Molecular Characterisation Of The Thioredoxins In The Parasitic Nematode *Haemonchus contortus*

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CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Irene Sotirchos.

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ABSTRACT

Thioredoxins are a family of small proteins conserved through evolution, essential for cellular homeostasis. The 'classic' thioredoxin, identified in most species, is a 12 kDa protein with a Cys-Gly-Pro-Cys (CGPC) active site. The thioredoxin system, composed of thioredoxin, thioredoxin reductase and peroxiredoxin, is essential to protect cells from metabolically produced reactive oxygen. This and the diversification of this system through evolution identified it as a target for the control of many diseases, including parasitic infections. This work characterises the thioredoxins of *Haemonchus contortus*, a parasite with increasing economic impact on sheep and wool production in Australia. Five thioredoxin proteins were identified, expressed and characterised (*Hc*Trx1-5).

H. contortus contained the classic thioredoxin (*Hc*Trx1), but the major thioredoxin was a 16 kDa protein (*Hc*Trx3) with a Cys-Pro-Pro-Cys (CPPC) active site, which is related to tryparedoxin, a unique protein in *Trypanosomes*. Both proteins were expressed through the lifecycle and both had a similar ability to reduce the disulphide bonds of insulin compared to the classic thioredoxins in *Escherichia coli* and sheep. Both proteins were regenerated by thioredoxin reductase, but unlike the ovine thioredoxin, both were also able to reduce oxidised glutathione, directly reduce hydrogen peroxide and indirectly reduce hydrogen peroxide coupled with *H.contortus* peroxiredoxin.

Two thioredoxin-like proteins were identified with homology to thioredoxins reported in human cells, a 31 kDa protein with a CGPC classic active site (*Hc*Trx2) and a 28 kDa protein with a Cys-Pro-Ala-Cys (CPAC) active site, a transmembrane domain and an endoplasmic reticulum localisation signal (*Hc*Trx4). These had different activities to the classic *Hc*Trx1 in that *Hc*Trx2 could not directly reduce insulin, but could when coupled to thioredoxin reductase. In contrast, *Hc*Trx4 could directly reduce insulin, but could not react with thioredoxin reductase. The results suggest that *Hc*Trx4 is not a thioredoxin, but acts as a protein-disulphide isomerase (PDI). Other characterised PDIs, contain at least two active sites, in contrast to the one active site in the *H.contortus* protein.

*Hc*Trx5 is a 20 kDa protein with a unique active site Cys-Arg-Ser-Cys (CRSC). Although this active site has a charge change, *Hc*Trx5 was able to reduce insulin, be regenerated by thioredoxin reductase and react with *H.contortus* peroxiredoxin. However, *Hc*Trx5 was also regenerated by glutathione reductase coupled with glutathione, showing it had the activity of a glutaredoxin as well as a thioredoxin, an activity not reported for any thioredoxin.

This study characterised the thioredoxins of a parasitic nematode. The differences identified may provide new drug targets for the control of many tropical diseases for which drug resistance is emerging as a major problem. Preliminary investigations showed increased *H.contortus* thioredoxin expression in a drug resistant strain. *Hc*Trx1 was highly increased in ivermectin resistant parasites and may provide a marker of drug resistance.

JOURNAL PUBLICATIONS

Irene M. Sotirchos, Amanda L. Hudson, John Ellis, Mary W. Davey. Thioredoxins of a parasitic nematode: Comparing the 16- and 12-kilodalton thioredoxins. *Free Radical Biology and Medicine*: **44** (12): 2026-33.

PRESENTATIONS

1. Presentation at the Meat and Livestock Australia Workshop, I. Sotirchos* Sydney, Australia
   2004: The Thioredoxins of *Haemonchus Contortus*
   2005: Characterisation of Thioredoxins in Parasitic Nematodes
   2006: Molecular Characterisation Of Thiol Metabolism In Parasitic Nematodes.
   2007: Evolution and Activity of the Thioredoxins of *Haemonchus Contortus.*

2. I. Sotirchos*, David Witcombe, Mary Davey and John Ellis (2005) Characterisation of Thioredoxins in Parasitic Nematodes. Poster presentation at UTS Research Forum, Sydney, Australia.

3. Irene Sotirchos*, David Witcombe, John Ellis and Mary Davey (2005) Characterisation of Thioredoxin(s) in Parasitic Nematodes. Presentation at the World Association for the Advancement of Veterinary Parasitology Conference (WAAVP), Christchurch, New Zealand.

4. Irene Sotirchos*, David Witcombe, John Ellis and Mary Davey (2006) Characterisation of Thioredoxin(s) in Parasitic Nematodes. Poster presentation 4 at the International Congress of Paraitology Association Conference (ICOPA), Glasgow, Scotland.

5. Irene Sotirchos*, John Ellis and Mary Davey (2007) Evolution and Activity of the Thioredoxins of *Haemonchus Contortus*. Poster presentation at the Australian Society for Parasitology conference (ASP), Canberra, Australia.

LIST OF ABBREVIATIONS

ABBREVIATION	FULL NAME
aa	Amino acid
APS	Ammonium persulphate
bp	Base pairs
BCIP	5-Bromo- 4-chloro- 3-indolyphosphate
BSA	Bovine Serum Albumin
cDNA	Chromosomal deoxyribonucleic acid
Ce	*Caenorhabditis elegans*
Cys	Cysteine
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
DTNB	5,5'-Dithio-bis (2-nitrobenzoic acid)
DTT	Dithiothreitol
Ec	*Esherichia coli*
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-Linked ImmunoSorbent Assay
EtBr	Ethidium bromide
FAD	Flavin-adenine dinucleotide
Fig	Figure
g	Centrifugal force (gravity)
GR	Glutathione reductase
Grx	Glutaredoxin
GSH	Reduced glutathione
GSSG	Oxidised glutathione
Hc	*Haemonchus contortus*
HEDS	2 hydroxyethyldisulphide
hr	Hour (s)
Hs	*Homo sapiens*
IPTG	Isopropylthiogalactopyranoside
kb	Kilobases
kDa	Kilodaltons

L1	First larval stage
L3	Third larval stage
LB	Luria Bertani Broth
M	Molar
Min	Minute (s)
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NBT	Nitro blue tetrazolium
NXN	Nucleoredoxin
Oa	*Ovine aries*
Oligo	Oligonucleotide
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pI	Isoelectric point
Prx	Peroxiredoxin
qPCR	Quantitative real time polymerase chain reaction
QS	Quackenbush
RACE	Rapid amplification of cDNA ends
RNA	Ribonucleic acid
S_2	Oxidised thiol
SDS	Sodium dodecyl sulphate
SH_2	Reduced thiol
TBE	Tris borate buffer with EDTA
tBOOH	Tert-butyl hydroperoxide
TBS	Tris buffered saline
TEMED	Tetramethylethylenediamine
TFB	Transformation buffer
TMX	Transmembrane thioredoxin
Tris	tris(hydroxymethyl)aminomethane
Trx	Thioredoxin
TrxR	Thioredoxin reductase

UV Ultraviolet

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CHAPTER THREE CHARACTERISATION OF *Hc*Trx1 & *Hc*Trx3

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