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Metabolomic composition of normal aged and cataractous human lenses

Yuri P. Tsentalovich [a, b, *], Timofey D. Verkhovod [a], Vadim V. Yanshole [a, b], Alexey S. Kiryutin [a], Lyudmila V. Yanshole [a, b], Anjella Zh. Fursova [c], Denis A. Stepakov [d], Vladimir P. Novoselov [d], Renad Z. Sagdeev [a, b]

[a] International Tomography Center SB RAS, Institutskaya 3a, Novosibirsk 630090, Russia
[b] Novosibirsk State University, Pirogova 2, Novosibirsk 630090, Russia
[c] Novosibirsk State Regional Clinical Hospital, Nemirovicha-Danchenko 130, Novosibirsk 630087, Russia
[d] Novosibirsk Regional Clinical Bureau of Forensic Medical Examination, Nemirovicha-Danchenko 134, Novosibirsk 630087, Russia

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ABSTRACT

Quantitative metabolomic profiles of normal and cataractous human lenses were obtained with the combined use of high-frequency nuclear magnetic resonance (NMR) and high-performance liquid chromatography with high-resolution mass-spectrometric detection (LC-MS) methods. The concentration of more than fifty metabolites in the lens cortex and nucleus has been determined. For the majority of metabolites, their concentrations in the lens cortex and nucleus are similar, which confirms low metabolic activity in the lens core. The difference between the metabolite levels in the cortex and nucleus of the normal lens is observed for antioxidants and UV filters, which demonstrates the activity of redox processes in the lens. A huge difference is found between the metabolomic compositions of normal and age-matched cataractous lenses: the concentrations of almost all metabolites in the normal lens are higher than in the cataractous one. The most pronounced difference is observed for compounds playing a key role in the lens cell protection and metabolic activity, including antioxidants, UV filters, and osmolytes. The results obtained imply that the development of the age-related cataracts might originate from the metabolic dysfunction of the lens epithelial cells.

1. Introduction

The transparency of the eye lens is provided by two main factors. First, the major part of the lens consists of metabolically inactive fiber cells, lacking nuclei and other organelles able to scatter the light. These cells have very high concentrations of structural proteins – crystallins, homogeneously distributed inside the cells. That provides high refractive index of the lens needed for focusing the incident light onto retina. Second, the lens does not have vascular system; the lens supply with nutrients occurs via the aqueous humor, surrounding the lens. The drawback of this design is that the crystallins in the lens core do not turnover. With aging, they accumulate numerous post-translational modifications, leading to the protein insolubilization, aggregation and coloration (van Heyningen, 1976; Bloemendal et al., 2004). Eventually, these processes result in the formation of large insoluble protein aggregates and give rise to the age-related nuclear cataract development (Bron et al., 2000; Sharma and Santhoshkumar, 2009). The long-term

List of abbreviations: 2OH-butyrate, 2-hydroxy-butyrate; 2OH-3Me-butyrate, 2-hydroxy-3-methyl-butyrate; 3OHKN, 3-hydroxykynurenine; 3OHKG, 3-hydroxykynurenine O-β-D-glucoside; AABA, α-aminobutyrate; ADP, adenosine diphosphate; AHBG, 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid O-β-D-glucoside; AHBDG, 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid O-β-D-diglucoside; Ala, alanine; Ala–Ala, alanine–alanine dipeptide; AMP, adenosine monophosphate; Arg, arginine; Asc, ascorbate; Asp, aspartate; Cys-3OHKG, cysteinyl-3-hydroxykynurenine O-β-D-glucoside; GABA, γ-aminobutyrate; Gln, glutamine; Glu, glutamate; Gly, glycine; GSH, glutathione reduced; GSH-KN, glutathionyl-kynurenine; GSH-3OHKN, glutathionyl-3-hydroxykynurenine; GSH-3OHKG, glutathionyl-3-hydroxykynurenine O-β-D-glucoside; GSSG, glutathione oxidized; His, histidine; Ile, isoleucine; KN, kynurenine; Leu, leucine; Lys, lysine; Met, methionine; NAD, nicotinamide adenine dinucleotide; PCA, pyroglutamate; Phe, phenylalanine; Pro, proline; SAH, S-adenosylhomocysteine; Tau, taurine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

* Corresponding author. International Tomography Center, 630090, Institutskaya 3a, Novosibirsk, Russia.
E-mail address: yura@tomo.nsc.ru (Y.P. Tsentalovich).

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integrity of the crystallin network is provided by metabolites, synthesized in the metabolically active lens epithelium or entering inside the lens through the lens epithelial layer from the aqueous humor. At the moment, a full list of metabolites required in the human lens nucleus remains unknown; however, two groups of compounds definitely play an important role in the crystallin protection against undesirable modifications. The first group consists of kynurenine (KN) and its derivatives (van Heyningen, 1971; Bova et al., 2001; Streete et al., 2004; Snytnikova et al., 2008). In the lens, these compounds act as UV filters, absorbing UV-A light in the 300–400 nm region and transforming the absorbed light energy into heat (Tsentalovich et al., 2005, 2011; Sherin et al., 2008, 2009). They protect the retina and the lens itself from UV-induced damages and improve our vision by reducing chromatic aberrations. The second group includes antioxidants, protecting the lens from the oxidative damages. Among them, the most abundant and important lens antioxidants are reduced glutathione (GSH) and ascorbate (Asc), able to deactivate reactive oxygen species, to reduce free radicals and other chemically active compounds, and to quench photoexcited molecules (Williams, 2006; Snytnikova et al., 2007).

The mechanism of the metabolite transport inside the lens is still poorly understood. It has been reported that the concentrations of most amino acids in the lens are higher than that in the aqueous humor (Kinsey, 1965; Reddy, 1973). The accumulation of metabolites in the lens against the concentration gradient testifies that the penetration of small molecules inside the lens is governed by active pumps, situated in the lens epithelial layer. It has been proposed (Reddy, 1973) that different compounds require different types of active pumps; the molecular mechanisms of some selective pumps have been reported (Lim et al., 2006). However, the mechanisms of the metabolite movement inside the lens are under debates (Donaldson et al., 2010; Beebe and Truscott, 2010; Dahm et al., 2011; Dobretsov et al., 2013). The disruptions of the lens transport system may have a deleterious effect for the lens nucleus due to the shortage of UV filters and antioxidants and the accumulation of oxidized products (Sweeney and Truscott, 1998; Truscott, 2005).

Thereby, the maintenance of the lens metabolic composition is essential for the correct functioning of the lens cells. It is important to notice that the content of specific metabolites in the lens may significantly vary under stressful conditions. For example, selenite and streptozotocin injections cause significant changes in the lens metabolomic composition (Fris et al., 2006; Kanth et al., 2009), leading to the elevated level of some amino acids and to the reduced level of other metabolites. UV irradiation *in vivo* also causes the alternation of the lens metabolomic content (Risa et al., 2004, 2005). One may expect that the development of the age-related cataracts is also accompanied by significant changes in the lens metabolomic composition. In the recent publications (Streete et al., 2004; Korlimbinis et al., 2007) it has been reported that the levels of UV filters in cataractous human lenses are significantly lower than in the normal lenses, while the concentrations of tryptophan and tyrosine are significantly higher. Very low level of GSH was found in the nuclei of cataractous lenses (Bova et al., 2001; Truscott and Augusteyn, 1977).

In our recent paper (Yanshole et al., 2014), we applied high-frequency ^{1}H NMR and high-resolution mass spectrometry with HPLC separation, electrospray ionization and time-of-flight detection (LC-MS) for the metabolomic profiling of rat lenses. It has been demonstrated that the combined application of these two methods is very fruitful for the quantitative metabolomic profiling of biological tissues: LC-MS analysis affords a long list of identified metabolites, while NMR method offers simple and reliable quantification. In the present paper, we applied the same approach to the metabolomic analysis of normal and cataractous human lenses, with some methodological modifications: the lens extracts were purified from lipids to improve the quality of NMR spectra, and an ion-pairing agent (Piraud et al., 2005) was used for a better HPLC separation. The major goals of this work are to measure the quantitative levels of a wide range of metabolites in the human lens, to compare the metabolomic content of the human lens cortex and nuclei, and to determine the differences in metabolomic profiles between normal and cataractous lenses.

2. Material and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (USA). Acetonitrile HPLC grade (ACN) was purchased from Panreac (Spain). D_2O 99.9% was purchased from Armar Chemicals (Switzerland). H_2O was deionized using Ultra pure water system (SG water, Germany) to the quality of 18.2 MOhm.

2.2. Sample preparation

Cataractous human lenses (6 lenses total) were obtained in the Novosibirsk Regional Hospital after surgical extracapsular removal from non-diabetic patients of the age from 59 to 71 years, the average age was 66 years. All six lenses had mature age-related nuclear cataracts. Normal human lenses of the age from 55 to 70 years (9 lenses total, the average age 62 years) were obtained from the Novosibirsk Regional Clinical Bureau of Forensic Medical Examination. The lenses were extracted within 24 h post mortem. Human tissue was handled in accordance with the tenets of the Declaration of Helsinki, with ethics clearance from International Tomography Center. The lenses were frozen immediately after extraction and stored at −70 °C until analyzed.

The lens nucleus was separated from the cortex by coring with a 4-mm home-made stainless steel borer along the optical axis followed by cutting off of approximately 1 mm from each end of the core. The procedure was performed with lenses taken from storage at −70 °C and warmed up to −18 °C for easier cutting, all tools were cooled down to −18 °C, and all manipulations were performed in a cold room at −5 °C. The nucleus and the cortex (the combined doughnut-shaped outer remainder of boring and the ends of the core) were weighed and then separately homogenized in 700 μL EtOH. Typically, the weight of nucleus was 35–45 mg, the weight of cortex from the normal lens was 130–180 mg, and the weight of cortex from the cataractous lens was 40–60 mg (part of the cortex from cataractous lenses was lost during the surgical removal from the patient's eye). The homogenate was placed in a shaker for 40 min, and then centrifuged (12 000 *g*, 30 min, 4 °C). The pellet was re-extracted twice with 700 μL 80% EtOH, all three supernatants were pooled together.

To remove lipids from the extract, 150 μL H_2O and 900 μL $CHCl_3$ was added to the combined supernatant, the sample was shaken for 15 min. Then 500 μL H_2O was added, the sample was shaken for 30 min, and left at −18 °C for 30 min. Then the sample was centrifuged (12 000 *g*, 30 min, 4 °C), and the aqueous (upper) fraction was collected. The procedure was repeated one more time. The obtained protein-free and lipid-free extract was divided into two parts in the ratio 2:1; the larger part was used for NMR measurements, and the lesser part – for LC-MS measurements.

The extract for NMR measurements was lyophilized and then re-dissolved in 500 μL of D_2O containing 2 μM sodium 3-trimethylsilylpropane-1-sulfonate (DSS) as internal standard (IS) and 20 mM deuterated phosphate buffer to maintain pH 7.1.

The extract for LC-MS analysis was lyophilized and then re-