Investigation into Peroxiredoxin and interactions in the Peroxiredoxin peroxide scavenging system

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Paul James

Abstract

Peroxiredoxins are a family of multifunctional enzymes that are able to protect the cell against oxidative stress. Peroxiredoxins form part of a recently discovered peroxide scavenging system along with thioredoxin, thioredoxin reductase and sulfiredoxin.

This study describes the purification of a recombinant human peroxiredoxin II from human erythrocytes. The original recombinant clone contained a point mutation at the fourth residue from glycine to valine and a number of problems were encountered with aggregation during purification. Reverting back to the original amino acid sequence allowed the protein to be purified and concentrated without aggregation, as well as leading to over-expression in the same oligomeric state as the native sample from blood.

This study also describes the over-expression and purification of the human peroxiredoxin II protein in the intermolecular disulfide form as well as the subsequent crystallisation and X-ray diffraction studies. The crystal structure for this form of the protein was obtained to 3.3 Å resolution revealing the peroxiredoxin to be in the decameric form. In addition conformational changes in the protein that are necessary for formation of the intermolecular disulfide between the peroxidatic (Cys52) and resolving cysteine (Cys172) have been observed. The structure also revealed that these movements did not interfere with the dimer:dimer interface as had been previously suggested. This then allows the disulfide to be seen within the decameric form of peroxiredoxin.

The production of covalent complexes formed between peroxiredoxin and sulfiredoxin, and peroxiredoxin and thioredoxin was also investigated. Complexes were stabilised by using DTNB to form a covalent bond between specific cysteine residues. The complex binding results from size exclusion chromatography showed that decameric peroxiredoxin bound to sulfiredoxin in a 1:5 ratio and decameric peroxiredoxin bound to thioredoxin in a 1:10 ratio.

Cloning, over-expression and purification of the selenocysteine containing enzyme thioredoxin reductase was achieved. A minimal selenocysteine insertion sequence was added to the 3’ end of the DNA sequence to drive selenocysteine insertion in place of the typical stop UGA codon. The activity of

this protein was found to be low but was greatly increased when co-expressed with a plasmid containing the *selA*, *selB* and *selC* genes. Although the activity of this co-expressed thioredoxin reductase was ~20% of the native enzyme activity, it was comparable to the activity of other recombinant forms of the enzyme.

These studies report the purification of all of the proteins necessary to reform the peroxiredoxin system and allow the production of a working assay for peroxiredoxin activity. Together with the first report for a structure of a decameric disulfide form of human peroxiredoxin II a greater insight into the peroxiredoxin system has been obtained.

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Abbreviations

3D	3-dimensional
APS	Ammonium persulfate
ATP	Adenosine triphosphate
BAM	Benzamidine
BASI	barley α-amylase/subtilisin inhibitor
BE	Adherent human colon carcinoma cells
CCP4	Collaborative computational project, number 4
DNA	Deoxyribonucleic acid
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
DTT	Dithiothreitol
EC	Enzyme commission
EDC	1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide
EDTA	Ethylenediaminetetraacetic acid
EM	Electron microscopy
ESI-MS	Electrospray ionization mass spectrometry
FAD	Flavin adenine dinucleotide
FFQ	Fast flow Q Sepharose
GF	Gel filtration
HEPES	N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
His-tag	Poly histidine tag
IPTG	Isopropyl-1-thio-β-D-galactopyranoside
LB	Luria-Bertani
LDAO	Lauryldimethylamineoxide
MAD	Multiple-wavelength anomalous diffraction
MWCO	Molecular weight cut off
NADPH	Nicotinamide adenine dinucleotide phosphate
NHS	N-hydrocysulfosuccinimide
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PDB	Protein data bank
PEG	Polyethylene glycol

PLP2	Yeast phosducin-like protein 2
PMSF	Phenylmethanesulfonylfluoride
Prx	Peroxiredoxin
RMS	Root mean square
ROS	Reactive oxygen species
SAD	Single-wavelength anomalous diffraction
SAXS	Small angle X-ray scattering
SDS	Sodium dodecyl sulfate
SECIS	Selenocysteine insertion sequence
SLIM	Site-directed ligase independent mutagenesis
SOD	Superoxide dismutase
SPR	Surface plasmon resonance
Srx	Sulfiredoxin
TCEP	Tris(2-carboxyethyl)phosphine
TEMED	N,N,N',N'-Tetramethyl-1-,2-diaminomethane
T_M	Melting temperature
TNB	5-thiobis-(2-nitrobenzoic acid)
Tris-HCl	Tris (hydroxymethyl) aminomethane
Trx	Thioredoxin
TrxR	Thioredoxin reductase
UV	Ultraviolet

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