OXIDATION OF ASCORBATE BY PROTEIN RADICALS IN SIMPLE SYSTEMS AND IN CELLS

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SUMMARY

Generation of peroxide groups in proteins exposed to a wide variety of reactive oxygen species (ROS) requires an initial formation of protein carbon-centred or peroxyl free radicals, which can be reduced to hydroperoxides. Both protein radicals and protein hydroperoxides are capable of oxidizing important biomolecules and thus initiate biological damage. In this study, we investigated the inhibition of protein hydroperoxide formation by ascorbate and GSH in gamma-irradiated HL-60 cells.

We used HL-60 cells as a model for general protection of living organisms by ascorbate (Asc) and glutathione (GSH) from the deleterious effects of protein hydroperoxides generated by radicals produced by gamma radiation. Measurement by HPLC indicated that incubation of HL-60 cells with Asc in the presence of ascorbate oxidase resulted in the accumulation of intracellular Asc. The intracellular Asc levels were lowered by irradiation, demonstrating intracellular consumption of Asc by the radiation-generated radicals. Exposure of HL-60 cells to increasing gamma irradiation doses resulted in increasing accumulation of protein peroxides in the cells. This was measured by the FOX assay. A significant decrease in intracellular protein hydroperoxides was noted when the cells were treated with ascorbic acid before irradiation. A dose-dependent protective effect of Asc was observed. Asc loading also provided strong protection from radiation-generated protein hydroperoxides independently of the composition of the external medium, showing that only the radicals formed within the cells were effective in oxidizing the cell proteins. Similarly, protein peroxidation was inhibited in cells with

enhanced levels of GSH and increased when the intracellular GSH concentration was reduced. These findings indicate that ascorbate and GSH are important antioxidants in protecting cells from oxidative stress associated with the generation of protein hydroperoxide.

DECLARTION

This thesis contains no material which has been presented or accepted for the award of any degree or diploma in any other university or institution.

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Chia-chi Liu

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ABBREVIATIONS

AAPH	2, 2’-azobis-(2-amidinopropane) dihydrochloride
ABTS	2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]
AD	Alzheimer’s disease
ALS	amyotrophic lateral sclerosis
AP-1	activator protein-1
Asc	ascorbate
BSA	bovine serum albumin
BSO	L-buthionine sulfoximine
Cg	cytosine glycol
CuZnSOD	copper/zinc superoxide dismutase
CYPs	cytochrome P-450 enzyme
DHA	dehydroascorbic acid
DMPO	5, 5-dimethyl-1-pyroline-1-oxide
DMSO	dimethylsulfoxide
DTNB	5-5’-dithiobis-(2-nitrobenzoic acid)
DTT	dithiothreitol
EC-SOD	extracellular superoxide dismutase
EDTA	ethylenediaminetetracetic acid
EPR	electron paramagnetic resonance spectroscopy
ESR	electron spin resonance
FCS	fetal calf serum

FMN	flavin mononucleotide
5-FoUra	5-formyluracil
GCS	γ-glutamylcysteine synthetase
GLUT	glucose transporters
GPx	glutathione peroxidases
GRD	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
GSTs	glutathione S-transferases
HNE	4-hydroxy-2-nonenal
5-HO-Cyt	5-hydroycytosine
8-HO-dG	8-hydroxy-2'-deoxyguanosine
5-HOMeUra	5-hydroxylmetyluracil
5-HO-Ura	5-hydroyuracil
HPLC	high performance liquid chromatography
HSA	human serum albumin
JUK	c-Jun N-terminal kinase
LDL	low-denisty lipoprotein
Lyz, LZ	lysozyme
Mb	myoglobin
MCO	metal-catalyzed oxidation
MDA	malondialdehyde
Mn-SOD	manganese superoxide dismutase

MPO	myeloperoxidase
MTT	3-(4,5)-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NAC	N-acetylcysteine
NADPH	nicotinamide-adenine dinucleotide phosphate
NF-κB	nuclear factor B
8-oxo-dG	8-oxo-2'-deoguanosine
PBN	α-phenyl-N-tert-butyl nitrone
PCA	Perchloric acid
PD	Parkinson disease
PDH	pyruvate dehydrogenase kinase
6-PGD	6-phosphogluconate dehydrogenase
PKA	protein kinase A
PUFA	polyunsaturated fatty acid
Ptx	pentoxifylline
RA	rheumatoid arthritis
ROS	reactive oxygen species
SCEs	sister chromatid exchanges
SLE	systemic lupus erythematosis
SOD	superoxide dismutase
TBA	thiobarbituric acid
TCA	trichloroacetic acid
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxy
TEMPOL	4-hydroxy-2 2,6,6-tetramethylpiperidine-1-oxy

Tg	thyroglobulin
$UQ^{\bullet -}$	ubisemiquinone anion radical
UV	ultraviolet
XO	xylenol orange

CONFERENCES & PUBLICTAIONS

The following contains a list of abstracts from scientific meetings and the publication from this study.

Conferences

Gebicki, J. M., Domazou, A., Nauser, T., Tweeddale, H, Liu, C. C., and Koppenol, W. H. Interaction of protein radicals with GSH and ascorbate in vitro and in cells. The 15th Annual Conference of the Society for Free Radical Research (Australasia), Perth, Australia, Dec 2006.

Gebicki, J. M., Domazou, A., Nauser, T., Tweeddale, H, Liu, C. C., and Koppenol, W. H. Oxidation of glutathione and ascorbate by protein radicals. 41st meeting of the Polish Biochemical Society, Bialystok, Poland, Sep 2006.

Liu, C. C. and Gebicki, J. M. Inhibition of protein peroxidation by ascorbate in gamma-irradiated HL-60 cells. The 13th Biennial Congress: International Society for Free Radical Research, Davos, Switzerland, Aug 2006.

Liu, C. C. and Gebicki, J. M. Oxidation of ascorbate by protein radicals. The 13th Annual Conference of the Society for Free Radical Research (Australasia), Christchurch, New Zealand, Dec 2005.

Publications

Gebicki, J. M., Domazou, A., Nauser, T., Tweeddale, H, Liu, C. C., and Koppenol, W. H. Oxidation of glutathione and ascorbate by protein radicals. Manuscript in preparation.

Liu, C. C. and Gebicki, J. M. The role of ascorbate and glutathione in protein peroxide formation in HL-60 cells. Manuscript in preparation.

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