Review Article

The proteasome and the degradation of oxidized proteins: Part II – protein oxidation and proteasomal degradation

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Here, we review the role of oxidative protein modification as a signal for recognition and degradation of proteins. It was clearly demonstrated that the ATP- and ubiquitin-independent 20S proteasome is playing a key role in the selective removal of oxidized proteins. Furthermore, the current knowledge of the substrate susceptibility on the degradation of oxidized proteins and the role of the immunoproteasome will be highlighted.

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Introduction

In the first part of this series we described the principle proteasomal structure and its regulatory complexes [1]. Here we focus on the role of protein oxidation in the substrate recognition of the proteasome.

Cellular metabolism is accompanied by constant formation of free radicals and oxidants. Depending on the cell type, some 200 different reactive species can be found in humans [2], physiological/pathophysiological conditions, cellular substrate turnover, specialization and the functional proteome, age and environmental factors the cellular radical formation may vary in a broad range.

Reactive oxygen (ROS) and nitrogen species (RNS) have been considered as the major cause of aging, aging associated cellular dysfunctions, and a main factor in many pathologies. Though, meanwhile it became clear that ROS are also integral mediators of cellular adaption, summarized under the term redox signaling. Nevertheless, ROS are able to oxidatively damage/modify cell structures as proteins, lipids or nucleic acids. Due to the abundance of proteins, a bulk of ROS-induced oxidative damage is taken by them, interestingly predominantly by cytosolic proteins. Usually, the nuclear compartment shows only very low amounts of oxidatively modified proteins or aggregates of oxidized proteins, even after phases of severe oxidative stress [3–5].

However, since the permanent oxidative modification of proteins is an inevitable by-product of metabolism in every living cell, several different "counteracting" systems have evolved. A few of them are specialized in the repair of oxidatively damaged proteins, but their capacity is very limited: the only two known amino acids that can be repaired in an enzymatic way in mammalian cells are cysteine and methionine, while the bulk of oxidative modification is irreversible. Fig. 1 gives an overview over the most common reversible and irreversible protein-modifications. If proteins become oxidatively modified/damaged in an irreversible way, cells need effective systems for recognition and removing. For this purpose a cell provides different systems for...
degradation of proteins, as the lysosomal system, mitochondrial proteases (mainly the Lon protease [6,7]), different calcium-dependent proteases and the proteasomal system [8]. The multienzyme 20S proteasome, an evolutionary very ancient system, has been described in detail in the first part of this series [1]. Proteasomes can be found in all three kingdoms of life: bacteria (in the archaea), plants, and animals.

A large body of evidence demonstrated that the 20S proteasome is the main proteolytic system removing oxidatively damaged proteins [1,9–12]. In contrast to the degradation of...
Degradation of oxidized proteins is mainly mediated by the 20S proteasome. Panel A shows the effect of proteasomal inhibition on the degradation of oxidized proteins. If cells are exposed to ROS/RNS (time of exposure highlighted in gray) a dramatic increase in the formation of oxidatively modified proteins takes place. These oxidized proteins (here measured as protein carbonyls) have to be recognized and degraded in order to maintain the functionality of the cell. According to experimental results, 80%–90% of all oxidatively damaged proteins are degraded via the 20S proteasomal pathway in an ATP-independent way [1,45–47]. Consequently, if the proteasome is inhibited such oxidized proteins are not removed from the protein pool (red dashed line) [5]. Experimental data show that the 20S proteasome is a very effective system that reduced the amount of protein carbonyls in a cell within several hours to the level found in unexposed/unstressed cells (blue continuous line) [5,41]. If the 20S proteasome is inhibited or knocked down by siRNA and ubiquitination, the amount of protein carbonyls might still decrease over time, but at a much lower rate, since the other cellular systems (for example the large diversity of lysosomal cathepsins) might be capable of degrading oxidized proteins, too, but show a much lower performance, or the proteasomal inhibition is not complete. Thus, proteasomal inhibition, a decrease of proteasomal activity or the loss of proteasomes promotes the accumulation of oxidized proteins. Panel B displays the contribution of the 26S proteasome or of the ubiquitination to the degradation of oxidized proteins. The 26S proteasome might be blocked by siRNA and ubiquitination by the usage of conditionally ubiquitin deficient cell lines [48,49]. After an E1-enzyme inactivation in such conditionally ubiquitin deficient cell lines, the amount of substrate polyubiquitination and thus labeling for 26S proteasomal degradation [1] significantly decreases. Neither of these modulations shows any significant impact on the degradation of oxidized proteins. In this panel the theoretical proteolytic response of cells to a given ROS concentration (here hydrogen peroxide) is demonstrated [12,15,48,50,51]. Elimination of the 26S proteasome or of the ubiquitination capacities do not have any influence on this response. Taken together, this demonstrates that the 20S proteasome is the main system removing oxidized proteins from the cellular protein pool, and that oxidized proteins do not have to be polyubiquitinylated in order to be degraded. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Degradation of oxidized proteins is mainly mediated by the 20S proteasome. Panel A shows the effect of proteasomal inhibition on the degradation of oxidized proteins. If cells are exposed to ROS/RNS (time of exposure highlighted in gray) a dramatic increase in the formation of oxidatively modified proteins takes place. These oxidized proteins (here measured as protein carbonyls) have to be recognized and degraded in order to maintain the functionality of the cell. According to experimental results, 80%–90% of all oxidatively damaged proteins are degraded via the 20S proteasomal pathway in an ATP-independent way [1,45–47]. Consequently, if the proteasome is inhibited such oxidized proteins are not removed from the protein pool (red dashed line) [5]. Experimental data show that the 20S proteasome is a very effective system that reduced the amount of protein carbonyls in a cell within several hours to the level found in unexposed/unstressed cells (blue continuous line) [5,41]. If the 20S proteasome is inhibited or knocked down by siRNA and ubiquitination, the amount of protein carbonyls might still decrease over time, but at a much lower rate, since the other cellular systems (for example the large diversity of lysosomal cathepsins) might be capable of degrading oxidized proteins, too, but show a much lower performance, or the proteasomal inhibition is not complete. Thus, proteasomal inhibition, a decrease of proteasomal activity or the loss of proteasomes promotes the accumulation of oxidized proteins. Panel B displays the contribution of the 26S proteasome or of the ubiquitination to the degradation of oxidized proteins. The 26S proteasome might be blocked by siRNA and ubiquitination by the usage of conditionally ubiquitin deficient cell lines [48,49]. After an E1-enzyme inactivation in such conditionally ubiquitin deficient cell lines, the amount of substrate polyubiquitination and thus labeling for 26S proteasomal degradation [1] significantly decreases. Neither of these modulations shows any significant impact on the degradation of oxidized proteins. In this panel the theoretical proteolytic response of cells to a given ROS concentration (here hydrogen peroxide) is demonstrated [12,15,48,50,51]. Elimination of the 26S proteasome or of the ubiquitination capacities do not have any influence on this response. Taken together, this demonstrates that the 20S proteasome is the main system removing oxidized proteins from the cellular protein pool, and that oxidized proteins do not have to be polyubiquitinylated in order to be degraded. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Degradation of proteins with oxidatively damaged sites is a very common process in many human cells and tissues. The oxidatively damaged proteins are primarily carbohydrates, lipids, and proteins. The oxidatively damaged proteins can be classified into two types: (1) oxidized proteins with a high degree of damage, such as cross-linked proteins or oxidized proteins with a high degree of damage, such as cross-linked proteins or oxidized proteins; and (2) oxidized proteins with a low degree of damage, such as cross-linked proteins or oxidized proteins. The oxidatively damaged proteins are primarily carbohydrates, lipids, and proteins. The oxidatively damaged proteins can be classified into two types: (1) oxidized proteins with a high degree of damage, such as cross-linked proteins or oxidized proteins; and (2) oxidized proteins with a low degree of damage, such as cross-linked proteins or oxidized proteins.
Increasing amount of oxidative modification

Native and functional protein

- Very low oxidation, exposure of hydrophobic inner structures, activity may be reduced
- Slight oxidation, (more) exposure of hydrophobic inner structures, activity may be (more) reduced
- Mediocre oxidation, no activity left, completely unfolded, hydrophobic structures maximal exposed
- Hydrophobic protein aggregate, no covalent cross-linking yet; still susceptible to proteases
- Heavily oxidized and covalently cross-linked protein aggregate (lipofuscin); resistant and inhibitory to proteases

1: Unfolded proteins, accessible for proteasomal degradation
2: Aggregate formation

Fig. 3. Increasing levels of oxidative protein damage affects the proteasomal degradation. The upper part of this figure shows the continuum of oxidative protein damage and the resulting modification and unfolding (according to Grune et al. [16]). Important to note, that oxidative modification of a protein is a continuous process covering a very broad range of successively increasing damage. An undamaged, natively folded protein (shown on the left in the upper row of images) is usually not recognized as a substrate by the 20S proteasome. After slight oxidative damage a protein might show only insignificant changes in folding or activity. Here the solubility of the protein increases due to additional charges that are brought in by oxidation. After further oxidative damage, hydrophobic sequences, normally buried in the inside of the protein are exposed more and more to its surface and thus the aqueous environment (third image from the left). The damage-induced conformational change causes significant loss of activity until the protein is completely unfolded (fourth image from the left). Such proteins are ideal substrates for proteasomal degradation and most of those proteins are actually recognized and removed via the 20S proteasomal pathway. However, the exposure of hydrophobic moieties to the protein surface holds the risk of a thermodynamically driven protein aggregate formation. So initial aggregate formation is mainly driven by thermodynamics: the exposed hydrophobic sequences of the damaged proteins tend to form clusters in order to minimize their interaction with the aqueous environment. These aggregates can still decompose again and be degraded as single unfolded proteins mainly by the 20S proteasome. Here, chaperones might play a role, but this is still not demonstrated conclusively. Though, under oxidizing conditions the probability of further protein oxidation and thus concomitant cross-linking of the aggregated proteins increases. Once they are covalently cross-linked, they become resistant to (proteasomal) proteolytic degradation. Such material has been shown to be resistant even to the most unspecific proteases [17]. Such a cross-linking might take place by direct protein–protein interaction, e.g. tyrosine radicals and dityrosine formation or protein carbonyl and amino groups via a Schiff’s-base formation. Furthermore, this cross-linking may be facilitated by products of lipid-peroxidation, mainly bifunctional aldehydes like MDA or HNE. Heavily cross-linked protein aggregates may show an autofluorescence that may cover a very broad spectrum of the visible range. One of the few already identified fluorescent structures is the pyridinium bisretinoid A2E, also a secondary product of lipid peroxidation [52] that can be excited using an Argon laser (458 nm) [53]; further structures are 1,4-dihydropyridine, that results from the reaction of MDA with glycine [54–56] as well as 2-hydroxy-3-imino-1,2-dihydropyrol derivatives that result from the cyclization of lysine-HNE Michael adducts [57]. This heavily oxidized material, containing both lipids and proteins, as well as low amounts of sugars is termed in the literature as “lipofuscin” [17,18,58–62]. Lipofuscin accumulates during aging, especially in postmitotic aging cells. However, lipofuscin can be formed with an increased rate during different pathologies, termed as “lipofuscinoses” or “ceroid-lipofuscinoses” [63,64]. These protein aggregates can incorporate redox-active transition metals (in mammalian cells mainly Fe2+ ) [45], thus catalyzing radical formation via Fenton-reaction, again increasing the rate of protein oxidation and lipofuscin-formation [59]. Interestingly, lipofuscin has been shown to be a potent proteasomal inhibitor [17]. Lipofuscin, the long-term product of oxidative stress, accumulates mainly in the lysosomal system, while only a very low amount (about 1%) is found free in the cytosol [18], where it may interact with the proteasomal system, slowing down the degradation of lipofuscin-precursor-material. The lower part of the figure summarizes the different effects of the exposure-time or the amount of oxidative stress applied to a cells on the proteolytic response. Curve 1 shows the amount of oxidized proteins that are accessible for proteasomal degradation. Over time or with increasing ROS/RNS-concentration this amount first increases due to a rising number of damaged proteins in the cell, but then decreases again, due to the rising amount of non-degradable covalently cross-linked material formed, that is very resistant to proteolytic attacks. (compare to Fig. 2B). Curve 2 depicts this increasing amount of protein aggregate formation that decreases the amount of damaged proteins accessible to proteasomal degradation shown in Curve 1.

Subunits was observed after exposure to modified proteins or oxidative stress [20,21]. Most interestingly, the immunoproteasome in combination with the 11S do have a higher activity towards the oxidatively modified proteins (Fig. 4) and degrade them with a higher efficiency. Since the induction of the immunoproteasome is fast compared with the turnover of the constitutive proteasome [22], the immunoproteasomal form might significantly contribute to the degradation of oxidized proteins. Therefore, this form of the proteasome is not only involved in antigen presentation (hence the name ‘immunoproteasome’), but seems to be involved in several cellular processes as an inducible proteasomal form.

Summary

The efficient degradation and removal of oxidized proteins is an absolute requirement for the maintenance of the cellular metabolism. It was demonstrated that the 20S proteasome is in an ATP- and ubiquitin-independent way responsible for the degradation of...
oxidized proteins. Furthermore, the substrate susceptibility is dependent on the degree of its oxidation. Interestingly, lately an involvement of the immunoproteasome and the 11S proteasomal regulator was proposed to play a role in the degradation of oxidized proteins.

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