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# Protein oxidation: basic view on characterization, detection and consequences

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Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive oxygene species (ROS) or indirectly by reactions with secondary byproducts of oxidative stress. Proteins have many specific functions. Oxidative modification of a protein leads to biochemical consequences. Different forms of oxidative modification have different functional consequences. ROS play a major role in the generation of acute and chronic inflammatory diseases There is no single universal marker for protein oxidation. The characterization, detection and consequences of proteins are rewieved.

**Key words:** Oxidized protein, markers of oxidized protein, oxidized proteins in diseases

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Reactive oxygen species and other damaging free radicals are generated in a wide spectrum of physiological and pathological processes.[1] Covalent modification of a protein induced by reactive oxygen intermadiates or by products of oxidative stress. Agents responsible for oxidative damage and protein oxidation are listed below.

- Chemical reagents ($H_2O_2$, $Fe^{+2}$, $Cu^{+}$, glutathione, HOCl, HOBr, $^1O_2$, $ONOO^-$)
- Activated phagocyte oxidative burst activity
- γ-irradiation in the presence of $O_2$
- UV light, ozone
- Lipid peroxides (HNE, MDA, acrolein)
- Mithocondrial electron transport chain leakage
- Oxidoreductase enzymes (xhantine oxidase, myeloperoxidase, P-450 enzymes)
- Drug and their metabolites.[2]

Results of studies demonstrated that modification of proteins is initiated mainly by reactions of the oxidative process. Collectively, these reactive oxygene species (ROS) can lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation.[3]

General types of protein modification;

- Sulfur oxidation (Cys disulfides, S-thiolation, Metsulfoxide)

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- Protein carbonyls (side chain aldehydes, ketones)
- Tyrosine crosslinks, chlorination, hydroxylation
- Tryptophanyl modifications
- Hydro(pero)xy derivatives of aliphatic amino acids
- Chloramines, deamination
- Aminoacid interconversions (e.g., His to Asn; Pro to OH-Pro)
- Lipid peroxidation adducts (MDA, HNE, acrolein)
- Aminoacid oxidation adducts (e.g., p-hydroxyphenylacetaldehyde)
- Glixocidation adducts (e.g., carboxymetillysine)
- Cross-links, aggregation, pepdide bound cleavage.[4,5]

## Biocemical Consequences of Protein Oxidative Modification

Proteins have many different and specific functions. Oxidative modification of a protein lead to biochemical consequences.[6]

- Loss or gain enzyme activity
- Loss of protein function
- Loss of protease inhibitor activity
- Protein aggregation
- Enhanced or diminished susceptibility to proteolysis
- Abnormal cellular uptake (e.g.,LDL)
- Modified gene transcription
- Increased immunogenicity

We showed that oxidatively modified fibrinogen had less binding activity than native fibrinogen to GpIIb/IIIa coated micro beads and to isolated human platelets.[6,7] Oxidation extent was followed by measuring carbonyl content and dityrosine formation in fibrinogen.[8] Blood plasma was treated by chloramin-T oxidative system *in vitro*. Levels of protein carbonyls were found to be significantly increased in oxidized plasma.[9]

Reactive oxygen species (ROS) play a major role in the generation of acute and chronic inflammatory diseases.[10] Levels of protein carbonyls were found to be statistically increased in rheumatoid arthiritis (RA) patients.[11] HOCl induced protein oxidation, serve as a marker for oxidants generated as part of the inflammatory responce.[12] Atherosclerosis and most neurodegenerative diseases are closely related to inflammatory processes.[13] Oxidized LDL is generated in the atherosclerotic tissue.[14] Oxidative processes play an essential role in Alzheimer disease[15] and other neurodegenerative diseases[16-18] as well as in aging.[15]

Diseases related to protein oxidation are:

- Atherosclerosis (LDL)
- Rheumatoid arthiritis (IgG, α-1-proteinase inhibitor)
- Ischemia reperfusion injury
- Emphysema (α-1-proeinase inhibitor, elastase)
- Neurodegenerative diseases (e.g, Alzheimer, Parkinson's)
- Muscular distrophy
- Aging (glutamine snthetase, carbonic anhydrase III, aconitase)
- Acute pancreatitis
- Cataractogenesis (α-crystallins)
- Cancer
- Chronic ethanol ingestion
- Adult respiratory distress syndrome

## Methods for Detection of Oxidative Protein Modifications

During the past years it was observed that the development of diseases is combined with the oxidation of proteins. Identical markers of oxidative reactions have been found enriched in plasma of

patients, with increase in carbonyls, dityrosines, disulfides and with other reaction products of reactive LPO products. These markers are useful to study detection of oxidative protein modifications of biological samples. Products were investigated after appropriate derivatization by gas chromatography (GC) and their structures were elucidated by electron impact mass spectrometry (EI/MS) and other instrumental methods (Table 1).[5,19-22] Detection of nitrotyrosine-containing proteins and carbonyl groups by a competition enzyme-linked immunosorbent assay (C-ELISA) in plasma.[23,24]

Among the various oxidative modifications of proteins formation of carbonyl groups is one of the early markers for protein oxidation[2,25] Human plasma proteins were reacted with dinitrophenylhydrazine and then proteins were non-specifically adsorbed to an ELISA plate (Table 1).[26]

Carbonyl groups of proteins are stable products. Present at low levels in most protein preparations (~1nmol/mgprotein ~1/300 amino acids). Protein carbonyls are elevated about 2-8 fold under conditions of oxidative stress *in vivo*. Carbonyl groups in proteins are induced by almost all types of oxidants *in vitro* (site specific metal catalyzed oxidation, γ-irradiation, HOCl, ozone, $^1O_2$, lipid peroxide adducts).[4,27,28]

There is no single universal marker for detection of protein oxidation. If source of oxidation is unknown several different assays are needed. If source of oxidation is known, the range narrows (metal catalyzed oxidation does not cause chlorination or nitration, HOCl does not cause lipid peroxidation adducts). Types of protein modification are revealed nature of oxidizing species. Due to the specificity of protein functions unique physiological consequences result. On the other hand, different forms of oxidative modifications have different functional consequences (e.g., carbonyls are often associated with disfunction of proteins but may require more stringent oxidative conditions where-

**Table 1**

*Methods for detection of oxidative protein modifications*[5,19-27]

| Modifications | Method of detection |
|---|---|
| Disulfides | SDS-gel electrophoresis, DTNB<br>Reaction with BIAM* or MPB** to SDS-PAGE |
| Glutathiolation | RP-HPLC/mass spectrometry<br>$S^{35}$-Cys/Chx→SDS-PAGE |
| Methionin sulfoxide | CNBr cleavage/amino acid analysis |
| Carbonyls | DNPH***-coupled assays:Spectroscopy<br>HPLC, Western blotting, ELISA,<br>Immunohistochemistry, Reduction with $NaB^3H_3$ |
| 2-oxo-Histidine | Amino acid analysis |
| Dityrosine | Spectrophotometric<br>Proteolysis or hydrolysis→HPLC |
| Chlorotyrosine | Hydrolysis/nitroso-naphtol/HPLC→GC/MS |
| Nitrotyrosine | Immunoassay, Hydrolysis→HPLC<br>HPLC/electrochemical detection |
| Tyrptophanyl modifications | Fluorescence, Amino acid analysis,<br>Proteolysis/MS |
| Hydroperoxides | KI/$I_3$-/Spectroscopy<br>$NaBH_4$/hydrolysis/OPA****-HPLC |
| Lipid peroxidation adducts | Immunoassays, DNPH, Hydrolysis/→GC/MS<br>$NaBH_4$/hydrolysis/OPA-HPLC |
| Amino acid oxidation adducts | NaCNBH3 reduction/hydrolysis/$H^1$-NMR/MS |
| Glycoxidation adducts | Derivization→GC/MS |
| Cross-linked aggregates, | SDS-gel electrophoresis |
| Fragments | HPLC |

***BIAM:** Biotinylated iodoacetamide, ****MPB:** Maleimido-propionyls biocytin, *****DNPH:** Dinitrophenylhidrazine, ******OPA:** O-phytaldialdehyde **RP:** Reverse phase, **IEF:** Isoelectric focusing, **CNBr:** Cyanogen bromide, **ELISA:** Enzyme linked Immunoassay, **HPLC:** High peformance liquid chromatography, **NaB3H3:** natrium borohydride, **HBr:** Hydrogen bromide, **GC/MS:** Gas chromatography/Mass spectrometry

as methionine highly susceptible to oxidation but its oxidation does not affect protein function).[29]

## How Can We Inhibit Protein Oxidation?

The transfer of radical reactions from one cell to an adjacent one is interrupted by antioxidants, such as glutathione. The antioxidant reacts with the radical by hydrogen abstraction, forming a new radical of the antioxidant. A compound is able to act as antioxidant and as a radical scavenger (probucol, spin traps, methionine) only if the generated antioxidant radicals have a sufficiently long lifetime to react with a second radical by forming a new molecule.[30-32]

The individual levels of the body's own antioxidants (e.g., glutathione, catalase, superoxide dismu-

tase, peroxiredoxins) depend on the ability of the corresponding enzymes and antioxidant enzyme mimics (e.g., ebselen, Tempol, TRAPS) to reproduce the scavenged molecules and is, therefore, tightly connected with the genetic predisposition. Augmentation of cellular antioxidant sytem leads to increased N-acetylcystein leading to increases in intracellular GSH levels. The ability of glutathione and other antioxidants regeneration decreases with aging.[4,33]

## References


1. Halliwel B, Gutteridge JM. Role of free radicals and and catalytic metal ions in human disease: an owerview. *Methods Enzymol* 1990; 186: 1-85.
2. Shacter E. Protein oxidative damage. *Methods Enzymol* 2000; 319: 428-36.
3. Baynes JW, Thorpe SR. Glycoxidation and lipoxidation in atherogenesis. *Free Radic Biol Med* 2000; 28: 1708–16.
4. Shacter E. Quantification and significance of protein oxidation in biological samples. *Drug Met Rev* 2000; 32: 307-26.
5. Çakatay U, Telci A. Oksidatif protein hasarı ve saptanmasında kullanılan marker'lar. *İ. Ü. Tıp Fakültesi Mecmuası*, 2000; 63: 314-7.
6. Tetik S, Yardimci KT. Oxidized protein and hemostasis. Congress of VIIth Thrombosis, Hemostasis and Angiology. Ed. Ulutin ON (ISBN: 978-9944-5184-1-3), 279-291, Çukurova University Medicine Faculty, Adana, 4-6 Mai 2007.
7. Shacter E, Williams JA, Levine RL. Oxidative modification of fibrinogen inhibits thrombin-catalyzed clot formation. *Free Radic Biol Med* 1995; 18: 815-21.
8. Kaya K. Examination of the structural alterations due to in vitro fibrinogen oxidation. Marmara University Institue of Health Science Up graduate Thesis, Istanbul, 2006 (Adviser: S. Tetik).
9. Kılıç K. Oxidized protein content of plasma. Marmara University Institue of Health Science Up graduate Thesis, Istanbul, 2006 (Adviser: S. Tetik).
10. Merry P, Winyard PG, Morris CS, Grootweld M, Blake DR. Oxygen free radicals, inflammation and synovitis: the current status. *Ann Rheum Dis* 1989; 48: 864-70.
11. Tetik S, Ersoy B, Dişbudak M, et al. Detection of oxidant stress in the plasma of patients with rheumatoid arthritis Congress of VIIth Thrombosis, Hemostasis and Angiology. Ed. Ulutin ON (ISBN: 978-9944-5184-1-3), 279-291, Çukurova University, Medicine Faculty, Adana, 4-6 Mai 2007.
12. Heinecke JW. Mechanisms of oxidative damage by myeloperoxidase in atherosclerosis and other inflammatory disorders. *J Lab Clin Med* 1999; 133: 321-5.
13. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherosclerogenesis: an overview. *Free Radic Biol Med* 2000; 28: 1815–1826.
14. Hazell LJ, van den Berg JJ, Stocker R. Oxidation of low-density lipoprotein by hypochlorite causes aggregation that is mediated by modification of lysine residues rather than lipid oxidation. *Biochem J* 1994; 302: 297-304.
15. Montine TJ, Neely JF, Quinn MF, et al. Lipid peroxidation in aging brain and Alzheimer's disease. *Free Radic Biochem Med* 2002; 33: 620–6.
16. Hald A, Lotharius J. Oxidative stress and inflammation in Parkinson's disease: is there a causal link?, *Exp Neurol* 2005; 193: 279–90.
17. Jenner P. Oxidative damage in neurodegenerative diseases. *Lancet* 1994; 344: 796–8.
18. Yao JK, Reddy RD, van Kammen DP. Oxidative damage and schizophrenia. *CNS Drugs* 2001; 15: 287–310.
19. Domingues MR, Domingues P, Reis A, Fonseca C, Amado FM, Ferrer-Correia AJ. Identification of oxidation products and free radicals of tryptophan by mass spectrometry. *J Am Soc Mass Spectrom* 2003; 14: 406-16.
20. Frankel EN. Lipid oxidation 2nd ed. Oily Press; Bridgewater, UK: 2005. p. 15–98.
21. Halliwell B. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo. *FEBS Lett* 1997; 411: 157-60.
22. Keller RJ, Halmes NC, Hinson JA, Pumford NR. Immunochemical detection of oxidized proteins. *Chem Res Toxicol* 1993; 6: 430-3.
23. Khan J, Brennan DM, Bradley N, Gao B, Brukdorfer R, Jacobs M. 3-Nitrotyrosine in the proteins of human plasma determined by an ELISA method. *Biochem J* 1998; 330: 795–801.
24. Buss H, Chan TP, Sluis KB, Domigan NM, Winterbourn CC. Protein carbonyl measurement by a sensitive ELISA method. *Free Radic Biol Med* 1997; 23: 361–6.
25. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta* 2003; 329: 23–38.
26. Levine RL. Carbonyl modified protens in cellular regulation, aging and disease. *Free Radic Biol Med* 2001; 32: 790–6.
27. Robinson CE, Kashavarzian A, Pasco DS, Frommel TO, Winshop DH, Holmes EW. Determination of protein car-

bonyl groups by immunoblotting. *Anal Biochem* 1999; 266: 48-57.

28. Stadtman ER, Levin RL. Protein oxidation. *Ann NY Sci* 2000; 899: 191-208.
29. Winerbaun CC, Kettle AJ. Biomarkers of myeloperoxidase –derived hypochlorus acid. *Free Radic Biol Med* 2000; 29: 403-9.
30. Sies H. Biochemistry of oxidative stress. *Angew Chem* 1986; 98: 1061–75.
31. Brigelius-Flohe R. Tissue specific functions of the individual glutathione peroxidases. *Free Radic Biol Med* 1999; 27: 951–65.
32. Imai H, Nakagawa Y. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx-GPx4) in mammalian cells Free Radic. *Biol Med* 2003; 34: 145–169.
33. Greenacre SA, Ischiropoulos H. Tyrosine nitration; localization, quantification, consequences for protein function and signal transduction. *Free Radic Res* 2001; 34: 541-81.