ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 1 & 2, 2006

Forum Review

Proteasome Function in Aging and Oxidative Stress: Implications in Protein Maintenance Failure

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ABSTRACT

Damage to cellular components by reactive oxygen species is believed to be an important factor contributing to the aging process. Likewise, the progressive failure of maintenance and repair is believed to be a major cause of biological aging. Cellular aging is characterized by the accumulation of oxidatively modified proteins, a process that results, at least in part, from impaired protein turnover. Indeed, oxidized protein buildup with age may be due to increased protein damage, decreased elimination of oxidized protein (i.e., repair and degradation), or a combination of both mechanisms. Since the proteasome has been implicated in both general protein turnover and the removal of oxidized protein, the fate of the proteasome during aging has recently received considerable attention, and evidence has been provided for impaired proteasome function with age in different cellular systems. The present review will mainly address age-related changes in proteasome structure and function in relation to the impact of oxidative stress on the proteasome and the accumulation of oxidized protein. Knowledge of molecular mechanisms involved in the decline of proteasome function during aging and in oxidative stress is expected to provide new insight that will be useful in defining anti-aging strategies aimed at preserving this critical function. *Antioxid. Redox Signal.* 8, 205–216.

INTRODUCTION

Increased accumulation of oxidatively modified proteins has been described as a hallmark of cellular aging (7, 8). This age-related buildup in damaged protein raises the problem of the efficacy of protein maintenance systems in charge of eliminating oxidized proteins. Indeed, the steady state level of oxidized proteins results from a balance between the rate of protein oxidative damage and the rate of oxidized protein elimination. Removal of oxidized protein is achieved by specialized degradation and repair pathways whose function is generally reported to be impaired with age (22, 50). In fact, only oxidative damage affecting sulfur-containing amino acids can be reversed by specific enzymes: thioredoxin/thioredoxin reductase can reduce disulfide bridges and cysteine sulfenic acids, while methionine sulfoxide reductases reduce methionine sulfoxide back to methionine (13, 66, 99). These repair systems are present in both cytosolic and mitochondrial compartments (84). For all other oxidative modifications of amino acids, no enzymatic repair mechanism has been evidenced; their elimination is achieved through oxidized protein degradation by various proteolytic pathways (Fig. 1). In mitochondria, oxidized protein degradation is achieved, at least in part, by the Lon protease (10). In the cytosol and the nucleus, the proteasome plays a major role in degradation of oxidized proteins (34, 59), although recent evidence has been provided that chaperone-mediated autophagy is activated upon oxidative stress (73). The proteasomal pathway is mainly responsible for intracellular protein degradation, including basal protein turnover and elimination of damaged or misfolded proteins, as well as control by the ubiquitin–proteasome system of processes essential for cell viability, such as the cell cycle, cell differentiation, signal transduction, DNA repair, and degradation of important rate-limiting enzymes in metabolic pathways (31). Since accumulation of oxidized proteins most likely results from impaired redox homeostasis and protein turnover associated with the aging process, the fate of the proteasomal system with age has been the subject of particular attention. The present review will describe and discuss studies from our and other lab-

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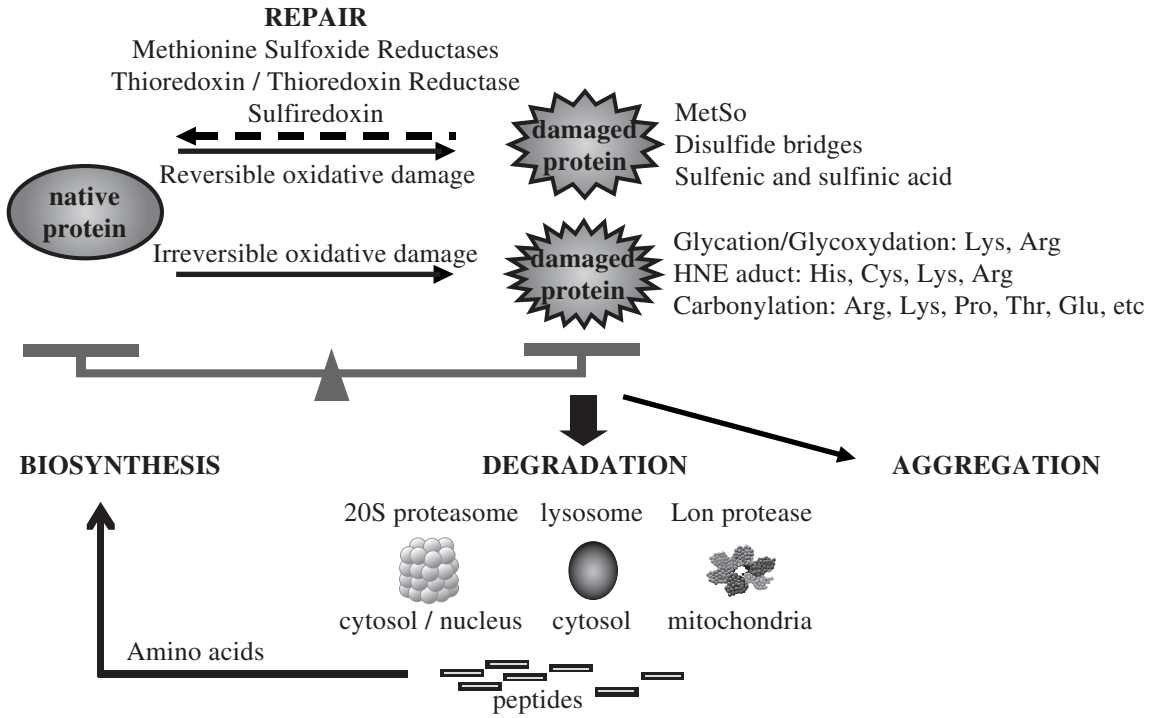

FIG. 1. Protein oxidative modifications and maintenance systems. The balance between native and oxidatively damaged proteins depends on rate of both protein biosynthesis, protein oxidative modifications, and oxidized protein elimination. Sulfur-containing amino acids oxidation products can be reversed within proteins either by the thioredoxin/thioredoxin reductase system or by the peptide methionine sulfoxide reductase system, whereas other irreversible amino acid oxidative modifications are eliminated by destruction of the protein. Degradation of oxidized proteins is mainly achieved by the 20S proteasome in the cytosol and by the Lon protease in mitochondria. The lysosomal pathway has also been implicated during oxidative stress through chaperone mediated autophagy and, when proteasome capacity is exceeded, upon activation of autophagin expression. Degradation and repair systems become less efficient during aging, leading to an increased intracellular load of damaged proteins and to the formation of protein aggregates.

oratories showing that proteasome function is impaired with age and during replicative senescence, as well as in certain situations of oxidative stress. The different mechanisms involved in such impairment of proteasome function will also be discussed. The outcome of these studies evokes the interesting possibility that the proteasome may play a central role in the onset of the aging phenotype. Preserving proteasomal function through stimulation or protection of its activity may therefore be considered as a potential means of delaying aging and increasing cell survival upon oxidative stress. First attempts in this direction will be presented.

OXIDATIVE DAMAGE TO PROTEINS

Protein oxidative damage is the result of oxidative insult derived from the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including the superoxide anion, hydrogen peroxide, the hydroxyl radical, peroxynitrite and nitric oxide (8, 35). These reactive species are generated through cellular aerobic metabolism at different levels in the cell, with organelles such as mitochondria and peroxisome being major sites for their production (7). Exogenous triggering of production of ROS is also achieved during situations of oxidative stress such as UV irradiation, inflammation, and ischemia-reperfusion. Basal production of ROS is part of normal cellular redox homeostasis, and antioxidants (enzymatic and nonenzymatic) regulate their level. However, when the balance between production and elimination of ROS is disrupted, increased damage to macromolecules (including lipids, nucleic acids, and proteins) occurs, leading to both reversible and irreversible modifications (63). Within proteins, all amino acids can be oxidized, with sulfur-containing (cysteine and methionine) and aromatic (tryptophan and tyrosine) amino acids being the most susceptible (115). In addition, tyrosine is a target for the reactive nitrogen species peroxinitrite, giving rise to nitrotyrosine. Oxidation of cysteine leads to disulfide bridges, mixed disulfides and cysteic acids [i.e. cysteine sulfenic, sulfinic and sulfonic acids (102)]. Formation of disulfide bridges, mixed disulfides, and cysteine sulfenic acids is reduced by thioredoxin and/or glutaredoxin (66, 80, 99). Oxidation of methionine leads to the formation of S and R diastereoisomers of methionine sulfoxide that are reduced by the peptide methionine sulfoxide reductase A (MsrA) and B (MsrB), respectively (56, 109).

Oxidation of other amino acids generally leads to the formation of hydroxyl and carbonyl derivatives. Detection of

protein-associated carbonyl groups is a commonly used method for assessing the extent of protein oxidation. Quantitative measurement of carbonyl groups within proteins is achieved after derivatization by 2,4–dinitrophenylhydrazine, and several methods, including immunochemical detection with an anti-dinitrophenyl antibody, have been described (78). Upon oxidation, proteins usually become less active, less thermostable, and more hydrophobic (33, 45). Exposure of hydrophobic amino acids at the surface of an oxidized protein can be easily monitored through increased binding of the hydrophobic fluorescent probe 8-anilino-1-naphthalene sulfonic acid (47). Protein damage can also originate from oxidation-derived reactions of amino acids such as lysine, arginine, histidine, and cysteine with sugar (e.g., carboxymethyl lysine, pentosidine) or lipid peroxidation products (e.g., 4-hydroxy-2-nonenal, malondialdehyde) (119). Adducts are formed on the protein that often bring in carbonyl groups and/or cross-links. The function of these modified proteins is generally impaired or completely inactivated.

Age-related increases in protein carbonyl content, taken as a signature of oxidative modifications, have been widely documented in different tissues and organisms: human dermal fibroblasts, human epidermal cells, human lenses, human erythrocytes, human brain, rat hepatocytes and whole *Drosophila* (80, 92). In human keratinocytes and lenses, we have shown that increased carbonyl detection is associated with increased immunoreactivity for antibodies recognizing, both glycoxidation and lipid peroxidation adducts (92, 126). Such an increase in oxidatively damaged protein is believed to affect cellular integrity, since it is associated with the impairment of key enzymes. Indeed, the age-related decline in glutamine synthetase and glucose-6-phosphate dehydrogenase has been suggested to be directly correlated with the oxidative modification of these two enzymes (116). Also, reversal of age-related loss of memory by chronic injection of the free radical scavenger tert-butyl-α-phenyl nitrone has been attributed to the treatment-induced reduction of oxidized proteins (21). Recent data argue for an age-related increase in protein carbonyl content such that elderly individuals would have one-third of their proteins carrying the modification (114). Whether all proteins are equally sensitive to oxidation, or whether only a restricted set is preferentially affected, remains to be defined. Indeed, Sohal and colleagues have already reported that in aging flies, two mitochondrial enzymes, aconitase and adenine nucleotide translocase, are specific targets for oxidative modification (134, 135). In addition, Jana *et al.* (68), in a study on plasma proteins from different animals, found that only a limited number of proteins undergo carbonylation, while Hamelin *et al.* (60) reported that the serum proteins albumin and transferrin are major targets for age-related glycoxidation. Moreover, using a proteomic approach, we recently reported that an age-related increase in protein glycation and modification by the lipid peroxidation product 4-hydroxy-2-nonenal (HNE) is restricted to preferential target proteins in peripheral blood lymphocytes (96, 97). Since 2D-gel electrophoresis of carbonylated proteins, derivatized by dinitrophenylhydrazine and revealed by anti-dinitrophenyl antibodies, has already proven useful for oxidized protein detection (120), it would be of interest to monitor age-related oxidative modifications in proteins in different tissue and cell systems in order to determine the number of protein targets for carbonylation.

REMOVAL OF OXIDIZED PROTEINS

Since repair of oxidized proteins is limited to a reduction in specific oxidation products of sulfur-containing amino acids, oxidation of all other amino acids within a protein will target it for degradation (22, 50). Indeed, only oxidation of cysteine to disulfide bridges, mixed disulfides and sulfenic acids is reversed by thioredoxin/thioredoxin reductase and/or glutaredoxin/glutathione/glutathione reductase, while cysteine sulfinic acid has been recently shown to be reduced by sulfiredoxin and sestrin, albeit within thioredoxin-dependent peroxidases, peroxiredoxins (9, 15, 66). Sulfiredoxin was first evidenced in mammalian cells and characterized in yeast (9, 133). Cysteine sulfinic acid is reduced back to cysteine at the catalytic site of peroxiredoxin in an ATP- and thioredoxin-dependent manner. Whether other proteins carrying cysteine sulfinic acids are repaired by sulfiredoxin remains to be elucidated.

Methionine sulfoxide is reduced by the peptide methionine sulfoxide reductase system. This system is composed of two classes of enzymes, MsrA and MsrB, that reduce methionine sulfoxide depending on whether the latter is present as the S- or R-diastereoisomer (56, 109). Complete reduction of methionine sulfoxides on a protein is therefore achieved by the combined action of MsrA and MsrB (84). These two classes of enzymes are present in most prokaryotes and eukaryotes. In mammalian cells, the MsrA protein is encoded by a single gene, and was found to be present in both cytosol and mitochondria (61, 128). Three different MsrB genes have been identified: MsrB 1 (SelR), located in the cytosol and the nucleus, MsrB2 (Cbs1), located in the mitochondria, and MsrB3, located in mitochondria or endoplasmic reticulum depending on its isoform (69, 74, 87). In addition to its repair role, the peptide methionine sulfoxide reductase system has been implicated in redox regulation of protein–protein interactions and protein function (6, 24, 118, 129), as well as in protection against oxidative stress by reducing the exposed methionine sulfoxides on protein that act as ROS scavengers (79). This protein repair system has been implicated in increased longevity upon overexpression of MsrA in *Drosophila* (106). Moreover, an age-related decrease in peptide methionine sulfoxide reductase activity and MsrA expression was monitored in rat organs, including kidney, liver and brain (93). Total peptide methionine sulfoxide reductase activity was decreased in senescent human fibroblasts, with a concomitant decrease in expression of both MsrA and MsrB (Cbs 1) (95). Taken together, these findings argue for the contribution of the peptide methionine sulfoxide reductase system to age-related impaired redox homeostasis and accumulation of oxidized protein.

Within mitochondria, which represent the major source of ROS in the cell, the ATP-stimulated Lon protease is thought to play a major role in degradation of oxidized proteins (82). Moreover, oxidized aconitase has been recently shown to be

preferentially degraded by the Lon protease (10). This protease is homologous to the bacterial Lon protease, which is a high molecular weight oligomer, the activity of which is stimulated by ATP (131). Studies addressing the fate of this protease during aging have shown that ATP-stimulated Lon-like protease activity is decreased in both murine skeletal muscle and rat liver, but this activity remains unchanged in rat heart (4, 11, 36, 77). This decreased activity was associated with decreased mRNA and protein expression in murine skeletal muscle (11, 77). No such decline in Lon protease expression was observed in rat liver, whereas a five-fold increase was monitored in heart, and the aconitase activity was decreased without changes in its expression levels (4, 36). Therefore, such age-related impairment of Lon protease function may also contribute to age-related accumulation of mitochondrial matrix oxidized protein and alterations in mitochondrial function.

In the cytosol and nucleus, most oxidized protein degradation has been attributed to the proteasome, an intracellular high molecular weight multienzymatic proteolytic complex (34, 55, 59). This complex is made up of a catalytic core, the 20S proteasome, that interacts with various regulators such as PA28, PA700 and PA200 (31, 91, 124, 127). The proteasome constitutes the main non-lysosomal proteolytic system implicated in protein degradation. The relative proportion of proteasome complexes greatly depends on the considered tissue (41, 42), cell type (104), and physiological state (3), nevertheless proteasomes are abundant and can represent up to 1% of soluble cellular proteins (121). This ubiquitous enzyme present in Archebacteria and Eukaryotes is made up of four stacked rings, each containing seven subunits classified into α and β subunits. The β subunits form the inner rings and carry the catalytic activity, while the α subunits form the apical rings of the complex (31). In the eukaryotic version of the 20S proteasome, only three β subunits carry catalytic activities: β1 for peptidyl glutamylpeptide hydrolase activity, β2 for trypsin-like activity, and β5 for chymotrypsin-like activity, that cleave peptide bonds after an acidic, a basic and an aromatic or hydrophobic amino acid, respectively (57). These activities can be individually assayed using fluorogenic synthetic peptides. Interestingly the subunit composition of the 20S proteasome can be modified, as inducible homologous subunits can be incorporated in the structure upon *de novo* synthesis: the iβ1, iβ2, and iβ5 subunits, respectively, replace their β1, β2, and β5 constitutive counterparts to form the immunoproteasome. Such replacement of proteasome subunits modify proteasome peptidase activities and lead to higher chymotrypsin-like and trypsin-like activities and lower peptidyl glutamylpeptide hydrolase activity, thus increasing production of peptides with higher affinity for MHC class I complex (51, 105, 122).

Proteins are degraded by the proteasome in a progressive manner resulting in short peptides (3 to 20 amino acids) that are further degraded by intracellular peptidases (54). Specific inhibitors have been developed, with the most widely used being natural compounds such as epoxomycin and lactacystin that act as irreversible inhibitors, or synthetic peptide aldehydes such as MG 132 (Cbz-Leu-Leu-leucinal) or MG101 (Cbz-Leu-Leu-Norleucinal), that are competitive inhibitors (58, 103). The 26S proteasome, which results from the association of the 20S proteasome with PA700 (or 19S), is an essential component of the ubiquitin–proteasome system implicated in degradation of polyubiquitinated proteins in an ATP-dependent manner (52). The 19S regulator is composed of at least eighteen subunits belonging to either the "lid" or the "base" of the complex. Among the nine base subunits, six of them are ATPases exhibiting a chaperone-like activity believed to contribute to unfolding of the protein before its degradation by the 20S catalytic core (12). The other subunits form the lid involved in recognition of polyubiquitinated protein substrates and recycling of ubiquitin through isopeptidase activity (37, 64). Polyubiquitination of a protein is a complex process that requires ATP and involves ubiquitin, a 76 amino acids proteins, and three enzymes (Fig. 2), E1 (ubiquitin-activating enzymes), E2 (ubiquitin-conjugating enzymes), and E3 (ubiquitin ligases), to ensure specific recognition of the protein substrate (27, 44, 64, 132). Besides eliminating misfolded and oxidized proteins, the proteasome is involved in ubiquitin-targeted degradation of regulatory proteins such as transcription factors, cyclins and important rate-limiting enzymes in metabolic pathways (for recent reviews, see 76, 94).

When a protein undergoes irreversible oxidative modifications and cannot be repaired (i.e., when amino acids other than sulfur-containing amino acids are modified), its elimination is achieved by degradation (50). Upon mild oxidation, proteins become more prone to proteolysis, whereas highly oxidized proteins usually become resistant to proteolysis because of the formation of intra- and intermolecular cross-links (46, 48). Oxidized proteins represent good substrates for degradation by the proteasome *in vitro,* and oxidized proteins have been shown to be preferentially degraded by the 20S proteasome in an ATP- and ubiquitin-independent manner in a variety of cell types (34, 59, 112). However, certain studies have reported that the ubiquitin-26S proteasome pathway is involved in degradation of oxidized protein from lens cells (108). Moreover, ubiquitination of proteins carrying glycation and lipid peroxidation adducts has also been documented (18, 83). The increased susceptibility of an oxidized protein to proteolysis has been correlated with exposure of hydrophobic amino acids at the surface of the protein that may represent a recognition signal for degradation by the 20S proteasomes (67). Alternatively, such exposure of residues that are normally hidden in the hydrophobic core of the protein may result from decreased thermodynamic stability of the oxidized protein that renders it more flexible, especially at the C-terminus and/or N-terminus end of the protein, hence making it more prone to progressive degradation by either the 20S or 26S proteasomes (54). Interestingly, recent evidence has been provided that chaperone-mediated autophagy of proteins carrying a KFERQ motif is activated upon oxidative stress, implying participation of this proteolytic pathway in elimination of some oxidized proteins (73). Moreover, it has been also recently reported that when proteasome capacity is exceeded, autophagin expression is induced (75) suggesting a physiological link between the lysosomal and proteasomal degradation systems.

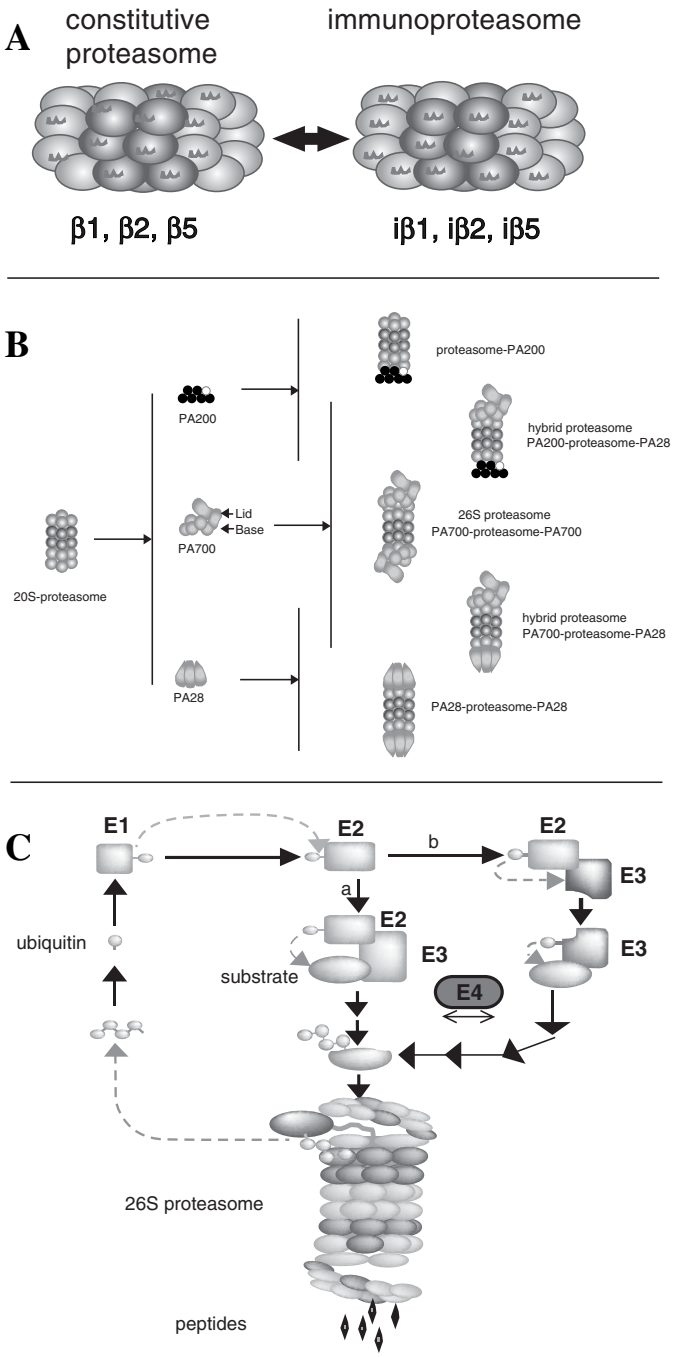

FIG. 2. Ubiquitin proteasome degradation pathway. (**A**) The 20S proteasome exhibit different subtypes, depending on the catalytic β subunits incorporated in its structure. Relative amount of core particle subtypes may vary upon physiological states and during aging. (**B**) Proteasomes exist as different oligomeric assemblies (20S, 26S, and immunoproteasome) which are defined by both the composition of the β catalytic subunits, and the type of regulatory complex associated with the core. PA700, a complex made up at least of 18 distinct subunits (among which six ATPases constitute its base) binds and unfold ubiquitin-modified proteins so that they can be degraded by the catalytic core. PA28 consists either in a heteroheptamer made of PA28α and PA28β proteins mainly expressed in the cytoplasm, or in a homoheptamer made of seven PA28γ nuclear proteins, particularly abundant in the brain. PA28αb complex is implicated in antigen processing. Recently a new PA200 complex has been identified as a large (200 kDa) nuclear protein that stimulates proteasomal hydrolysis of peptides. (**C**) The ubiquitin proteasome degradation pathway: the E1 enzyme (ubiquitin activating enzyme) activates ubiquitin in presence of ATP. The ubiquitin molecule is then transferred to an E2 enzyme (ubiquitin conjugating enzyme). Frequently an E3 enzyme (ubiquitin ligase) is required, and binds to both the E2 and the protein target (*a*), on which the ubiquitin molecule is transferred. Ubiquitin can also bind to the E3 enzyme before being transferred either directly or via ancillary protein to the targeted protein (*b*). An E4 enzyme (an elongation factor of the ubiquitin chain), may be necessary for the target protein carrying a short polyubiquitin chain (at least four residues), which is recognized by the 26S proteasome. The ubiquitin forms of protein are then degraded into small peptides by the 26S proteasome, whereas ubiquitin molecules are recycled. E2 and E3 enzymes both constitute classes of large enzymes family, thus leading to multiple possible combinations, with or without ancillary protein association, and are responsible for the diversity and specificity of the controlled proteasomal degradation pathway.

THE AGE-RELATED DECLINE IN PROTEASOME FUNCTION

The age-related accumulation of oxidatively modified and ubiquitinated proteins, and the general decline in protein turnover, have raised the possibility that proteasome function is impaired with age (22, 49). Pioneering studies from our group and that of Ward provided evidence that proteasome proteolytic activity is affected with aging (28, 110, 111). Indeed, we showed that the 20S proteasome from rat liver exhibited a 50% decrease for the peptidyl glutamylpeptide hydrolase activity when purified from old animals compared with young ones, while Ward and collaborators reported a 40% decrease in the same peptidase activity when activated by SDS and assayed in crude homogenates (28, 110, 111). Interestingly, we also reported that dietary self-selection of nutriments, which leads to decreased protein uptake, can compensate for the age-related decrease in the rat liver 20S proteasome observed with standard diet (1). We suggested that this result is related to the beneficial effects associated with dietary restrictions in calories and proteins, including

decreased macromolecular damage, increased expression of antioxidant enzymes and increased longevity. Subsequently, we and other groups have reported that proteasome activity declines with age in a variety of tissues (5, 20, 62, 70, 86, 92, 98, 126), although some studies have shown that this decline may not be universal. Such a decline in proteasome activity is believed to contribute to the age-associated buildup of oxidized protein.

We have shown that in human keratinocytes (92), human fibroblasts (25), and rat cardiomyocytes (20), the age-related decline in proteasome activity might be explained by decreased expression of proteasome subunits. Interestingly, fibroblasts from healthy centenarians exhibited proteasome activity and proteasome subunit expression levels closer to those of younger individuals than older ones, suggesting that sustained proteasome activity contributed to the successful aging of these individuals (25). In a recent study, exhaustive analysis of proteasome subunit expression in senescent WI 38 human fibroblasts indicated that only the expression of catalytic β-subunits is decreased, and that less 20S proteasome is assembled while certain α-subunits are found in a free state (26). Moreover, exposure of WI 38 young fibroblasts to sublethal doses of the proteasome inhibitor epoxomycin resulted in a senescent-like phenotype. Transcriptome analysis using microarrays performed on both mouse skeletal muscle and human fibroblasts has shown decreased expression of several 20S and 26S proteasome subunits (77, 81). In both analyses, performed with either postmitotic or mitotic cell types, fewer than 2% of the genes monitored showed age-related altered expression, with very little overlap except for proteasome components. In the earlier study, the gene expression profile observed with dietary-restricted old animals led the authors to propose that the anti-aging effect associated with dietary restrictions may have originated from stimulation of protein turnover and decreased accumulation of macromolecular damage (77). Evidence for changes in the proteasome composition has been provided in certain age-related neurodegenerative diseases (125). Of particular interest is Huntington disease where a concomitant increased of chymotrypsin-like and trypsin-like activities of the proteasome and an overexpression of the iβ1 and iβ5 inducible subunits were observed in the affected brain regions, indicating changes in the 20S core particle subunit composition may play a role in neurodegeneration (38, 39). More recently Ferrington *et al*. reported in aged muscle a two- to three-fold increased of immunoproteasome, whereas 20S proteasome expression was decreased. Moreover the low proteasome activity was resulting from a 75% reduced amount of PA700 and PA28 complexes, suggesting that in aged muscle, the endogenous content of proteasome activators is inadequate for complete activation of the 20S proteasome (43).

In addition to decreased and or modified proteasome subunits expression, as an explanation for the age-related decline in proteasome activity, our initial finding of decreased peptidyl glutamyl peptide-hydrolase-specific activity of proteasome purified from aged rat liver was indicative of direct inactivation of the proteasomes (1, 28). Further studies on proteasome purified from rat liver or cardiomyocytes and human epidermis showed decreased proteasome proteolytic activity coupled with subunit replacement and/or post-translational modifications, as evidenced by comparison of two-dimensional gel electrophoretic patterns of proteasome subunits (1, 16, 20). In the spinal cord of Fisher 344 rats, the age-related decrease of proteasome activity was associated with both a decreased level of proteasome expression and an increased level of HNE modified β subunits (71). In more recent studies, 26S proteasome was purified from human peripheral blood lymphocytes obtained from donors of different ages, and the patterns of proteasome subunits modified by either glycoxidation or conjugation with a lipid peroxidation product were analyzed by 2D gel electrophoresis followed by specific immunodetection of the carboxymethyl lysine or HNE adducts (23). These modifications were analyzed, since treatment of purified proteasome with either glyoxal or HNE can inactivate the proteasomes (17, 18, 29). Interestingly, only a restricted number of 20S proteasome subunits were modified with age, while PA700 subunits were unmodified (23). The question as to why some proteasome subunits are more prone to modifications than others remains to be elucidated, but the age-related increased load of modifications in certain proteasome subunits might be related to the observed inactivation of proteasome peptidase activities. Finally, we analyzed the fate of the proteasome in the human eye lens and observed an age-related decline in all three peptidase activities (126). This finding was consistent with the previous report of Wagner and Margolis (130) indicating an age-related decline in proteasome peptidase activities in the bovine lens. This decline could be explained, at least in part, by decreased proteasome content with age (126). However, among the three peptidase activities, the peptidylglutamyl peptide hydrolase activity was more strongly inactivated than the other two, indicating that this peptidase activity was the target of an inactivation process. Although this finding was only correlative and may not be related to the observed inactivation, the increased presence of glycoxidative modifications in the proteasome was evidenced with age.

Proteasome activity has been reported to be directly inhibited by highly modified proteins such as cross-linked proteins generated upon incubation with the lipid peroxidation product HNE (47). Indeed, the model protein glucose-6-phosphate dehydrogenase (G6PDH), when treated with HNE, becomes less susceptible to proteolysis by the 20S proteasome, in contrast to oxidized G6PDH that becomes more sensitive to degradation. Moreover, it was subsequently found that these cross-linked proteins inhibited, in a noncompetitive manner, degradation of an oxidized protein by the proteasomes (48). Therefore, such cross-linked proteins, if present in cellular extracts of elderly individuals, could therefore act as inhibitors of the proteasome. Evidence for such an inhibitory mechanism has been provided: indeed, introduction of artificial lipofuscin (a ceroid pigment that accumulates in aged cells) inhibited proteasome function (113). More recently, accumulation of lipofuscin has been shown to result in proteasome inhibition which can induce apoptosis through the increase of proteasome regulated proapoptotic proteins (100). Conversely, proteasome inhibition can promote lipofuscin formation, suggesting that insufficient proteasomal function may contribute to lipofuscinogenesis by a compensatory increase in the amount of proteins that are directed for lysosomal degradation (123). Since proteasome inhibition also in-

duces alteration of mitochondrial homeostasis in neural cells (65, 117), the appearance of increased level of lipofuscin suggest that impairments in mitochondrial turnover may occur following proteasome inhibition. Of additional interest is the observation that proteasome peptidase activities that were strongly inhibited in rat heart homogenates from old animals, were partially restored when assayed on the purified proteasome, suggesting that endogenous inhibitors were eliminated during the purification process (20). Finally, depending on the cellular system investigated, the age-related decline in proteasome activity appears to be due, at least in part, to the combined effects of: (a) decreased proteasome subunits expression; (b) direct inactivation upon modification of proteasome subunits; and (c) the presence of endogenous inhibitors such as cross-linked proteins.

PROTEASOME INACTIVATION UPON OXIDATIVE STRESS

The proteasome appears to be a key actor in protein damage elimination and other regulatory processes, and oxidative damage to protein has been implicated in age- and disease-related impairment of cellular functions. Therefore, the fate of the proteasome during oxidative stress has received particular attention. Indeed, peptidyl glutamyl peptide hydrolase and trypsin-like activities are readily inactivated upon exposure of the 20S proteasome to metal-catalyzed oxidation *in vitro* (28, 30). However, these alterations depend on whether the proteasome is in its active or latent state, a finding that may be related to the differential susceptibility to oxidative stress of the 26S and 20S proteasomes (101). Moreover, *in vitro* treatment of the 20S and 26S proteasomes with nitric oxide or HNE was found to inactivate certain peptidase activities (29, 53). Of particular interest is the fact that the proteasome is a target for modifications by oxidative processes *in vivo* that can lead to either its transient or irreversible inactivation. We first reported that FAO hepatoma cells, treated with iron and ascorbate in order to promote metal-catalyzed oxidation, exhibited decreased peptidyl glutamyl peptide hydrolase and trypsin-like activities, indicating that the proteasome was a target for inactivation upon oxidative stress (30). Interestingly, both Hsp 90 and α-crystallin were found to protect proteasomes against oxidative insults *in vitro,* while depletion or overexpression of Hsp 90 in FAO cells resulted in either decreased or increased protection of proteasome trypsin-like activity. This chaperone-mediated protection of proteasome activity during oxidative stress may be related to other antioxidant properties described, especially for small heat shock proteins (2). In addition, neural SH-SY5Y cells stably transfected with human HDJ-1, a member of the HSP40 family, were shown to retain a greater preservation of proteasome activity following oxidative injury (40). Taken together the data suggest that heat shock proteins may confer resistance to oxidative stress, at least in part, by preserving proteasome function. Oxidative stress induced *in vivo* by treatment with ferric nitriloacetate in kidney and ischemia-reperfusion in brain induced impairment of proteasome function correlated with the appearance of HNE-modified proteasomes (72, 90). Upon coronary occlusion-reperfusion, inactivation of trypsin-like activity has been associated with specific modification by HNE of three proteasome subunits (17). In contrast, upon UV irradiation of cultured keratinocytes leading to a decline in proteasome activity, no modification of the proteasome was observed when the proteasome was purified from irradiated cells (19). Proteasome inhibition resulted from the UV-induced increased load of damaged proteins, such as HNE modified proteins. In neural cells, inhibition of mitochondrial complex I by rotenone and 1-methyl-4-phenylpyridinium was found to increase the production of ROS and to inactivate proteasomes, most likely through oxidative damage and ATP depletion (65, 107). Upon treatment of neuroblastoma cells with rotenone, the drastic reduction in proteasome activity was suggested to originate from direct modification of 20S proteasome subunits by acrolein and the presence of aggregated acrolein-modified proteins co-immunoprecipitated with the proteasomes (107). Conversely, proteasome inhibition has been shown to decrease complex I and complex II activities and to increase oxygen free radical production, indicating that as a result of proteasome inhibition, mitochondrial homeostasis is altered, oxidative stress is triggered, and cell vulnerability to oxygen free radicals is increased (65, 117). This finding underscores the critical importance of the interplay of the different protein maintenance systems implicated in cellular redox homeostasis, protection against oxidative stress and oxidized protein removal.

CONCLUSIONS

Failure of protein maintenance (i.e., degradation and repair) appears to be an important factor in aging and in the associated increased susceptibility to oxidative stress. This feature is particularly relevant for the age-related build-up of damaged protein in postmitotic tissues such as heart or brain. In fact, aging represents the main risk factor for neurodegenerative diseases which are almost always associated with the accumulation of protein aggregates (125). In addition, besides achieving efficient protein turnover and elimination of altered proteins, proteasome integrity seems to play an equally important role in preventing the increase intracellular oxidative stress through mitochondrial cross-talk and the regulation of pro-apoptotic proteins level.

The age-related slow-down in protein turnover is accompanied by a decline in functioning of all three of the main proteolytic pathways implicated in oxidized protein degradation: proteasomes, but also ATP-stimulated mitochondrial proteases and lysosomes (4, 10, 14, 22, 32, 34, 36, 85). In the present review, we have summarized evidence concerning impairment of proteasome function with age and upon oxidative stress; and we have described the mechanisms by which proteasome activity may be affected at the molecular level. Whether strategies can be elaborated to protect proteasome function against oxidative insults, or even stimulate its activity, is a critical issue that may have important implications in both protection against oxidative stress and anti-aging intervention. This question has been recently addressed in a pioneering study aimed at protecting proteasome activity against UV-induced inhibition in keratinocytes (88, 89). Since low

molecular weight compounds such as SDS or certain fatty acids have been shown to stimulate proteasome activity *in vitro*, cellular extracts from algae and plants have been assayed for their ability to enhance proteasome activity in keratinocyte cultures. We found that an extract from *Phaeodactylum tricornutum* was able to stimulate the activities of all three proteasome peptidases and, as a consequence, to decrease the intracellular load of oxidized proteins, especially after UV irradiation. In another study, global activation of proteasome activity was monitored upon overexpression of the β1 and β5 catalytic subunits in WI38 SV40 fibroblasts resulting from enhanced biosynthesis of all other proteasome subunits (26). In addition, activation of proteasomal function was found to promote an increased capacity to cope with various forms of stress, since the transfected cells were more resistant to treatment both with the proteasome inhibitor epoxomycin and with hydrogen peroxide. Thus, these findings illustrate that the proteasome may be used as a valid target for increasing cellular resistance to oxidative stress, and may serve as a basis for further development of anti-aging strategies based on protection and/or activation of proteasome proteolytic capacity and its maintenance.

ABBREVIATIONS

G6PDH, glucose-6-phosphate dehydrogenase; HNE, 4-hydroxy-2-nonenal; Msr, methionine sulfoxide reductase; ROS, reactive oxygen species; RNS, reactive nitrogen species.

ACKNOWLEDGMENTS

The work from our laboratory is supported by funds from MENRT, and European 5th and 6th grants (QLK6-CT1999–02193, FOOD-CT-2003–506850). Luc Farout has been appointed as postdoctoral fellow of National Institutes of Health Grant 5 R01 AG-19357–03.

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Received after final revision May 10, 2005; accepted July 9, 2005.

This article has been cited by:

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