NANOPOROUS SAMPLE HOLDERS FOR MULTIFREQUENCY/HIGH-FREQUENCY EPR OF FULLY HYDRATED MACROSCOPICALLY ALIGNED SPIN-LABELED MEMBRANE PROTEINS

A.I. Smirnov, A.M. Alaouie (NCSU, Chemistry); J. van Tol, L.-C. Brunel (NHMFL, EMR)

Introduction

Studies of liquid aqueous samples with EPR encounter a problem of high dielectric losses occurring in water at microwave frequencies 2-3 GHz and above. The losses result in non-resonant (magnetic field independent) energy absorption and a rather short penetration depth of electromagnetic field into an aqueous sample. This dictates special arrangements for aqueous samples and specialized sample holders constructed in a way to minimize the Eddy currents through the sample. The latter could be achieved by positioning an aqueous sample within a plane of the magnetic field component of the mm-wave field (*i.e.*, at the electrical field node). In high field/high frequency EPR spectrometers that employ Fabri-Perot resonators this is accomplished by sandwiching an aqueous sample between thin quartz or mylar discs. For example, for water at 20 ^{o}C and 250 GHz the sample thickness should be about 18 μm. Such a sample could be configured by putting together two thin quartz discs with one of the disc having a groove etched in. However, it might be difficult to maintain the 18 μm thickness uniformly throughout the sample. Additional problems arise when macroscopically aligned membrane protein samples are studied with HF EPR. For example, positioning of planar bilayers with director vector perpendicular to magnetic field require a different resonator design. None of the designs described so far allow for convenient examination of physically the same macroscopically aligned membrane protein sample with, for example, at 240 and 9 GHz EPR.

Experimental

Lipid bilayer were prepared from DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine), doped with 1 mol% of spin-labeled phospholipid 5PC (1-palmitoyl-2-stearoyl-(5-doxyl)-sn-glycero-3 phosphocholine) or spin-labeled ion channel gramicidin A. Hydrated lipid samples were deposited onto 60 μm-thick nanoporous anodic aluminum oxide discs (Whatman, Ltd) to form a macroscopically aligned lipid nanotubular structure. The samples were sealed with a polyvinylidene chloride film with a thickness of 25 μm. 240 GHz EPR spectra were recorded with multi-purpose transient EMR spectrometer equipped with a Fabri-Perot resonatore (NHMFL). Consequently, the same samples were examined with a conventional Varian 9 GHz EPR spectrometer (NCSU).

Results and Discussion

Figure 1 shows a representative room temperature 240 GHz EPR spectrum from fully hydrated DMPC/spin-labeled gramicidin A (100:1) aligned by nanoporous AAO. Similar well-aligned spectra were observed from DMPC doped with 1mol% of 5PC. Physically the same AAO discs were used for consequent examination with conventional X-band EPR (not shown). It was determined that polyvinylidene chloride film provides an exceptional moisture seal allowing for several hours of experimentation. We also found that these samples remained to be macroscopically aligned for a period of more than 1 month when stored in a refrigerator at 100 % humidity.

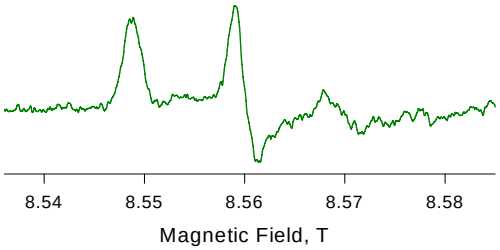

Figure 1. A representative 240 GHz EPR spectrum from DMPC/gramicidin A.

Conclusions

The new technology we describe here allows one to study hydrated and macroscopically aligned membrane proteins with both high frequency/high field and conventional EPR for the first time. Sample handling is also significantly simplified.

Acknowledgements

Support of NHMFL Visiting Scientist Program as well as NIH and DOE (to A.I.S) are gratefully acknowledged.