Oxidized Proteins As a Biomarker of Oxidative Stress in Aging and Diseases

Yaşlanma ve Hastalıklarda Oksidatif Stres Biyomarkörü Olarak Okside Proteinlerin Rolü

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ABSTRACT Oxidative stress is an inevitable process of the aerobic life. Among the effects of oxidative stress, protein oxidation takes an important place because of the high protein abundance in the organism. The degradation of non-functional, oxidized proteins is an essential function of intra- and extracellular proteolytic systems. However, severely oxidized proteins are poor substrates for degradation and may accumulate. This process of accumulation of oxidized, cross-linked protein material is involved in the physiology and pathophysiology of aging and many diseases.

Key Words: Oxidative stress; aging

ÖZET Oksidatif stres, günlük yaşamda oksijenli solunumun kaçınılmaz sonuçlarındandır. Oksidatif stresin etkileri arasında protein oksidasyonu, organizmadaki yüksek protein oranından dolayı, önemli yer almaktadır. Fonksiyonel olmayan, okside proteinlerin degradasyonu intra ve ekstraselüler proteolitik sistemlerin önemli bir fonksiyonudur. Bununla birlikte, ağır derecede okside olan proteinler degradasyon için zayıf substratlardır ve akümüle olabilirler. Bu okside ve kovalent bağlı protein materyalinin akümülasyonu süreci, yaşlanma ve birçok hastalığın fizyolojisi ve patofizyolojisinde rol oynamaktadır.

Anahtar Kelimeler: Oksidatif stress; yaşlanma

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Increased formation of free radicals and other oxidants leads to cellular damage involving the oxidation of biomolecules. Proteins are recognized as the main structures in the cells and tissues and are, therefore, potentially major targets for oxidative damage in addition to DNA and lipids.[1] Reactions of free radicals with proteins leads to the formation of different characteristical products and subsequently loss of function. The degradation of non-functional, oxidized proteins is an essential part of the antioxidant defense in the cells.[2] Experimantal evidence from several studies shows that many of the alterations during aging and the progression of certain diseases are the result of the occurence of protein oxidation products and decrease in the degradation of oxidized proteins.[3]

OXIDATIVE MODIFICATIONS OF PROTEINS

Protein oxidation is often mediated by free radicals, usually in a site specific manner.[3] As biomarkers of protein oxidative damage, both the oxidation of the amino acid residue side chains and the oxidation of peptide backbones have been studied

by different laboratories. Amino acid side chains are known to be the primary sites of free radical damage.[4] Several products are formed during the oxidation of the residues. Especially cysteine and methionine residues are even under mild conditions susceptible to many reactive oxygen species (ROS). Direct oxidation of lysine, arginine, proline and threonine residues may produce carbonyl derivatives. Carbonyl groups are most commonly used markers of protein oxidation, which are thought to be main products.[5-8] However, carbonyl groups are generally used in the quantification of protein oxidation because of the relative early formation and the stability of carbonylated proteins and therefore determined in the tissues and cells during a number of diseases and aging.[3] For this purpose several sensitive methods have been developed to determine and quantify protein carbonyls.[5]

In addition to modification of amino acid side chains, oxidation reactions can also lead to the formation of protein cross-links and protein fragmentation as a result of protein backbone oxidation.[2,4,5,9,10] Fragmentation is the result of the cleavage of the peptide bond by either the diamide or α-amidation pathways. Fragments can be derived from the N-terminal or C-terminal side. The α-hydrogen atom of an amino acid residue is abstracted from the polypeptide backbone to form a carbon-centered radical.[11] The carbon centered radical may react with another carbon-centered radical to form a protein-protein cross-linked derivative.[5] One of the most thoroughly investigated cross-links is the formation of a 2,2'-biphenyl cross-link by two tyrosyl radicals.[12] As a consequence of cross-link formation, insoluble protein aggregates can be fromed. Besides covalent cross-linking a large part of protein aggregation is due to hydrophobic and electrostatic interactions.[10,13] The accumulation of the large aggregates are known to be often toxic to cells and poor substrates for proteases, which results in their accumulation within cells.[14] This aggregate accumulation has been reported for many experimental models, as measured by several markers for protein oxidation.[4,10]

Damage to proteins can occur by direct attack of reactive species or by secondary damage involving attack by products of lipid peroxidation, such as isoketals, MDA (malondialdehyde) and HNE (4-hydroxynonenal). Proteins can also be damaged by glycation/glyoxidation.[15] These secondary reactions with products resulting from oxidation of non-protein cellular constituents are in part responsible for the introduction of carbonyl groups into the protein pool.[2]

Some protein damage is reversible, such as peroxiredoxin inactivation, methionine sulphoxide formation, s-nitrosylation. Other damage, for example oxidation of side-chains to carbonyl residues, appears ireversible and the protein is destroyed and replaced.[15]

ANTIOXIDATIVE MECHANISM AGAINST PROTEIN OXIDATION

In the cellular defence, intracellular oxidation products formed are either repaired or removed.[14] For the most part, oxidatively modified proteins are not repaired and must be removed by proteolytic degradation.[3] The degradation of abnormal, oxidized and unfolded proteins is a physiological process required to maintain normal cellular function.[9,16] Therefore, cells have developed highly regulated intracellular proteolytic systems responsible for the removal of such non-functional proteins before they begin to aggregate,[4,10] whereas disulfide bonds and methionine sulfoxides can be repaired enzymatically by thioredoxin/thioredoxin reductase system, protein disulfide isomerase and methionine sulfoxide reductases.[14] Cells also contain a number of proteins such as shock or stress proteins with the ability to reconstitute the tertiary structure of oxidized proteins.[2,14]

Mammalian cells include several pathways for general protein breakdown comprises membrane proteases, lysosomal cathepsins, mitochondrial proteases (including the lon protease), calpains, caspases, and the proteasomal system.[2,14] Several intracellular proteins, foreign proteins from outside and proteins from various organelles are degraded within lysosomes.[2] However, in oxidative stress conditions, proteasomes play the major role in the protein turnover of the cells.[2,9,14] Beside protein degradation also several vital cellular functions including cell differentiation, cell cycle dependent cyclin turnover, cell division, antigen processing and NF-κB activation have been shown to require the action of the proteasome.[16,17]

For understanding of cellular protein maintenance the distribution of the proteasomes in the cell and their response upon oxidative stress is of great interest.[9] The proteasome, known to be localized in the cytosol and in the nuclei of mammalian cells and furthermore attached to the endoplasmic reticulum and the cell membrane, is mainly composed of 20S core proteasome and various regulatory components like 19S (PA700) and 11S (PA28).[16,18] Binding of 11S and 19S on the core proteasome influences the activity of 20S proteasome.[9] However, it is generally believed that ATP and ubiquitin

independent degradation of 20S proteasome core complex is sufficient for degradation of oxidized proteins.[19,20] The 20S proteasome is composed of 28 subunits and these subunits form a cylindrical particle of four rings. The protein substrates enter the cylinder via the opening the outer rings and are cleaved on the carboxyl side of basic, hydrophobic and acidic amino acids (trypsin like-, chymotrypsin like-, and peptidylglutamyl-peptide-hydrolase activity).[2] The 26S proteasome complex is formed by binding the two 19S regulators on both side of the core and characterized by different substrate specificity and a selective degradation process which is ATP and ubiquitin dependent.[9]

To examine the nuclear maintenance, several studies focused on the role of the proteasomal degradation in the nuclei[2,21,22] and it was suggested that the nuclear proteasome selectively degrades oxidatively damaged proteins in the nucleus.[2,9,14,23] The results also showed that following oxidative stress in the nucleus, the PARP is activated and the activated PARP is able to increase the nuclear proteasome activity that facilitates selective degradation of oxidatively damaged histones.[21,23]

Several experimental procedures show the necessity of proteasome in proteolytic degradation of oxidized proteins.[13,24-26] The importance of proteasome was confirmed by proteasome immunoprecipitation, antisense treatment and inhibitor treatments which cause a dramatic decrease in the ability of cell lysates to degrade oxidized proteins.[24,25] These studies indicate that the proteasome is responsible for about 70–80% of protein degradation after oxidant exposure.

It has been shown that the proteasomal degradation increases following mild oxidation of the substrate, whereas severe oxidative damage causes decrease in the proteasomal activity.[24,25] The strong correlation between the increase of proteolytic susceptibility and the amount of oxidant at moderate oxidant concentrations is due to a permanent increase in the hydrophobicity of the protein substrate following oxidation.[12,27] It is suggested that oxidation of proteins cause an exposure of hydrophobic moieties from the protein core to the surface. Since it was shown that the proteasome has a preference to bind hydrophobic and aromatic amino acids, the recognition of these hydrophobic unfolded patches by the proteasome was proposed. It was demonstrated that a further oxidation causes a decrease in proteasomal proteolytic susceptibility due to protein aggregation and cross-linking. These aggregates are thought to inhibit proteasome activity by clogging up the outer ring of proteasomal cylinder. At this point, it can be concluded that the removal of minimally oxidized proteins is an essential function for maintaining cellular homeostasis and to prevent the accumulation of highly oxidized and cross-linked proteins, which are no longer degradable.[2,28]

PROTEIN OXIDATION IN AGING AND DISEASES

Protein oxidation may be a biomarker of numerous diseases and aging in several ways. The biomedical literature is full of claims that reactive species are involved in human diseases. Some diseases are probably caused by oxidative stress. However, in most diseases, oxidative stress is a consequence and not a cause. Oxidative stress and protein oxidation contributes to tissue injury in some diseases, so that therapeutic intervention with antioxidants should be beneficial, provided that the agents used actually do decrease the oxidative damage.[29]

Reactive species can be a cause or a consequence of cancer and also in the chemotherapy nuclear defence was hypothesized to be strong with high proteasome activity in the nucleus against the oxidative nucleoprotein damage caused by chemotherapeutic drugs. Recently it was shown that the rapid activation of 20S proteasome in response to oxidative stress is accompanied by the poly (ADP-ribosyl)ation.[23] Also a PARP-1 independent mechanism of nuclear proteasome activation after glyoxal treatment was demonstrated.[30] This was based on partially higher proteasome content in nucleus.

Recently, aging studies related to oxidative stress are of great interest. The free radical theory of aging was introduced in 1956 by Denham Harman, who proposed that ageing results from random deleterious damage to tissues by free radicals.[29] Measurements of biomarkers in humans and other animals suggest that oxidative damage increase with age. Indeed both lon proteinase and proteasome activities decline with age in some animal tissues especially in brain. In mice the decline in Lon in liver was associated with increased levels of oxidized mitochondrial proteins, including aconitase.[31,32] Methionine sulphoxide reductase was also shown to be important by increasing the lifespan with overexpression in Drosophila.[33] As an interesting aside, an age related increase in the protein carbonyl content was found in rat hepatocytes,[34] human brain,[35] human red blood cells[36] and eye lens.[37] One of the highlights put forward to support the free radical theory of aging was the presence of age pigments such as lipofuscin, ceroid or AGE-pigment like fluorophores. Lipofuscin is thought to be conjugates of MDA and protein thiol groups deduced from the flu-

orescence character[38] and it was recently shown by several groups that the presence of such material influences the proteasomal activity.[39, 40] These aggregated cross-linked material will be autophagozytosed resulting in a major accumulation of this material in lysosomes. The observed age-related accumulation of oxidized cross-linked material may be the result of both increased protein oxidation followed by aggregation and/or decline protein breakdown and a malfunction of the proteasomal system.[2]

Specifically human skin undergoes an aging process, which is characterized by a loss of elasticity and wrinkle formation. These symptoms of skin aging are caused by intracellular changes and an enhanced degradation of collagen fibers. For example the activity of collagen degrading matrix metalloproteinases is increased in skin aging and photoaging caused by UV exposure. Photoaging is a growing problem recently which many studies are focused on. Activation of MMP expression after UVA irradiation was shown to be mediated by singlet oxygen formation.[41-43] Also the increase in matrix metalloproteinase and collagen degradation was seen to be in correlation with protein oxidation and proteasomal degradation in the ongoing study in our laboratory.

CONCLUSION

The available literature suggest that free radical formation is the most abundant case studied in human diseases and aging. In the oxidative modifications of biomolecules, protein oxidation takes an important place. Although, proteolytic systems exist removing oxidized proteins, protein oxidation is an excellent biomarker of oxidative stress due to the relative long half-life of such oxidized proteins. Following severe oxidative stress, the decrease in the proteolytic degradation and accumulation of non-folded proteins may be the cause and/or the consequence of many disorders and aging. All data until now shows the importance of protein oxidation and the functionality of the proteasomal system in the lifespan and seem to be crucial for further studies in the field.

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