Multi-Frequency EPR on Oxalate Decarboxylase Reveals a Mn(II) Site with Large Fine Structure

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Introduction

Oxalate Decarboxylase (OxDC) from *Bacillus subtilis* is a Mn-dependent enzyme that contains two structurally similar Mn-binding domains. The N-terminal Mn site has been shown by X-ray crystallography to be solvent exposed through a flexible region in the amino acid sequence which can act as a lid while the C-terminal site does not show any apparent solvent channel at pH 8 but its Mn(II) may be pentacoordinated to three His, one Glu, and one water molecule [1]. Prior multi-frequency EPR experiments revealed at least two Mn^{2+} ions in the enzyme with medium-sized fine structure parameters of $|D|$ = 1200 (site 2) and 2700 MHz (site 1), respectively [2]. Here we report on the observation of a third site with very large $|D|$.

Experimental

Preparation of OxDC was performed as reported before [2]. Experiments were carried out using the high-field EPR facilities at the NHMFL with OxDC in overlapping buffer systems to avoid artifacts from buffer switching. The buffers used in the experiments were approx. 200 mM each of N,N'-dimethylpiperazine, piperazine, BIS-TRIS, and TRIS.

Results and Discussion

Fig. 1A shows the results of high-field EPR experiments of OxDC at various pH values in frozen solution on the low-field side of the main Mn(II) sextet. Apart from the relatively broad signals that are due to the higher electron spin manifolds of sites 1 and 2 one can see two relatively narrow lines near 14.46 T. They belong to the central +1/2 ↔ –1/2 sextet transitions of a third site (3) with a much larger fine structure splitting of approximately |D| =10,600 MHz and |E| = 1,300 MHz (see simulation in fig. 1B). Similarly high fine structure values have previously been observed in Mn-superoxide dismutase and arise from pentacoordinated Mn(II) [3]. This suggests that site 3 arises from the C-terminal Mn(II).

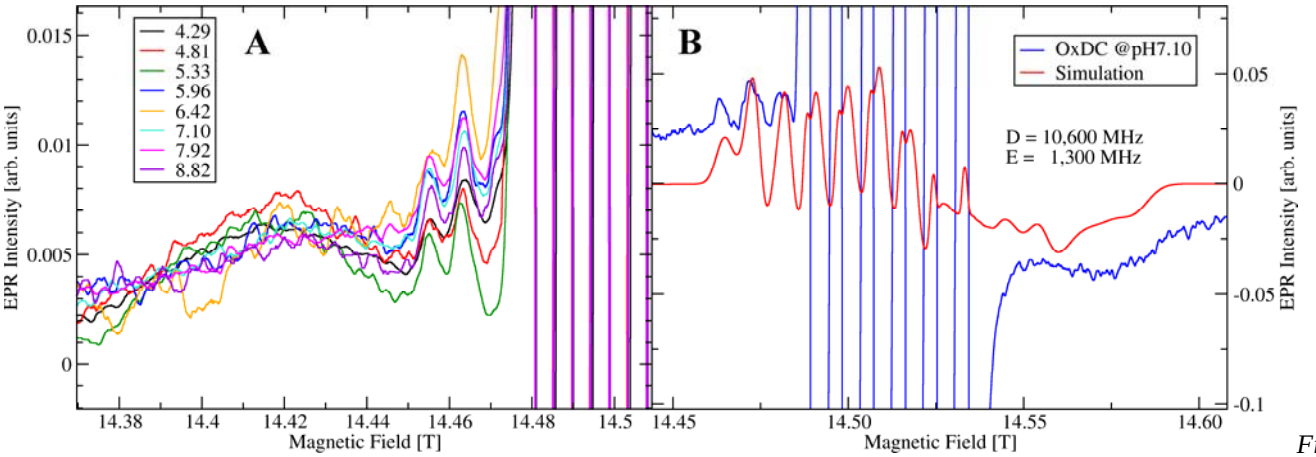

Fig. 1: A: pH Dependence of the low field wings of Mn(II) in OxDC with 20 G modulation amplitude, 406.4 GHz and 20 K. B: Simulation of a more highly resolved spectrum at pH7.10.

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References

[1] Just, V.J., *et al.*, Journal of Biological Chemistry **279**, 19867-19874 (2004).
[2] Angerhofer, A., *et al.*, Journal of Physical Chemistry **B 111**, 5043-5046 (2007).
[3] Tabares, L.C., et al., Biochemistry **45**, 1919-1929 (2006)