Characterizing DNP Mechanisms at High Magnetic Fields to Enable Membrane Protein NMR Studies

A.N. Smith, M.A. Caporini, G.E. Fanucci, J.R. Long (University of Florida)

Introduction

Elucidation of membrane protein structure is an integral part of understanding their diverse and highly specific mechanisms of action. However, due to the difficulty in sample preparation (e.g. producing high enough concentrations for NMR or X-ray crystallography experiments) many membrane proteins remain uncharacterized. Magic angle spinning solid state NMR (MAS ssNMR) has shown promise as a key technique in solving membrane protein structure problems. However, the problem of producing enough material for relatively insensitive NMR characterization remains. Recently there has been much interest in utilizing dynamic nuclear polarization (DNP) in conjunction with MAS ssNMR experiments to enhance the sensitivity of the NMR experiments. DNP transfers the high polarization of an unpaired electron in a magnetic field to NMR-active nuclei of interest, thereby greatly enhancing the sensitivity of the NMR experiments [1]. To date, much of the development of DNP techniques for enhancing MAS ssNMR has utilized nitroxide biradicals as the source of electron polarization as they have proven to be efficient enhancement agents at 5-9.4 T via the cross effect. The current belief is that the advantages of these biradicals are the enhanced electron-electron dipolar coupling due to their proximity imposed by molecular tethering and that the g-tensors of the electrons in the most successful explored biradicals are perpendicular to one another. However, mixtures of monoradicals have shown almost the same level of enhancements, via the solid effect, when using radicals with appropriate properties [2,3]. With the initial development of higher field DNP instrumentation at 14.1-18.8 T and 395-527 GHz, the DNP enhancements via biradicals is much less dramatic; enhancements via monoradicals has not to date been published. In addition to the lack of understanding of the DNP mechanism at play at higher fields, the nitroxide biradicals typically used poorly partition into lipid bilayers. Observations of DNP enhancements within lipid membranes for samples incorporating the biradicals are not as dramatic as for protein fibrils and soluble proteins, likely due to the heterogeneous nature of membrane protein samples and the partitioning properties of the biradicals developed [4]. These observations underscore the need to understand DNP mechanisms at high magnetic fields and to develop alternative DNP polarization agents for the study of membrane proteins.

Results and Discussion

One of the confounding factors in developing strategies for sample preparation to maximize DNP enhancements is that many of the samples have not been characterized by conventional EPR experiments at mid to high magnetic fields. Knowing basic properties of the unpaired electrons---their spin concentration, g-tensors, dipolar couplings, relaxation properties etc. will radically improve our understanding of DNP mechanisms at high magnetic fields and allow us to optimize new sample preparation strategies. To date we have begun obtaining high field MAS ssNMR DNP data of liposomes with various combinations of monoradicals. These initial experiments have given lipid NMR signal enhancements of approximately 10. In addition we have begun collecting W-band EPR data on the same liposome/monoradical samples. At present was have only obtained echo detected field sweep data that has allowed us to begin analyzing the g-tensor orientations of the radicals in our liposomes (Fig. 1). We are also in the process of obtaining T_{1e} measurements on the same samples, with plans to make the same measurements at higher fields (336 GHz).

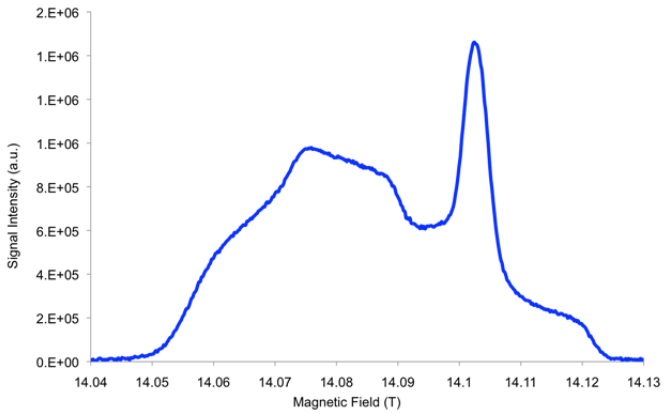

Figure 1: Echo detected field sweep of liposome sample with a wide line and narrow line radical mixture.

References

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