Effect of Dioxygen Exposure on Hydrogen Peroxide Production by *Bacillus subtilis* Oxalate Decarboxylase in the Absence of Substrate

Megan Booth and Dr. Alexander Angerhofer

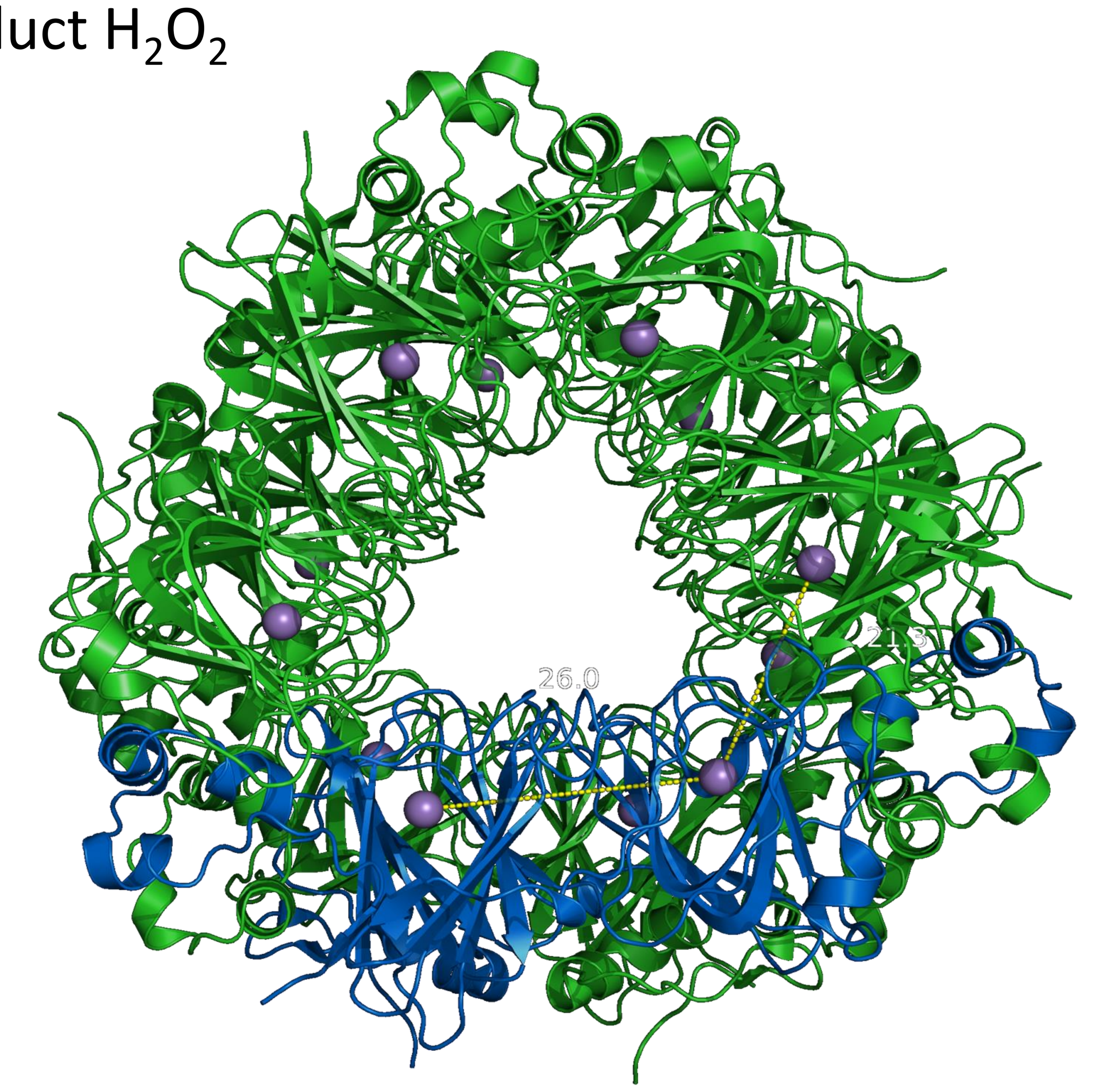
Department of Chemistry, University of Florida, Gainesville FL 32611-7200, USA

Introduction

Oxalate decarboxylase (OxDC) from *Bacillus subtilis* is a manganese dependent enzyme that catalyzes the breakdown of oxalate into CO_2 and formate using bound O_2 as a cofactor.[1,2] It is known that Mn(III) drives the reaction; however, the curious dual dependence on O_2 has garnered attention, including questions such as

- Where does O_2 bind?
- Is there a LRET process involved between bound O_2 and the catalytically active Mn?
- Is O_2 only required to generate Mn(III) or does it actively participate in decarboxylation?

The answer to this first question awaits further study; however, new evidence presented herein supports the theory that O_2 binding drives Mn oxidation, acting as an electron sink to form superoxide and its acid catalyzed dismutation product H_2O_2



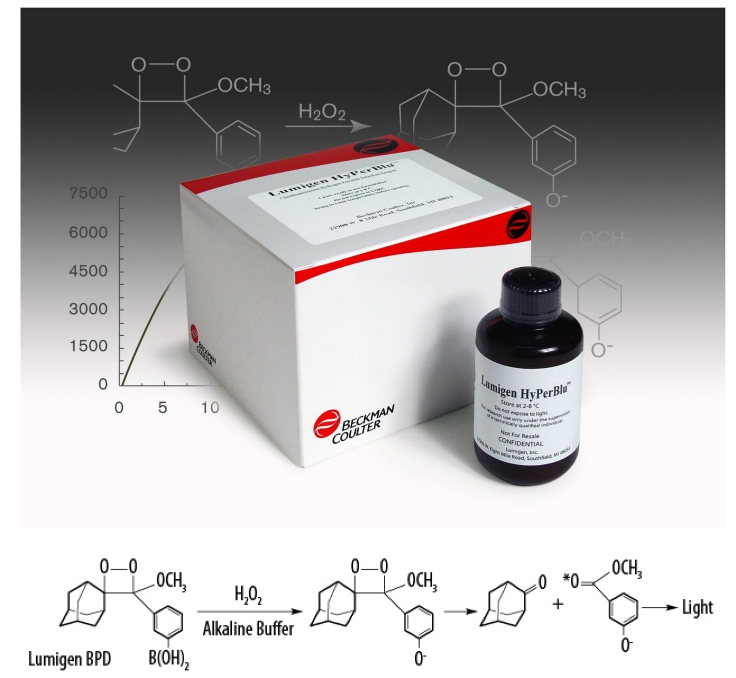
Unpublished crystal structure of wild type OxDC. Manganese centers are shown in purple. Monomer is denoted in blue.

Methodology

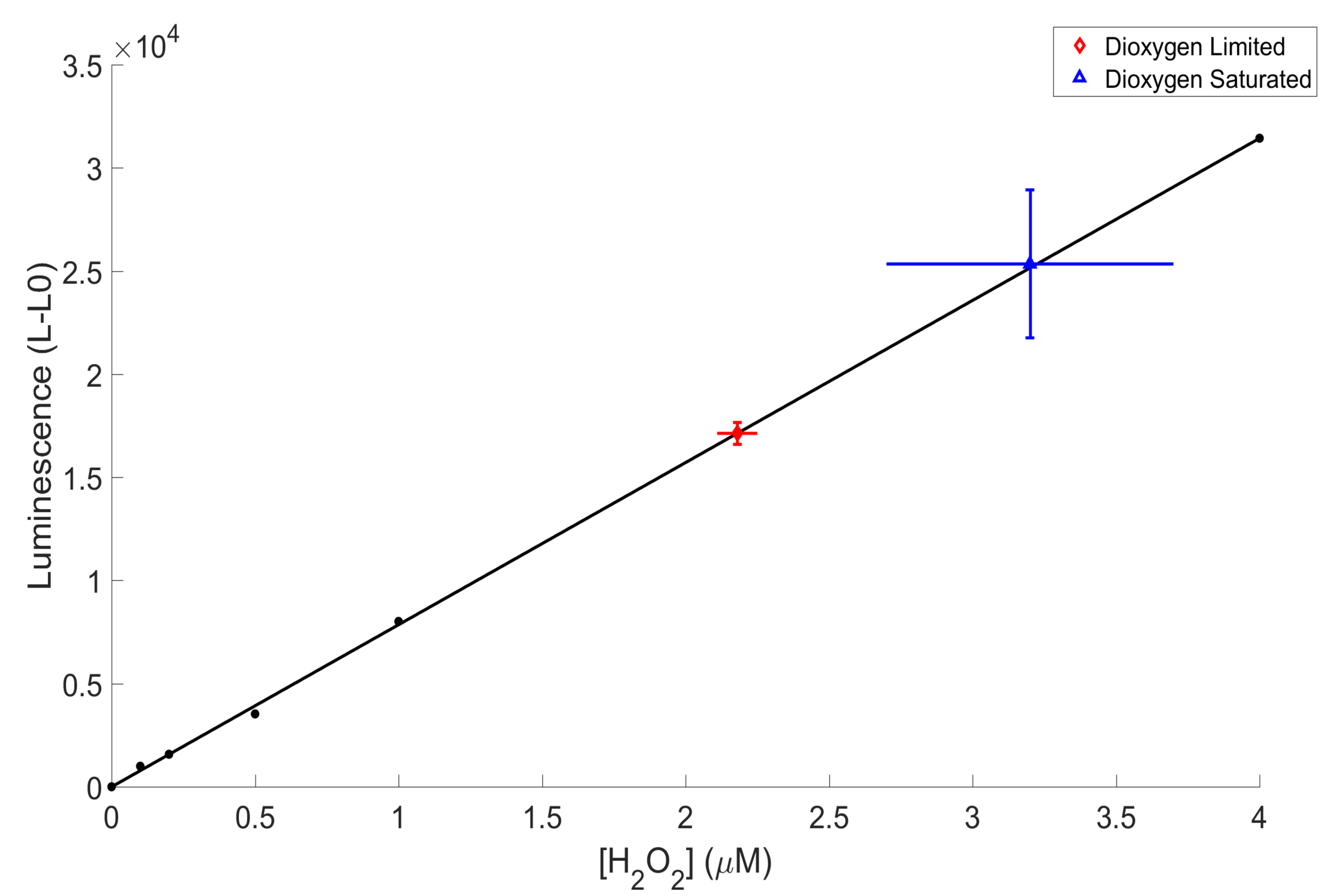
Enzyme expression, purification, and concentration

Anaerobic dialysis from pH 8.5 to 4.0 and reintroduction of O_2

HyPerBlu chemiluminescent assay to determine extent of H_2O_2 production



Results



	Dioxygen Limited	Dioxygen Saturated
Final [OxDC]	390 ± 30 μM	340 ± 30 μM
$[H_2O_2]$ Produced	2.18 ± 0.07 μM	3.22 ± 0.45 μM
$[H_2O_2]/[OxDC]$	5.6 ± 0.5 ($\times10^{-3}$)	9.5 ± 1.5 ($\times10^{-3}$)
% Difference in $[H_2O_2]/[OxDC]$	70%	

Conclusions

- A 70% increase in H_2O_2 production was observed upon saturating OxDC with O_2 suggesting a direct correlation between O_2 binding and Mn oxidation
- Future experiments will be focused on establishing a true baseline for H_2O_2 by ensuring O_2 is eliminated from the system. This will allow further studies to see if activity is solely dependent on Mn oxidation or if O_2 plays an active role in addition to facilitating oxidation

References

1. Tanner, A. et al. (2001) *J. Biol. Chem.* **276**: 43627–43634.
2. Twahir, U et al. (2016) *Biochemistry.* **55**: 6506-6516.

Funding

Funding for this project was provided by the University of Florida's University Scholars Program