360 nm.

| Sample layers | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R             | −0.16 | −0.32 | 0.129 | 0.337 | 0.379 | 0.614 | 0.676 | 0.195 | 0.261 |
| P-values      | 0.480 | 0.165 | 0.547 | 0.079 | **0.047** | **0.000** | **0.000** | 0.311 | 0.266 |

Values <0.05 were considered to be statistically significant (bold-faced values).
Absorption of the lens capsules

Owing to the small number of the samples, the averaged absorption coefficients of the lens capsules were calculated only in five different age groups: 7–19 years, 20–39 years, 40–49 years, 50–59 years and above the age of 60. Again, for statistical analysis, the age was considered as a continuous value and no age groups were formed. Regardless of age, we found that the posterior capsules show higher absorption than the anterior ones (Fig. 8) but further statistical analysis (ANCOVA) showed that this difference is not significant \( P = 0.198 \). The age showed no significant effect on absorption coefficients at 280 nm \( P = 0.18 \).

DISCUSSION

We examined the age-related UV absorption changes of the various parts of the human lens in an expanded age range (7–81 years), and it was found that there is an age-independent absorption gradient in the anteroposterior direction inside the lens. Only the youngest lenses showed slightly different tendency in the UV-C range which can be explained by the remodeling of the lens in this age (20) when the aspect ratio significantly changes and the primary fiber cell shortening happens. The results of the statistical analysis showed a significant correlation between the absorption coefficient taken at 280 nm and the age only in the case of the posterior layers 6 \( R = 0.371, P = 0.022 \), 7 \( R = 0.49, P = 0.002 \) and 9 \( R = 0.363, P = 0.041 \). At 360 nm, significant correlation was found between age and absorption of nuclear layers 5 \( R = 0.379, P = 0.047 \), 6 \( R = 0.614, P = 0.000 \) and 7 \( R = 0.676, P = 0.000 \). The anterior capsules have lower absorption coefficients than the posterior ones in each age groups; however, this difference is not significant and no age-related changes were found in these cases. This indicates that the age has no effect on the absorption properties of the lens capsules.

Most studies treat the cortex as a whole, since the anterior and posterior sides consist of the same elongated fibers. However, several results suggest that there is a difference in protein expression levels along the visual axis. Spectroscopically obtained Carr-Purcell-Meiboom-Gill data suggest spatial and age-dependent variations in crystallin content (23). Water content in the posterior pole is lower than in the anterior pole (24), indicating higher protein concentration in the posterior part. Levels of alpha-crystallin A and beta-crystallin B1 chains are higher in the posterior layers of the lens (17). The concentration of alpha-crystallin also increases with age at the expense of beta- and gamma-crystallins (25). These findings suggest that the age-related changes of the absorption at 280 nm are strongly related to the age-dependent protein expression levels. The water soluble protein fraction decreases after middle age, resulting in decreased

![Absorption coefficients at 360 nm of the three age-dependent sample layers are shown as function of age. Significant R and P-values are indicated.](image1)

![Averaged absorption coefficients of the capsules as function of wavelength. The curves are related to the indicated age groups.](image2)
absorption at 280 nm wavelength (26) which can explain the plateau around the age of 60 in the absorption coefficient of the posterior cortex and of the nucleus (Fig. 4). The UV-filter compounds, such as 3-hydroxykynurenine glucoside (3OHKG) or 3-hydroxykynurenine (3OHKyn) are responsible for the absorption in the UV-A and -B ranges. The concentration of all the tryptophan metabolites shows a decreasing tendency with age (27), except the glutathione adduct of 3OHKG. On the other hand, the levels of protein bound UV-filters especially that of 3OHKG in the nucleus, are significantly increased after middle age (28). These age-dependent changes of 3OHKG are in agreement with the enhanced absorption of the lenses older than 50 years (Figs. 5 and 6) and can explain the significant correlation.

The age-related results found in the literature are taken from the entire lens or lens homogenates, except the study of Gaillard et al. (2). To determine the UV transmission of the whole lens, the averaged cumulative absorbance was calculated in each age groups. The absorption values of the discarded segments were considered to be equal to the absorbance values of the samples taken in front of the unused sections. The measured absorbance resulted from the absorption and scattering processes together. Although the samples were placed between quartz plates in a drop of isotonic saline, the amount of light scattering through the lens sections is presumably still higher than in the case of the measurements related to the whole lens. When the cumulative absorbance is calculated, the effect of a relatively small difference in the scattering process can be highly enhanced due to the large number of lens sections (n = 60–70 with 60 μm thickness). In order to take into account this process, 2% of the incoming radiation was considered as scattered light in each step and the absorbance was reduced according to this. Then the transmission was calculated using the following formula:

\[ T = 100 \times 10^{(-A_{bc})} \]

where \( T \) is the percent transmission and \( A_{bc} \) is the corrected cumulative absorbance.

Figure 9 shows the transmission of the whole lens without the capsules in the case of seven different age groups. It can be seen that the youngest lenses transmits about 2% of the incoming light above 310 nm, while the older lenses allow very little transmission (approximately 0.1%) in the near-UV range. These absorption changes in the UV-B range is presumably caused by the age-dependent concentration of UV-filter molecules (27,28) and are in agreement with the data found in the literature (3,29,30). It can be also noticed that transmission of the lenses in the 7–19 years old age group shows a fine peak at wavelength of 320 nm. This corresponds to the local minimum in the absorbance spectrum of young lenses (1 and 2). The transmission of lenses with different age shows similar features (30). Both Gaillard et al. (2) and Cooper and Robson (22) found that the peak at 360 nm shifts toward the shorter wavelengths with age and the minimum vanishes which can explain the above phenomena.

The same changes can be observed in the absorption spectrum of lens sections derived from individual lenses (Figure S1) and the averaged curves also show similar tendency (Fig. 3). To prove whether any age-dependent changes occur at this wavelength, statistical analysis was performed and it was found that almost all the nuclear layers show significant correlation with age (Table 3). These findings suggest that the absorption of the aged nucleus is increased at this wavelength; therefore, the transmission window can be observed in the case of the 7–19 years old group only. Interestingly, other studies (3,14) found that the transmission of the lens is always increased around 320 nm, independently of age. This discrepancy could be due to either the different measurement techniques (absorbance of lens sections compared to the transmission of the whole lens) or the detection limit of the spectrophotometer which was calibrated to 280 nm peak value.

The averaged transmission curves are in good agreement with the data found in literature (3,30). On the other hand, the measured transmission of individual lenses shows high variability. These differences shall be reflected by the absorbance of the lens sections too. Both in the 7–19 and 50–59 years age groups, the layers derived from individual lenses with the same age have significantly different absorption capacities (see Figure S2). Standard deviation (SD) values of absorption coefficients taken at 280 nm as well as at 360 nm also confirm that the variability is relatively high within the groups, especially that of the anterior layers at the longer wavelength. The SD values together with the means are presented in Tables S1 and S2.

Averaged transmission of lens capsules was calculated as well but no compensation was made for light scattering. In Fig. 10, it can be seen that below 300 nm, the anterior and posterior capsules absorb approximately 30–40% and 20% of the incident UV

![Figure 9. Calculated transmission of the full lens as function of the wavelength.](image)

Note the increased transmission of very young lenses at wavelength above 310 nm. The older lenses possess very low capacity to transmit near UV light.

| Sample | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R      | 0.992 | -0.042 | 0.033 | 0.422 | 0.044 | 0.720 | 0.706 | 0.247 | 0.119 |
| P-value | 0.692 | 0.853 | 0.091 | **0.020** | **0.018** | **0.000** | **0.000** | 0.197 | 0.586 |

Values <0.05 were considered to be statistically significant (bold-faced values).
Light, respectively. Older anterior capsules are able to transmit less light, presumably due to their increased thickness (31).

As the lens grows continuously, it is evident that the absorbance of the whole lens becomes higher with age. On the other hand, here, we demonstrated the age-dependent changes of the absorption coefficient which is a more characteristic parameter. The statistical analysis showed that the age affects significantly the absorption properties of the posterior layers while the changes of the anterior ones are less notable. Previously, it was found that the greater UV absorption in the posterior cortex is accompanied by higher levels of Alpha-crystallin A and Beta-crystallin B1 chains (17) and alpha-crystallin was suggested to play an important role in the significant correlation between absorption and age in the case of posterior lens sections. At longer wavelengths, only posterior nuclear layers showed age-dependent changes. These are presumably associated with the increased level of the bound UV-filters in the old nucleus.

These results suggest that the absorbance of the old lens is higher not only due to its greater thickness but also the increased absorption capacity of the posterior layers. Although the capsules show no age-related changes, the thicker anterior capsule also increases the total absorbance of the aging lens. These findings could help better understand the process of the age-related or UV-induced cataract formation. Data related to the absorption of lens capsules could be also important in cataract surgery and add valuable information to the design of intraocular lenses.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. Absorption coefficients of individual lens sections. Note the high variability of the spectra of lenses with the same age.

Table S1. Standard deviation of absorption coefficients taken at 280 nm together with mean values in three age groups.

Table S2. Standard deviation of absorption coefficients taken at 360 nm together with mean values in three age groups.

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