Amino Acids (2007) 33: 51–56
DOI 10.1007/s00726-006-0410-2
Printed in The Netherlands

Amino Acids

Detecting oxidative post-translational modifications in proteins

Mini-Review Article

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Received August 18, 2006
Accepted August 30, 2006
Published online October 6, 2006;

Summary. Oxidative stress induces various post-translational modifications (PTM); some are reversible in vivo via enzymatic catalysis. The present paper reviews specific procedures for the detection of oxidative PTM in proteins, most of them including electrophoresis. Main topics are carbonylated and glutathionylated proteins as well as modification of selected amino acids (Cys, Tyr, Met, Trp, Lys).

Keywords: Oxidative post-translational modifications – Proteomics – Electrophoresis – Carbonylation – Glutathionylation

1. Introduction

In a previous study, our group has reviewed in vitro redox modifications of proteins useful for an optimal resolution of all components in a complex sample, or for the structural characterization of selected components/pure proteins (Wait et al., 2005). Conversely, the present paper reviews biochemical procedures for the detection, and in some instances for the identification, of in vivo (post-translationally) redox-modified proteins.

Reactions with proteins of a variety of free radicals and reactive oxygen and nitrogen species (ROS and RNS) lead to oxidative modifications such as formation of protein hydroperoxides, hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, oxidation of sulfhydryl groups, oxidation of methionine residues, conversion of some amino acid residues into carbonyl groups, cleavage of the polypeptide chain and formation of cross-linking bonds. Aromatic and sulfur-containing residues are particularly susceptible to oxidative modification, with formation of L-DOPA from tyrosine, ortho-tyrosine from phenylalanine; sulphoxides and disulphides from methionine and cysteine; and kynurenines from tryptophan. The occurrence of valine and leucine hydroxides, reduced from hydroperoxide intermediates, has been reported (key information in Packer, 1984; Packer and Glazer, 1990; a recent review in Griffiths, 2000).

Endogenous production of ROS is mainly linked to the one-electron carriers in the mitochondrial respiratory chain and to oxidase enzymes (including peroxidases secreted by eosinophils and polymorphonuclear (PMN) leukocytes that inactivate plasma proteinase inhibitors and enhance the activity of PMN serine proteases in the inflammatory foci) (Dean et al., 1997).

Adverse effects from exposure to UV light or ionizing radiation and toxicity by a number of metals and xenobiotics, including drugs for therapeutic use, are linked to production of free radicals/ROS (Berlett and Stadtman, 1997); for metals the mechanism involves redox-cycling reactions, depletion of glutathione and bonding to sulfhydryl groups (Valko et al., 2005).

Radical scavengers, such as nitric oxide, and antioxidant enzymes, chiefly superoxide dismutase, catalase and glutathione peroxidase, constitute the primary antioxidant defense system of the organism (Dean et al., 1997). Part of the oxidative damage to cysteine and methionine residues can be repaired by various enzymatic systems that

catalyze the reduction of cysteine disulfides, sulfenic and sulfinic acids as well as of methionine sulfoxide (Mary et al., 2004).

Unless repaired or removed from cells, these oxidized proteins are often toxic and can threaten cell viability (Berlett and Stadtman, 1997), as oxidatively modified proteins can form large aggregates due to covalent cross-linking or increased surface hydrophobicity as aromatic and bulky aliphatic residues are exposed during the oxidative rearrangement of secondary and tertiary protein structure (Grune et al., 2003). Indeed, oxidatively damaged proteins undergo selective proteolysis, primarily by the 20S proteasome that recognizes hydrophobic amino acid residues in an ubiquitin- and ATP-independent way. However, HOIL-1 (heme-oxidized IRP2 ubiquitin ligase-1) is an E3 ligase that recognizes a protein oxidized by iron (Iwai, 2003). The 26S proteasome is not very effective in degrading oxidized proteins. Relatively mild oxidative stress rapidly (but reversibly) inactivates both the ubiquitin activating/conjugating system and 26S proteasome activity, but does not affect 20S proteasome. More severe oxidative stress causes extensive protein oxidation, resulting in protein fragments, and cross-linked and aggregated proteins, that become progressively resistant to proteolytic digestion and actually bind to the 20S proteasome to act as irreversible inhibitors (Davies, 2001; Dunlop et al., 2002; Grune et al., 2003).

Current investigation is addressing the issue of 'redox regulation' by reversible oxidative PTM, namely the hypothesis that under physiological conditions the ratio between various forms of cysteine oxidation (thiol-disulfide balance but also oxidation to sulfinic and sulfenic acids, and formation of mixed disulfides with small-molecular-weight thiols, including cysteine and glutathione) in key proteins may control signal transduction and metabolic pathways (reviewed in Ghezzi et al., 2005). In this framework, ROS would act as intracellular messengers and redox-regulated proteins as redox sensors.

Conversely, a vast literature already exists on the harmful effects of irreversible oxidative PTM as a result of oxidative stress i.e. the production of ROS in excess to tissue antioxidants. Oxidatively-modified proteins have been shown to correlate with ageing (age of an organism or its tissues; an increase in susceptibility to experimentally induced protein oxidation depends on the maximum lifespan potential of the species) (Linton et al., 2001). Oxidative modifications of proteins leading to loss of their function (enzymatic activity), accumulation and inhibition of their degradation have been observed in several human degenerative diseases (such as cancer (Valko et al., 2006), atherosclerosis, Alzheimer's dementia, Parkinson disease and ALS (Moreira et al., 2005)). However, there is no general consensus on whether excess ROS is cause or consequence of tissue injury, or both (Juranek and Bezek, 2005). Very disappointing and intriguing, in contrast to in vitro observations and to indirect evidence from epidemiological/nutritional surveys, clinical tests of antioxidant strategies i.e. diet supplementation with high doses of vitamins and other natural antioxidant products, all performed poorly (see e.g. Fisher and Naughton, 2005; Frank and Gupta, 2005).

Electron spin resonance techniques directly evaluate free radical activity (Jackson, 1999). More commonly, the effects of ROS are assessed by quantitating the degradation products of the affected structures. Various measures of lipid oxidation (thiobarbituric acid reactive substances, exhaled pentane/ethane, low-density lipoprotein resistance to oxidation, isoprostanes) and DNA oxidation (oxidized DNA bases such as 8-OHdG, autoantibodies to oxidized DNA, modified comet assay) are in common use (Mayne, 2003). For proteins, a general feature connected with oxidation is the induction of a characteristic fluorescence (excitation 360 nm, emission 454 nm) (Jones and Lunec, 1987). Specialized techniques, able to detect individual oxidative PTM and in some instances allowing at the same time the identification of the affected protein(s), will be dealt with in the following.

2. Specific procedures

2.1 Carbonylated proteins

Protein carbonyls are biomarkers of the presence and the action of hydroxyl radicals. In the literature, 'protein oxidation' appears to be used as a synonym for 'protein carbonylation'; a widely used commercial kit to detect carbonylated proteins is indeed named OxyBlot®. γ-Glu-

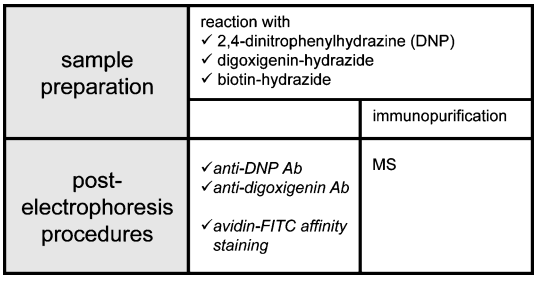

Fig. 1. Outline of the procedures for investigating carbonylated proteins in a proteomic experiment. Italics denote analytical procedures, regular fonts (micro) preparative procedures (allowing for MS-based identification)

tamyl semialdehyde (GGS) and 2-amino-adipic semialdehyde (AAS) from arginine, proline and lysine are the main products of the oxidation process.

For their study (Fig. 1), in most instances carbonyls are derivatized with 2,4-dinitrophenylhydrazine (DNP), and the resulting hydrazones are then detected via immunological procedures. Proteins are reacted with DNP before electrophoresis (Talent et al., 1998) or better, for 2-DE runs, after the IEF step (Reinheckel et al., 2000). After electrophoresis and electroblotting, whole protein pattern is stained with Ponceau Red (Rottoli et al., 2005), or other photometric reagent (BLOT-FastStain®, with a lower detection limit of approximately 0.3 ng/band) (Talent et al., 1998) or with a fluorescent dye (Korolainen et al., 2002), then carbonylated proteins are immunodetected with anti-DNP antibodies.

The anti-DNP antibodies may also be used to immunoprecipitate carbonylated proteins; proteins in the purified/enriched fraction are then fractionated by 2-DE and identified by MALDI-TOF MS fingerprinting (England and Cotter, 2004). In a high-throughput approach the DNP-tagged proteins are immunoprecipitated and digested with trypsin. The peptides are separated by stepwise ion exchange chromatography followed by reverse phase chromatography (2D-LC), and analysed by nano-HPLC coupled online to an ESI-Quad-TOF mass spectrometer (Kristensen et al., 2004).

An alternative tag to DNP is biotin: biotinylation of carbonylated proteins is carried out with biotin-hydrazide prior to 1-DE or 2-DE. The biotinylated species are then detected using avidin-FITC affinity staining, affording a sensitivity five times higher than silver staining, with a detection limit of approximately 0.64 pmol of protein-associated carbonyls (Yoo and Regnier, 2004). Biotin may also be the basis for affinity enrichment using monomeric avidin affinity chromatography columns (Mirzaei and Regnier, 2005); for high-throughput functional proteomics, the hydrazide biotin-streptavidin methodology is coupled with liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis (Soreghan et al., 2003).

A further possibility is labelling carbonyls with digoxigenin-hydrazide and detecting on blots with an anti-digoxigenin antibody conjugated to alkaline phosphatase. This assay covers a range of sensitivity from 1.26 to 126 pmoles of carbonyl groups (Bautista and Mateos-Nevado, 1998).

2.2 *Glutathionylated proteins*

Different detection procedures are listed in Fig. 2. The most general procedure for assessing protein glutathionylation involves the metabolic labelling of the intracellular glutathione pool with ^{35}S-cysteine while inhibiting protein synthesis (Rokutan et al., 1991; Thomas et al., 1991). After separation under non-reducing conditions, labelled proteins are detected in dried 1-DE or 2-DE gels and analyzed by MALDI-TOF (Fratelli et al., 2002, 2003). This approach applies to in vitro cell culture studies, and cannot be extended to ex vivo samples. Control experiments include: evaluation of constitutive glutathionylation, i.e. in the absence of oxidative stress; reversibility of labelling upon reduction with DTT; discrimination from cysteinylation by showing that labelling is inhibited by blocking GSH synthesis with BSO. Biotin-cysteine may be used in in vitro cultures as an alternative to radiolabelling (Humphries et al., 2002).

The use of biotin-labelled glutathione (Eaton et al., 2002) or of its membrane-permeant analog, biotinylated glutathione ethyl ester (Sullivan et al., 2000), does not require protein synthesis inhibition, however target selectivity based on tag size has been reported (Eaton et al., 2002). Biotinylation of glutationylated proteins may be obtained in vitro: glutaredoxin specifically reduces glutathionylated protein thiols that can then be tagged with N-ethylmaleimide-biotin; this approach is most suitable to monitor baseline PTM (Lind et al., 2002). Glutaredoxin,

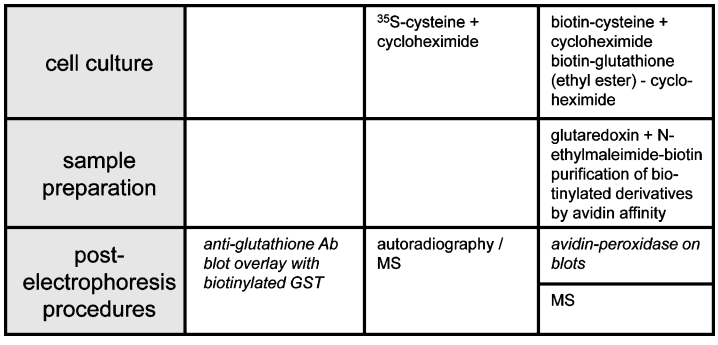

Fig. 2. Outline of the procedures for investigating glutathionylated proteins in a proteomic experiment. Legend: same as in Fig. 1

however, has variable affinity for various S-glutathiolated substrates (Chai et al., 2003). S-glutathionylation in bulk may be measured biochemically by glutaredoxin-stimulated release of GSH from precipitated proteins (Caruso et al., 2005). GSH may be released chemically by $NaBH_4$ treatment (Demasi et al., 2003).

Biotin-labelled proteins may be detected on blots with streptavidin-HRP or may be affinity-purified with streptavidin-agarose for analysis as highly enriched fraction. After trypsinisation, avidin-affinity purification and analysis by HPLC-MS/MS of the tagged peptides have been proposed for high-throughput identification of the S-glutathionylation sites of proteins (Hamnell-Pamment et al., 2005).

Direct purification of glutathionylated proteins by affinity chromatography on glutathione-agarose is also possible (Eaton et al., 2002; Niture et al., 2005).

On blots, S-glutathionylated proteins may be detected by glutathione S-transferase (GST) overlay: biotinylated GST binds to the glutathione moiety of S-glutathionylated proteins (Cheng et al., 2005). Anti-glutathione antibodies are commercially available, but suffer from aspecific background staining.

2.3 Miscellaneous oxidation products

Cysteine

The redox balance −SH/-S-S- may be assessed with a number of reagents. If N-ethylmaleimide is used to block thiol proteins and dithiothreitol is then added to reduce the disulfide proteins, labelling with 5-iodoacetamidofluorescein tags the oxidized forms (Baty et al., 2002). Conversely, derivatization with monobromobimane tags the reduced forms; the thioether derivatives are visualized through their fluorescent emission (Awasthi et al., 1998). Reagents unable to cross plasma cell membranes are of special relevance for the evaluation of exofacial thiols; examples of this class are the fluorochrome N-(7-dimethyl-amino-4-methyl-coumarinyl) maleimide (DACM) (Seppi et al., 1991) and N-(biotinoyl-N-(iodoacetyl)ethylendiamine (BIAM) (Laragione et al., 2003).

Sulfinic/sulfonic acids produced from cysteine residues under oxidative stress conditions may be identified by MS after standard trypsin digestion (Rabilloud et al., 2002). Overoxidation of cysteines however also introduces additional cleavage points for digestion with peptidyl-Asp metalloendopeptidase (AspN). While the mass of the novel N-terminus peptide is independent of the oxidation state, the m/z of the C-terminus peptide varies with the number of bound oxygen atoms (Wagner et al., 2002). For the detection of sulfinylated peroxiredoxins an antibody could be produced that recognizes both sulfinic and sulfonic forms of the protein and can be used in a simple immunoblot assay (Woo et al., 2003); no such antibody exists for the detection of cysteic acid in a context-independent setting.

Tyrosine

3-Nitrotyrosine is considered a biomarker of peroxynitrite exposure. An anti-nitrotyrosine antibody is commercially available. However, to reveal false immunopositive spots, blot membranes (PVDF) should be stripped and chemically reduced with sodium dithionite to convert nitrotyrosine to aminotyrosine, extensively washed and probed again with the anti-nitrotyrosine antibody (Casoni et al., 2005; Miyagi et al., 2002). Dityrosine is another product of tyrosine oxidation (via a tyrosyl radical), and a specific antibody is commercially available. The addition of tyramine coupled to the succinimidyl ester of (fluorescein-5 (and-6)-carboxamido) hexanoic acid to a cell culture medium under conditions of oxidative stress results in the formation of dityrosine bonds. This is reflected by the linkage of the fluorescent tyramine to proteins as detected by immunoblotting with anti-fluorescein antibody (Czapski et al., 2001). An alternative reagent for this protocol is acetyl-tyramine-fluorescein (Sakharov et al., 2003).

Methionine

No antibody against methionine sulfoxide is available; accumulations of MetO in proteins may be monitored as inhibition of CNBr degradation (Moskovitz and Stadtman, 2003).

Tryptophane

The formation of N-formylkynurenine by dioxygenation of tryptophane in proteins separated by 2DE is associated with a significant peptide score using a Q-TOF mass spectrometer, with an increase in peptide mass of 32 mass units, and a fragmentation pattern showing loss of oxidized tryptophane (Moller and Kristensen, 2006).

Lysine adducts with aldehydes

Peroxidation of fatty acids containing three or more double bonds produces malondialdehyde (MDA); that of ω-6 polyunsaturated fatty acid produces 4-hydroxynonenal (HNE). Adducts with lysine residues in proteins may be detected by immunoblotting (Moreau et al., 2003; Tiku et al., 2000).

3. Conclusions

Solving the still open questions about redox regulation and oxidative stress in the fields of physiology and pathology is worth a compound strategy, relying on classical techniques as well as on recent developments of proteomic procedures. The number of entries in the body of this review spells the complexity of the 'protein oxidation' issue and of the technical approaches to its study.

Acknowledgements

Proteomic investigation at DSF is supported by grants from MIUR (FIRB) and from Association Française contre les Myopathies. E.G. is thankful to Maddalena Fratelli, Pietro Ghezzi and Valentina Bonetto for first introducing her to the field of redox proteomics.

References

Awasthi S, Gyurasics A, Knight SA, Welty SE, Smith CV (1998) Protein oxidation biomarkers in hyperoxic lung injury in rats: effects of U-74389. Toxicol Lett 95: 47–61

Baty JW, Hampton MB, Winterbourn CC (2002) Detection of oxidant sensitive thiol proteins by fluorescence labeling and two-dimensional electrophoresis. Proteomics 2: 1261–1216

Bautista J, Mateos-Nevado MD (1998) Immunological detection and quantification of oxidized proteins by labelling with digoxigenin. Biosci Biotechnol Biochem 62: 419–423

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272: 20313–20316

Caruso RL, Upham BL, Harris C, Trosko JE (2005) Biphasic lindane-induced oxidation of glutathione and inhibition of gap junctions in myometrial cells. Toxicol Sci 86: 417–426

Casoni F, Basso M, Massignan T, Gianazza E, Cheroni C, Salmona M, Bendotti C, Bonetto V (2005) Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: Possible multifunctional role in the pathogenesis. J Biol Chem 280: 16295–16304

Chai YC, Hoppe G, Sears J (2003) Reversal of protein S-glutathiolation by glutaredoxin in the retinal pigment epithelium. Exp Eye Res 76: 155–159

Cheng G, Ikeda Y, Iuchi Y, Fujii J (2005) Detection of S-glutathionylated proteins by glutathione S-transferase overlay. Arch Biochem Biophys 435: 42–49

Czapski GA, Avram D, Wirtz KW, Pap EH, Strosznajder JB (2001) Detection of protein oxidation in endothelial cells by fluorescently labelled tyramine. Med Sci Monit 7: 606–609

Davies KJ (2001) Degradation of oxidized proteins by the 20S proteasome. Biochimie 83: 301–310

Dean RT, Fu S, Stocker R, Davies MJ (1997) Biochemistry and pathology of radical-mediated protein oxidation. Biochem J 324: 1–18

Demasi M, Silva GM, Netto LE (2003) 20S proteasome from *Saccharomyces cerevisiae* is responsive to redox modifications and is S-glutathionylated. J Biol Chem 278: 679–685

Dunlop RA, Rodgers KJ, Dean RT (2002) Recent developments in the intracellular degradation of oxidized proteins. Free Radic Biol Med 33: 894–906

Eaton P, Wright N, Hearse DJ, Shattock MJ (2002) Glyceraldehyde phosphate dehydrogenase oxidation during cardiac ischemia and reperfusion. J Mol Cell Cardiol 34: 1549–1560

England K, Cotter T (2004) Identification of carbonylated proteins by MALDI-TOF mass spectroscopy reveals susceptibility of ER. Biochem Biophys Res Commun 320: 123–130

Fisher AE, Naughton DP (2005) Why nutraceuticals do not prevent or treat Alzheimer's disease. Nutr J 4: 14

Frank B, Gupta S (2005) A review of antioxidants and Alzheimer's disease. Ann Clin Psychiatry 17: 269–286

Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M, Bonetto V, Mengozzi M, Duffieux F, Miclet E, Bachi A, Vandekerckhove J, Gianazza E, Ghezzi P (2002) Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. Proc Natl Acad Sci USA 99: 3505–3510

Fratelli M, Demol H, Puype M, Casagrande S, Villa P, Eberini I, Vandekerckhove J, Gianazza E, Ghezzi P (2003) Identification of proteins undergoing glutathionylation in oxidatively stressed hepatocytes and hepatoma cells. Proteomics 3: 1154–1161

Ghezzi P, Bonetto V, Fratelli M (2005) Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. Antioxid Redox Signal 7: 964–972

Griffiths HR (2000) Antioxidants and protein oxidation. Free Radic Res 33 [Suppl]: S47–S58

Grune T, Merker K, Sandig G, Davies KJ (2003) Selective degradation of oxidatively modified protein substrates by the proteasome. Biochem Biophys Res Commun 305: 709–718

Hamnell-Pamment Y, Lind C, Palmberg C, Bergman T, Cotgreave IA (2005) Determination of site-specificity of S-glutathionylated cellular proteins. Biochem Biophys Res Commun 332: 362–369

Humphries KM, Juliano C, Taylor SS (2002) Regulation of cAMP-dependent protein kinase activity by glutathionylation. J Biol Chem 277: 43505–43511

Iwai K (2003) An ubiquitin ligase recognizing a protein oxidized by iron: implications for the turnover of oxidatively damaged proteins. J Biochem (Tokyo) 134: 175–182

Jackson MJ (1999) An overview of methods for assessment of free radical activity in biology. Proc Nutr Soc 58: 1001–1006

Jones AF, Lunec J (1987) Protein fluorescence and its relationship to free radical activity. Br J Cancer [Suppl] 8: 60–65

Juranek I, Bezek S (2005) Controversy of free radical hypothesis: reactive oxygen species: cause or consequence of tissue injury? Gen Physiol Biophys 24: 263–278

Korolainen MA, Goldsteins G, Alafuzoff I, Koistinaho J, Pirttila T (2002) Proteomic analysis of protein oxidation in Alzheimer's disease brain. Electrophoresis 23: 3428–3433

Kristensen BK, Askerlund P, Bykova NV, Egsgaard H, Moller IM (2004) Identification of oxidised proteins in the matrix of rice leaf mitochondria by immunoprecipitation and two-dimensional liquid chromatography-tandem mass spectrometry. Phytochemistry 65: 1839–1851

Laragione T, Bonetto V, Casoni F, Massignan T, Bianchi G, Gianazza E, Ghezzi P (2003) Redox regulation of surface protein thiols: identification of integrin α-4 as a molecular target by using redox proteomics. Proc Natl Acad Sci USA 100: 14737–14741

Lind C, Gerdes R, Hamnell Y, Schuppe-Koistinen I, von Lowenhielm HB, Holmgren A, Cotgreave IA (2002) Identification of S-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. Arch Biochem Biophys 406: 229–240

Linton S, Davies MJ, Dean RT (2001) Protein oxidation and ageing. Exp Gerontol 36: 1503–1518

Mary J, Vougier S, Picot CR, Perichon M, Petropoulos I, Friguet B (2004) Enzymatic reactions involved in the repair of oxidized proteins. Exp Gerontol 39: 1117–1123

Mayne ST (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 133 [Suppl] 3: 933S–940S

Mirzaei H, Regnier F (2005) Affinity chromatographic selection of carbonylated proteins followed by identification of oxidation sites using tandem mass spectrometry. Anal Chem 77: 2386–2392

Miyagi M, Sakaguchi H, Darrow RM, Yan L, West KA, Aulak KS, Stuehr DJ, Hollyfield JG, Organisciak DT, Crabb JW (2002) Evidence that light modulates protein nitration in rat retina. Mol Cell Proteomics 1: 293–303

Moller IM, Kristensen BK (2006) Protein oxidation in plant mitochondria detected as oxidized tryptophan. Free Radic Biol Med 40: 430–435

Moreau R, Heath SH, Doneanu CE, Lindsay JG, Hagen TM (2003) Age-related increase in 4-hydroxynonenal adduction to rat heart α-ketoglutarate dehydrogenase does not cause loss of its catalytic activity. Antioxid Redox Signal 5: 517–527

Moreira PI, Smith MA, Zhu X, Nunomura A, Castellani RJ, Perry G (2005) Oxidative stress and neurodegeneration. Ann N Y Acad Sci 1043: 545–552

Moskovitz J, Stadtman ER (2003) Selenium-deficient diet enhances protein oxidation and affects methionine sulfoxide reductase (MsrB) protein level in certain mouse tissues. Proc Natl Acad Sci USA 100: 7486–7490

Niture SK, Velu CS, Bailey NI, Srivenugopal KS (2005) S-thiolation mimicry: quantitative and kinetic analysis of redox status of protein cysteines by glutathione-affinity chromatography. Arch Biochem Biophys 444: 174–184

Packer L (ed) (1984) Oxygen radicals in biological systems. Methods Enzymol 105: 3–600

Packer L, Glazer AN (eds) (1990) Oxygen radicals in biological systems. Part B: Oxygen radicals and antioxidants. Methods Enzymol 186: 1–855

Rabilloud T, Heller M, Gasnier F, Luche S, Rey C, Aebersold R, Benahmed M, Louisot P, Lunardi J (2002) Proteomics analysis of cellular response to oxidative stress: evidence for in vivo overoxidation of peroxiredoxins at their active site. J Biol Chem 277: 19396–19401

Reinheckel T, Korn S, Mohring S, Augustin W, Halangk W, Schild L (2000) Adaptation of protein carbonyl detection to the requirements of proteome analysis demonstrated for hypoxia/reoxygenation in isolated rat liver mitochondria. Arch Biochem Biophys 376: 59–65

Rokutan K, Thomas JA, Johnston RB Jr (1991) Phagocytosis and stimulation of the respiratory burst by phorbol diester initiate S-thiolation of specific proteins in macrophages. J Immunol 147: 260–264

Rottoli P, Magi B, Cianti R, Bargagli E, Vagaggini C, Nikiforakis N, Pallini V, Bini L (2005) Carbonylated proteins in bronchoalveolar lavage of patients with sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis. Proteomics 5: 2612–2618

Sakharov DV, Bunschoten A, van Weelden H, Wirtz KW (2003) Photodynamic treatment and H_2O_2-induced oxidative stress result in different patterns of cellular protein oxidation. Eur J Biochem 270: 4859–4865

Seppi C, Castellana MA, Minetti G, Piccinini G, Balduini C, Brovelli A (1991) Evidence for membrane protein oxidation during in vivo aging of human erythrocytes. Mech Ageing Dev 57: 247–258

Soreghan BA, Yang F, Thomas SN, Hsu J, Yang AJ (2003) High-throughput proteomic-based identification of oxidatively induced protein carbonylation in mouse brain. Pharm Res 20: 1713–1720

Sullivan DM, Wehr NB, Fergusson MM, Levine RL, Finkel T (2000) Identification of oxidant-sensitive proteins: TNF-α induces protein glutathiolation. Biochemistry 39: 11121–11128

Talent JM, Kong Y, Gracy RW (1998) A double stain for total and oxidized proteins from two-dimensional fingerprints. Anal Biochem 263: 31–38

Thomas JA, Park EM, Chai YC, Brooks R, Rokutan K, Johnston RB Jr (1991) S-thiolation of protein sulfhydryls. Adv Exp Med Biol 283: 95–103

Tiku ML, Shah R, Allison GT (2000) Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation: possible role in cartilage aging and the pathogenesis of osteoarthritis. J Biol Chem 275: 20069–20076

Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12: 1161–1208

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160: 1–40

Wagner E, Luche S, Penna L, Chevallet M, Van Dorsselaer A, Leize-Wagner E, Rabilloud T (2002) A method for detection of overoxidation of cysteines: peroxiredoxins are oxidized in vivo at the active-site cysteine during oxidative stress. Biochem J 366: 777–785

Wait R, Begum S, Brambilla D, Carabelli AM, Conserva F, Rocco Guerini A, Eberini I, Ballerio R, Gemeiner M, Miller I, Gianazza E (2005) Redox options in two-dimensional electrophoresis. Amino Acids 28: 239–272

Woo HA, Kang SW, Kim HK, Yang KS, Chae HZ, Rhee SG (2003) Reversible oxidation of the active site cysteine of peroxiredoxins to cysteine sulfinic acid: immunoblot detection with antibodies specific for the hyperoxidized cysteine-containing sequence. J Biol Chem 278: 47361–47364

Yoo BS, Regnier FE (2004) Proteomic analysis of carbonylated proteins in two-dimensional gel electrophoresis using avidin-fluorescein affinity staining. Electrophoresis 25: 1334–1341

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