Protein Bound 3,4-Dihydroxyphenylalanine as a Signal for Enhanced Antioxidant Defences

by

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Publications Arising from this Thesis

Peer Reviewed Journal Articles:

Nelson, M. A., Foxwell, A. R., Tyrer, P. C. and Dean, R. T. (2007) Protein-bound 3,4-dihydroxy-phenylalanine (DOPA), a redox active product of protein oxidation, as a trigger for antioxidant defences. *International Journal of Biochemistry and Cell Biology*, **39**: 879-889

Conference Presentations and Posters:

Nelson, M. A. (2004) The influence of DOPA on the expression and function of specific lysosomal and proteasomal proteins. Oral presentation, *Divisional Research Institute Corroboree*, University of Canberra, Canberra, Australia

Nelson, M. A., Foxwell, A. R., Tyrer, P. C., Kyd, J. M. and Dean, R. T. (2005) Influences of DOPA on protein degradation and cellular antioxidant defences. Poster presentation, *The Canberra Hospital Annual Research Meeting – Health and Medical Research in the Canberra Region*, The Canberra Hospital, Canberra, Australia

Nelson, M. A. (2005) Physiological functions of DOPA, the good and the bad. Oral presentation, *Divisional Research Institute Corroboree*, University of Canberra, Canberra, Australia

Nelson, M. A., Foxwell, A. R., Tyrer, P. C., Kyd, J. M. and Dean, R. T. (2005) Potential signalling properties of DOPA. Poster presentation, *University of Canberra Showcase*, University of Canberra, Canberra, Australia

Nelson, M. A., Foxwell, A. R., Tyrer, P. C., Kyd, J. M. and Dean, R. T. (2005) Potential signalling properties of PB-DOPA. Poster presentation, *Australian Society for Medical Research, ACT Young Investigators Forum*, Australian National University, Canberra, Australia

Nelson, M. A., Foxwell, A. R., Tyrer, P. C. and Dean, R. T. (2008) Regulation of the cellular antioxidant defence system by protein-bound 3,4-dihydroxyphenylalanine. Oral presentation, *Australian Society for Medical Research, ACT Young Investigators Forum*, The Canberra Hospital, Canberra, Australia

Journal Articles in Preparation:

Nelson, M. A., Foxwell, A. R., Tyrer, P. C. and Dean, R. T. (2008) Radical sequestration and cellular protection against oxidative stress induced by free and protein-bound 3,4-dihydroxyphenylalanine (DOPA). Based on results presented in chapter 4.

Nelson, M. A., Foxwell, A. R., Tyrer, P. C. and Dean, R. T. (2008) Differential protein expression in response to protein-bound 3,4-dihydroxyphenylalanine (DOPA). Based on results presented in chapter 7.

Abbreviations

AAPH	2,2'-Azobis(amidinopropane) dihydrochloride
AP-1	Activator protein-1
α-syn	α-Synuclein
BAG-1	Bcl-2-associated athanogene
BCA	Bicinchoninic acid
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
BSC	Biodegradable counting scintillant
CA-2	Carbonic anhydrase 2
CAP-1	Adenylyl cyclase-associated protein 1
CHIP	Carboxyl terminus of Hsc70-interacting protein
CREB	cAMP response element binding protein
CSF	Cerebrospinal fluid
DAPI	4,6-Diamidino-2-phenylindole
DCPIP	2,6-Dichlorophenolindophenol
DMEM	Dulbecco's Modified Eagles medium
DOPA	3,4-Dihydroxyphenylalanine
DTT	Dithiothreitol
EMSA	Electromobility shift assay
EpRE (ARE)	Electrophile response element (Antioxidant response element)
ERK	Extracellular signal-regulated kinase
FBS	Foetal bovine serum
FCS	Foetal calf serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GRP78	78 kDa glucose regulated protein
GSH	Reduced glutathione
GSSG	Oxidised glutathione
GTPase Ran	GTP-binding nuclear protein Ran
H_2O_2	Hydrogen peroxide
Hsp	Heat shock protein
IEG	Immediate early gene
IPG	Immobilised pH gradient
LDH	Lactate dehydrogenase

MALDI-TOF	Matrix assisted laser desorption ionisation-time of flight
MAPK	Mitogen activated protein kinase
MEM	Eagle's minimal essential medium
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NADH	Nicotinamide adenine dinucleotide
NBT	Nitroblue tetrazolium
NDK A	Nucleoside diphosphate kinase A
NFκB	Nuclear factor kappa enhanced binding protein
NGF	Nerve growth factor
NQO	NAD(P)H:quinone oxidoreductase
Nrf2	Nuclear factor-erythroid 2 related factor 2
6OHDA	6-Hydroxydopamine
P13K	Phosphatidylionsitol-3-kinase
PB-DOPA	Protein-bound 3,4-dihydroxyphenylalanine
PBS	Phosphate buffered saline
PD	Parkinson's disease
PDI	Protein disulfide-isomerase
PMA	Phorbol 12-myristate 13-acetate
PMF	Peptide mass fingerprint
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAPK	Stress-activated protein kinase
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
-SOH	Sulphenic acid
TCA	Trichloroacetic acid
TCP	T-complex protein
TEMED	N,N,N',N'-Tetramethylethylenediamine
TRE	12-*O*-tetradecanoylphorbol 13 acetate (TRA)-response element
WT1	Wilms tumor-1
WTAP	Wilms tumor 1-associating protein

Abstract

Protein-bound 3,4-dihydroxyphenylalanine (PB-DOPA), a long-lived, redox-active product of protein oxidation, is capable of functioning as both a pro- and anti-oxidant. A number of *in vitro* and *in vivo* studies have demonstrated a toxic, non-toxic or even beneficial effect of free DOPA, however little investigation has examined the physiological activity of PB-DOPA. Furthermore, as free DOPA is currently the major treatment available for Parkinson's disease, most studies have focused on the effect of DOPA within neurological cells or tissues, although the presence of PB-DOPA in other locations, for example within atherosclerotic plaques, suggests that broader research is needed to fully understand the physiological effects of both free and PB-DOPA.

The hypothesis presented in this thesis is that under physiological conditions, when little redox active transition metal is available, PB-DOPA can function as a redox signalling molecule, triggering an enhancement of cellular antioxidant defences, with a potentially specific role in the regulation of defences targeted against protein oxidation. Physiological levels of PB-DOPA are very low, however the level on individual proteins can change to a proportionally large degree during oxidative stress, an appropriate property for a signalling molecule. In addition, remarkably elevated levels occur in some pathologies, including atherosclerosis. As an initial and commonly formed product of protein oxidation, PB-DOPA is well placed for a signalling role, promoting a significant up-regulation of antioxidant defences in the early stages of oxidative stress, before extensive damage has occurred. As an initiator of antioxidant defences, PB-DOPA would be potentially useful as a therapeutic for the treatment of diseases involving oxidative stress or the accumulation of oxidative damage.

The main objective of this thesis was, therefore, to examine the effect of PB-DOPA on the cellular antioxidant defence system using monocytic and macrophage-like cells, key cells involved in the formation of atherosclerotic plaques. The incorporation of free DOPA into protein during protein synthesis, a process previously shown to occur both *in vitro* and *in vivo*, was used to generate PB-DOPA. Neither free nor PB-DOPA were found to be toxic to monocytic or macrophage-like cells in culture, but rather were both capable of protecting these cells from oxidative stress. Free DOPA was shown to be capable of directly scavenging radicals, a process that was thought to be in part responsible for the protection induced during oxidative stress. The presence of free and PB-DOPA up-regulated the activity of catalase and

NAD(P)H:quinone oxidoreductase, two enzymatic antioxidants, however the activity of superoxide dismutase and the concentration of oxidised and reduced glutathione were not affected. Whilst it was thought that PB-DOPA would have a specific effect on the activity of antioxidant defences targeted against protein oxidation, proteolysis and bulk chaperone activity were not affected by a combination of free and PB-DOPA. Oxidatively-induced protein aggregation, however, was inhibited by the presence of free and PB-DOPA, suggesting that a more specific chaperone regulation may be taking place.

The regulation of gene and protein expression was thought to be one possible mechanism by which PB-DOPA could function as a signalling molecule. To test this hypothesis, the effect of free and PB-DOPA on transcription factor activation and protein expression were investigated. Free and PB-DOPA did not induce the expression or activation of Nrf2, AP-1 or NFκB, three transcription factors thought to be involved in the expressional regulation of genes involved in the antioxidant defence system. However, the expression of a number of proteins, including antioxidants, chaperones and proteins involved in cell cycle progression, were regulated in monocytic and macrophage-like cells following the administration of free DOPA under conditions that resulted in either a high or low level of PB-DOPA generation. The regulated proteins differed between the two conditions, suggesting that the level of PB-DOPA may be a key factor in determining the specific defences targeted.

The results presented in this thesis support the hypothesis that PB-DOPA can function as a signalling molecule, triggering an enhancement of cellular antioxidant defences, with a specific role in the regulation of the chaperone system, a key defence targeted against protein oxidation. This thesis may provide the basis for the potential use of free or PB-DOPA as a therapeutic for diseases known to involve oxidative stress or oxidative damage, however more research will be required to determine if the effects demonstrated in this thesis are also capable of occurring *in vivo*.

1. Literature Review

Title page