Manganese Redox States In *Bacillus subtilis* Oxalate Decarboxylase

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Introduction

Oxalate Decarboxylase (OxDC) from *Bacillus subtilis* is a Mn-dependent enzyme that catalyzes the degradation of oxalate to carbon dioxide and formate. OxDC is a member of the bicupin superfamily of enzymes, with a mononuclear Mn(II) coordinated in each of its two domains. The reaction is redox-neutral but it has been proposed that oxygen is a co-catalyst necessary to drive an initial proton-coupled electron transfer step in the catalytic cycle, suggesting Mn(III) is present during the enzymatic mechanism. Mn(III) has been observed in the related oxalate oxidase [1] where the Mn ion cycles through oxidation states during turnover. We have previously observed the EPR signal of Mn(III) in chemically oxidized OxDC in parallel mode in the X-band. Low-field can only conclusively report on the N-terminal site because of the large fine structure of the C-terminal Mn(II). Here we report on high-field EPR spectra of redox-cycled OxDC with the goal to distinguish between the N- and C-terminal Mn sites and to find out whether only one or both can be oxidized.

Experimental

WT-OxDC was prepared as previously described [2]. Samples studied were dialyzed into respective buffers, and hexachloroiridate was used as a mild oxidant. It was added to the samples in a stepwise fashion until no further spectral changes were observed. Spectra were collected at the EMR facility on a 15/17 T SC magnet.

Results and Discussion

Due to sensitivity issues with the enzyme only the relatively sharp sextet of lines near g = 2 were observed for Mn(II). We did not find any spectral features that could be interpreted as Mn(III). However, the intensity of the Mn(II) species was affected by oxidation and could be used as an indication of the production of oxidized Mn. Fig. 1A (left panel) shows a partially split sextet indicative of the two different Mn(II) sites in the enzyme [3]. Upon oxidation of OxDC at low pH with hexachloroiridate both signals vanish to almost zero intensity while reduction of the same sample with ascorbate revives them, albeit with different linewidths and somewhat different splitting patterns. Panel B of fig. 1 shows the same experiment at high pH with essentially the same result, except that at this pH the C-terminal Mn(II) is not visible because of its large zero field splitting parameter of more than 10 GHz. Careful inspection of the spectrum of the oxidized species reveals an additional signal that may possibly be due to a tyrosyl radical which has been observed under oxidative conditions at high pH before [4].

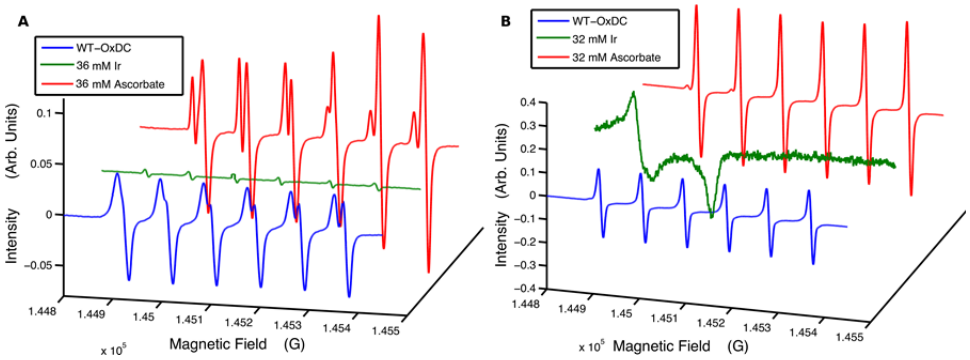

Fig. 1: Redox cycling of Mn centers in WT-OxDC, at 406.4 GHz and 20 K. (A) pH 4.0 in 50 mM succinate buffer (B) pH8.0 in 50 mM phosphate buffer.

Conclusions

From this study it is apparent that both sites have the ability to be reversibly oxidized to Mn (III). This indicates that the C-terminal could play more than a structural role, and may be involved in catalysis.

Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy. An NSF grant no. CHE-121440 to A. Angerhofer is also acknowledged.

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