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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.



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# Abstract

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Doctor of Philosophy

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.

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# Table of Contents

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<b>Abstract</b> .....	vii
<b>Acknowledgments</b> .....	ix
<b>Table of Contents</b> .....	xi
<b>List of Figures</b> .....	xv
<b>List of Tables</b> .....	xvii
<b>Abbreviations</b> .....	xix
<b>Related Publications</b> .....	xxi
<b>CHAPTER 1. Introduction</b> .....	1
1.1. Superoxide chemistry .....	1
1.1.1. Electronic structure .....	1
1.1.2. Redox reactions of superoxide .....	2
1.1.3. The dismutation reaction in aqueous solution .....	4
1.2. Superoxide in biochemical systems .....	4
1.2.1. Production of superoxide <i>in vivo</i> .....	5
1.2.2. Superoxide as a damaging species .....	5
1.2.3. Cellular defence from oxidative damage .....	8
1.2.4. The regulatory role of superoxide in <i>E. coli</i> .....	8
1.2.5. Interactions with enzymes other than superoxide dismutases .....	9
1.3. Superoxide dismutase .....	9
1.3.1. Historical perspective .....	10
1.3.2. The copper-zinc superoxide dismutases .....	11
1.3.3. The manganese, iron and nickel superoxide dismutases .....	11
1.3.3.1. Transition metal binding .....	11
1.3.3.2. Metal-specific superoxide dismutases .....	12
1.3.3.3. Metal-non-specific superoxide dismutases .....	12
1.3.3.4. Inactivation of FeSOD by hydrogen peroxide .....	13
1.3.4. Evolution .....	13
1.4. Structural aspects of Mn and Fe superoxide dismutases .....	14
1.4.1. Primary structure .....	14
1.4.2. Secondary and tertiary structure .....	14
1.5. Kinetics and the catalytic mechanisms of Fe and MnSODs .....	17

1.5.1.	Kinetics of MnSODs .....	17
1.5.2.	Kinetics of FeSODs.....	19
1.5.3.	Kinetics of cambialistic SODs .....	22
1.5.4.	The role of reduction-oxidation potentials on catalysis .....	22
1.5.5.	pH dependence of activity and inhibition.....	24
1.6.	<i>E. coli</i> iron and manganese superoxide dismutases.....	25
1.6.1.	Hybrid superoxide dismutases.....	26
1.6.2.	Manganese superoxide dismutase properties and features.....	26
1.6.2.1.	Metal ion .....	26
1.6.2.2.	Redox forms.....	26
1.6.2.3.	Ligands.....	27
1.6.2.4.	Functional inequivalence of Mn and Fe forms .....	28
1.7.	Research aims .....	28

**CHAPTER 2. Crystal Structure of *Escherichia coli* Manganese Superoxide Dismutase at 2.1 Å Resolution .....** 31

2.1.	Introduction.....	31
2.2.	Structure determination.....	32
2.2.1.	Purification .....	32
2.2.2.	Crystallisation.....	32
2.2.3.	Data collection and processing.....	33
2.2.4.	Structure solution .....	35
2.2.5.	Model building and refinement .....	35
2.3.	Structure description and discussion.....	36
2.3.1.	Model validation.....	36
2.3.2.	Polypeptide chain conformation.....	38
2.3.3.	Quaternary structure .....	40
2.3.4.	The active site.....	41
2.3.5.	The wider active-site region .....	44
2.3.6.	Solvent regions adjoining the active site .....	44
2.3.7.	The significance of the dimer .....	45
2.3.8.	Comparison with <i>E. coli</i> FeSOD.....	47
2.4.	Structure determination of an azide derivative.....	52
2.5.	Conclusions.....	53

**CHAPTER 3. Crystal Structure of Iron-substituted *Escherichia coli* Manganese Superoxide Dismutase at 2.2 Å Resolution .....** 55

3.1.	Introduction.....	55
3.2.	Structure determination.....	56
3.2.1.	Purification .....	56
3.2.2.	Crystallisation.....	56
3.2.3.	Data collection and processing.....	58
3.2.4.	Structure solution .....	61
3.2.5.	Model building and refinement .....	61
3.3.	Structure description .....	63
3.3.1.	Model validation.....	63
3.3.2.	Secondary, tertiary and quaternary structure.....	64
3.3.3.	Active site of azide-free Fe <sub>2</sub> MnSOD .....	65

3.3.4.	Comparison with native MnSOD <i>E. coli</i> .....	67
3.4.	Fe <sub>2</sub> MnSOD-azide complex.....	72
3.5.	Discussion.....	73
3.6.	Conclusions.....	76

**CHAPTER 4. Crystal Structures of Active-site Mutants of *Escherichia coli* Manganese Superoxide Dismutase .....** 77

4.1.	Introduction.....	77
4.1.1.	Rationale of mutants.....	78
4.1.2.	Characterisation of mutants of Tyr34.....	79
4.1.3.	Characterisation of mutants of Gln146.....	81
4.2.	Structure determination of Y34F.....	83
4.2.1.	Crystallisation.....	83
4.2.2.	Data collection and processing.....	83
4.2.3.	Structure solution, model building and refinement.....	83
4.3.	Structure determination of Q146L.....	83
4.3.1.	Data collection and processing.....	83
4.3.2.	Structure solution, model building and refinement.....	85
4.4.	Structure determination of Q146H.....	86
4.4.1.	Data collection and processing.....	86
4.4.2.	Structure solution, model building and refinement.....	86
4.5.	Structural description of Y34F.....	86
4.5.1.	Structural effects of the Y34F mutation.....	86
4.5.2.	Tertiary structure.....	88
4.6.	Structural description of Q146H.....	90
4.6.1.	Structural effects of the Q146H mutation.....	90
4.6.2.	Tertiary structure.....	92
4.6.3.	Comparison with natural His146 MnSOD from <i>P. shermanii</i> .....	93
4.7.	Structural description of Q146L.....	94
4.7.1.	Structural effects of the Q146L mutation.....	94
4.7.2.	Tertiary Structure.....	96
4.8.	Discussion.....	96
4.8.1.	Y34F.....	96
4.8.2.	Q146H.....	98
4.8.3.	Q146L.....	99
4.9.	Conclusions.....	100

**CHAPTER 5. Crystal Structures of Gateway Mutants in *Escherichia coli* Manganese Superoxide Dismutase .....** 101

5.1.	Introduction.....	101
5.2.	Structure determination of H30A.....	102
5.2.1.	Crystallisation.....	102
5.2.2.	Data collection and processing.....	102
5.2.3.	Structure solution, model building, and refinement.....	102
5.3.	Structure determination of Y174F.....	104
5.3.1.	Data collection and processing.....	104
5.3.2.	Model building and refinement.....	105
5.4.	Structural description of H30A.....	106

5.4.1.	Structural effects of the H30A mutation .....	106
5.5.	Structural description of Y174F .....	107
5.5.1.	Structural effects of the Y174F mutation .....	107
5.5.2.	NCS analysis .....	111
5.5.3.	Comparisons of Y174F with native.....	114
5.5.4.	Crystal packing.....	116
5.6.	Discussion.....	118
5.6.1.	H30A.....	119
5.6.2.	Y174F.....	119
5.7.	Conclusions.....	120

**CHAPTER 6. Atomic Resolution Crystal Structure of *Escherichia coli* Y174F Manganese Superoxide Dismutase .....** 123

6.1.	Introduction.....	123
6.2.	Data collection and processing .....	124
6.2.1.	Crystal mounting and freezing .....	124
6.2.2.	Data collection and processing <i>in-house</i> .....	125
6.2.3.	Collection and processing of <i>synchrotron</i> data .....	126
6.3.	Synchrotron X-ray data quality.....	130
6.3.1.	Accuracy of cell dimensions .....	130
6.3.2.	Diffraction limits and effective resolution .....	133
6.4.	Benefits of atomic resolution data of <i>E. coli</i> Y174F MnSOD.....	133
6.4.1.	In investigations of Mn and Fe superoxide dismutases.....	133
6.4.2.	Advancement of crystallographic techniques and protocols.....	135
6.4.3.	<i>Ab initio</i> structure solution .....	135

**CHAPTER 7. Perspectives.....** 137

7.1.	Concluding remarks.....	137
------	-------------------------	-----

**Appendix A: Selected active-site bond lengths and angles .....** 139

**Appendix B: Surface Area of Interface Residues .....** 142

**Appendix C: Mn and FeSOD Sequence Alignments .....** 143

**Bibliography .....** 153

# List of Figures

<b>CHAPTER 1.</b>	1
Figure 1-1. Molecular-orbital diagrams for oxygen and superoxide	2
Figure 1-2. Ionizations of dioxygen, superoxide and hydrogen peroxide	3
Figure 1-3. Schematic diagram of the <i>E. coli</i> MnSOD monomer	15
Figure 1-4. Proposed reaction scheme for MnSOD-catalysed superoxide dismutation	18
Figure 1-5. Reaction mechanism for MnSOD proposed by Whittaker <i>et al.</i> 1996	19
Figure 1-6. Scheme for the enzyme-catalyzed dismutation of superoxide as proposed by Lah <i>et al.</i> 1995	21
Figure 1-7. Reduction potential tuning of metal ions by SOD enzyme structure	23
Figure 1-8. Reaction scheme for Fe-substituted MnSOD from <i>S. marcescens</i>	25
<b>CHAPTER 2.</b>	31
Figure 2-1. Multiple Ramachandran plot for native <i>E. coli</i> MnSOD	37
Figure 2-2. Stereo C $\alpha$ plot for the native <i>E. coli</i> MnSOD dimer	39
Figure 2-3. Stereo view of the metal site in native <i>E. coli</i> MnSOD	42
Figure 2-4. Stereodiagram of the solvent structure in the substrate-access funnel of MnSOD from <i>E. coli</i>	45
Figure 2-5. Glutamate bridge linking the two active sites of the MnSOD dimer	46
Figure 2-6. A structure-based sequence alignment of <i>E. coli</i> manganese and iron superoxide dismutases	47
Figure 2-7. Surface comparisons of the <i>E. coli</i> FeSOD and MnSOD dimers	49
Figure 2-8. A model of the interaction of standard B-DNA with the dimer groove of native <i>E. coli</i> MnSOD	50
Figure 2-9. Stereodiagram of the metal environment of native MnSOD and FeSOD from <i>E. coli</i>	51
<b>CHAPTER 3.</b>	55
Figure 3-1. Multiple Ramachandran plot for <i>E. coli</i> Fe <sub>2</sub> MnSOD	64
Figure 3-2. An overlay of the C $\alpha$ -traces of the A and B subunits of <i>E. coli</i> Fe <sub>2</sub> MnSOD	65
Figure 3-3. A stereodiagram showing an overlay of the two active-sites of the Fe <sub>2</sub> MnSOD dimer	66
Figure 3-4. A stereodiagram showing electron density around the disordered Tyr34 in subunit A of Fe <sub>2</sub> MnSOD	68
Figure 3-5. A stereodiagram showing an overlay of the Fe <sub>2</sub> MnSOD dimer on the AB dimer of native MnSOD	69
Figure 3-6. A stereodiagram showing an overlay of the Fe <sub>2</sub> MnSOD subunit B active-site on that of native MnSOD	69
Figure 3-7. Stereodiagrams showing simulated-annealing omit maps of selected residues in the active-sites of Fe <sub>2</sub> MnSOD	71



Figure 3-8.	A stereodiagram showing the movement of waters in the substrate-access funnel of Fe <sub>2</sub> MnSOD relative to those of native MnSOD ...	72
<b>CHAPTER 4.</b>		<b>77</b>
Figure 4-1.	A stereo diagram showing an overlay of Y34F and the native structure in the region of the mutation .....	87
Figure 4-2.	A stereo diagram of the waters in the substrate-access funnel of Y34F MnSOD .....	89
Figure 4-3.	A stereo plot of the C $\alpha$ main-chain trace of the native and Y34F AB dimers, overlaid using the A subunit of each dimer .....	90
Figure 4-4.	A stereo diagram showing an overlay of Q146H and the native structure in the region of the mutation .....	91
Figure 4-5.	A stereo plot of the C $\alpha$ main-chain trace of the native, Q146H, and Q146L AB dimers.....	93
Figure 4-6.	A stereo diagram showing an overlay of Q146L and the native structure in the region of the mutation .....	95
<b>CHAPTER 5.</b>		<b>101</b>
Figure 5-1.	A stereo plot showing an overlay of H30A and the native structure in the region of the mutation .....	107
Figure 5-2.	A stereo plot of the C $\alpha$ main-chain trace of the native, H30A and Y174F dimers, overlaid using the A subunit of each dimer .....	108
Figure 5-3.	A stereo plot showing an overlay of Y174F and the native structure in the region of the mutation .....	109
Figure 5-4.	Plot of temperature factor of C $\alpha$ atoms by residue number for the two chains of Y174F.....	112
Figure 5-5.	Standard deviation of phi and psi plotted as a function of residue number for Y174F.....	113
Figure 5-6.	Sigma of the chi1 and chi2 angles plotted as a function of residue number .....	115
Figure 5-7.	Schematic diagram of the packing of dimeric MnSOD Y174F within the crystal lattice .....	117
Figure 5-8.	A schematic diagram showing the packing of the dimers into planes of identical subunits .....	118
<b>CHAPTER 6.</b>		<b>123</b>
Figure 6-1.	Completeness of data plotted against resolution for the three synchrotron data sets of Y174F MnSOD.....	132
Figure 6-2.	R <sub>merge</sub> plotted against resolution for the three synchrotron data sets of Y174F MnSOD.....	134
<b>CHAPTER 7.</b>		<b>137</b>

# List of Tables

<b>CHAPTER 1.</b>	.....	1
Table 1-1.	X-ray crystal structures of Fe and Mn superoxide dismutases .....	16
Table 1-2.	Summary of general properties of MnSOD .....	27
<b>CHAPTER 2.</b>	.....	31
Table 2-1.	Data collection and reduction statistics for native <i>E. coli</i> MnSOD .	34
Table 2-2.	Refinement and model statistics for native <i>E. coli</i> MnSOD .....	36
Table 2-3.	RMS superpositions for subunits of native <i>E. coli</i> MnSOD .....	38
Table 2-4.	Hydrogen bonding interactions in the dimer interfaces of Mn and FeSODs from <i>E. coli</i> .....	41
Table 2-5.	Summary of potential azide derivative structures .....	53
<b>CHAPTER 3.</b>	.....	55
Table 3-1.	Crystallisation conditions in the ‘PEG screen’ in which crystalline material was produced .....	56
Table 3-2.	Crystallisation conditions in the ‘Crystal screen’ in which crystalline material was produced .....	57
Table 3-3.	Crystallisation methods and conditions of Fe <sub>2</sub> MnSOD crystals.....	58
Table 3-4.	Data collection and reduction statistics for <i>E. coli</i> Fe <sub>2</sub> MnSOD .....	59
Table 3-5.	Effects of different cryoprotectants on diffraction of Fe <sub>2</sub> MnSOD ..	60
Table 3-6.	Effects of different NCS weighting schemes .....	62
Table 3-7.	Refinement and model statistics for native <i>E. coli</i> MnSOD .....	63
Table 3-8.	Selected B-factors (Å <sup>2</sup> ) at the active site of Fe <sub>2</sub> MnSOD.....	67
<b>CHAPTER 4.</b>	.....	77
Table 4-1.	Catalytic activities of wild-type and iron-substituted MnSOD and their mutants from <i>E. coli</i> at various values of pH .....	79
Table 4-2.	Anion affinities of wild-type and iron-substituted MnSOD and their Y34F mutants from <i>E. coli</i> at various values of pH .....	80
Table 4-3.	Data collection and reduction statistics for <i>E. coli</i> Y34F, Q146H and Q146L MnSOD .....	84
Table 4-4.	Refinement and model statistics for <i>E. coli</i> Y34F, Q146H and Q146L MnSOD .....	85
Table 4-5.	Selected inter-atomic contacts associated with His146 in <i>E. coli</i> Q146H MnSOD and <i>P. shermanii</i> MnSOD .....	94
<b>CHAPTER 5.</b>	.....	101
Table 5-1.	Data collection and reduction statistics for <i>E. coli</i> H30A and Y174F MnSOD .....	103
Table 5-2.	Refinement and model statistics for <i>E. coli</i> H30A and Y174F MnSOD .....	104
Table 5-3.	Summary of refinement protocol of the 1.35 Å model of Y174F MnSOD .....	106

Table 5-4.	Temperature factor ( $\text{\AA}^2$ ) statistics for the two chains of Y174F....	111
Table 5-5.	Details of the crystal packing contacts for the primitive orthorhombic lattice of Y174F.....	116
<b>CHAPTER 6.</b>		<b>123</b>
Table 6-1.	Data collections of Y174F MnSOD with various oxidation states and ligands .....	124
Table 6-2.	Data collection and reduction statistics for <i>E. coli</i> Y174F MnSOD. Data were collected on an RaxisIIC <i>in-house</i> .....	126
Table 6-3.	Data collection parameters for data collected on beamline X11 at DESY .....	128
Table 6-4.	Data collection and reduction statistics for native oxidation state, and reduced-MnSOD-N3-, Y174F MnSODs from <i>E. coli</i> .....	129
Table 6-5.	Data collection and reduction statistics for native and reduced oxidation states of Y174F MnSOD and for a Y174F reduced-MnSOD-N3- derivative from <i>E. coli</i> .....	131
<b>CHAPTER 7.</b>		<b>137</b>

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## Abbreviations

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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 <sub>2</sub> MnSOD	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

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## Related Publications

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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.