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Lipid domains in intact fiber-cell plasma membranes isolated from cortical and nuclear regions of human eye lenses of donors from different age groups

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ABSTRACT

The results reported here clearly document changes in the properties and the organization of fiber-cell membrane lipids that occur with age, based on electron paramagnetic resonance (EPR) analysis of lens membranes of clear lenses from donors of age groups from 0 to 20, 21 to 40, and 61 to 80 years. The physical properties, including profiles of the alkyl chain order, fluidity, hydrophobicity, and oxygen transport parameter, were investigated using EPR spin-labeling methods, which also provide an opportunity to discriminate coexisting lipid domains and to evaluate the relative amounts of lipids in these domains. Fiber-cell membranes were found to contain three distinct lipid environments: bulk lipid domain, which appears minimally affected by membrane proteins, and two domains that appear due to the presence of membrane proteins, namely boundary and trapped lipid domains. In nuclear membranes the amount of boundary and trapped phospholipids as well as the amount of cholesterol in trapped lipid domains increased with the donors' age and was greater than that in cortical membranes. The difference between the amounts of lipids in domains uniquely formed due to the presence of membrane proteins in nuclear and cortical membranes increased with the donors' age. It was also shown that cholesterol was to a large degree excluded from trapped lipid domains in cortical membranes. It is evident that the rigidity of nuclear membranes was greater than that of cortical membranes for all age groups. The amount of lipids in domains of low oxygen permeability, mainly in trapped lipid domains, were greater in nuclear than cortical membranes and increased with the age of donors. These results indicate that the nuclear fiber cell plasma membranes were less permeable to oxygen than cortical membranes and become less permeable to oxygen with age. In clear lenses, age-related changes in the lens lipid and protein composition and organization appear to occur in ways that increase fiber cell plasma membrane resistance to oxygen permeation.

1. Introduction

Fiber-cells, which form tightly packed layers in the eye lens, lose their organelles soon after they are formed (Bassnett et al., 2011; Rafferty, 1985; Wride, 2011); the plasma membrane becomes the only membranous structure of matured fiber-cells. The lipid bilayer portion of the membrane determines bulk membrane properties (including diffusion barriers) (Borchman and Yappert, 2010; Subczynski et al., 2012) and can affect the properties of membrane proteins (Epand, 2005; Gonen et al., 2005; Reichow and Gonen, 2009; Tong et al., 2012, 2013). Age-related changes in the plasma membranes of human eye lens fiber-cells are much greater than age related changes in the membranes of other organs and tissues. Phospholipid (PL) composition changes drastically with age, with the increase of sphingolipid content (Borchman et al., 1994; Deeley et al., 2008; Yappert and Borchman, 2004; Yappert et al., 2003) and depletion of phosphatidylcholine (Borchman et al., 1994; Deeley et al., 2008; Yappert and Borchman, 2004; Yappert et al., 2003). The saturation levels of PL alkyl chains also

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increase (Deeley et al., 2008; Li et al., 1985; Yappert et al., 2003). Most characteristic is the increase in cholesterol (Chol) content up to the Chol/PL ratio of 4 (Li et al., 1985, 1987; Rujoi et al., 2003; Zelenka, 1984; Mainali et al., 2015). Aged fiber-cell membranes are loaded with integral proteins (Bassnett et al., 2011; Gonen et al., 2004; Kistler and Bullivant, 1980), the organization of which, including the formation of domains, arrays, and other structures, also changes with age (Buzhynskyy et al., 2007; Costello et al., 1989; Dunia et al., 2006; Zampighi et al., 2002). Regardless of these changes, the lens normally remains transparent. Due to a lack of turnover (Lynnerup et al., 2008), cells in the center of the nucleus of an adult human lens are as old as the individual, and membrane proteins that perform several functions in young human lenses likely perform the same functions in older lenses with altered lipid compositions. Thus, homeostasis of the fiber-cell plasma membrane and fiber cell itself should be maintained throughout the entire human life. We believe that the fiber-cell plasma membrane, with its unique structure and properties, helps to maintain cell homeostasis.

Previously, we have investigated the organization, properties, and dynamics of eye lens lipid membranes made of total lipids extracted from eyes of different species, donors of different age, and different regions of the eye lens (Mainali et al., 2012, 2013b; Raguz et al., 2008, 2009; Widomska et al., 2007). These works were summarized in the review (Subczynski et al., 2012) where we identified the significant functions of Chol specific to the fiber-cell plasma membrane. In human fiber-cell plasma membranes, Chol not only saturates the bulk PL bilayer (Mainali et al., 2013b) but also induces formation of immiscible pure cholesterol bilayer domains (CBDs) within the membrane (Jacob et al., 1999; Mainali et al., 2013b). The saturating Chol content keeps the bulk physical properties of lens-lipid membranes consistent and independent of changes in PL composition. Thus, the CBD helps to maintain lens membrane homeostasis by providing the buffering capacity for Chol concentration in the surrounding PL bilayer, keeping it at a constant saturation level. This is especially significant for human lenses because among mammalian lenses, those from humans have the longest life span, and changes in lens PL composition with age are most pronounced (Estrada et al., 2010). Other functions of Chol include the formation of a hydrophobic barrier and the alteration of membrane lipid rigidity (Subczynski et al., 1994).

Our most recent studies have focused on intact lens membranes isolated from human (Raguz et al., 2014) and porcine (Mainali et al., 2012) lenses. We hypothesized that we should find the presence of the four purported lipid domains, namely bulk, boundary, and trapped lipids, as well as the pure CBD. We were able to confirm the existence of three of them: bulk, boundary, and trapped lipid domains (Mainali et al., 2012; Raguz et al., 2014). CBDs have been observed in bilayers containing lipids isolated from fiber cells membranes (Mainali et al., 2013b), but have not been observed in intact membranes. We also made an effort to quantitatively evaluate the relative amounts of PLs and Chol in lipid domains in intact human eye lens membranes (Raguz et al., 2014). All of these measurements were obtained using samples pooled from about twenty clear lenses.

In the studies reported here, we have extended our work in order to study the properties of the lipid bilayer portion of the intact fiber-cell plasma membranes of donors in age groups ranged from 0 to 20, 21 to 40, and 61 to 80 years, which compliment those obtained earlier from donors of the age group from 41 to 60 years (Raguz et al., 2014). Results were obtained for cortical and nuclear intact membranes isolated from clear lenses in each age group, which has allowed us to assess changes in the organization of lipids that occur with age. The data indicate that the amount of lipids in domains uniquely formed due to the presence of integral membrane proteins is greater in nuclear membranes than in cortical membranes and in nuclear membranes increases significantly with age. These studies will guide future work in which we are planning to assess changes that occur during cataract formation.

2. Materials and methods

2.1. Materials

Doxylstearic acid spin labels (*n*-SASL, $n = 5, 7, 9, 12,$ or 16) and androstane spin label (ASL) (see Fig. 1 in Raguz et al., 2014 for their structure) were purchased from Molecular Probes (Eugene, OR). Other chemicals of at least reagent grade were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Isolation of intact membranes from cortical and nuclear fiber cell membranes

Twenty clear human lenses from donors in each of three groups, ranging in age from 0 to 20 (average age of 17.5 years, 4 female, 16 male, and all Caucasian donors), 21 to 40 (average age of 30.8 years, 4 female, 16 male, 18 Caucasian, and 2 African American donors), and 61 to 80 years (average age of 67.4 years, 6 female, 14 male, 19 Caucasian, and 1 African American donor), were obtained from the Lions Eye Bank of Wisconsin. Separately, seven pairs of clear lenses from donors of different ages (all male and all Caucasian) were also obtained from the same Eye Bank and were used for single donor and single lens measurements. Lenses were removed *in situ* from refrigerated bodies within an average time frame of 9 h postmortem. All of the lenses were stored at −80 °C until intact membrane isolations were performed. Lenses were examined using a binocular microscope and were evaluated for color and opacities to determine the presence or absence of cataractous changes. Usually lenses were accumulated over four months, intact membrane isolations were then performed. The cortical and nuclear regions of these lenses were separated based on differences in tissue consistency (Estrada and Yappert, 2004; Rujoi et al., 2003). Cortical and nuclear intact membranes were isolated based on minor modifications of the method developed by Bloemendal et al. (1972), as reported earlier (Cenedella and Fleschner, 1992; Chandrasekher and Cenedella, 1995; Lim et al., 2005). Each time special care was taken to produce a uniform suspension by repeatedly aspirating the solution through a syringe fitted with an 18-gauge needle. Finally, the pellet was washed and re-suspended with buffer (0.1 M borate, pH 9.5) and stored at −20 °C. The same preparation procedures were used for isolation of cortical and nuclear intact membranes from three pairs of lenses from three different donors and for isolation of cortical and nuclear intact membranes from left and right eye lenses from four different donors.

2.3. Preparation of samples for EPR measurements

Spin labeling of intact membranes using *n*-SASLs and ASL was performed as described earlier (Ligeza et al., 1998; Mainali et al., 2012). The film of *n*-SASL was prepared on the bottom of a test tube by drying the appropriate amount of spin label in chloroform (to achieve the molar ratio of spin label:total lipids of 1:100 in each sample). Only one type of spin label was present in each sample. Intact membrane suspensions (~0.2 mL) were added to the test tubes and shaken for about 2 h at room temperature. Control measurements demonstrated that this incubation time was sufficient to incorporate nearly all of the spin labeled molecules into the membranes. Spin labeled membrane suspensions were centrifuged twice using an Eppendorf centrifuge (16,000 g, 20 min, 4 °C), and