High-Field EPR of Wild-Type Oxalate Decarboxylase Immobilized on IMAC Resin

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Introduction

Oxalate decarboxylase catalyzes the interesting redox-neutral conversion of oxalate into formate and carbon dioxide utilizing molecular dioxygen as a co-catalyst. Prior EPR studies of the two distinct Mn centers in the enzyme showed a pH dependence of the magnetic parameters but quantitative analysis was hampered by low signal-to-noise ratios due to solubility problems of the enzyme at low pH. Here, we describe a protocol to immobilize the enzyme on a Zn-loaded IMAC resin which not only allows us to study samples of high enzyme concentration of the order of 100 mg/mL, but also facilitates reuse of the enzyme for sequential spectra at different pH values.

Experimental

Uncharged Profinity IMAC resin (#156-0121) was purchased from BioRad. Zn ions were loaded onto approx. 200 μL of starting resin slurry according to the procedures in the instruction manual. 400 μL of approx. 20 mg/mL solution was repeatedly flowed over the freshly metal-loaded resin contained in a custom tube fitted with a polypropylene filter for 5 μm particles. The tube is used to hold the enzyme-loaded resin while buffers of different pH can be flowed through the solution, and it also serves as the sample holder for low-temperature EPR.

Results and Discussion

Fig. 1 shows the high-field EPR spectra of immobilized WT OxDC at various pH levels taken at a temperature of approx. 3 K. At this temperature the main signal arises from transitions between the higher spin manifolds, *i.e.*, m_S = -5/2 to -3/2. There are small shifts of the lines with pH but more significantly, the relative intensity decreases with lower pH. The spectrum at pH 8.5 was taken after the sample had been fully cycled through lower pH values. Since its intensity almost matches that of the spectrum at pH 8.0, loss of enzyme during the low pH cycle appears to be a minor issue. While quantitative EPR is still difficult given the fact that the whole EPR probe has to be removed from the cryostat every time a new sample is to be measured, the data shows a qualitative decrease of the Mn(II) concentration at low pH. This could be due to the pH dependence of the redox potentials of the Mn ions in the protein.

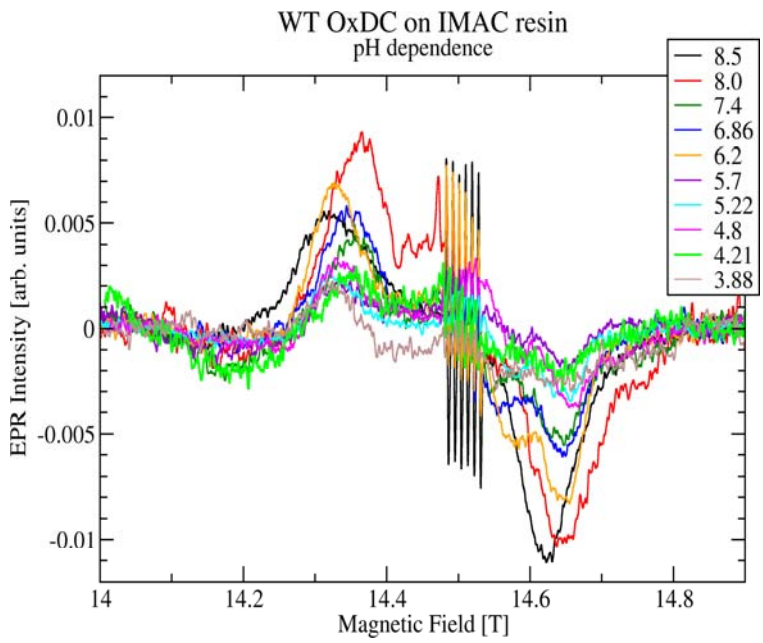

Figure 1: High-field EPR of WT OxDC on Zn-loaded IMAC resin as a function of pH. Please note that the spectra were taken on two samples that were successively stepped down in pH. At the end of the experiment, one sample's pH was raised to 8.5 (black trace).

Conclusion

We have succeeded in immobilizing OxDC which leads to higher signal-to-noise ratios for our preparations which we will utilize for a full quantitative EPR study of the pH dependence in order to clarify the contributions of the two distinct Mn species.

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References

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