

Expression, purification and characterisation of  
typical 2-Cys peroxiredoxins from Southern bluefin  
tuna (*Thunnus maccoyii*), Atlantic salmon (*Salmo  
salar*) and Yellowtail kingfish (*Seriola lalandi*)

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# Table of Contents

Summary	i
Declaration	iii
Acknowledgements	iv

## Chapter 1

### Introduction

1.1 Reactive oxygen species	2
1.2 Sources of ROS	2
1.3 Role of ROS in cell damage and disease	6
1.4 Role of ROS in normal cell function	8
1.5 Decomposition of ROS	10
1.6 Oxidative stress in fish	16
1.7 Oxidative stress in farmed finfish and its impact on the industry	17
1.8 Enzymatic antioxidant defences in finfish	18
1.9 Aims and scope of this study	19

## Chapter 2

### Common materials and methods

2.1 <i>Escherichia coli</i> strain descriptions	22
2.2 pET-30a vector map	22
2.3 <i>Escherichia coli</i> growth conditions	24
2.4 Cryopreserved <i>Escherichia coli</i> cell stock preparation	24
2.5 DNA purification	24
2.6 Agarose gel electrophoresis of DNA	25
2.7 Polymerase Chain Reaction (PCR) - based amplification of DNA	26
2.8 DNA sequencing	26
2.9 <i>Escherichia coli</i> transformation	26
2.10 Polymerase Chain Reaction (PCR) - based colony screening	27
2.11 Recombinant protein expression	28
2.12 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis	29
2.13 Immunoblotting	31
2.14 Nickel affinity chromatography purification of recombinant proteins	31
2.15 Peroxiredoxin enzyme assays	33
2.16 Gel filtration chromatography	34

2.17 Differential centrifugation	34
2.18 Protein crystallisation	35
2.19 Protein crystal harvesting and X-ray crystallography analysis	36

### Chapter 3

Expression, purification, and characterisation of a peroxiredoxin 2 from southern bluefin tuna (*Thunnus maccoyii*)

3.1 Introduction	38
3.2 Materials and Methods	40
3.2.1 Gene construct	40
3.2.2 Sequencing of pET-30a-SBT-Prx construct	40
3.2.3 Optimisation of recombinant SBT Prx 2 protein expression	41
3.2.4 Large scale recombinant SBT Prx 2 protein expression	41
3.2.5 Ni-affinity purification of SBT Prx 2 protein	42
3.2.6 SBT Prx 2 kinetic assays	42
3.2.7 Gel filtration chromatography of SBT Prx 2 protein	42
3.2.8 SBT Prx 2 Cys mutant cloning, expression and purification	43
3.2.9 SBT Prx 2 antibody preparation and evaluation	45
3.2.10 Crystallisation of SBT Prx 2 protein	47
3.3 Results	52
3.3.1 Sequencing of pET-30a-SBT Prx construct	52
3.3.2 SBT Prx protein sequence analysis	52
3.3.3 Optimisation of recombinant SBT Prx 2 protein expression	59
3.3.4 Large scale recombinant expression of SBT Prx 2 protein	61
3.3.5 Ni-affinity purification of SBT Prx 2 protein	65
3.3.6 Development of the Prx kinetic assay	67
3.3.7 SBT Prx 2 kinetics	73
3.3.8 Gel filtration chromatography of SBT Prx 2 protein	77
3.3.9 SBT Prx 2 Cys mutant cloning, expression, purification and characterisation	81
3.3.10 SBT Prx 2 antibody preparation and evaluation	103
3.3.11 Crystallisation of SBT Prx 2 protein	107
3.4 Discussion	119

## Chapter 4

Expression, purification, and characterisation of a peroxiredoxin 1 from Atlantic salmon (*Salmo salar*)

4.1 Introduction	138
4.2 Materials and Methods	140
4.2.1 Gene Construct	140
4.2.2 Sequencing of pET-30a-AS Prx construct	140
4.2.3 Optimisation of recombinant AS Prx 1 protein expression	141
4.2.4 Large scale recombinant AS Prx 1 protein expression	141
4.2.5 Ni-affinity purification of AS Prx 1 protein	142
4.2.6 AS Prx 1 kinetic assays	142
4.2.7 Gel filtration chromatography of AS Prx 1 protein	142
4.2.8 Crystallisation of AS Prx 1 protein	143
4.3 Results	144
4.3.1 Sequencing of pET-30a-AS Prx construct	144
4.3.2 AS Prx protein sequence analysis	144
4.3.3 Optimisation of recombinant AS Prx 1 protein expression	151
4.3.4 Large scale recombinant expression of AS Prx 1 protein	153
4.3.5 Ni-affinity purification of AS Prx 1 protein	157
4.3.6 AS Prx 1 kinetics	159
4.3.7 Gel filtration chromatography of AS Prx 1 protein	161
4.3.8 Crystallisation of AS Prx 1 protein	163
4.4 Discussion	168

## Chapter 5

Expression, purification and characterisation of a peroxiredoxin 4 from yellowtail kingfish (*Seriola lalandi*)

5.1 Introduction	174
5.2 Materials and Methods	176
5.2.1 Gene Construct	176
5.2.2 Sequencing of pET-30a-YTK Prx 4 construct	176
5.2.3 Site-directed mutagenesis of pET-30a-YTK Prx 4 construct	177
5.2.4 Preparation of constructs with truncated YTK Prx 4 inserts	178
5.2.5 Expression and purification of YTK Prx 4-197, 231 and 264 proteins	180

5.2.6 Gel filtration chromatography of YTK Prx 4-197 and 231 proteins	181
5.3 Results	182
5.3.1 Sequencing of pET-30a-YTK Prx 4 construct	182
5.3.2 Site directed mutagenesis of pET-30a-YTK Prx 4 construct	184
5.3.3 Sequence analysis of predicted N-terminal YTK Prx 4 signal peptides	186
5.3.4 Preparation of constructs with truncated YTK Prx 4 inserts	187
5.3.5 Large scale expression of YTK Prx 4-197, 231 and 264 protein	189
5.3.6 Ni-affinity purification of YTK Prx 4-197, 231 and 264 proteins	194
5.3.7 Gel filtration chromatography of YTK Prx 4-197 and 231 proteins	198
5.4 Discussion	203
<b>Chapter 6</b>	
General discussion and conclusion	210
<b>References</b>	216
<b>Appendix</b>	
Crystallisation suite composition tables	
1.1 Qiagen® NeXta® Classic Suite	234
1.2 Qiagen® NeXta® PEGs Suite	239
1.3 Qiagen® NeXta® MPD Suite	243
1.4 Hampton Research Crystal Screen	247
1.5 Hampton Research Crystal Screen 2	250

## Summary

The typical 2-Cys peroxiredoxins (Prxs) are a family of highly conserved proteins that are ubiquitously distributed throughout all phyla. Within the cell, they detoxify various peroxide substrates and thus contribute to regulation of the redox environment. The function and structure of typical 2-Cys Prxs has been well characterised in mammals, yeast, protists and bacteria but not in fish. In the present study, I present the functional and structural characterisation of a selection of typical 2-Cys Prxs from three finfish species that are important in Australian aquaculture. These finfish species include *Thunnus maccoyii* (southern bluefin tuna, SBT), *Salmo salar* (Atlantic salmon, AS) and *Seriola lalandi* (yellowtail kingfish, YTK).

The SBT and AS Prx deduced amino acid sequences contain features including peroxidatic and resolving Cys residues that are highly conserved among typical 2-Cys Prxs. Phylogenetic analysis of these amino acid sequences revealed that the AS Prx was most likely a Prx 1 isoform while the SBT Prx was most likely a Prx 2 isoform. Previously, the YTK Prx deduced amino acid sequence was found to contain the highly conserved features of typical 2-Cys Prxs in addition to an N-terminal extension. The N-terminal amino acid extension is a defining feature of Prx 4 isoforms and encodes an endoplasmic reticulum signal peptide. Analysis of the YTK Prx 4 N-terminal amino acid extension revealed that the putative signal peptide was most likely encoded by the first 33 amino acids. The deduced amino acid sequences of all three finfish Prx isoforms also contained the GGLG motif associated with the sensitivity of eukaryotic typical 2-Cys Prx proteins to overoxidation and consequent inactivation by peroxide substrates.

Functional characterisation of purified recombinant SBT Prx 2 and AS Prx 1 revealed that both proteins exhibited thioredoxin-dependent peroxidase activity. At low concentrations of peroxide substrate ( $\leq 120 \mu\text{M}$ ), the SBT Prx 2 protein

displayed positive cooperativity, a unique feature among typical 2-Cys Prxs. In contrast, at increasing high concentrations of peroxide substrate (0.5-2.0 mM), the peroxidase activity of the SBT Prx 2 and AS Prx 1 proteins was progressively lost. Consistent with other eukaryotic typical 2-Cys Prx 1 and Prx 2 isoform comparisons, the AS Prx 1 protein displayed a greater susceptibility to inactivation by increasing high concentrations of peroxide substrate compared to the SBT Prx 2 protein.

Analysis of the native molecular mass of purified recombinant SBT Prx 2, AS Prx 1 and YTK Prx 4 proteins revealed differences in oligomeric structure that were influenced by changes in the redox environment and protein concentration. Under reducing and oxidising conditions, the native molecular mass of the SBT Prx 2 and YTK Prx 4 proteins (minus its predicted N-terminal signal peptides) remained relatively unchanged. However, prolonged storage of the SBT Prx 2 protein promoted the formation of large aggregates consistent with a dimer of decamers. In contrast to the SBT Prx 2 and YTK Prx 4 proteins, the oligomeric properties of the AS Prx 1 protein were strongly influenced by the redox environment. Under reducing conditions, the AS Prx 1 protein existed predominantly as decamers while under oxidising conditions its structure was destabilised, resulting in the formation of insoluble aggregates. At comparable protein concentrations, the AS Prx 1 and YTK Prx 4 (minus its predicted N-terminal signal peptides) existed predominantly as decamers while the SBT Prx 2 existed predominantly as dimers. However, at a higher protein concentration, the native molecular mass of the SBT Prx 2 shifted from dimer to higher order oligomeric structures including decamers.

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