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X-ray Crystallographic Investigations of the Structure and Function of Oxidoreductases

by

Ross Andrew Edwards

A dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in the

Institute of Fundamental Sciences, Chemistry

at

MASSEY UNIVERSITY, NEW ZEALAND

1999
X-ray Crystallographic Investigations of the Structure and Function of Oxidoreductases

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This thesis is dedicated to

The Venerable Fredrick John Ford

in tribute to his role in inspiring thoughtfulness and for sharing a fine understanding of the enjoyment of this adventure that is life.
Abstract

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Massey University, New Zealand

1999

Associate Professor Geoffrey B. Jameson, Professor Edward N. Baker, Supervisors

The structure and function of oxidoreductases were studied using the manganese-containing superoxide dismutase of *Escherichia coli* as the model system. The technique of single-crystal X-ray crystallography was used to determine the three-dimensional structure of this system. The structures of derivatives of this system, including iron-substituted manganese superoxide dismutase, and the five mutants Y34F, Q146H, Q146L, H30A and Y174F, were also determined. Analysis of these structures on a near-atomic scale revealed new structural aspects to the catalytic mechanism of this group of enzymes.

A structural basis for the inactivity of *E. coli* Fe-substituted MnSOD has been determined in the altered geometry of the metal site on substitution of the non-native metal. The change in geometry from active five-coordinate trigonal-bipyramidal to inactive six-coordinate distorted octahedral modifies both the kinetics and thermodynamics of superoxide dismutation at the enzyme’s metal centre.

Gln146 is not essential for activity, but has an important role in optimising the reaction. Unlike the naturally active His146-containing MnSOD enzymes, the mutation of *E. coli*
MnSOD Gln146 to histidine largely inactivates the enzyme. The inactivity may be a consequence of the greater inflexibility of the mutated histidine when compared with its natural counterparts.

The lack of any change in both the primary and secondary coordination shells of the H30A mutant active-site, coupled with a 70% reduction in catalytic activity, indicate an important role for His30 in optimising the catalytic mechanism.

It is likely that the 60% reduction in catalytic activity of the Y174F mutant is due to a different orientation, and possibly different effective pKa of His30, although a loss of activity due to the slight differences of the primary and secondary coordination spheres can not be entirely ruled out.

Structural evidence supports a role for Tyr174 in orienting and possibly also modifying the pK of His30. The association of His30, in particular via its ND1 nitrogen, with aspects of the catalytic mechanism including interaction or protonation of substrate, can be postulated based on its structural behaviour.
This thesis is not the work of an individual, merely the end result of the influence of many, and I recognise those contributions here with gratitude.

I thank my supervisor, Associate Professor Geoffrey Jameson for generous contributions both scientifically and financially. Over the course of my PhD Geoff has only ever been supportive, open-minded and insightful. I wish to thank my secondary supervisor Professor Ted Baker, for his contributions, both financial and scientific. I acknowledge the foresight of my undergraduate Professor, Ward T. Robinson, who set me on this path over four years ago. This work was done in close collaboration with Drs. Jim and Mei Whittaker, who provided excellent biochemical and chemical results, enzyme and crystals.

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Abbreviations

ADP    adenosine diphosphate
ANIS   SHELXL specific command, refine atoms anisotropically
ATP    adenosine triphosphate
CCP4   Collaborative Computational Project, Number 4
CoSOD  cobalt superoxide dismutase
CuSOD  copper superoxide dismutase
CuZnSOD copper, zinc superoxide dismutase
DESY   Deutsches Elektronen-Synchrotron
DMF    dimethylformamide
DNA    deoxyribonucleic acid
DORIS  Double Ring Storage (for synchrotron radiation)
EDTA   Ethylenediaminetetraacetic acid
EMBL   European Molecular Biology Laboratory
EPR    electron paramagnetic resonance
EXAFS  extended X-ray absorption fine structure
Fe₂MnSOD iron-substituted manganese superoxide dismutase
FeSOD  iron superoxide dismutase
HOPE   SHELXL specific command, refine anisotropic scaling parameters
ISOR   SHELXL specific command, 'approximately isotropic' restraints
MPD    2-methyl-2,3-pentanediol
MnSOD  manganese superoxide dismutase
NADH   reduced form of nicotinamide adenine dinucleotide
<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>NADPH</td>
<td>reduced form of nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NCS</td>
<td>non-crystallographic symmetry</td>
</tr>
<tr>
<td>NHE</td>
<td>normal hydrogen electrode</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NiSOD</td>
<td>nickel superoxide dismutase</td>
</tr>
<tr>
<td>PDB</td>
<td>protein data bank</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>RES</td>
<td>resolution</td>
</tr>
<tr>
<td>RMS</td>
<td>root mean square</td>
</tr>
<tr>
<td>SO</td>
<td>superoxide</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
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The results presented in chapters two and three have been published in peer-reviewed journals.
