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

by

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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

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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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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.

Acknowledgments

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.

I would also like to thank the numerous friendships made over the years both in and out of the Massey environment, that have shaped my experiences and given my life meaning:

Justin Corey, a most constant and great friend

Aaron Mikaere, David Fairley and Grant Samuels

Bryan, Andrew, Richard, Neil, Jakki and Paul, Rose, Catherine, Heather, Paul, Phil, Maria, Bin, Julian, Rebecca, Richelle, Rochelle, Todd

Dr. Stan Moore (the rudest Moose in the woods, mentor and fishing buddy)

Puck, of singular importance, thanks for keeping me sane in Hamburg - and for being yourself!

Katja, a close friend and great travelling companion, and Steffi

Esther

Saeid Miri, a generous friend

my flatmates, Sandy, Farah, Jeniffer, Carman, Adrian, Vitri and Sia who together share many great memories of 11 Keiller Place

John Ford (to whom this work is dedicated)

I especially thank my family for all their love and support:

Gloria, Graham, Peter and Nicola

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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
Fe_2MnSOD	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

NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
NCS	non-crystallographic symmetry
NHE	normal hydrogen electrode
NMR	nuclear magnetic resonance
NiSOD	nickel superoxide dismutase
PDB	protein data bank
PEG	polyethylene glycol
RES	resolution
RMS	root mean square
SO	superoxide
SOD	superoxide dismutase
SWAT	SHELXL specific command, refine diffuse solvent parameter
UV	ultra-violet
WT	wildtype
apoFeSOD	iron-free iron superoxide dismutase
apoMnSOD	manganese-free manganese superoxide dismutase
hMnSOD	human mitochondrial manganese superoxide dismutase
redMnSOD	reduced manganese superoxide dismutase

Related Publications

The results presented in chapters two and three have been published in peer-reviewed journals.

R. A. Edwards, H. M. Baker, M. M. Whittaker, J. W. Whittaker, G. B. Jameson, and E. N. Baker. Crystal structure of *Escherichia coli* manganese superoxide dismutase at 2.1 Å resolution. *Journal of Biological Inorganic Chemistry*, **3**:161–171, 1998.

R. A. Edwards, M. M. Whittaker, J. W. Whittaker, G. B. Jameson, and E. N. Baker. Distinct metal environment in Fe-substituted manganese superoxide dismutase provides a structural basis of metal specificity. *Journal of the American Chemical Society*, **120**(37):9684-9685, 1998.