GD(III)-NITROXIDE INTERACTIONS: A MULTIFREQUENCY EPR STUDY

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Introduction

Distance measurements using site-directed spin labeling (SDSL) and EPR are based on magnetic interactions of a nitroxide spin-label with another paramagnetic center. The second center could be another nitroxide label or a paramagnetic metal ion. Previously, distance-dependent relaxation effects of Cu$^{2+}$ (1) and Gd$^{3+}$ (2) on nitroxides were measured with X-band (9 GHz) EPR and analyzed using Leigh’s treatment (3). We are interested in extending the well-established method of SDSL EPR to high magnetic field experiments in order to fully utilize advantages of HF EPR. Here we report on investigation of the mechanism of nitroxide-Gd$^{3+}$ interactions in viscous solutions at magnetic fields from ca. 0.3 to 8 T (corresponding frequencies from 9.5 to 220 GHz) in order to determine relative contributions of dipole-dipole and exchange interactions in nitroxide-Gd$^{3+}$ pairs and to establish which stochastic process modulates dipolar interaction.

Experimental

Slow (as compared with other paramagnetic metal ions) electronic relaxation of Gd$^{3+}$ at magnetic fields above 3 T and highest possible for an ion electronic spin state (S=7/2) results in easily observable relaxation enhancement effects for the nitroxide labels. T$_2$ relaxation effects were measured for continuous wave EPR signal from deuterated-Tempo (PDT). Effects were determined as a slope of linewidth vs. concentration of the relaxant in solutions of various viscosities and as a function of temperature and magnetic field. Gd$^{3+}$ aqua-ion and two Gd$^{3+}$ complexes, Gd-DTPA and Gd-DTPA were chosen as relaxants in our studies. Measurements were done using 95 and 220 GHz EPR spectrometers at NHMFL and conventional 9 GHz EPR at NCSU.

Results and Discussion

We have observed no measurable broadening effect on PDT linewidth when Dy$^{3+}$ was used instead of Gd$^{3+}$. Also, relaxation effects induced by Gd$^{3+}$ were the same for aqua ion and both complexes. These two observations support our hypothesis that Heisenberg exchange contribution to Gd-nitroxide interaction is negligible and that the line width broadening is dominated by dipole-dipole interaction. Absence of micro viscosity effects and strong dependence of broadening upon magnetic field of experiments indicates that dipole-dipole interactions between PDT and Gd$^{3+}$ in 50/50 buffer/glycerol solutions are modulated by electronic relaxation of Gd ions. As a result of an increase in the electronic relaxation rate of Gd$^{3+}$ with increasing the magnetic field, the observed broadening induced by Gd-complex increased from 47 mG/mM at 9.5 GHz to is 84 mG/mM at 95 GHz to 108 mG/m at 220 GHz, illustrating that we can manipulate the magnitude of the dipole-dipole interactions by varying the frequency of the experiment.

Conclusions

Reported results suggest that it would be possible to extend the range of distances measurable in biological systems utilizing Gd$^{3+}$-nitroxide pairs by conducting EPR experiments at high magnetic fields.

Acknowledgements

This work is supported by ACS PRF 40771-G4 and the NHMFL Visiting Scientist Program.

References