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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B.Med.Sci., B.Sc. (Hons)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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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 (≤ 120 µM), 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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