High-Field EPR on the Mn(II) Centers in Oxalate Decarboxylase

Angerhofer, A., Montoya, A. (UF, Chemistry); Ozarowski, A. (NHMFL)

Introduction

Oxalate Decarboxylase (OxDC) is a Mn-dependent enzyme that utilizes dioxygen, apparently as a co-catalyst, in the redox-neutral decarboxylation of oxalate into carbon dioxide and formate. It contains two Mn ions which are essential for catalysis, the so-called N- and C-terminal Mn even though only the N-terminal ion participates directly in the reaction. E280Q is a "single-site" mutant in which only the N-terminal Mn(II) binding pocket is occupied while the C-terminal one is empty.[1] It can therefore be used as a model for small molecule binding in the wild-type. A recent low pH crystal structure demonstrated that the N-terminal Mn binds acetate.[2] X-band EPR demonstrated that bound acetate, succinate, and formate affect the magnetic parameters of the oxidized enzyme where the N-terminal Mn is in the +3 oxidation state.[3]

Experimental

We have carried out pH dependent high-field EPR experiments with the OxDC E280Q mutant as well as wild type OxDC in the presence and absence of small molecules as Mn ligands. Sample preparations were carried out as described before.[1,3] The pH was poised at 4.6 for the low-pH experiments and at 8.5 for the high pH measurements. The sample was frozen to 20 K for each measurement. The multi-frequency EPR instrument based on the 15/17 T SC magnet at the EMR Facility was used for the experiments and multiple frequencies were used in order to obtain more information about the Mn(II) fine structure parameters.

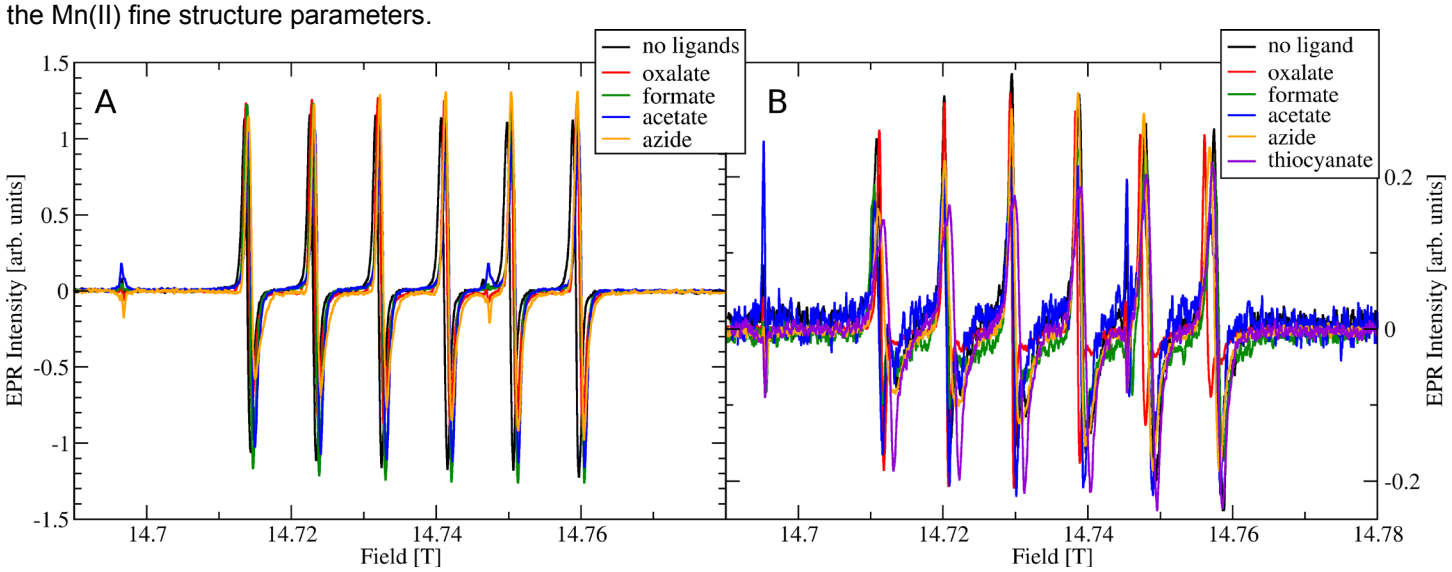

Fig.1 (A) High pH (8.5). (B) Low pH (4.6). High field EPR spectra of OxDC E280Q in the presence of ligands at 20 K.

Results and Discussion

Fig.1 shows the results of the experiments on E280Q at high (panel A) and low (panel B) pH. One can see small differences in the hyperfine coupling and small shifts in the g-value (as a horizontal shift of the center of gravity of the sextet of lines) when small molecules bind to the N-terminal Mn(II) ion. The spectral changes are more pronounced at low pH, particularly for oxalate which shows a smaller hyperfine splitting between the sextet signals indicating bidentate binding. On the other hand, thiosulfate binding leads to both a smaller hyperfine coupling and a shift to higher fields (lower g-values). These spectra will have to be analyzed more carefully for the magnetic parameters *A* (hyperfine coupling), *g*, and the fine structure parameters *D* and *E* to fully characterize the binding events of small ligand molecules. Theoretical calculations of the magnetic parameters based on binding models from single crystal data are currently under way.

Acknowledgements

The National High Magnetic Field Laboratory is supported by the National Science Foundation through NSF/DMR-1157490/1644779 and the State of Florida.

References

[1] Moomaw, E.W., *et al.*, *Biochemistry*, **48**, 6116-6125 (2009).
[2] Angerhofer, A., *et al.*, PDB ID: 5VG3.
[3] Twahir, U.T., *et al.*, *Biochemistry* **55**, 6505-6516 (2016).