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Reprint of "Accumulation of modified proteins and aggregate formation in aging"

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ABSTRACT

Increasing cellular damage during the aging process is considered to be one factor limiting the lifespan of organisms. Besides the DNA and lipids, proteins are frequent targets of non-enzymatic modifications by reactive substances including oxidants and glycating agents. Non-enzymatic protein modifications may alter the protein structure often leading to impaired functionality. Although proteolytic systems ensure the removal of modified proteins, the activity of these proteases was shown to decline during the aging process. The additional age-related increase of reactive compounds as a result of impaired antioxidant systems leads to the accumulation of damaged proteins and the formation of protein aggregates. Both, non-enzymatic modified proteins and protein aggregates impair cellular functions and tissue properties by a variety of mechanisms. This is increasingly important in aging and age-related diseases. In this review, we will give an overview on oxidation and glycation of proteins and the function of modified proteins in aggregate formation. Furthermore, their effects as well as their role in aging and age-related diseases will be highlighted.

1. Accumulation of modified proteins and aggregate formation in aging: an introduction

Aging is a physiological and irreversible, progressive process involving changes in the ability to maintain cellular functionality, affecting tissues, organs and the whole organism and thus finally causing death. It is accepted that a series of complex reactions causes the aging process, however, the exact molecular mechanisms are not yet fully understood. During the last decades, over 300 different theories of aging have been described (Medvedev, 1990). These theories can be grouped into those emphasizing that aging is genetically programmed and into the so-called damage or error theories which consider the accumulation of cellular damage due to metabolism and/or environmental factors as a general cause of the aging process (Jin, 2010). Besides the DNA and lipids, it has been shown that proteins are primary targets of such damaging agents. Proteins become modified by different chemical reactions and accumulate in aged individuals (Stadtman, 2006). Moreover, modified proteins were identified to influence physiological pathways and, therefore, to play an important role in aging and age-related diseases including cardiovascular (Simm, 2013; Stocker and Keaney, 2004) and neurodegenerative diseases (Grimm et al., 2011; Li J. et al., 2012) and diabetes mellitus (DM) (Goh and Cooper, 2008). According to the free radical theory of aging (Harman, 1956), reactive oxygen species (ROS) are proposed to be the major source of damage to proteins. Glycation is another important non-enzymatic modification of proteins leading to the formation of altered proteins, known as advanced glycation end products (AGEs). Not only the protein function is impaired due to these chemical modifications, proteolytic degradation can also be affected leading to protein accumulation or aggregate formation during aging. To avoid accumulation of damaged, non-functional proteins, cells contain different proteolytic systems. However, there is persuasive evidence that the removal of damaged proteins declines during the aging process, probably due to the decreased activity of these proteolytic systems (Bulteau et al., 2002; Sitte et al., 2000a). Recently, López-Otín et al. categorized common aspects of aging and defined nine different hallmarks, among them the loss of proteostasis (Lopez-Otin et al., 2013). They reviewed that different stress factors, endogenous as well as exogenous, cause protein modifications associated with protein unfolding. Unfolded proteins are either refolded by proteins of the heat-shock family or degraded by the ubiquitin-proteasome or endosomal–lysosomal system. Failure in these processes finally causes protein aggregation. In addition to the defective or declined degradation of modified, unfolded proteins, it was observed that there is an age-related decrease in protein synthesis (Rattan,

Abbreviations: AD, Alzheimer's disease; AGE, advanced glycation end product; AMD, age-related macular degeneration; CML, Nε-(carboxymethyl) lysine; DM, diabetes mellitus; ECM, extracellular matrix; LDL, low density lipoprotein; PD, Parkinson's disease; RAGE, receptor for advanced glycation end products; RNS, reactive nitrogen species; RPE, retinal pigment epithelium; ROS, reactive oxygen species; RS, reactive species; SOD, superoxide dismutase.

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1996). Therefore, replacement of new functional proteins is reduced which in turn increases the ratio of damaged to functional proteins in cells. All mentioned factors cause or further accelerate aggregate formation. This review focuses on the accumulation of modified proteins and their involvement in aggregate formation during the aging process. Protein modifications, especially reactions involving oxidation and glycation processes, are primary, causal events leading to increasing concentrations of damaged proteins. Moreover, the potential function of oxidized and glycated proteins and related protein aggregates, how they influence physiological pathways and contribute to age-related diseases, will be discussed in the following.

2. Protein oxidation

Damage to proteins, particularly oxidative damage, is proposed to play an essential role in aging. Especially organisms living in an aerobic environment are continuously exposed to ROS. ROS are either formed as a by-product during metabolic processes of molecular oxygen or are generated due to exogenous sources including radiation, air pollutants, cigarette smoke or drugs. The intracellular production of ROS occurs enzymatically and non-enzymatically in different cell organelles, but four enzyme systems, the NADPH oxidase (Bedard and Krause, 2007), xanthine oxidase (Harrison, 2002), uncoupled endothelial nitric oxide synthase (Landmesser et al., 2003), and the mitochondrial electron transport chain (Liu et al., 2002) predominate (Fig. 1). In the mitochondria, ROS are generated by components of the respiratory chain, especially complex I (Brandt, 2006) and III (Hunte et al., 2008), or other mitochondrial enzymes such as NADPH oxidase 4 (Bedard and Krause, 2007) and monoamino oxidase (Edmondson et al., 2009; Grivennikova and Vinogradov, 2013). ROS include among others oxygen-containing free radicals, e.g. superoxide anion radical ($O_2^{\bullet-}$) or hydroxyl radical ($^{\bullet}OH$), and non-radical molecules such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) or hypochlorous acid (HOCl). $O_2^{\bullet-}$, the product of the reduction of oxygen, is one of the most important free radicals and a precursor of many ROS. $O_2^{\bullet-}$ is reduced to H_2O_2 in a spontaneous or by superoxide dismutase (SOD) catalyzed reaction. Since $O_2^{\bullet-}$ is able to reduce iron, it also accelerates the production of highly reactive $^{\bullet}OH$ via the Fenton reaction. Moreover, $O_2^{\bullet-}$ reacts with nitric oxide ($^{\bullet}NO$) which leads to the formation of peroxynitrite ($ONOO^-$), a very strong oxidant which belongs to the reactive nitrogen species (RNS). RNS are closely related to ROS and include, besides $^{\bullet}NO$ and higher oxide radicals of nitrogen, non-radical species such as nitroxyl anion (NO^-), nitrosonium cation (NO^+), nitrous acid (HNO_2) or dinitrogen tetroxide (N_2O_4). To summarize ROS and RNS, the term reactive species (RS) is used in the following. RS are known to play a dual role: low levels of RS are indispensable for physiological cellular processes whereas high levels cause cellular damage. Beneficial effects of RS rely on their function e.g. in cell signaling (Hancock et al., 2001) and regulation or pathogen defense (Torres et al., 2006). It has been shown that low RS levels are induced when cells are stimulated by cytokines or growth factors (Sauer et al., 2001). Thus, some RS act as secondary messengers regulating cellular functions, including proliferation on one side and apoptosis on the other. However, RS are predominantly related to cellular damage so that cells evolved various antioxidant systems to delay, prevent or eliminate oxidative damage to the target molecules. On the one hand, there are proteins such as transferrin and albumin that decrease RS formation by minimizing the availability of metal ions. On the other hand, there are pathways, e.g. glutathione metabolism, SOD or catalase, which catalyze the removal of RS. Beside the elimination of RS, cells are able to repair or remove damaged molecules preventing their accumulation and aggregation. One of the most important proteolytic systems for the degradation of modified proteins is the proteasomal system. It was reported that about 70–90% (Lee and Goldberg, 1998; Rock et al., 1994) of misfolded, damaged proteins are degraded by the proteasome. The proteasomal system, essential for the physiological cell function, includes the 20S core unit and several regulator proteins which can bind to the 20S proteasome resulting in different proteasomal complexes such as the immunoproteasome or 26S proteasome (Jung et al., 2009). Protein modifications may change the conformation of the protein structure and cause the rearrangement of hydrophobic amino acids from the protein interior to the surface, and thus, modified proteins are recognized by the 20S proteasome (Grune et al., 2003). When the antioxidative defense capacity is lower than the RS flux rate, the term oxidative or respectively nitrosative stress is used. The term oxidative stress was introduced by Helmut Sies in the 1980s (Sies, 1985) and extended 2006 by Jones (2006) by including

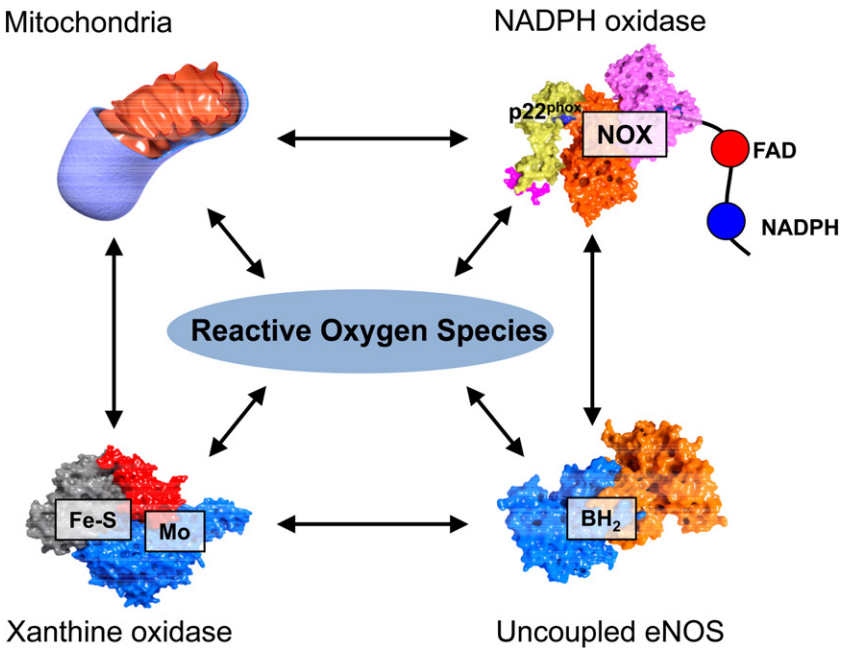

Fig. 1. Enzymatic generation of ROS, modified according to Dikalov (2011). Among many others, there are four major intracellular enzyme systems which cause the formation of ROS: NADPH oxidase, xanthine oxidase, uncoupled endothelial nitric oxide synthase and the mitochondrial electron transport chain. Activation of one ROS source can lead via ROS-mediated activation, stimulation or uncoupling of the others to increased ROS formation.