Amino Acids (2007) 32: 527–534
DOI 10.1007/s00726-006-0428-5
Printed in The Netherlands

Amino Acids

The nuclear proteasome and the degradation of oxidatively damaged proteins

Review Article

P. Voss and **T. Grune**

Research Institute of Environmental Medicine, Heinrich Heine University, Duesseldorf, Germany

Received May 28, 2006
Accepted September 1, 2006
Published online November 15, 2006;

Summary. The accumulation of oxidized proteins is known to be linked to some severe neurodegenerative diseases like Alzheimer's, Parkinson's and Huntington's disease. Furthermore, the aging process is also accompanied by an ongoing aggregation of misfolded and damaged proteins. Therefore, mammalian cells have developed potent degradation systems, which selectively degrade damaged and misfolded proteins. The proteasomal system is largely responsible for the removal of oxidatively damaged proteins form the cellular environment. Not only cytosolic proteins are prone to oxidative stress, also nuclear proteins are readily oxidized. The nuclear proteasomal system is responsible for the degradation of these proteins. This review is focused on the specific degradation of oxidized nuclear proteins, the role of the proteasome in this process and the regulation of the nuclear proteasomal system under oxidative conditions.

Keywords: Protein oxidation – Protein degradation – Proteasome – Nucleus – Histone – PARP

Abbreviations: AD, Alzheimer's disease; ADP, adenosinediphosphate; AGE, advanced glycosilation end products; ATP, adenosinetriphosphate; DNA, desoxyribonucleic acid; EGF, epidermal growth factor; ER, endoplasmic reticulum; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HD, Huntington's disease; HNE, 4-hydroxynonenal; IFγ, interferon γ; MDA, malondialdehyde; MHC-I, major histocompatibility complex class I; NAD^+, nicotinamide adenosine dinucleotide; NLS, nuclear localization signal; pADPR, poly-(ADP-ribose); PARP, poly-(ADP-ribose) polymerase; PD, Parkinson's disease; RNS, reactive nitrogen species; ROS, reactive oxygen species

1. The proteasome and oxidative stress

Accumulation and aggregation of oxidatively damaged proteins is known to be linked to some severe neurodegenerative diseases like Alzheimer's (AD), Parkinson's (PD) and Huntington's disease (HD) (Barnham et al., 2004; Bossy-Wetzel et al., 2004; Ciechanover and Brundin, 2003; Emerit et al., 2004; Goldberg, 2003; Willner, 2004) as well as they are involved in the aging process (Keller et al., 2005; Sitte et al., 2000a–c; Viteri et al., 2004). Therefore, mammalian cells have developed potent degradation systems, which selectively degrade damaged and misfolded proteins.

Beside its multiple functions in antigen processing, signal transduction, transcription, cell cycle progression and apoptosis, the proteasomal system is largely responsible for the removal of the oxidatively damaged proteins from the cellular environment (Carrard et al., 2002; Coux et al., 1996; Davies, 2001). As not only cytosolic proteins are prone to oxidative stress, the nuclear proteasomal system is responsible for the degradation of oxidatively damage histones and other nuclear proteins.

This review is focused on the specificity of the degradation of oxidized nuclear proteins, the role of the proteasome in this process and the regulation of the nuclear proteasomal system under oxidative conditions.

1.1 Structure of the proteasomal system

Multicatalytic proteases like the proteasome can be found in all forms of life; including bacteria, archaea and eukaryotes. The eukaryotic proteasomal system consists out of the 20S proteasome which is the core particle and different regulators like the 11S and the 19S, which can bind to the 20S proteasome and, therefore, modify the activity and the selectivity of the degradation process (Volker and Lupas, 2002).

Four stacked rings of the 20S core enzyme form a hollow barrel. Each ring itself consists out of seven subunits, the outer rings contain seven homologous α-, and the inner rings seven homologous β-subunits. Three of the β-subunits in each ring bare the different catalytic activities so that the six catalytic active centers are hidden in a chamber inside the barrel between the two β-rings (Zwickl, 2002).

Binding of 11S or 19S regulators on one or both sides of the core proteasome lead to further variants of proteasomal proteases, including the 26S proteasome with a different substrate specifity and degradation pattern. The 26S proteasome consists out of the core with a 19S regulator on each side. It is mainly responsible for the recognition and ATP dependent degradation of polyubiquitinated proteins. Combination of the proteasomal core with two 11S regulators leads to an enhanced peptidase activity and an increase of small peptide products, which are suitable to be presented by the MHC-I complex. Also a hybrid form containing one 11S and one 19S regulator bound to the 20S proteasome has been identified (Glickman and Maytal, 2002; Hill et al., 2002).

In order to fulfill the multiple reactions in which the proteasomal system is involved, proteasomes are localized in the cytosol and the nucleus as well as attached to the endoplasmic reticulum (Peters et al., 1994; Rivett, 1998; Enenkel et al., 1998).

1.2 Removal of oxidized proteins

The first step of a proteolytic process is the recognition of the substrate. In the case of the degradation of damaged or misfolded proteins this is a selective process. As oxidized and misfolded proteins expose more hydrophobic amino acid residues to the surface than native proteins do, these hydrophobic patches serve as first recognition-sites for the 20S proteasome (Giulivi et al., 1994; Lasch et al., 2001; Pacifici et al., 1993). Damaged proteins are at least partially unfolded and the polypeptide chain enters the proteasome through the narrow opening (α-annulus) of one α-ring. Within the catalytic chamber between the two β-rings all polypeptide chains are cut by the six active centers into smaller polypeptides ranking from 4 to 25 residues with an average length of 7–9 residues which leave the protease through the opening of the second α-ring (Groll et al., 1997; Löwe et al., 1995; Voges et al., 1999; Wenzel and Baumeister, 1995). In contrast to the 20S proteasome, which degrades proteins in an ATP and ubiquitin independent manner the 26S protease processes predominantly proteins that are marked for degradation by polyubiquitination (Braun et al., 1999). The resulting oligopeptides are further hydrolyzed by several intracellular peptidases to single amino acids (Tamura et al., 1996; Tomkinson, 1999; Walz et al., 1997) or are presented as antigens by the MHC-I complex (Tanaka and Kasahara, 1998).

1.3 Regulation of the proteasomal system

The regulation of the proteasomal activity is very complex as a multitude of factors have an influence on the degradation process. First of all there are the two regulators: 11S and 19S. Both of them are able to bind to each end of the 20S core proteasome. The 19S regulator is responsible for the recognition and ATP dependent unfolding of polyubiquitinated proteins as well as for the recycling of the ubiquitin. In contrast thereof the 11S regulator only opens the channel of the α-rings and, therefore, facilitates the entry of the unfolded polypeptide chain as well as the removal of the small oligopeptides out of the protease (Whitby et al., 2000).

Interferon-γ (IFγ) induces the production of the 11S regulator as well as the expression of three alternative β-subunits which exchange catalytic active units in the β-rings and, therefore, modify the catalytic activity of the proteasome which is by several authors then called immunoproteasome as it is now a part of the immune defense of the cell (Aki et al., 1994; Rechsteiner et al., 2000).

Beside this, some non-proteinogenic factors like phosphorylation of proteasomal subunits (Bose et al., 2004) as well as N-acetylation of subunits (Claverol et al., 2002) and glutathionylation (Demasi et al., 2003) are able to influence the proteasomal activity. In vitro incubation of proteasomes with polylysine, fatty acids, low detergent concentrations (Saitoh et al., 1989), repeated freeze-thaw cycles (Bajorek and Glickman, 2004) and mild head shock (Beedholm et al., 2004) activate the proteasome by opening the channel through structural changes in amino acid chains of the α-subunits.

Up to now there are a lot of specific and non-specific inhibitors known which lower the degradation rate of the proteasomes either reversible or irreversible. Among these MG-132, MG-115 (Lee and Goldberg, 1996), peptide vinyl sulfones, α′,β′-epoxyketones, chlormethyl ketones, diazomehtyl ketones and 4,4-dichloroisocoumarin (Harper and Powers, 1985; Savory et al., 1993; Spaltenstein et al., 1996; Palmer et al., 1995) are the most prominent.

Lactacystin, epoxymicin and TMC-95 A-D are natural inhibitors with a high specifity towards the proteasome (Fenteany et al., 1995; Groll et al., 2000; Kohno et al.,

2000). Also whole protein complexes like PI31 or the HIV Tat protein inhibit the proteasome (Chu-Ping et al., 1992; Li and Etlinger, 1992). The PI31 forms multimers, which interact with the 20S core proteasome and inhibit it thereby. Tat is able to increase the proteolytic activity of the 20S proteasome and additionally it inhibits the interaction of the core with the 11S regulator. By this way the formation of the immunoproteasome and, therefore, the antigen presentation is reduced (Seeger et al., 1997).

1.4 Proteasomal regulation during oxidative stress

One of the main tasks of the proteasome is the removal of oxidatively damaged proteins. Therefore, oxidative stress regulates the proteasomal activity. Salo et al. (1988, 1990) observed that oxidatively damaged superoxide dismutase is degraded faster by cell free extracts of bovine erythrocytes. This is one of the first hints for an enhanced degradation of oxidatively modified proteins by the proteasomal system and has been proven by several experiments in vitro as well as in vivo.

The proteasomal degradation of aconitase (Grune et al., 1998), ferritin (Grune et al., 2001), crystallins (Sommerburg et al., 1998), glucose-6-phosphat dehydrogenase (G6PD) (Ullrich et al., 1999a; Friguet et al., 1994a, b), glyceraldehyde-3-phosphat dehydrogenase (GAPDH) (Buchczyk et al., 2003), lysozyme (Shringapure et al., 2003; Wang et al., 1999) as well as nuclear histones (Ullrich et al., 1999a–c) has been shown to be raised due to mild oxidation. In contrast thereof leads stronger oxidative damage to a decrease in the proteasomal protein degradation.

It seems that in vitro the 20S core proteasome is sufficient to degrade the oxidatively damaged proteins, as ATP and ubiquitin are not necessary for the protein degradation. This is supported by the fact that the 26S activity declines after oxidative stress while the 20S activity remains unchanged or is even activated (Reinheckel et al., 1998, 2000; Strack et al., 1996). Furthermore, the in vitro observations are supported by a number of experiments exploring different cell lines. Whole cells of the human hematopoetic cell line K562 (Reinheckel et al., 2000; Ullrich et al., 1999b; Ullrich and Grune, 2001), Clone 9 liver cells (Grune et al., 1995, 2002), BV-2 cells (Mehlhase et al., 2000), MCR-5 fibroblasts (Sitte et al., 1998) and RAW-cells (Gieche et al., 2001; Mehlhase et al., 2005) or cell lysates were used to investigate the proteasomal degradation after oxidative stress. Several different chemicals and systems were used as oxidants for these investigations, ranking from simple incubation of the proteins or cells with reactive oxygen species like H_2O_2 (Reinheckel et al., 1998; Ullrich et al., 1999b, c; Gieche et al., 2001), singlet oxygen (Grune et al., 1996, 2001) to complex radical generating systems like the xanthine/xanthine-oxidase system (Grune et al., 1995; Salo et al., 1988). Besides reactive oxygen species the influence of other oxidatively active substances like peroxynitrite (Buchczyk et al., 2003; Grune et al., 2001) and hypochlorite (Reinheckel et al., 1998; Ullrich et al., 1999a) were used to oxidize the proteins and test the influence of the extent of oxidation on proteolytic susceptibility. Lipid peroxidation products like 4-hydroxynonenal (HNE) and malondialdehyde (MDA) are also able to influence the proteasomal degradation of proteins (Grune et al., 1995; Grune and Davies, 2003; Shringarpure et al., 2000).

2. The proteasome in the nucleus

The proteasome is required for a variety of cellular processes, like cell cycle progression, removal of oxidized proteins, antigen processing, signal transduction and transcription. As some of them, like the transcription, are located in the nucleus, it is not astonishing that the proteasomal system can be found within the nucleus and not only in the cytosol. Especially rapid dividing cells such as in early stages of embryogenesis and tumorigenic cells show a high accumulation of proteasomes in the nucleus (Klein et al., 1990; Knuehl et al., 1996).

Among the multitudes of proteasomal functions within the nucleus the proteasome has an additional protective function as it is also responsible for the degradation of oxidized proteins. Oxidized nuclear proteins including histones could damage the integrity of the chromatin structure and, therefore, lead to irreversible and perhaps lethal abnormalities for the cells.

All parts of the proteasomal system are localized as well in the cytoplasm and the nucleus of cells. Whereas the 20S core proteasome and the 19S regulator are distributed in all compartments, the IFγ inducible 11S-α and -β subunits are mainly located in the cytosol and a special nuclear 11S-γ subunit exists, which is not induced by IFγ (Brooks et al., 2000; Tanahashi et al., 1997). Approaches using fluorescence labeled proteasomes show that they can diffuse rapidly within either the nucleus or the cytoplasm. In contrast, thereof, is the transport between these two compartments slowly and unidirectional from the cytoplasm to the nucleus (Reits et al., 1997).

Rivett (1998) postulated that nuclear localization signals and tyrosine phosphorylation have an impact on the transport of the proteasome into the nucleus. These findings were supported by the experiments of Evdonin et al.

(2001). These authors reported that the distribution of the proteasomes is changed upon addition of epidermal growth factor (EGF) to the cells. After the supplementation the proteasomes are concentrated in the nuclei.

Kloetzel and coworkers also investigated the import of proteasomes into the nucleus. It was found that the 20S proteasome is imported as precursor, which contain α-subunits and unprocessed β-subunits, inside the nucleus before the proteasome is finally matured (Kruger et al., 2001; Lehmann et al., 2002). Some of the 20S α-subunits carry so-called nuclear localization signals (NLS) by which the corresponding protein is marked to translocate into the nucleus (Knuehl et al., 1996). The involvement of the NLS dependent transport is supported by the findings of the group of Tsuruo who discovered an accumulation of proteasomes in the nucleus after upregulation of the NLS dependent transport (Ogiso et al., 2002). Further they described an up regulation of proteasomal activity within the nucleus under glucose starvation or hypoxic conditions in different cancer cell lines (Kim et al., 1999; Ogiso et al., 1999).

3. Oxidative stress in the nucleus

Oxidative stress within the nucleus can result from different origins. As the nuclear pores retain only high molecular weight molecules cytosolic generated reactive oxygen species (ROS) and reactive nitrogen species (RNS) with longer half-lifes are able to diffuse into the nucleus and damage the nuclear structures like DNA and proteins. Ionizing irradiation and ultraviolet (UV) irradiation can lead to ROS formation directly inside of the nucleus (von Sonntag, 1987; Wei et al., 1987). In the case of UV irradiation this leads to the formation of thymine dimers, which can inhibit the correct transcription and replication of the damaged genes (Vodenicharov et al., 2005).

Third origins of oxidative stress for nuclear proteins are drugs or chemicals developing their oxidative properties in the nucleus. Such chemicals include several environmental toxins and numerous anti-tumor drugs like cytosine arabinoside, isothiocyanates, daunorubicin, doxorubicin and epidoxorubicin (Kanno et al., 2004; Taatjes and Koch, 2001; Wold et al., 2005; Zhang et al., 2005a). The anti-tumor treatment is often accompanied by severe nuclear oxidative stress since such pharmaceuticals are used to specifically stress the rapidly-dividing tumor cells while intact cells should be affected minimally. The internal iron complexes of daunorubicin, doxorubicin and epidoxorubicin for example are able to form ROS and formaldehyde. This enhanced oxidative stress leads to DNA-adduct formation, fragmentation and apoptosis (Khan et al., 2006). Another side-effect of the antitumor treatment with these cytostatics is the downregulation of the antioxidative enzymes activity including superoxide dismutase and glutathione peroxidase (Zhang et al., 2005b).

But some tumor-cells have developed resistance against the treatment with these oxidative chemicals and, therefore, it is important to elucidate how these cells escape the treatment to develop new and more effective pharmaceutically active substances against tumor-cells (Ullrich and Grune, 2003).

4. Proteasomal degradation of oxidized proteins in the nucleus

In general oxidative stress in the nucleus is causing DNA damage and protein modifications. DNA damage has to be repaired as fast as possible to avoid severe mutation-damage to the organism. Repair proteins are required and need to be removed after they have done their work. This is also true for transcription factors, which are only used for a certain time and then need to be removed by the nuclear proteasomal system.

To fulfill their function as a proteome repair system, the proteasome within the nucleus has to deal with the degradation of free proteins as well as with DNA-protein complexes like histones, which are bound to the DNA to protect the chromatin structure. Free oxidatively modified histones are excellent substrates for the 20S proteasome (Ullrich et al., 1999b). As for the most cytosolic proteins also strong oxidized histones decrease the proteolytic activity of the proteasome. However, the proteasome is also able to degrade oxidatively damaged histones, which are still DNA-bound (Ullrich et al., 1999c). This ability seems to be important to prevent the build up of cross-linked histone-DNA complexes. Such complexes are able to inhibit the proteolysis in a concentration dependent manner (Arnold and Grune, 2002; Ullrich et al., 1999b).

The importance of the ability of the removal of oxidized proteins from the nucleus for cellular survival is underlined by the stability of this system during the aging process. While the overall proteolysis of cells is declining with age and also the slight age dependent increase in number of proteasomes does not compensate their lower activity, the nuclear proteasomal activity remains relatively stable during senescence of MRC-5 fibroblasts (Merker et al., 2000, 2003).

It is known that glycoxidative stress up regulates the nuclear proteasomal activity, while the total cellular proteasome activity is slightly decreased. This upregulation is

achieved by both increased proteasomal activity and increased number of proteasomes (Cervantes-Laurean et al., 2005). The decrease of the proteasomal activity in the cytosol may be explained by the inhibitory effect of advanced glycation end products (AGEs) and lipofuscin, which accumulate predominantly in the cytosol and not in the nucleus (Merker et al., 2003). The specific up regulation of the nuclear proteasomal system may serve further as a protection against the accumulation of oxidized histones which would lead to severe diseases or premature aging (Cervantes-Laurean et al., 2005).

5. Regulation of the proteasome in the nucleus by oxidative stress – the activatory role of PARP

As oxidative stress damages not only the DNA but also the DNA-bound proteins, an activated proteasome is required for a quick and efficient removal of damaged chromatin proteins. Beside the already mentioned mechanisms by which the proteasomal activity can be regulated in general (Sections 1.3 and 1.4) the nuclear proteasome can additionally be activated upon oxidative stress by the poly-(ADP-ribose) polymerase (PARP).

PARP transfers ADP-ribose from NAD^+ to histones and other nuclear proteins and it interacts in a non-covalent manner with the proteasome and activates it by this way. The usage of PARP inhibitors as well as immunoprecipitation experiments elucidated the role of PARP in the ROS induced activation of the proteasome (Ullrich et al., 1999b, 2001). It could be shown that interactions between poly-(ADP-ribose) (pADPR), PARP and the 20S proteasome enhance the activity of the enzyme (Mayer-Kuckuk et al., 1999). As there were no direct interactions of PARP with the proteasome detected, the PARP has first to be poly-(ADP-ribosyl)ated by an autocatalytic pathway and then this activated PARP increase the proteolytic activity of the 20S proteasome by non-covalent interactions (Fig. 1). Whether there is also a covalent pADPR modification of the proteasome remains to be elucidated.

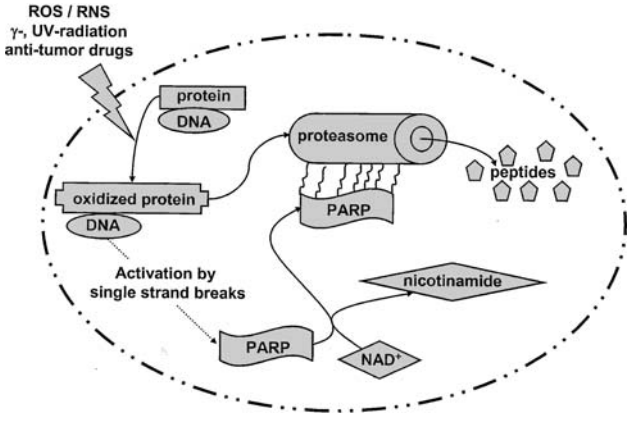

Fig. 1. PARP and the proteasome. In addition to several different regulatory mechanisms the nuclear proteasome is activated by catalytically-active PARP. Oxidative stress damages chromatin proteins and mark them for the proteasomal degradation. PARP is activated by DNA single-strand breaks, leading to PARP auto-poly(ADP)ribosylation. This activated PARP binds non covalent to the 20S proteasome and, therefore, increase the proteasomal activity leading to an enhanced degradation of oxidized nuclear proteins

Beside this effect on the proteolytic activity of the proteasome, PARP itself is also involved in the repair of oxidative DNA damage as automodified PARP binds to histones next to oxidative DNA lesions and liberate them from the damaged DNA. Therefore, the decondensation of the chromatin might be facilitated and the DNA repair machinery has easier access to the damaged DNA.

On the other hand PARP is activated by single strand breaks, which can be the result of oxidative DNA damage. Therefore, PARP is most likely a cross-point of a coordination of DNA-repair and removal of oxidized proteins (Arnold and Grune, 2002).

6. Outlook

The nuclear proteasomal system is responsible for the removal of oxidatively damaged nuclear proteins and the 20S proteasome within the nucleus is activated by PARP and pADPR during oxidative stress. The proteasome has several different functions in the nucleus and is involved in some important cellular processes. Several questions still remain open, for example the impact of the 26S in the nuclear proteolysis during oxidative stress. The knowledge how and why the 20S and the 26S proteasome are regulated differentially under oxidative stress can be of great importance for anti-tumor strategies. As tumorigenic cells have an elevated concentration of proteasomes in their nucleus the proteasomal system is also at least a co-candidate for an anti-tumor therapy. More information on the function and regulation of the proteasomal system within the nucleus are required to elucidate all these questions.

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Authors' address: Tilman Grune, Institute of Biological Chemistry and Nutrition, University of Hohenheim, 70593 Stuttgart, Germany,
Fax: +49-711-45923386, E-mail: grune@uni-hohenheim.de