OXIDATIVE STRESS IN PLANTS AND THE MALARIAL PARASITE *PLASMODIUM FALCIPARUM*

By

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Table of contents

Summary

This thesis is divided into three main parts. Part A of the project aimed to investigate the effects of the mixed-culture of wheat (*Triticum aestivum* L.) with white lupin (*Lupinus albus* L.) on the availability of soil-P to the wheat and to study the mechanisms by which white lupin mobilises P from unavailable soil-P pools making it more available to the wheat crop partner. P limitation is a major problem for many agricultural systems, particularly in the tropics and subtropics. White lupin is well-known for its ability to mobilise P from a P pool that is normally unavailable to plants. In this study, white lupin was grown in a mixed culture with wheat and the effects of the mixed culture on the availability of P to the wheat was investigated using a novel leaching system. The results show that while lupin was capable of mobilising the P that was locked up in the soil, as the level of the immobile P pool was significantly lower in the presence of white lupin compared to the wheat monoculture. This ability of white lupin to mobilise the unavailable P was demonstrated to have beneficial effects on the growth and P uptake of the wheat when they were grown in a mixed-culture.

Part B and C of this study focuses on the 2-Cys peroxiredoxin proteins of malarial parasite *Plasmodium falciparum*; *Pf*Prx-1 and *Pf*Prx-2 respectively. Malaria parasites are highly exposed to oxidative burden due to a large amount of reactive oxygen and nitrogen species produced by haemoglobin degradation in the food vacuole of the parasite and the immune effects of the host in response to parasite infection. Therefore, inside erythrocytes, the ability of the parasite to defend itself against

oxidative damage is of vital importance for parasite survival. Such defences are thus expected to be potential targets for malaria control strategies. This study focuses on the 2-Cys peroxiredoxin (Prx) system of *P. falciparum*; these enzymes are mainly responsible for the detoxification of hydrogen peroxide and other organic peroxides in the parasite, thereby protecting the parasite against oxidative stress.

Part B of the project aimed to study the factors influencing the oligomeric states of the *P. falciparum Pf*Prx-1 protein recombinantly expressed in *E. coli* and to investigate its three dimensional structure using X-ray crystallography. Firstly, the *Pf*Prx-1 gene was successfully expressed in *E.coli* for protein production. Following high-level expression, the *Pf*Prx-1 protein was purified to homogeneity. The purified *Pf*Prx-1 protein was studied for its Prx activity and its oligomeric structure. The *Pf*Prx-1 protein exists in both decameric and dimeric forms. High pH (8.5) and high concentrations of urea (2.8 M) seem to favour the formation of dimers. Attempts at crystallization of the protein yielded crystals, of which one diffracted to around 6-7 Å. Eventhough these data were not enough to sovle the structure of the protein, they could serve as a ground for further studies in order to obtain the full details of *Pf*Prx-1 3-D structure enabling inhibitor studies for structure-based drug design.

Part C of this study aimed to develop a *Pichia pastoris* expression system for the *P. falciparum Pf*Prx-2 protein and to investigate the oligomeric states and the subcellular localization of the recombinant *Pf*Prx-2 protein. The *P. pastoris* expression system was demonstrated to be able to translate this *P. falciparum* A+T rich sequence and recognise the mitochondrial targeting sequence of the protein.

This production system could be up-scaled to obtain sufficient quantities of recombinant *Pf*Prx-2 protein to enable structural and functional studies for structure-based drug design. Evidence for the production by *P. pastoris* of the *Pf*Prx-2 protein as an oligomer (decamer and larger) was obtained by gel filtration chromatography, SDS-PAGE and Western blotting. It was found that purification of the functionally active *Pf*Prx-2, by Ni-affinity chromatography, resulted in the protein being "locked" in the dimeric state and it could not be converted to the monomer by a variety of reducing agents as indicated by SDS-PAGE.

Declaration

"I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any University; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text".

Suong T. T. Cu

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Publications

Part of the work in this thesis has been published or is in preparation for submission for publication. The publications are listed below:

Cu STT, Hutson J, Schuller KA (2005) Mixed culture of wheat (*Triticum aestivum* L.) with white lupin (*Lupinus albus* L.) improves the growth and phosphorus nutrition of the wheat. **Plant and Soil**, 272(1-2): 143-151

Suong T.T. Cu, R. Ian Menz and Kathryn A. Schuller (in preparation) Expression, purification, crystallization and preliminary X-ray analysis of *Pf*Prx-1, a cytosolic 2-Cys peroxiredoxin from *Plasmodium falciparum. Journal of Biochemistry.*

Suong T.T. Cu, R. Ian Menz and Kathryn A. Schuller (in preparation) Expression, purification and characterisation of a recombinant *Plasmodium falciparum* mitochondrial 2-Cys peroxiredoxin in *Pichia pastoris*. *Protein Expression and Purification.*

Cu, S TT and Schuller, KA (2005) Intercropping lupin and wheat for better phosphorus acquisition. Oral presentation at the XV International Plant Nutrition Colloquium, Beijing, China, September 14-20.

List of symbols and abbreviations

Å	angstrom (10^{-10} m)
Amp	ampicillin
AOP	antioxidant protein
AOX	alcohol oxidase
A_x	absorbance at x nm
ATP	adenosine triphosphate
βME	β-mercapto ethanol
BMGY	buffered minimal glycerol
BMMY	buffered minimal methanol
bp	base pair
BPB	bromophenol blue
BSA	bovine serum albumin
BT	Bis-Tris, bis (2-hydroxyethyl)imino-tris(hydroxymethyl)methane
cDNA	complementary DNA
CHAPS	3-(3-Cholamidopropyl)dimethylamino-1-propanesulphonate
Ch	chloramphenicol
Cys	cysteine
Da	dalton
dH_2O	milliQ water
DMSO	dimethylsulphoxide

DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotides triphosphate
dsDNA	double-stranded DNA
DTT	dithiothreitol
EDTA	ethylendiaminetetraacetate
eg	for example
EGTA	ethylene glycol-bis(2-aminoethylether)-N,N,N,N-tetra acetic acid
ESI-MS	electrospray ionisation mass spectrometry
Fig.	figure
FPLC	fast performance liquid chromatography
g	gram
g	acceleration of gravity
GF	gel filtration chromatography
GPx	glutathione peroxidase
GSH	glutathione
GSR	glutathione reductase
GST	glutathione S-transferase
h	hour
H_2O_2	hydrogen peroxide
HEPES	N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid

HPLC	high performance liquid chromatography
His_6-tag	$(polyhistidine)_6$ tag
IPTG	isopropyl β-D-1-thiogalactopyranoside
Kan	kanamycin
kb	kilobase
kDa	kilodalton
lacI	gene encoding the lac repressor
l	litre
LB	Luria-Bertani
m	milli (10^{-3})
μ	micro (10^{-6})
M	molar
MALDI	matrix assisted laser desorption ionization
MCS	multiple cloning site
MES	2-(N-morpholino)ethane sulphonic acid
min	minute
mRNA	messenger RNA
MS	mass spectrometry
MW	molecular mass
NADH	nicotinamide adenine dinucleotide (reduced form)
NADPH	nicotinamide adenine dinucleotide phosphate

NMR	nuclear magnetic resonance spectroscopy
NKEF	natural killer enhancing factor
n	nano (10^{-9})
$O_2^{\bullet-}$	superoxide
$ONOO^-$	peroxynitrite
OD_x	optical density at x nm
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PDB	protein data bank
PEG	polyethyleneglycol
pI	isoelectric point
Prx	peroxiredoxin(s)
PVDF	polyvinylidene fluoride
RBC	red blood cell
RNA	ribonucleic acid
RNase	ribonuclease
rmp	revolutions per minute
ROS	reactive oxygen species
s	second

SAP	shimp alkaline phosphatase
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SOD	superoxide dismutase
ssDNA	single-stranded DNA
Taq	*Thermus aquaticus*
TAE	Tris-acetate-EDTA buffer
TB	Tris buffer
TBE	Tris-borate- EDTA buffer
TCA	Trichloroacetic acid
TE	Tris-EDTA buffer
TEMED	N,N,N,N-tetramethylendiamine
TPx	thioredoxin peroxidase
Tris	Tris(hydroxymethyl)aminomethane
tRNA	transfer RNA
Trx	thioredoxin
TrxR	thioredoxin reductase
TSA	thiol-specific antioxidant
UV	ultraviolet
V	volt
vol	volume

v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
wt	wild-type
YEP	yeast extract peptone
YNB	yeast extract nitrogen base

List of figures

List of tables