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Forum Review

Proteasomal Defense of Oxidative Protein Modifications

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ABSTRACT

The proteasome has an important role in the degradation of normal, damaged, mutant, or misfolded proteins. This includes the degradation of normal and regulatory proteins in the cellular metabolism and additionally the removal of damaged proteins as a stress response. The two well-described proteasome regulators, the 11S and the 19S regulators, forming together with the 20S 'core' proteasome various forms of the proteasome, including the ATP-stimulated 26S proteasome. As a result of aerobic metabolism, reactive oxygen species (ROS) are constantly generated during the lifetime of biological organisms. Consequently a permanent generation of oxidative damage takes place. This includes the formation of oxidatively modified proteins. These oxidized protein derivatives tend to aggregate, and accumulation of these aggregates may lead to cell death. To prevent this, such oxidatively modified proteins are selectively recognized and either repaired or degraded by the proteasome. The current knowledge of the repair systems and the degradation mechanism is reviewed here. The possible interactions between the ubiquitin–proteasome-system, the chaperone system, the protein repair mechanisms, and other antioxidative defense strategies are highlighted. *Antioxid. Redox Signal.* 8: 173–184.

INTRODUCTION

Cells possess several major pathways for protein degradation: lysosomal proteases, calcium-dependent proteases, and the proteasomal system. The proteasome has a key role in the degradation of intracellular proteins (33, 69).

Numerous cellular pathways are regulated by the timely coordinated removal of critical proteins [e.g., proteins involved in the cell cycle, DNA repair, and stress response (14, 44)]. On the other hand, proteins undergo, as other cellular components, a permanent damaging process and these abnormal folded proteins have to be degraded. The majority of proteins to be degraded are first covalently attached to a polymeric chain of ubiquitin molecules. This modification is recognized by the proteasome, which binds to the polyubiquitin and hydrolyzes the target protein into amino acids and small peptides (14, 44, 61). During this process the ubiquitin is released again. The proteasomal form involved in the degradation of ubiquitinated proteins is the 26S proteasome, a 2 MDa holoenzyme, divided into three major subcomplexes: the 20S core particle, and two 19S regulator particles (16, 34). This degradation pathway is ATP-stimulated (28). But that pathway degrades not all proteins. Our work (35, 39, 40, 93), and the work of others (18, 27, 52, 80, 87) postulate a degradation of unfolded oxidized proteins via the 20S proteasomal form. Additionally, the 26S proteasome is also able to degrade some not polyubiquitinated proteins (51). However several posttranslational modifications are accompanied by a decline in the proteolytic susceptibility of the substrates, leading to an accumulation of this nonfunctional protein debris. These modifications include at least severe oxidation (35, 39, 40, 94) and nonenzymatic glycation (75). The accumulation of nonfunctional proteins above a threshold level may lead to disturbance of cellular function, cellular death, aging, and disease.

THE PROTEASOMAL SYSTEM AND ITS STRUCTURE

The proteasomal system is a multicomponent enzymatic system consisting of several regulatory factors and particles and the 20S 'core' proteasome (700 kDa). This 20S proteasome

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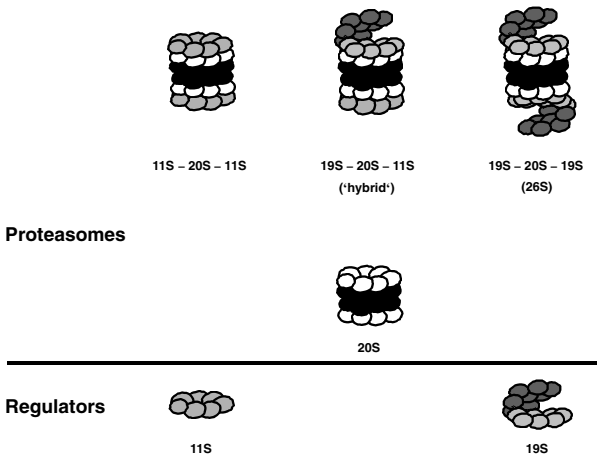

FIG. 1. Different proteasomal forms. The 20S 'core' proteasome is interacting with the two known regulators, the 11S and the 19S regulators. The 11S-20S-11S, 19S-20S-19S (26S proteasome) or the "hybrid" proteasome (19S-20S-11S) are formed. The 20S proteasome exists also as a free particle in the cell.

is a barrel-shaped structure, made up of four rings of seven subunits each. The inner rings contain β-subunits containing the proteolytic active sites located inwards in a sequestered proteolytic chamber (67), the two outer rings are made up of the so-called α-subunits. Three of the β-subunits can be replaced by inducible subunits upon interferon-γ induction that alter the proteolytic specificities of the proteasome.

There are two well-described regulators of the proteasomal system: the 11S and the 19S. The regulators are able to attach to the surface of the outer α-rings; two 19S regulators form the so-called 26S proteasome (Fig. 1). The 19S itself can be further dissected into two multisubunit substructures, a lid and a base (30). The base consists of six homologous ATPases and three non-ATPase subunits. The function of the ATPases is most probably the unfolding of substrates and the translocation of them into the core particle (8). The lid (400 kDa) is made up of eight non-ATPase subunits which can be released from the proteasome or rebind under certain conditions. The lid is required for degradation of polyubiquitinated proteins (29).

The 11S regulator consists in general of PA28α and PA28β subunits (45, 101). The binding of the 11S regulator to the 20S proteasome results in a 3- to 25-fold increase in the degradation of fluorogenic peptides (45). Since several subunits of the proteasome are phosphorylated (49, 55, 70), it can be suggested that proteasomal function is also regulated via phosphorylation.

THE MULTIPLE FUNCTIONS OF THE PROTEASOMAL SYSTEM

The proteasomal system realizes its functions in the cellular metabolism via the degradation of proteins. As stated above, the majority of proteins to be degraded are covalently attached to a polyubiquitin chain. The substrates of this process are involved in a large number of cellular processes and include cyclins, cyclin-dependent kinases (CDK), transcription factors, cell surface receptors, and structural proteins (14, 61). Therefore, the ubiquitin-proteasome pathway is fundamental to synchronized continuation of many cellular processes, for example, cell cycle progression, stress response, and cell differentiation (14, 111).

The ubiquitin–proteasome-system is also primarily responsible for the degradation and clearance of damaged, mutant, or misfolded proteins in eukaryotic cells (91). So it might play a role in the protection against the prion protein, since inhibition of the proteasomal pathway generates a highly aggregation-prone, cytotoxic form of the prion protein, involved in neurodegeneration (83). The degradation of functional incompetent nuclear proteins is also an essential role of the proteasomal system, which is also located within the nucleus (34). Recent studies demonstrated that the ubiquitin–proteasome pathway is involved in the regulation of the nucleotide excision repair (NER) in yeast (111).

To be degraded several specific signals for recognition exist which can be switched on or off. This includes structural dismantling of signal sequences, phosphorylation, or hydroxylation. These signals are recognized by a specific constellation of ubiquitinating enzymes that covalently link the carbonyl group at the C-terminus of ubiquitin via an amide bond with the amino group of the substrate and therefore catalyze the ubiquitination of the target molecule.

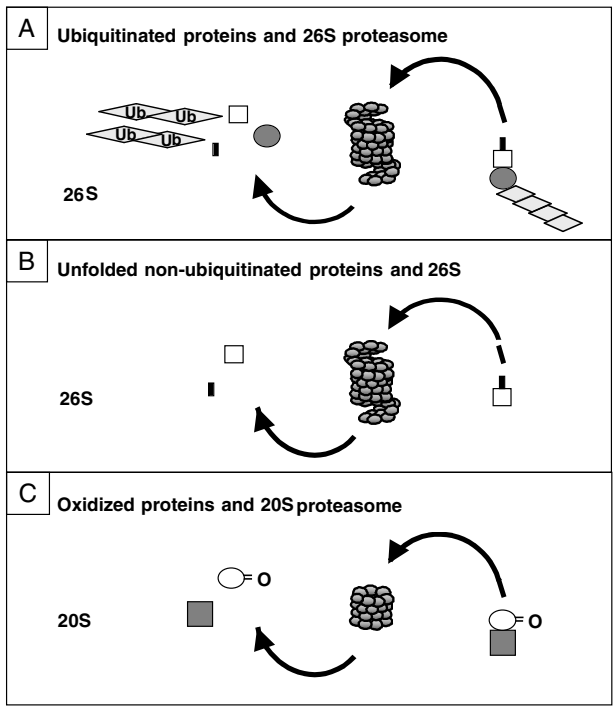

FIG. 2. Proposed mechanisms for the proteasomal protein degradation. (**A**) Most proteins are degraded via the ubiquitin–proteasome-system. The proteins to be degraded are first covalently attached to a chain of ubiquitin molecules recognized by the 26S proteasome, and hydrolyzed into small peptides. (**B**) The 26S proteasome has the ability to degrade some unfolded nonubiquitinated proteins. (**C**) Oxidized proteins are degraded by the 20S proteasome.

Simple versions of the proteasome are present in archaic bacteria; however ubiquitin and the ubiquitin conjugating systems are not, indicating that the proteasome has at least in these organisms the ability to degrade nonubiquitinated proteins (67). Furthermore, the isolated 20S proteasome, purified from prokaryotes and eukaryotes, can hydrolyze a number of nonubiquitinated unfolded proteins *in vitro* (60). Additionally, the eukaryotic 26S can bind and degrade nonubiquitinated, unfolded proteins (8) (Fig. 2).

OXIDATIVE STRESS AND PROTEIN OXIDATION

Oxidative stress

As a consequence of aerobic metabolism, small flux rates of reactive oxygen species (ROS) are constantly generated in tissues and organs. Cellular antioxidants act in concert to detoxify these molecules, but when the balance of formation of prooxidants and primary antioxidative defense mechanisms is disrupted, cells undergo oxidative stress. Under these conditions lipid peroxidation, DNA damage, and protein oxidation take place (19). Oxidative stress has been implicated in a number of diseases, including neurological disorders and neurodegenerative diseases. Oxidative stress is able to mediate protein oxidation in various cell lines and as a consequence increase the protein turnover in those cells (35, 40, 108). This was demonstrated for a number of chemically-induced oxidative stress variants, including bolus additions or fluxes of hydrogen peroxide (40), superoxide anion radical (40), hypochloric acid (108), peroxynitrite (35), and various combinations of these.

Oxidative protein modification

The degree of protein oxidation caused by a given oxidant depends on many factors, including the nature, relative location, and flux rate of the oxidant and the presence of detoxifying antioxidative mechanisms (34). Oxidation of proteins results primarily in modification of amino acid side chains or polypeptide backbone fragmentation. Proteins contain thiol-bearing cysteine residues that are sensitive to oxidation, and this may interfere with biological function either as damage or in the context of oxidant-dependent signal transduction (6). Cross-linking via S–S bonds due to free radical action is common. Further protein modifications are the methionine sulfoxide formation or the formation of free carbonyl groups as an intermediate step in the oxidation process of numerous amino acid side chains. The occurrence of these groups is frequently used as a measure of protein oxidation processes (65). The large variety of further protein modifications by oxidants was extensively reviewed recently (78).

It should be mentioned that during oxidative stress, proteins in cells also undergo secondary oxidative modifications. Very often products formed during reactions of free radicals with other cellular constituents, such as lipids, carbohydrates, and nucleic acids, are modifying proteins. Lipid-, carbohydrate-, and nucleic acid-radicals are able to react with proteins and form a wide range of adducts. Nonradical oxidized products such as peroxides, aldehydes, and ketones will readily form adducts with proteins (34). Furthermore, reactive intermediate protein oxidation products are able to perform further reactions. This includes carbonylic protein oxidation products and protein hydroperoxides (20).

Accumulation of oxidized/cross-linked material

It was already mentioned that changes in the protein backbone might lead to dramatic changes in the molecular weight of the formed protein oxidation product. Due to oxidative stress fragmentation of polypeptide chains, intra- and intermolecular cross-linking occur (18, 109); the latter results in the formation of protein aggregates (18). Besides the already mentioned disulfide-mediated protein cross-linking, one of the best investigated cross-links is the formation of a 2,2'-biphenyl cross-link by two tyrosyl radicals (27). Protein aggregation is triggered often by free radical reactions (34, 66). Aggregates are first formed out of an initially noncovalent basis, mainly mediated by electrostatic and hydrophobic interactions. These initial aggregates tend to form covalent cross-links due to reactions between carbon-, oxygen-, and nitrogen-centered radicals of amino acid side chains. Nonradical groups and components such as carbohydrates and oxidized lipids are also able to react with these aggregates and act as cross-linking agents. This leads to an increasingly growing mass of oxidized material (26) (Fig. 3). The accumulation of oxidized proteins that lack their function is introducing a pool of cellular debris into the cells. Several diseases, and the aging process, are accompanied by accumulation of cross-linked proteins. The accumulated, cross-linked material has effects on cellular functions, sometimes it even results in a cellular metabolic malfunction (39, 94) (Fig. 4).

Protein aggregation results in a decline of proteolytic susceptibility of the involved proteins (39). This is also true for

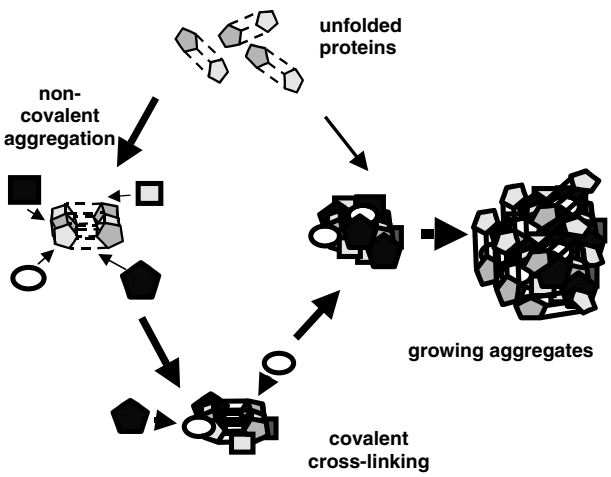

FIG. 3. Formation of protein aggregates. Aggregates are formed out of an initial noncovalent basis, based on electrostatic and hydrophobic interactions. These aggregates tend to form covalent cross links due to numerous reactions. Noncovalent components can also react to the growing oxidized mass of material. The aggregates grow bigger in time.

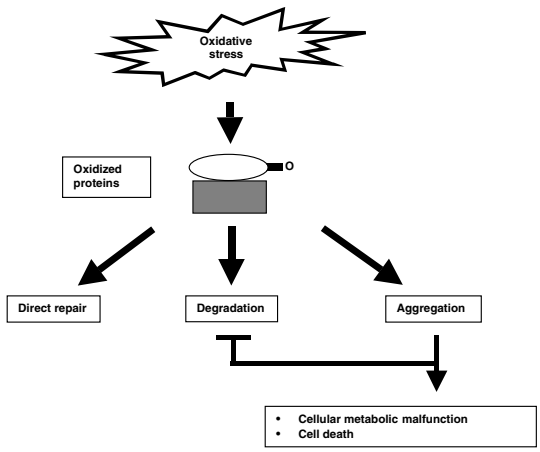

FIG. 4. The fate of oxidized proteins. There are two mechanisms to remove oxidatively damaged proteins: the direct repair or the degradation by the proteasome. Some of the damaged proteins are not repaired or degraded, these are forming aggregates. The formation of aggregates results in a lower proteolytic susceptibility of this protein material. Aggregates are able to inhibit the protein degradation machinery of the cell, consequently leading to disturbances of the cellular metabolism and in some cases to cell death.

the highly cross-linked oxidized protein aggregates. However, as already mentioned, the accumulation of mutant proteins and the formation of aggregates of misfolded proteins also take place and are characteristic for diverse neurodegenerative diseases, including the polyglutamine diseases. Several studies have suggested that polyglutamine protein aggregates impair the ubiquitin–proteasome system (46). Moreover, prevention of polyglutamine oligomerization by Congo red ameliorated polyglutamine-induced decrease in proteasome activity, suggesting that the inhibitory effect of polyglutamine proteins is due to their self-association properties (89).

CELLULAR RESPONSE

Repair

Cells possess several ways to rescue the oxidatively damaged proteins and restore their original function. To decrease the burden of oxidized proteins and the potential possibility to form aggregates, two principal ways exist to remove these damaged proteins: the direct repair or the degradation/removal of the damaged proteins (Fig. 4). Very often protein degradation is the only way to remove damaged proteins, since the number of protein repair functions is limited to some oxidative protein modifications (Table 1). Usually cells possess repair systems for sulfur-containing amino acids. This includes the thiol repair systems; these systems require either glutathione (66) or thioredoxin (47) and the methionine sulfoxide repair with the methionine sulfoxide reductases (109). Besides the mentioned direct repair systems, other enzymes are involved: disulfide isomerase facilitates the disulfide exchange reactions in large inactive protein substrates, heat shock proteins, including HSP90 and HSP70, and other stress proteins are assumed to stabilize the unfolded oxidized proteins to prevent their aggregation, and if possible to assist the refolding of the oxidized proteins (62, 88).

Recently, a new enzymatic system directed towards the repair of thiols oxidized to sulfenic acid was described (6). Sulfenic acids are generally unstable, either forming disulphides with another nearby located thiol or being further oxidized to a stable sulfinic acid. Normally disulfides are reduced by glutathione or thioredoxin in biological systems, but cysteine-sulfinic acid derivatives have been treated as irreversible protein modifications (6). Woo *et al*. (115) described in mammalian cells that the sulfinic form of peroxiredoxin I, produced during the exposure of cells to H_2O_2, is rapidly reduced to the catalytically active thiol form (Fig. 5). Peroxiredoxins are antioxidative proteins containing a redox-active thiol. They are reducing hydroperoxides and therefore, control hydroperoxide-mediated signaling pathways in mammalian cells (6). Woo *et al.* (115) concluded that the ability of mammalian cells to reduce protein sulfinic acids might serve as a mechanism to repair oxidatively damaged proteins or represent a new type of cyclic modification by which the function of various proteins is regulated. Biteau *et al.* (6) identified a protein named sulfiredoxin (13 kDa) that is conserved in eukaryotes and is responsible for reducing cysteine-sulfinic acid in the yeast peroxiredoxin Tsa1. The sulfiredoxin reaction is ATP- and magnesium-dependent. It was concluded that sulfiredoxin is important for the antioxidant function of peroxiredoxins, and is involved in the repair of proteins containing cysteine-sulfinic acid modifications, and in signaling pathways involving protein oxidation. A hypothetical model of a multistep reduction process of the cysteine-sulfinic acid of Tsa 1 (peroxiredoxin) by sulfiredoxin was proposed (Fig. 5). In this model sulfiredoxin acts as a specific phosphotransferase and thioltransferase.

TABLE 1. DIRECT PROTEIN REPAIR MECHANISMS FOR OXIDIZED PROTEINS

Thiol repair	glutathione/thioltransferase system thioredoxin/thioreductase system
Methionine sulfoxide repair	methionine sulfoxide reductase methionine reductase for free methionine
Cysteine sulfenic acid repair	sulfiredoxin

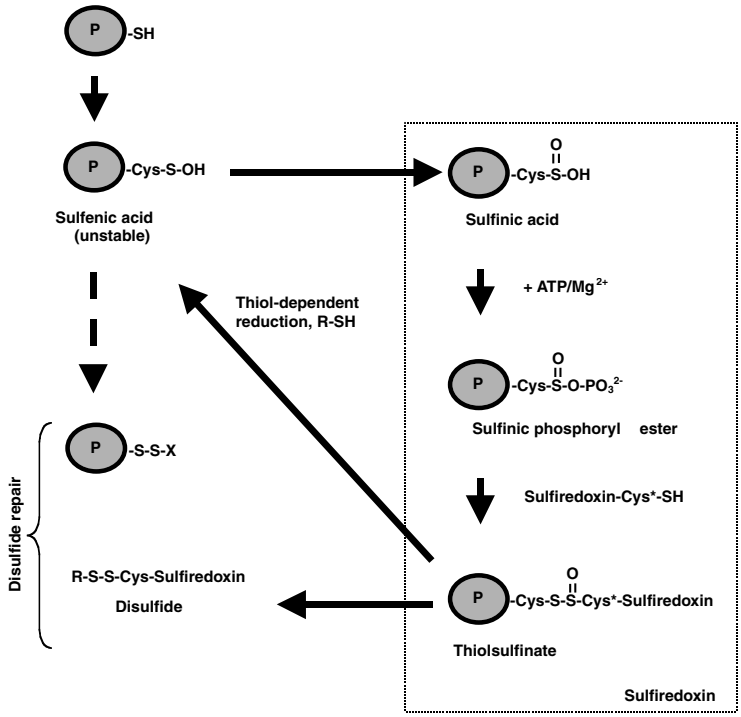

FIG. 5. Proposed mechanism of the reduction of the cysteine-sulfinic acid of peroxiredoxin by sulfiredoxine [adapted from Biteau *et al.,* 2003 (6)]. The oxidation of thiols is leading to unstable sulfenic acids, which are decomposing either to disulfides or sulfinic acid. Biteau *et al.* proposed that sulfiredoxin catalyzes the reduction in a multistep process by acting both as a specific phosphotransferase and as a thioltransferase. The sulfiredoxin requires ATP/Mg^{2+} in the first catalytic step, the second step leads to a transient formation of an intermolecular disulfide between sulfiredoxin 1 and peroxiredoxin. In the last step the thiolsulfinate is thiol-dependent reduced. It was proposed that this mechanism is not only true for the reduction of peroxiredoxin sulfinic acid.

DEGRADATION OF OXIDATIVE MODIFIED PROTEINS

There are three most important pathways for protein degradation: the lysosomal pathway, the proteasomal system, and the calpains.

The lysosomal pathway is mainly used for the removal of extracellular and autophagositized material. The cell takes up foreign protein material, cellular proteins to be degraded are transported into the lysosomes, and are there exposed to cathepsins. Perhaps the lysosomal system lacks selectivity once the protein material is inside the lysosomes; therefore it has not been often considered as a possible system in cells for the removal of oxidized proteins (59). Aging has an impact on the lysosomal pathway. Recently there was evidence that oxidation of substrates of chaperon-mediated autophagy is accompanied by an enhanced translocation into lysosomes (59). Chaperon-mediated autophagy itself is activated during oxidative stress (59).

DEGRADATION OF INTRACELLULAR OXIDIZED PROTEINS

Rivett (87) demonstrated the nonlysosomal degradation of oxidized glutamine synthetase by a cytosolic protease; later this was identified as the proteasome. Numerous studies have been performed to demonstrate that the proteasome has a key role in the degradation of oxidized proteins (26, 27, 35, 40, 80, 87, 109). Oxidative stress promotes protein misfolding (63, 106) and unfolding of proteins is accompanied by an exposure of hydrophobic moieties to the surface (80). It was reported that the proteasome has a preference to bind hydrophobic and aromatic amino acids (50). ATP inhibited the degradation of oxidized proteins in cell free lysates by 10–20% (34). Oxidized proteins alter their proteolytic susceptibility in dependence of the intensity of oxidative damage. Mild oxidation increases proteolysis, whereas extensive damage causes a decrease in proteolysis, due to cross-linking, aggregation, and decreased solubility (18, 27, 39). Therefore, the increase in proteolytic susceptibility is limited to a certain oxidation state (90). Consequently, an 'optimal' oxidative damage exists and excessive oxidation leads to a decline in the proteolytic susceptibility of proteins.

Evidence of the involvement of the proteasomal system in degradation of oxidized proteins in living cells was given by antisense treatments and proteasome inhibitors (38, 40, 41). In living cells the proteasomal system is not acting by itself, but in tight interaction with the chaperone system. A linkage between HSP90 function and the rates of protein degradation involves both the stabilization of partially unfolded proteins as well as a direct modulation of protein degradation by the proteasome (4, 10, 71). A high affinity interaction between HSP90 and the 20S proteasome was described (15, 23, 52, 54, 76, 110). Inhibition of HSP90 modulates the rates of protein degradation and inhibits the activity of the proteasome in cellular assays (15, 71). HSP90 selectively recognizes oxidized calmodulin and promotes its degradation by the 20S proteasome (113).

DEGRADATION OF EXTRACELLULAR OXIDIZED PROTEINS

The degradation of extracellular oxidized proteins was studied to a lesser extent in comparison to that of intracellular

and therefore, the knowledge about the fate of oxidatively modified extracellular proteins is rather limited.

Endocytosed *in vitro* oxidized ApoB from low-density lipoproteins was poorly degraded and accumulated in the lysosomes of macrophages (56). The half-life of endocytosed albumin was found to be longer than that of the native protein (32). Due to the extensive level of protein oxidation used in these studies, this supports the results on the poor degradation of extensively oxidized intracellular proteins (40). Consequently, in studies using highly aggregated protein material or lipofuscin, no degradation of this material could be found, but an inhibitory effect on the proteasomal system was described (94, 96).

More recently these questions were addressed by studying oxidized laminin and myelin basic protein in a system of microglial or RAW cells (105). Both cell types were able to internalize and to remove oxidized forms of these proteins in dependence of the oxidation state of the substrate proteins. Whereas proteins were internalized independently of their oxidation status, moderately oxidized proteins are degraded, strongly oxidized accumulate within these cells (105) (Fig. 6). Activation of these microglial and macrophageal cells enhances the degradation of moderately oxidized proteins (105). Both the lysosomal and the proteasomal system are involved in the degradation of these up-taken extracellular proteins (105). Also more complex oxidized material such as apoptotic bodies is taken up by these cells and is degraded (104).

PROTEASOMES IN OXIDATIVE STRESS

There are numerous studies reporting the degradation of oxidized proteins after oxidative stress (18, 27, 40, 64, 87, 114). Since the proteasome by itself is also a protein, it is obvious that it should be also damaged by oxidative stress. The 26S proteasome was in general more sensitive than the 20S proteasome to oxidants such as H_2O_2, hypochlorite, and $ONOO^-$ (85). The activity of the 20S proteasome after moderate oxidative stress did not change significantly (37, 85, 86). The 20S proteasome activity remained unchanged after H_2O_2 exposure of up to 2 m*M*, while the 26S proteasome was completely inhibited under these conditions. The decline of the 26S proteasome activity was demonstrated directly after sublethal oxidative stress, followed by a recovery of the 26S proteasomal activity in the after-stress period (85). Therefore, clearly an oxidation dependent regulation of the proteasomal activity takes place, although the function of this regulation still remains unknown. Interestingly, no up- or downregulation of proteasomal subunits or changes in the

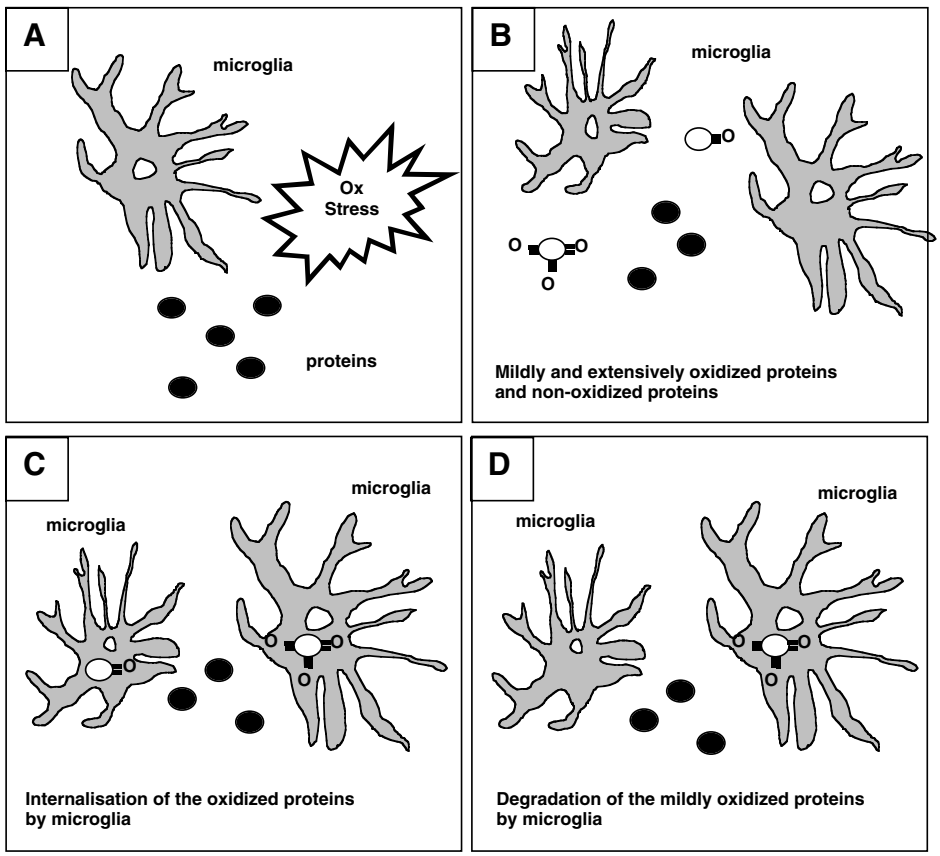

FIG. 6. Internalization and degradation of oxidized proteins by microglial cells. Intracellular proteins and also extracellular proteins are undergoing oxidative changes. Moderately and strongly oxidized proteins are internalized by microglial cells. Within cells moderately oxidized proteins are degraded, whereas strongly oxidized are accumulating within cells.

total amount of proteasome was detected during oxidative stress (40).

Other components of the proteasome–ubiquitin system are influenced by oxidative stress (90). Several enzymes of the ubiquitination cascade are oxidation sensitive due to the thiol group involved in their function. Therefore, it is not surprising that cells which harbor temperature-sensitive components of the ubiquitination cascade are under restrictive conditions still able to remove oxidized proteins efficiently (93). All these data together show clearly that the 20S proteasome core complex might be actively involved in the degradation of oxidized proteins in cells (35, 40, 85, 93). Since the heat shock and chaperone protein systems might be also influenced by oxidative stress, an intense regulation of the proteasome-HSP-interactions under oxidative conditions might be suggested. HSP90 has been suggested to bind to the α-subunits of the 20S proteasome and to act as a regulator of proteasome function (15, 23, 68). The association between HSP90 and the 20S proteasome has been implicated in modulating the dynamic exchange of the 11S and 19S regulatory subunits to affect proteasome function, as an allosteric activator of the proteasome, and to play a critical role in protecting the 20S proteasome against oxidative inactivation (25, 53, 68).

A distinct activation of the nuclear proteasome was described during oxidative stress. Since nuclear proteins are also subject to oxidation, an efficient removal of oxidized nuclear proteins, including histones (107), is required. The rapid, transient and effective activation of the nuclear proteasome is mediated by the poly(ADP)ribose polymerase (PARP). This activation is directed towards the efficient and rapid removal of oxidized nuclear proteins (107). Most likely this quick response of the proteasomal system in the nucleus is required for an efficient DNA repair after oxidative conditions (1).

PROTEASOMES IN AGING

Aging is defined as a progressive decline in physiological functions with a significantly increased risk of developing cancer, neurodegenerative, and cardiovascular diseases (92). Harman proposed the Free Radical Theory of Aging (43), which suggested that free radicals cause damage, resulting in aging and death (43). This theory was several fold revised and modified, but the rational—the damaging effect of oxidizing agents and the progressive accumulation of damaged product—is still accepted (73). Among these damaged products, of course, also oxidized proteins are present tending to aggregate and form during the aging process lipofuscin or other age-related oxidized protein aggregates. Whether this accumulation of oxidized protein waste is the result of increased oxidative damage to proteins, either by an increased exposure of cells to oxidants or by a malfunctioning of the primary antioxidative defense, or whether this is the consequence of a decline of the efficiency of the proteolytic removal of oxidized proteins remains obscure. Age-related alterations in proteolysis are believed to contribute to the elevations in protein oxidation, protein aggregation, and neurodegeneration evident in the aged CNS (58).

We demonstrated that the turnover of oxidized proteins progressively declines during senescence of proliferating (95, 197) as well as nondividing human fibroblasts (94, 96). An inverse correlation between the accumulation of oxidized or cross-linked proteins and the decline in proteasome activity was found (Fig. 7). It could be clearly shown that the decline in proteasome activity is associated with a decrease in the intracellular protein turnover during *in vitro* senescence. Although we found a marked decline in all three proteasome activities (trypsin-like, chymotrypsin-like, and peptidyl-glutamyl-hydrolyzing activities) (41, 95, 143), the proteasome content and the transcription level of proteasome subunits was unchanged. Our conclusion was that the proteasome was being inhibited by the accumulated oxidized or cross-linked aggregates, leading to a progressively diminishing cellular ability to degrade oxidized proteins (94–97). This was supported by studies demonstrating the inhibition of the proteasome by artificial lipofuscin and oxidized protein aggregates (94). Other authors also found a decline in the function of major cytosolic proteolytic system (5). Bulteau *et al.* (9) demonstrated a decline in proteasome activity during skin photo-aging. Chondrogianni *et al.* (13) have shown an impaired function of the proteasome: senescent cells have reduced proteolytic activities and less proteasome content. In this study it was demonstrated that inhibition of the proteasome by a specific inhibitor induces a senescence-like phenotype in young WI38 fibroblasts (13). The induction of a senescence-like phenotype following treatment with proteasome inhibitors seems to be a common feature of primary human fibroblasts.

The group of Keller (12) deleted UMP1 in yeast, a gene necessary for 20S proteasome biogenesis. There were no influence on cellular viability under normal growth conditions, but affected the ability of cells to survive under stationary phase conditions. During this phase, the level of oxidized proteins increased more rapidly and to higher levels in the mutant (12). This suggests a role for impaired proteasome-mediated protein degradation in increased protein oxidation and cell death noted during the aging process.

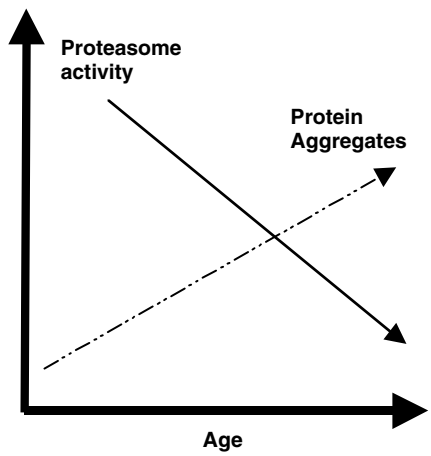

FIG. 7. Inverse correlation of proteasome activity and aggregate accumulation during senescence.

PREVENTION

Antioxidantive substances are compounds that either prevent the formation of oxidants or interfere with the oxidation-driven chain reactions and thereby delay or prevent oxidative modifications of cellular components, including proteins. Antioxidants are able to prevent to a certain extent the oxidative stress-induced changes in protein oxidation and proteolysis. OLN 93 oligodendrocyte cells, myelin-forming cells in the brain, are highly susceptible to oxidative stress due to their low antioxidative defense system and high metabolic rate. The effects of α-lipoic acid and coenzyme Q_{10} were tested (24, 81). Both showed an antioxidative effect and protected cellular proteins from oxidation-induced damage. Other antioxidative compounds such as trolox and stobadine, flavonoids of *Ginkgo biloba* and Pycnogenol, agents more or less effectively prevent protein oxidation, although most of the used compounds are more effective in the prevention of lipid peroxidation (48).

The lipid algae extract (*Phaeodactylum tricornutum*) stimulated the 20S proteasome peptidase activity (79). Human keratinocytes and stratum corneum skin cells (obtained by stripping) from human volunteers treated with *Phaeodactylum tricornutum* extract showed after UVA and UVB irradiation a reduced level of carbonyls and exhibited a sustained level of proteasome activity. It was concluded that this algal extract contains stimulating and/or protective compounds for the proteasomal system, resulting in a reduced level of carbonyls.

The protection of the intracellular protein pool by antioxidants was thought to be a promising way to prevent the protein oxidation and the accumulation of oxidized protein aggregates in aging and neurodegenerative diseases (77). To achieve this, different antioxidants were proposed. It was shown that vitamin E is able to prevent lipofuscin accumulation in mouse brain cells (36), lipid oxidation in aged at kidneys (84), and protein oxidation in brain cells and lymphocytes (82). Vitamin E appears to stabilize various homeostatic functions in elderly individuals (74). Lipoic acid shows beneficial effects on age-related changes in mitochondrial function (42). A number of nutritional supplements have been reported to exert anti-aging properties, for example, L-carnitine and acetyl-L-carnitine decrease the accumulation of single-strand break in DNA (7). The spin-trap *N-tert*-butyl-phenyl-nitrone (PBN) has been used as an antioxidant in a number of animal studies. It was found to be effective in preventing or reducing the formation of protein carbonyls during aging (22, 102, 103), and to prevent the age-related decline of glutamine synthase active (116). The group of Griffiths (11) investigated vitamin C supplementation effects on immunoglobulin oxidation and total plasma protein sulfhydryls in healthy human volunteers. It was demonstrated that dietary vitamin C supplementation can reduce certain types of oxidatively protein damage correlated with a low basal antioxidant level, but no difference in plasma sulfhydryl content was noticed.

An alternative way to prevent oxidative damage might be the blockage of the actual formation of free radicals. One method to reduce the rate of overall free radical generation in organisms is thought to be the restriction of dietary input of calories (41). Caloric restriction is the only experimental intervention that consistently has been shown to slow the rate of aging and to increase mean and maximum lifespan in a variety of species (100, 112, 117). Lifelong caloric restriction decreases mitochondrial superoxide and H_2O_2 production (2, 3), lipid peroxidation (72), oxidative damage to DNA (98), and also protein oxidation (21, 57). Dietary restriction and moderate long-term exercise seem to restore higher proteasome activity and turnover rate of proteins in aged animals (31).

CONCLUSIONS

The important role of the proteasomal system in the removal of oxidized proteins is not questioned any more. Whether all oxidized proteins are not ubiquitinated, what are possible signals for the recognition of oxidized proteins in living cells, and how is the proteasomal system regulated during oxidative stress remain to be clarified. Especially, a deeper understanding of the recognition of oxidized proteins and the regulation of the proteasomal system under oxidizing conditions might reveal new strategies for the prevention of the accumulation of oxidized protein aggregates in aging and disease. Here the interaction of the ubiquitin–proteasome-system with chaperone proteins is of special importance. The direct repair of oxidized proteins may become an important topic of research. The recent discovery of the sulfiredoxin function makes it clear that other unknown repair enzymes or mechanisms might be present in the cell. Further studies are needed to understand the complexity and coherences of the protein oxidation defence network.

ABBREVIATIONS

ApoB, apolipoprotein B; ATP, adenosine triphosphate; CDK, cyclin-dependent kinase; CNS, central nervous system; HSP, heat shock protein; NER, nucleotide excision repair; ROS, reactive oxygen species.

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