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Mini Review

Mitochondrial protein oxidation and degradation in response to oxidative stress and aging

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Abstract

Mitochondria are a major source of intracellular reactive oxygen species (ROS), the production of which increases with age. These organelles are also targets of oxidative damage. The deleterious effects of ROS may be responsible for impairment of mitochondrial function observed during various pathophysiological states associated with oxidative stress and aging. An important factor for protein maintenance in the presence of oxidative stress is enzymatic reversal of oxidative modifications and/or protein degradation. Failure of these protein maintenance systems is likely a critical component of the aging process. Mitochondrial matrix proteins are sensitive to oxidative inactivation and oxidized proteins are known to accumulate during aging. The ATP-stimulated mitochondrial Lon protease is a highly conserved protease found in prokaryotes and the mitochondrial compartment of eukaryotes and is believed to play an important role in the degradation of oxidized mitochondrial matrix proteins. Age-dependent declines in the activity and regulation of this proteolytic system may underlie accumulation of oxidatively modified and dysfunctional protein and loss in mitochondrial viability.

Keywords: Mitochondrial protein degradation; Lon protease; Aging; Oxidative stress; Ischemia–reperfusion

1. Introduction

Functional integrity and rapid and appropriate responses to physiological and pathophysiological stimuli are required for mitochondria to meet cellular energy demands. In addition, mitochondria participate in cellular Ca^{2+} homeostasis, signaling cascades, and under certain conditions, can initiate cell death. Nevertheless, the mitochondrial respiratory chain is one of the main sources of endogenous reactive oxygen species (ROS) and mitochondrial proteins represent targets for oxidative modification and loss in function. Mitochondrial dysfunction has been implicated in the aging process as well as a number of age-associated diseases such as Parkinson's disease and Alzheimer's disease associated with increased levels of mitochondrial derived free radicals and oxidative damage. Enzymatic repair of oxidative modification and protein degradation have been recognized as important factors for the maintenance of cellular homeostasis and survival. Failure of protein maintenance systems is considered a critical component of the aging process. In the cytosol, the proteasome constitutes the main proteolytic machinery involved in the elimination of oxidized protein (Shringarpure et al., 2001), however, this proteolytic complex is not present in the mitochondria. Pioneering studies have shown that mitochondrial matrix from liver and heart contains proteolytic activity that degrades oxidized, dysfunctional, and misfolded protein (Marcillat et al., 1988). More recent work supports a role for the mitochondrial matrix Lon protease in eliminating oxidatively modified mitochondrial proteins (Bota and Davies, 2002), similar to the role of the proteasome in the cytosol.

2. Oxidized protein degradation by the mitochondrial matrix Lon protease

In the mitochondria, the steady state level of oxidatively modified protein is dependent on five major determinants: (1) ROS production, (2) ROS removal, (3) susceptibility of proteins to ROS mediated modification, (4) oxidized protein repair and (5) oxidized protein degradation. Protein degradation constitutes the final step by which oxidatively modified proteins can be eliminated. Mammalian mitochondria contain

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three major ATP-dependent proteases, Lon, Clp-like and AAA proteases. Clp-like and AAA proteases are hetero-oligomeric complexes located in the matrix and inner mitochondrial membrane, respectively (Van Dyck and Langer, 1999; Kaser and Langer, 2000). As evidenced by various mutational studies, these proteases contribute to the degradation of misfolded and damaged proteins and/or the maintenance of mitochondrial genome stability. In addition, both proteolytic systems appear to exert chaperone activity. The physiological function of Clp-like protease, however, is yet to be determined. Currently, information regarding the regulation of each of the ATP-dependent proteases and/or the identities of specific protein substrates is limited. Nevertheless, exposure of hydrophobic residues is likely a common recognition element and both chaperone and proteolytic functions participate in prevention of the accumulation of aggregated material (Friguet et al., 1994; Friguet and Szweda, 1997; Grune et al., 1997). The Lon protease plays a critical role in the removal of oxidized protein. Aconitase, a Krebs cycle enzyme known to be susceptible to oxidative inactivation, has been shown to be a substrate of the Lon protease when the enzyme is inactivated upon treatment with oxygen radicals (Bota and Davies, 2002). Indeed, the matrix proteolytic activity responsible for the selective degradation of oxidatively modified aconitase was strongly stimulated by ATP and inhibited by the serine protease inhibitor PMSF. In addition, the same proteolytic activity co-purified with the Lon protease after size exclusion chromatography and affinity chromatography of the mitochondrial matrix fraction. Moreover, both purified Lon protease and mitochondrial matrix extracts exhibited similar activation and inhibition profiles. Treatment with anti-sense oligonucleotides in WI-38 human lung fibroblasts resulted in decreased Lon protease content and activity while causing an accumulation of oxidatively modified aconitase (Bota and Davies, 2002). More recently, it has been shown that down regulation of the human Lon protease results in disruption of mitochondrial structure, loss in function, and cell death, with the majority of cells undergoing caspase 3 activation and apoptosis within 4 days (Bota et al., 2005). Electron microscopy performed on Lon-deficient cells revealed aberrant mitochondrial morphology and the presence of electron dense inclusion bodies in the mitochondrial matrix thought to represent oxidatively modified and aggregated protein. Taken together, these findings argue for an important role of the Lon proteolytic system for the degradation of oxidized protein within the mitochondrial matrix and for the maintenance of mitochondrial structural and functional integrity.

3. Properties of the Lon mitochondrial protease

As are numerous mitochondrial proteins, the Lon protease is encoded by the nuclear genome. The lon gene encodes a 963 amino acid protein (Wang et al., 1993) homologous to the bacterial protease La. This protease is active as an homo-oligomeric complex composed of six monomers in *Escherichia coli* and seven monomers in eukaryotes such as yeast with a molecular weight of approximately 106 kDa. The Lon protease is composed of three domains (Fig. 1) that are conserved within the different species (Iyer et al., 2004; Mogk et al., 2004). The N-terminal domain (domain N) is capable of interacting with protein substrates in concert with the second domain, also termed the AAA+ module. The AAA+ module is composed of two segments, one involved in ATP binding (α/β domain) and the other in ATP hydrolysis (α domain). A third domain (P domain) carries the Serine and Lysine active site residues that form the catalytic dyade for proteolytic activity (Amerik et al., 1990). The identity of these active site residues was first evidenced by comparison of the different Lon protease protein sequences and further confirmed by site directed mutagenesis analysis and elucidation of the three-dimensional structure of the *E. coli* Lon protease P domain (Amerik et al., 1991; Besche and Zwickl, 2004; Botos et al., 2004a,b). Interestingly, the isolated P domain does not exhibit any proteolytic activity towards protein substrates such as casein but is capable of degrading small peptides such as melittin (Rasulova et al., 1998). The three-dimensional structure of both the N and AAA+ domains of the *E. coli* Lon protease has also been recently solved (Botos et al., 2004a,b; Li et al., 2005). While

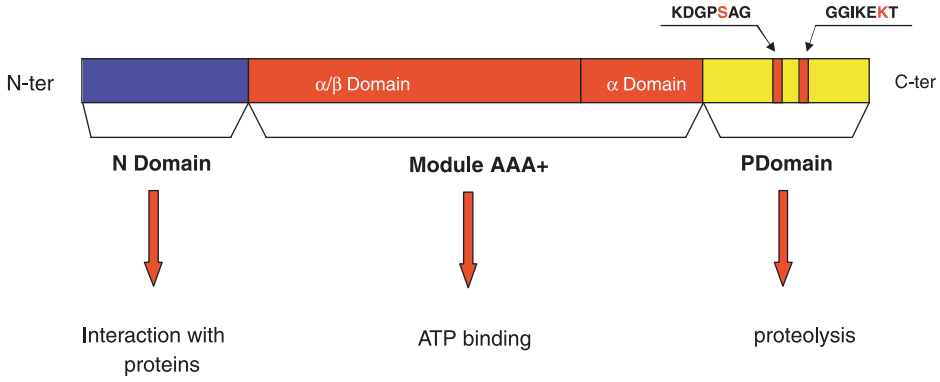

Fig. 1. Schematic representation of domain organization of human Lon protease. Consensus sequences are shown by arrows and the two catalytic residues (Ser and Lys) are indicated in red (adapted from Mogk et al., 2004) (for interpretation of the reference to color in this legend, the reader is referred to the web version of this article).