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Optical properties of the human lens constituents

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ABSTRACT

The absorption and fluorescence properties of the metabolomic (MET), water-soluble and urea-soluble protein fractions from the middle-age, aged, and cataractous human lenses have been measured. At 280 nm and 300 nm the major lens absorbers are crystallins, which absorb more than 90% of light in the UV-B region (280–315 nm). In middle-aged lenses, the absorption at 360 nm is mostly provided by UV filters contained in the MET fraction. With aging, and especially with the cataract development, the absorption of MET fraction in UV-A region (315–400 nm) decreases due to the drop of the UV filter concentration, while the absorption of protein fractions increases due to the accumulation of post-translational modifications. Consequently, the contribution of the MET fraction into the total lens absorption at 360 nm decays from 63% in middle-aged lenses to 25% in aged lenses to 3% in cataractous lenses. The fluorescence yield of the MET fraction from cataractous lenses also significantly increases. Therefore, the protection of the lens tissue against UV radiation in aged and cataractous lenses weakens: the absorption of UV-A light is mostly provided by modified crystallins and non-UV-filter metabolites, which are photochemically more active than the UV filters. The obtained data indicate that the aged and cataractous human lenses are more vulnerable to UV-A light than the middle-aged lenses.

1. Introduction

Solar UV radiation penetrating inside the eye is one of the major factors causing oxidative stress in the eye lens [1,2]. It is believed [3–5] that the photochemical reactions inside the lens can induce post-translational modifications of the lens proteins, resulting in the protein coloration, aggregation and insolubilization. Eventually, these processes can lead to the cataract development.

A human lens mostly consists of organelle-free fiber cells filled with the specific lens proteins – crystallins. The protein content in the lens is approximately 30–40%, which is higher than in any other human tissue. Such design – absence of organelles and vascular system, high protein concentration – provides lens transparency, high refractive index and flexibility needed for the lens accommodation. Besides focusing the incident light onto retina, the lens also fulfills an important task of filtering out the UV-A (315–400 nm) component of the solar radiation [6–8]. The protection against UV radiation is performed by molecular UV filters contained in the lens, including kynurenine (KN), 3-hydroxykynurenine (3OHKN), 3-hydroxykynurenine *O*-β-D-glucoside (3OHKG), 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glycoside (AHBG), and glutathionyl-3-hydroxykynurenine glycoside (GSH-3OHKG). The chemical structures of UV filters are shown in SI. These compounds are characterized by high absorption in the UV-A region and low quantum yields of reactive triplet states and photodecomposition, transforming most of the light energy into heat [9–11]. It has been shown that the protective abilities of “primary” UV filters KN, 3OHKN and 3OHKG are better than that of “secondary” filters AHBG and GSH-3OHKG [11], and that the abundance of “primary” UV filters in the human lens decreases with age, while the concentration of “secondary” filters increases [7].

The absorption of the lens proteins in UV-A and visible regions increases with age [12,13]. The intact crystallins do not absorb in the UV-A region; however, the crystallins taken from aged lenses have yellow colour and absorb in the region of 315–400 nm due to the post-translational modifications accumulated in proteins during the whole lifespan [12]. The modifications leading to the protein coloration include the covalent attachment of UV filters to the protein chain [14–20], the oxidation of aromatic amino acid residues [21,22], the formation of advanced glycation endproducts (AGEs) [23–25], and others. It is important to notice that the chromophores incorporated into the protein chain could be significantly better photosensitizers than the free UV filters. In particular, the triplet quantum yields of the protein-bound UV

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filters are much higher than that of free ones [20]; the oxidized residues can generate reactive species with very high efficiency [2]. Therefore, one can assume that the age-related increase of the protein absorption weakens the lens UV protection.

The fluorescence properties of the lens also change with age. It has been reported that the fluorescence quantum yield from the whole lens increases from 0.002 (30-year-old lens) to 0.017 (60-year-old lens) to 0.121 (80-year-old lens) [26]. The major lens fluophor is a tryptophan residue [27] present in crystallins and absorbing in UV-B region. The tryptophan fluorescence is sensitive to the environment; it can be used for the early noninvasive diagnosis of pathogenic changes in the lens structure [28,29]. Besides tryptophan fluorescence, the emission has also been found at longer wavelengths (400–550 nm); the contribution of non-tryptophan fluorescence increases with age [30,31]. One of the reasons of this effect is the accumulation of post-translational modifications in the lens proteins, causing the increase of the protein absorption in the UV-A region and of the protein fluorescence. Therefore, study of the fluorescence from the whole lens and from the different lens constituents gives information on the age-related and pathogenic processes in the lens.

The present study is aimed at the determination of contributions from small molecules, water-soluble (WS) and urea-soluble (US) proteins into the total lens absorption and fluorescence. The measurements have been performed for three types of the human lenses: normal middle-aged lenses, normal aged lenses, and cataractous lenses. The goal of the work is to determine the age-related and cataract-related changes in the optical properties of the constituents of the human lens, which may have a significant effect on the lens protection against the UV radiation.

2. Materials and Methods

2.1. Materials

H_2O was distilled and deionized to 18 MΩ. Urea and Tris buffer were purchased from Bio-Rad Laboratories (Hercules, USA).

2.2. Sample Preparation

All procedures of this study were subjected to the Declaration of Helsinki – ethical principles for medical research involving human subjects, with the ethical approval from International Tomography Center and written informed consent from patients.

Three types of lenses – middle-aged, aged, and cataractous lenses – were used in this study. The middle-aged human lenses (34–40 year-old, the average age 37 years, 6 lenses total) and the aged human lenses (57–81 year-old, the average age 65 years, 6 lenses total) were obtained from cadavers in the Novosibirsk Regional Clinical Bureau of Forensic Medical Examination. The lenses were extracted within 10–14 hour post-mortem. The cataractous human lenses (69–84 year-old, the average age 78 years, age-related cataracts at 4–5 stages, 6 lenses total) were obtained from the Novosibirsk Regional Hospital after surgical removal. All lenses were immediately placed at −70 °C and stored frozen until analyzed.

Frozen human eye lenses (three lenses of each type) were separately homogenized in Tris buffer (50 mM, pH 7.4, 1.5 mL) in the presence of the protease inhibitor cocktail (Sigma/Aldrich, in a ratio 1 mL per 20 g of wet tissue). The obtained homogenate was centrifuged at 13200*g* for 30 min at 4 °C. The water-soluble (WS) supernatant was collected. The obtained pellet was re-extracted four times with 1 mL of Tris buffer, after every extraction the sample was centrifuged (13,200*g*, 30 min, 4 °C), and the supernatants were collected. All water-soluble supernatants containing metabolites and water-soluble proteins were pooled together. The water-insoluble (WIS) pellet was dissolved in urea (6 M, 5 mL) and centrifuged (13,200*g*, 50 min, 4 °C). The supernatant containing US protein fraction was collected, while the pellet containing urea-insoluble proteins was discarded.

Pooled water-soluble supernatant was separated into metabolomic (MET) fraction and WS protein fraction with the use of Millipore Amicon Ultra filters (Tullagreen, Ireland) with 3 kDa molecular weight cut-off (10,000*g*, 7–8 min, 4 °C). The protein fraction was washed 2 times with Tris buffer (1.5 mL) to remove small molecules from the solution. All metabolite-containing filtrates from the same lens were pooled together for further analysis.

Additionally, three lenses of each type have been used for the determination of the content of WS and WIS proteins in lenses. The lens homogenates were separated into WS and WIS fractions as described above, vacuum dried, and then weighed. The mass of the buffer salts in the samples was determined by the evaporation of clean buffer solution with the following weighting; this value was subtracted from the measured fraction weight. The weight percentage of WS and WIS proteins in the lens was calculated by dividing the fraction weight by the lens weight, the obtained values were averaged.

2.3. Steady-state Absorption and Fluorescence Measurements

UV–visible electronic absorption spectra were recorded with an 8453 spectrophotometer (Agilent, USA). Blank spectra were obtained for the pure solvents used for sample measurements (i.e. Tris buffer for MET and WS fractions and 6 M urea for US fraction). Fluorescence emission spectra were measured by a FLSP920 (Edinburgh Instruments, UK) spectrofluorimeter with a Xe-lamp as an excitation source. Before optical measurements, the solutions were centrifuged to remove the remains of scattering particles, and diluted to the desirable optical density. All measurements were carried out in a 10 × 10 mm^2 quartz cell. The obtained fluorescence spectra were corrected for the wavelength-dependent sensitivity of the detection. All samples for fluorescence measurements were prepared with the optical densities ~0.2 at the excitation wavelength.

3. Results

The measurements of the WS and WIS protein content in middle-aged, aged, and cataractous human lenses were performed for three lenses of each type. The results are presented in Table 1. As expected, the percentage of WS proteins in the lens decreases with aging and with cataract, while the percentage of WIS proteins increases. We should note that all cataractous lenses lacked a significant part of cortex (mostly from equatorial and posterior areas) lost during the surgical lens extraction; therefore, the masses of the cataractous lenses are almost twice lower than the masses of normal lenses.

Typical absorption spectra of MET, WS and US fractions from the

Table 1
Lens weights and the content of WS and WIS proteins in lenses of different types.

Lenses	Lens weight, mg	WS proteins		WIS proteins	
		Weight, mg	Percentage	Weight, mg	Percentage
Middle-aged	234 ± 33	60 ± 7	25.9 ± 0.9	10 ± 3	4.6 ± 1.8
Aged	224 ± 55	44 ± 5	20.0 ± 2.9	24 ± 7	10.6 ± 0.3
Cataract	127 ± 14	11 ± 2	8.9 ± 1.3	31 ± 3	24.2 ± 1.8