Experimental Eye Research 140 (2015) 179–186

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

Amounts of phospholipids and cholesterol in lipid domains formed in intact lens membranes: Methodology development and its application to studies of porcine lens membranes

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ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
29 August 2015
Accepted in revised form 14 September 2015
Available online 16 September 2015

Keywords:
Cholesterol
Membrane domain
Fluidity
Hydrophobic barrier
Oxygen permeation
EPR
Spin labeling

ABSTRACT

An electron paramagnetic resonance spin-labeling method has been developed that allows quantitative evaluation of the amounts of phospholipids and cholesterol in lipid domains of intact fiber-cell plasma membranes isolated from cortical and nuclear regions of eye lenses. The long term goal of this research is the assessment of organizational changes in human lens fiber cell membranes that occur with age and during cataract development. The measurements needed to be performed on lens membranes prepared from eyes of single donors and from single eyes. For these types of studies it is necessary to separate the age/cataract related changes from preparation/technique related changes. Human lenses differ not only because of age, but also because of the varying health histories of the donors. To solve these problems the sample-to-sample preparation/technique related changes were evaluated for cortical and nuclear lens membranes prepared from single porcine eyes. It was assumed that the differences due to the age (animals were two year old) and environmental conditions for raising these animals were minimal. Mean values and standard deviations from preparation/technique changes for measured amounts of lipids in membrane domains were calculated. Statistical analysis (Student's *t*-test) of the data also allowed determining the differences of mean values which were statistically significant with $P \leq 0.05$. These differences defined for porcine lenses will be used for comparison of amounts of lipids in domains in human lens membranes prepared from eyes of single donors and from single eyes. Greater separations will indicate that differences were statistically significant with ($P \leq 0.05$) and that they came from different than preparation/technique sources. Results confirmed that in nuclear porcine membranes the amounts of lipids in domains created due to the presence of membrane proteins were greater than those in cortical membranes and the differences were larger than the differences observed for human intact fiber cell membranes [Raguz, M. Mainali, L., O'Brien, W.J., and Subczynski, W.K. (2015) Exp. Eye Res.]. Lipids in porcine nuclear fiber cell plasma membranes were more rigid and less permeable to oxygen than in human nuclear membranes. Most likely the significant differences in the lipid composition were responsible for the observed differences.

1. Introduction

Lens fiber cells lose their intracellular organelles soon after they are formed (Bassnett et al., 2011; Rafferty, 1985; Wride, 2011), and the plasma membrane together with the cytoskeleton is the only supramolecular structure of the matured fiber cell. The plasma membrane accounts for essentially all lipids of the matured fiber cell. Thus, the fiber cell plasma membrane and its lipid bilayer component likely play significant roles in the maintaining fiber cell viability as well as maintaining lens homeostasis thus preventing lens opacification and development of cataract. To understand these functions of fiber cell plasma membrane it is necessary to understand functions of membrane components at the molecular level.

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http://dx.doi.org/10.1016/j.exer.2015.09.006
0014-4835/

Using EPR spin-labeling approaches we investigated in detail the organization and properties of lens lipid membranes made of the total lipid extracts from cortical and nuclear regions of lenses from different species of animals at different ages (Mainali et al., 2011a, 2013, 2015; Raguz et al., 2009). We were able to discriminate membrane domains, including the cholesterol bilayer domain (CBD), and to show that for old human donors (from age group from 61 to 70 years) nuclear membranes contain cholesterol crystals (Mainali et al., 2015). We were also able to identify cholesterol and CBD functions specific to fiber cell plasma membranes (Subczynski et al., 2012). The saturating Chol content keeps the bulk physical properties of lens lipid membranes consistent and independent of changes in phospholipid (PL) composition. Thus, 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 saturating level. Maintaining lens membrane homeostasis during aging is not an easy task because the lens membrane phospholipid composition changes dramatically with age (Borchman and Yappert, 2010; Li et al., 1987). Another significant conclusion from our work with lens lipid membranes is that the high Chol content, formation of CBDs, and formation of Chol crystals should not be considered as major predetermining factors for the development of age-related cataracts (Mainali et al., 2015). Investigation of lens lipid membranes (membranes without intrinsic proteins) is a necessary step in the investigation of the properties and organization of the lipid-bilayer portion of fiber-cell plasma membranes. Without this research, it is not possible to understand clearly the mechanisms by which intrinsic and extrinsic proteins affect the properties of the lipid bilayer (see (Subczynski et al., 2012) for more discussion).

All the studies cited above were completed using lens lipid membranes, thus, membranes without membrane integral proteins which should significantly alter the organization, structure, and dynamics of the lipid bilayer portion of the membrane. 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 change 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; Peterson and Delamere, 1992), 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.

Recently, we have extended our work in order to study the properties of the lipid bilayer portion of intact fiber-cell plasma membranes isolated from human (Raguz et al., 2014, 2015) and pig (Mainali et al., 2012) lenses. We were able to confirm the existence of three distinct lipid domains: bulk lipid domain, which appears minimally affected by membrane proteins, and two domains that appear due to the presence of membrane proteins, namely boundary lipid and trapped lipid domains. We also have made efforts to quantitatively evaluate the relative amounts of PLs and Chol in lipid domains of intact human eye lens membranes (Raguz et al., 2014, 2015). Based on our work (Raguz et al., 2014, 2015), the PL analog spin label 12-SASL is the best to show the distribution of PLs between membrane domains discriminated in intact membranes. This spin label can be inserted into intact membranes from its dry film without the use of damaging solvents (Ligeza et al., 1998) and, thus, with a minimal disturbance to the membrane structure. Among examined spin-labeled cholesterol analogs only androstane spin label (ASL) can be inserted into intact membranes from its dry film (Mainali et al., 2012). Structures and approximate locations of these two spin labels in the lipid bilayer are shown in Fig. 1. The unique distributions of these spin labels between domains in lens lipid and intact membranes (Fig. 2) allow us to obtain unique quantitative information about the relative amounts of PLs and Chol in lipid domains. Fig. 2 also provides the guideline for our experiments and interpretation of data. Quantitative results obtained for cortical and nuclear intact membranes isolated from clear human lenses of different age groups have 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. However, all of these measurements were obtained using samples pooled from about twenty clear lenses.

Data from Fig. 6 of (Raguz et al., 2015) also contains preliminary data for the quantitative evaluation of the relative amounts of PLs and Chol in lipid domains in cortical and nuclear human intact membranes from three pairs of clear lenses from three different donors and for cortical and nuclear intact membranes from left and right eye clear lenses from four different donors. These preliminary data allowed us to conclude that the differences observed between the left and the right eye of the same donor were much smaller that the scattering of data from different donors of a similar age. Thus, the significant methodological problem need to be solved before this quantitative method can be widely applied to assess organizational changes in fiber cell membranes occurring with age and during cataract development, namely: what is the contribution of the preparation/technique related experimental error? The separation of the age/cataract related changes from preparation/technique related changes cannot be performed based on measurements on single human lenses because the human lenses are different not only because of age, but also because of varying health history of the donors. Here we evaluated the preparation/technique related changes from sample-to-sample (changes due to the sample preparations and measurements) for cortical and nuclear lens membranes prepared from single porcine eyes assuming that the differences due to the age (animals were two-year-old) and environmental conditions for raising these animals (obtained from the same meat factory) are minimal. Statistical analysis of the data allowed us to evaluate preparation/technique related changes. Greater changes can be assumed to come from factors connected with health history of the donors, like age or cataract development.

2. Materials and methods

2.1. Materials

Doxylstearic acid spin label (12-SASL) and spin-labeled cholesterol analog (androstane spin label [ASL]) 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

Porcine eyes from two-year-old animals were obtained on the day of slaughter from Johnsonville Sausage, LLC (Watertown, WI). The eyes were dissected, and the lenses were collected. The cortical and nuclear regions of each lens were separated based on differences in tissue consistency (Estrada and Yappert, 2004; Rujoi et al.,