Research Highlight #153

Spin-label W-band EPR with a seven-loop–six-gap resonator: Application to lens membranes derived from eyes of a single donor

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Introduction & Method: Spin-label W-band (94 GHz) electron paramagnetic resonance (EPR) with a five-loop–four-gap resonator (LGR) was successfully applied to study membrane properties.[1] In that study, samples were equilibrated with the selected gas mixture outside the resonator in a sample volume ~100 times larger than the sensitive volume of the LGR and transferred to the resonator in a quartz capillary. A seven-loop–six-gap W-band resonator has since been developed (Fig. 1) and is described in a manuscript published at the very end of last reporting period.[2] This new resonator permits measurements on aqueous samples of 150 nL volume positioned in a polytetrafluoroethylene (PTFE) gas permeable sample tube. Samples can be deoxygenated or equilibrated with an air/nitrogen mixture inside the resonator, which is required in saturation-recovery measurements and in spin-label oximetry.

Results: This approach was tested for lens lipid membranes derived from lipids extracted from two porcine lenses (single donor). Profiles of membrane fluidity and the oxygen transport parameter were obtained from saturation-recovery EPR using phospholipid analog spin-labels. Cholesterol analog spin-labels allowed discrimination of the cholesterol bilayer domain and acquisition of oxygen transport parameter profiles across this domain. Results were compared with those obtained previously for membranes derived from a pool of 100 lenses and demonstrate that EPR at W-band can be successfully used to study aqueous biological samples of small volume under controlled oxygen concentration.

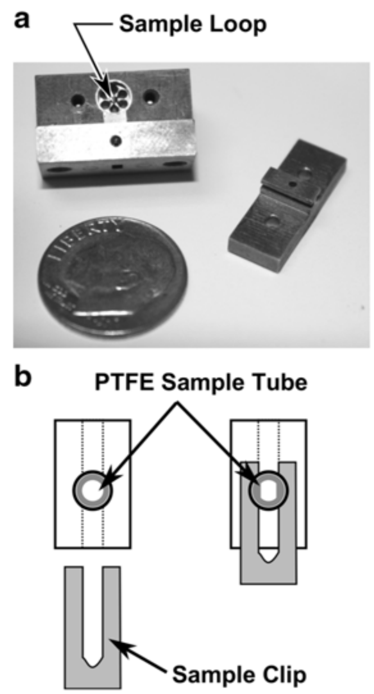

Fig. 1. a) New Seven-Loop–Six-Gap LGR resonator with gas exchange port and sample holder. Highlighted here is the sample loop 0.825 mm ID, located in the center of the Seven-Loop–Six-Gap geometry. b) New sample holding mechanism for PTFE samples uses a clip that compresses the sample tube and holds it in place.

Implications & Discussion: This published work highlights major benefits for eye lens research that are offered by this new EPR approach at W-band. As is the case with many human age-related diseases, age-related cataract formation appears to be unavoidable. Thus, future strategies for slowing cataract formation may depend on a detailed examination of people with age-matched cataractous and normal lenses, as well as those who retain clear lenses into their eighth and ninth decades. For this examination, the individual (single donor) experimental approach is critical, as it will allow acquisition of results for the cortex and nucleus as a function of age and health history of donors. Many agents such as mutations, diet, radiation, drugs, and toxic chemicals, as well as certain diseases, can induce lens opacification. The new approach allows us to include these agents in the donor health history and opens opportunities in eye lens research. The single donor approach combined with EPR spin-labeling methods will help to elucidate the biophysical basis of lens transparency in the human eye at the cell membrane and molecular level and to determine the causes and mechanisms of age-related changes in the lens that lead to cataract formation.

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[1] Mainali L, Hyde JS, Subczynski WK. (2013) Using spin-label W-band EPR to study membrane fluidity in samples of small volume. J Magn Reson. 226:35-44. PMCID: PMC3529815

[2] Mainali L, Sidabras JW, Camenisch TG, Ratke JJ, Raguz M, Hyde JS, Subczynski WK. (2014) 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(12)1343-58. PMCID: PMC4273494.