TUNABLE-FREQUENCY, HIGH -FIELD EPR OF A HIGH-SPIN FERRIC HEME PROTEIN: MET-MYOGLOBIN

J. Krzystek, D. Smirnov (NMHFL); J.M. Nocek, B.M. Hoffman (Northwestern U., Chemistry);
J. Telser (Roosevelt U., Chemistry)

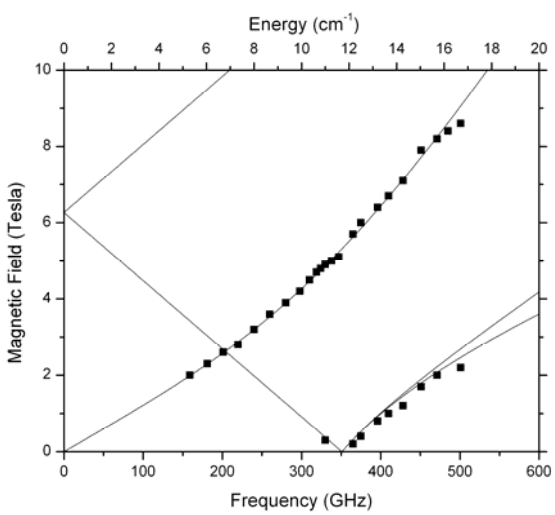
Introduction

Iron is one of the most important elements in biochemistry, comprising the active site in a number of important proteins, such as heme oxygenase and cyochrome P450, the many cytochromes involved in electron transport, and hemoglobin (Hb) and myoglobin (Mb) respectively for oxygen transport and storage.[1] It is very desirable to characterize the ground spin state (typically: $S = 5/2$) of these enzymes using EPR, and this has indeed been done for a long time. However, conventional low-frequency and -field EPR does not deliver much information on spin Hamiltonian parameters of the $S = 5/2$ state because of the typically large zero-field splitting in that state. We have previously showed a method to obtain relevant information somewhat indirectly, using fixed-frequency HFEPR.[2] In this report we present a direct way of obtaining the same parameters.

Experimental

Experiments were performed using the Mm and Sub-mm Wave Spectroscopy Facility and the 25-Tesla resistive "Keck" magnet. The samples were sperm whale metmyoglobin (met-Mb; distal H_2O ligand), and its fluoridated analog (F-met-Mb; distal F^- ligand), both in pH 7 phosphate buffer at ca. 5 mM concentration.

Results and Discussion



Using the tunable-frequency version of HFEPR, we were able to detect inter-Kramers transitions in F-met-Mb (see Figure 1). These are transitions *between* different $\pm M_S$ multiplets (where $M_S = 1/2$, 3/2 and 5/2), in addition to conventionally observable resonances within the $\pm 1/2$ spin multiplet. These inter-Kramers resonances allow direct determination of *intrinsic* (as opposed to effective) spin Hamiltonian parameters for the ground state of the protein. In the case presented here, the parameters are: $S = 5/2$, $D = +5.85$ cm^{-1}, $E = 0$, $g_{iso} = 2.00$. An analogous experiment with met-Mb remains inconclusive, since the zero-field transition from which the inter-Kramers resonances originate is estimated to appear above 600 GHz, i.e., near the limit of the BWO sources. New effort to detect resonances in that system using a new high-frequency BWO source is in progress.

Figure 1. 2-D field/frequency (energy) map in F-met-Mb at 4.2 K. Squares are experimental points. Curves were simulated using spin Hamiltonian parameters as in text.

Conclusions

High-energy BWO sources allow direct determination of zfs in an important metalloprotein, fluoro-met-myoglobin. There is great potential for similar studies on the many other heme proteins.

Acknowledgements

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References

[1] Bertini, I., et al., *Bioinorganic Chemistry*, University Science Books: Mill Valley, CA (1994).
[2] Krzystek, J., et al., 2002 Annual NHMFL Research Review, p. 74.