

Research Highlight #150

Lipid domains in Intact Fiber-Cell Plasma Membranes: structure, dynamics, and function

Witold K. Subczynski^a, Marta Pasenkiewicz-Gierula^{a,b}, Marija Raguz^{a,c}, Laxman Mainali^a, James S. Hyde^a
^aNational Biomedical EPR Center, Dept. of Biophysics, Medical College of Wisconsin; ^bDept. of Biophysics & Biotechnology, Jagiellonian University, Krakow, Poland; ^cDept. of Medical Physics & Biophysics, University of Split, Croatia

Introduction: The major direction of our research is the analysis of age-related changes in membranes of human eye lens fiber-cells in order to elucidate major differences occurring in transparent and cataractous lenses. During the last year we focused our efforts to improve methodology of membrane studies from the use of pooled samples (usually 20 lenses) to the single lens level, which will allow us to consider donor health history information provided by the Eye Bank. Significant progress in our studies at the single lens level was possible because of the state-of-the-art EPR techniques available at the National Biomedical EPR Center.

Methods: X-band and W-band EPR spectrometers with saturation recovery (SR) capabilities were used. Development of a new loop-gap resonator (LGR) at W-band [1] and new approaches at X-band [2] has allowed us to perform measurements not only on samples prepared from pools of 20 lenses but also from one or two lenses from a single donor.

Results: Age is the number one risk factor associated with cataract formation, and therefore we decided to study this parameter. In a first experiment, cortical and nuclear membranes were prepared from the total lipid extracts from two groups of donors: 41-60, and 61-70 years of age. In clear lenses, cholesterol (Chol) crystals were found only in the nuclear membranes of the oldest group. We also prepared cataractous samples from 61-70 age donors and compared results with the age matched clear lenses. In cataractous membranes Chol crystals were not detected. Formation of cholesterol bilayer domains (CBDs) was observed in both clear and cataractous lenses in both cortical and nuclear membranes. These results [3] are schematically summarized in Fig. 1. In a second experiment, membranes were prepared from extracts that contained both lipids and proteins. Age groups were 0-20, 21-40, 41-60 and 61-80) years. Results are shown in Fig. 2, and compared with results from single-donor—single-lens samples [4].

Conclusions: Although results suggest that the high Chol content, formation of CBDs, and formation of Chol crystals should not be regarded as major predispositions for the development of age-related cataracts, these results establish that samples prepared from a single lens and from pooled lenses are comparable.

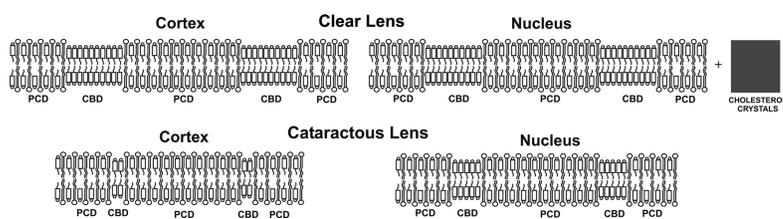


Fig. 1. Schematic drawing of clear (a) and cataractous (b) cortical and nuclear lens-lipid membranes containing bulk phospholipid-cholesterol bilayers (PCD) and the pure cholesterol bilayer domains (CBD), with cholesterol crystals (for the clear nuclear lens-lipid membrane).

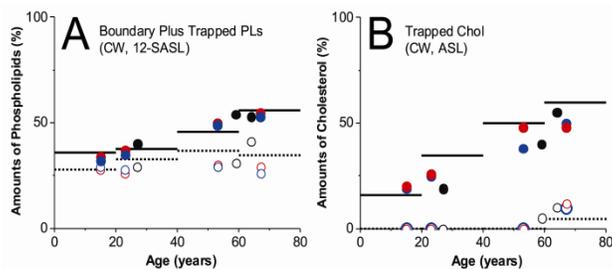


Fig. 2. Amounts of PLs (A, % of total PLs) and Chol (B, % of total Chol) in domains uniquely formed due to the presence of membrane proteins in human intact cortical (.....) and nuclear (—) lens membranes (data for pools of ~20 clear lenses). Data obtained for single donors (○, ○) and single lenses (○, ○) right and (○, ○) left eye are included. Filled symbols are for nuclear and open for cortical membranes.

[1] Mainali, L., Sidabras, J. W., Camenisch, T. G., Ratke, J. J., Raguz, M., Hyde, J. S., Subczynski, W. K.: Spin-label W-band EPR with seven-loop-six-gap resonator: Application to lens membranes derived from eyes of a single donor. *Appl. Magn. Reson.* 45:1343-1358, 2014.

[2] Raguz, M., Mainali, L., O'Brien, W.J., Subczynski, W. K.: Lipid-protein interactions in plasma membranes of fiber cells isolated from the human eye lens. *Exp. Eye Res.* 120:138-151, 2014.

[3] Mainali, L., Raguz, M., O'Brien, W.J., Subczynski, W. K.: Properties of membranes derived from the total lipids extracted from the clear and cataractous human lenses of 61- to 70-year-old donors. *Eur. Biophys. J.* (DOI 10.1007/s00249-014-1004-7), 2015.

[4] Raguz, M., Mainali, L., O'Brien, W.J., Subczynski, W. K.: Lipid Domains in Intact Fiber-Cell Plasma Membranes Isolated from Cortical and Nuclear Regions of Human Eye Lenses of Donors from Different Age Groups. *Exp. Eye Res.* (DOI: 10.1016/j.exer.2015.01.018), 2015.