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Research article

Spatial distribution of metabolites in the human lens

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ABSTRACT

Spatial distribution of 34 metabolites along the optical and equatorial axes of the human lens has been determined. For the majority of metabolites, the homogeneous distribution has been observed. That suggests that the rate of the metabolite transformation in the lens is low due to the general metabolic passivity of the lens fiber cells. However, the redox processes are active in the lens; as a result, some metabolites, including antioxidants, demonstrate the "nucleus-depleted" type of distribution, whereas secondary UV filters show the "nucleus-enriched" type. The metabolite concentrations at the lens poles and equator are similar for all metabolites under study. The concentric pattern of the "nucleus-depleted" and "nucleus-enriched" distributions testifies that the metabolite distribution inside the lens is mostly governed by a passive diffusion, relatively free along the fiber cells and retarded in the radial direction across the cells. No significant difference in the metabolite distribution between the normal and cataractous human lenses was found.

1. Introduction

The transparency of the eye lens depends on the integrity of the major lens proteins, crystallins. Inside the lens fiber cells, crystallins form a complex 3-dimensional network, and their high concentration and ordered distribution inside the cells provide high refractive index of the lens. Crystallins practically do not turn over, and under oxidative stress they undergo coloration, aggregation and insolubilization. These processes eventually lead to the formation of large insoluble protein aggregates and to the clouding of the lens. The protection of the lens cells is performed by small-molecular-weight compounds, including antioxidants, UV filters, and osmolytes. Since the metabolic activity in the lens fiber cells is very low, most of these compounds either enter the lens from the surrounding aqueous humor, or are synthesized in the metabolically active lens epithelial cells. During the metabolite movement inside the lens, they undergo enzymatic and non-enzymatic transformation. Thus, the analysis of the metabolite spatial distribution inside the lens may shed light on the dynamics of metabolic processes: the homogeneous metabolite distribution would indicate a low rate of the metabolite consumption, while the compounds undergoing intensive transformation would show a gradient in the spatial distribution.

The lens lacks the vascular system, and the transport of metabolites inside the lens is governed by several mechanisms (Dahm et al., 2011), including the passive diffusion, active transport driven by Na^+ ion currents (Mathias et al., 2007), membrane-based transport of small molecules from the extracellular space into the fiber cytoplasm (Lim et al., 2006, 2007), gap-junctional intercellular transport (Goodenough, 1992), and vesicle-mediated transport (Bassnett et al., 1994). The contribution of each mechanism is unknown; in particular, the roles of the passive diffusion and metabolite transport driven by ion currents have been discussed (Donaldson et al., 2010; Beebe and Truscott, 2010). The Fluid Circulation Model postulates the existence of a fluid flow through the cytoplasm of the lens fiber cells with the influx at the lens poles and the efflux at the equatorial zone (Mathias and Rae, 1985, 2004; Mathias et al., 2007) (Fig. 1A). This circulation is driven by Na^+/K^+ pumps concentrated at the equator. The existence of the flow was

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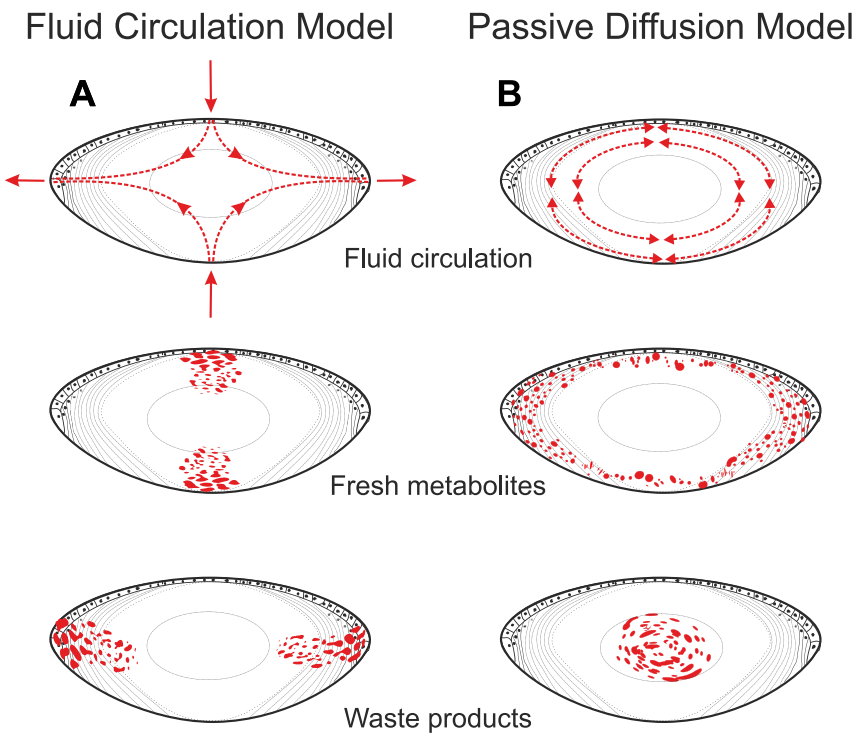

Fig. 1. Schematic presentation of fluid movement, spatial distribution of fresh metabolites entering the lens from the aqueous humor, and spatial distribution of waste products formed inside the lens according to fluid circulation model (A) and passive diffusion model (B).

proposed based on the observation of electrical currents outside the lens (Mathias and Rae, 1985), and has recently been supported by MRI measurements (Vaghefi et al., 2009, 2011) and by the direct observation of the fluid movement (Candia et al., 2012). In contrast, the passive diffusion does not imply a specific direction of the fluid flow; however, the movement of water and metabolites is highly anisotropic (Fig. 1B), being relatively free along the fiber cells and substantially restricted by cell membranes in the radial direction (Moffat and Pope, 2002). The contributions of fluid current and passive diffusion into the spatial metabolite distribution inside the lens are unknown. One can expect that if the fluid circulation is much faster than the passive diffusion, and the metabolite consumption is sufficiently high, the concentration of fresh metabolites would be the highest at the lens poles and lowest at the equator, while the waste products would concentrate in the equatorial zone. For metabolites produced in the epithelial cells, their concentration at the anterior pole would be higher than that at the posterior pole (Fig. 1A). In opposite, if the diffusion rate is comparable or faster than the circulation rate, a concentric metabolite distribution is expected, with the highest concentration of fresh metabolites in the outer cortex and the accumulation of the waste products in the lens nucleus (Fig. 1B).

In the present work, we applied ion-pairing HPLC separation and high resolution mass spectrometric detection (LC-MS) for the mapping the metabolite distribution in the human lens along the optical and equatorial axes. Due to the high sensitivity of LC-MS method, it is possible to determine the levels of a wide range of metabolites in relatively small pieces of biological tissue. The goals of the work are: (i) to develop a method for the measurement of the metabolite spatial distribution in a biological tissue; (ii) to determine which metabolites are actively consumed in the lens metabolic processes, (iii) which compounds are produced inside the lens, (iv) how the consumable metabolites and metabolic products are distributed within the lens; (v) to compare the metabolite distribution in the normal and cataractous human lenses.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile HPLC grade (ACN) was purchased from Scharlau (Hamburg, Germany). H_2O was deionized using Ultra pure water system (SG water, Barsbuettel, Germany) to the quality of 18.2 MOhm.

2.2. Sample preparation

Normal human lenses (32–55-year-old) were obtained from the Novosibirsk Regional Clinical Bureau of Forensic Medical Examination. The lenses were extracted within 24 h (typically – 12–14 h) post-mortem. Cataractous human lenses (mature age-related nuclear cataracts, 63–73-year-old) were obtained in the Novosibirsk Regional Hospital after surgical extracapsular removal from non-diabetic patients. Human tissue was handled in accordance with the tenets of the Declaration of Helsinki, with ethics clearance from International Tomography Center. All lenses were frozen immediately after extraction and stored at −70 °C until analyzed.

To obtain the lens slices along the optical axis, the lens was warmed up to −15 °C, placed in a cold Petri dish, and bored along the optical axis with a cold 3.5-mm home-made stainless steel borer. The obtained cylinder of 3.5 mm diameter and of approximately 4 mm high was vertically mounted on an ice cylinder of 8 mm diameter, and gently coated with supercooled water. The resulting ice block with the lens cylinder frozen inside was fixed on the specimen head of a CryoStar NX70 cryostat (Thermo Scientific,