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Modulating cellular aging in vitro: Hormetic effects of repeated mild heat stress on protein oxidation and glycation

P. Verbeke, B.F.C. Clark, S.I.S. Rattan*

Danish Centre for Molecular Gerontology, Laboratory of Cellular Ageing, Department of Molecular and Structural Biology, University of Aarhus, Gustav Wieds Vej 10-C, DK-8000 Aarhus, Denmark

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Abstract

Intracellular and extracellular proteins are subject to a variety of spontaneous non-enzymatic modifications which affect their structure, function and stability. Protein oxidation and glycation are tightly linked and are implicated in the development of many pathological consequences of aging. Although multiple endogenous pathways in the cell can prevent the formation of oxidized and glycated proteins, and repair and degrade abnormal proteins, such abnormal proteins do accumulate during aging. The heat shock response involving the family of stress-proteins or the so-called heat shock proteins (HSP), represents the quickest and highly conserved response to proteotoxic insults. Since repeated mild heat stress is able to prevent the onset of various age-related changes during cellular aging in vitro, we suggest that treatments which increase HSP expression should reduce the extent of accumulation of abnormal proteins during aging. Such modulation of aging is an example of hormesis, which is characterized by the beneficial effects resulting from the cellular responses to mild repeated stress.

Keywords: Anti-aging; Gerontogenes; Heat shock; Hormesis; Protein modification; Stress

1. Introduction

Cells aged in vitro or isolated from aged organisms have increased levels of abnormal proteins as compared with young cells. Most of these damaged proteins are formed by post-translational modifications (Rattan, 1995, 1996). Of these, oxidation and glycation produce the most noxious crosslinks and apparently no cellular systems are able to completely repair the damage (Dean et al., 1997; Baynes, 2000). These modifications

* Corresponding author. Tel.: +45-89-425034; fax: +45-86-123178.
E-mail address: rattan@imsb.au.dk (S.I.S. Rattan).

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result in misfolding and denaturation of proteins, and confer increased resistance to proteolysis, leading to their accumulation in the cell. However, cells have various systems to prevent the formation of oxidized and glycated proteins, and to repair or degrade the weakly damaged proteins. The highly conserved heat shock (HS) response involving the HS family of stress proteins (HSP) is the immediate cellular response to stress, especially proteotoxic insults. Since HSP are known to act as chaperones and protect proteins from misfolding and denaturation, we are investigating the link between HS response and protein oxidation and glycation.

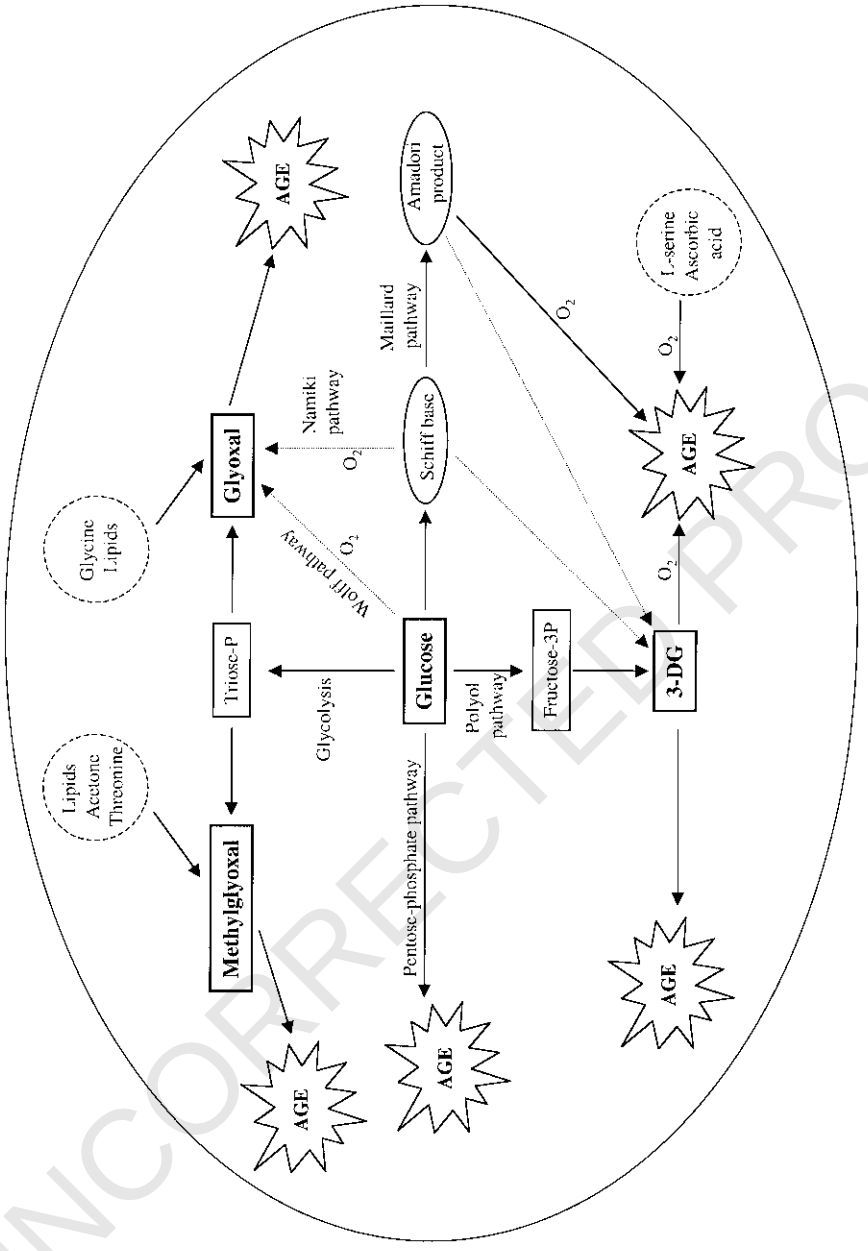
In a pilot study from our laboratories, it has been shown that mild repeated heat stress (MRHS) prevents the onset of several age-related changes in human fibroblasts undergoing aging in vitro (Rattan, 1998). Such modulation of aging is an example of hormesis, which is characterized by the beneficial effects resulting from the cellular responses to mild repeated stress (Rattan, 2000). One of the main effects of MRHS was the prevention of age-related cell enlargement, which is generally due to the accumulation of abnormal and inactivated proteins (Rattan, 1995). We are now investigating whether MRHS affects the processes of protein oxidation and glycation during cellular aging in vitro. In this article, we describe the biochemistry of protein oxidation and glycation, and discuss our approach of utilizing MRHS for modulating cellular aging.

2. Protein oxidation

Reactive oxygen species (ROS) and protein oxidation have been implicated in cancer, cell death and the loss of various homeodynamic functions during aging (Dean et al., 1997). Potentially, ROS may attack any amino acid but sulfur-containing (methionine, cysteine), aromatic (tyrosine, tryptophan), and basic (lysine, arginine) amino acid residues are most prone to oxidation (Berlett and Stadtman, 1997). Amino acid-oxidation generates the formation of oxo-, sulfo-, hydroxy-, chloro- and nitro-derivatives, which can lead to the formation of protein–protein crosslinks and protein fragmentation (Berlett and Stadtman, 1997). Several amino acids may yield a common oxidative modification, forming carbonyl derivatives. Carbonyl has been used as a marker of ROS-mediated protein oxidation although glycoxidation and lipoxidation are also source for this damage.

Aerobic cells have a complex network of antioxidants, such as glutathione (GSH), ascorbate, urate, quinols and vitamins A, C and E, and enzymatic systems such as superoxide dismutase (SOD), catalase, glutathione-peroxidase and phospholipid-peroxidase to scavenge or detoxify the ROS (Dean et al., 1997). In eucaryotes, enzymatic repair systems can reverse some colvalent protein damages. For example, thiol-protein disulfide exchange enzymes (protein disulfide isomerase, thioredoxin reductase and thiol transferase) reduce the unnatural disulfide bonds (Grune et al., 1997). Methionine sulfoxide reductase and the peptide methionine sulfoxide reductase (pMSR) reduce either the free

Fig. 1. Potential major pathways of cytosolic and glycoxidation. Glucose and its secondary metabolites glyoxal, methylglyoxal and 3-deoxyglucosone (3-DG) react with proteins to form advanced glycation endproducts (AGE). Glyoxal and methylglyoxal are also formed from the metabolism of amino acids, lipids and acetone. AGE can also be formed by direct interaction of oxidized products of L-serine and ascorbic acid.



methionine sulfoxide or the one present in the protein. Methionine itself is supposed to be an important antioxidant because it can be preferentially oxidized without affecting the protein activity (Levine et al., 1999). The proteolytic systems are the last alternative to remove damaged proteins. The ATP-ubiquitin-independent 20S-"core" proteasome recognizes the protein hydrophobicity and denaturation, and is responsible for 70–80% of the degradation of the cytosolic oxidized proteins (Grune et al., 1997; Grune, 2000). Protein oxidation is also a targeting signal for polyubiquitin conjugation and degradation by the ATP-ubiquitin-dependent 26S proteasome or by ubiquitin specific hydrolases (Grune et al., 1997).

During aging, the levels of various markers of protein-oxidation, such as carbonyl, o-tyrosine and dityrosine have been reported to increase in insects, nematodes, rodents and human tissues (Berlett and Stadtman, 1997). Similarly, the level of carbonyls has been reported to increase in cultured cells from patients suffering from premature aging syndromes (Oliver et al., 1987). Age-related loss of activity of several enzymes and an accumulation of abnormal proteins during aging is most frequently considered to be due to oxidation. The increase of the oxidative stress observed during aging is due to both a decrease of the antioxidant (redox) status of the cell, and the alteration in enzymatic pathways related to the redox status. The level of antioxidative defenses and especially antioxidants enzymes increase, decrease or remain unchanged with age depending on the organism, the tissue and the cell type studied (Rattan, 1995). Similarly, the increase of protein oxidation observed during aging could be due to a decrease of protein degradation. A large reduction of the transcription of genes implicated in the protein turnover, such as ubiquitin-proteasome pathway (UPP), has been reported (Lee et al., 1999). Furthermore, there is a decline in both the 20S- and 26S-proteasome inducible activity or some protease-like activities of this complex in cells and tissues during aging (Conconi et al., 1996; Hayashi and Goto, 1998). With regard to enzymatic repair systems, the activity of thiol-protein disulfide exchange enzymes appears to be unchanged during aging (Rikans and Hornbrook, 1998), but the specific pMSR activity is suggested (Sun et al., 1999) to decrease.

3. Protein glycation

Glycation products are formed in vitro by the binding of sugars or of intermediary products formed during metabolism to the accessible free ε-NH2 group of the amino acid constituents of a protein (Miyata et al., 1999; Baynes, 2000). Glycoxidation products are formed by sequential glycation and oxidation reactions (Miyata et al., 1999). The end-result of these processes of glycation and glycoxidation is the formation of the so-called advanced glycation/glycoxidation end products (AGE). Some of the main AGE are Nε-carboxymethyl-lysine (CML), pentosidine, imidazolones and α-oxoaldehydes-lysine dimers. In fact, two types of glycation processes occur in vivo. The extracellular process, which is dependent on a high level of glycemia, uses the Maillard pathway as the main route of glycation. The intracellular process is more complex due to the fact that the potential sources of AGE are multiple in the cytosol (Fig. 1). Glucose and the Maillard pathway are certainly not the main source for the intracellular formation of AGE, except

under pathological conditions where there is a misregulation of the glucose metabolism. The highly reactive α-oxoaldehydes (glyoxal, methylglyoxal, and 3-deoxyglucosone or 3-DG) are thought to be more important in the cytosolic glycation because these result from multiples metabolisms (Fig. 1). Due to the complexity of this intracellular glycation process, several pathways can lead to the formation of the same AGE, such as Nϵ-carboxymethyl-lysine (CML) and pentosidine.

A short period of hyperglycemia due to impaired glucose tolerance observed during aging may be sufficient to divert glucose from glycolysis and reroute this fuel into other pathways (Fig. 1). These pathways promote oxidative stress and generate α-oxoaldehydes sharing the ability to promote mutagenesis, to accelerate the AGE formation and to induce some cytotoxic effects as well as apoptotic cell death (Thornalley et al., 1999). Furthermore, glycation could inhibit both ubiquitin conjugation and ubiquitin-mediated degradation due to the fact that both processes share the common protein-conjugating site. Also, glycation and oxidation are tightly linked processes since the process of glycation generates ROS and AGE-proteins themselves are a source of ROS.

To prevent glycation, several cytosolic enzymes (2-oxaloaldehyde dehydrogenase, aldo–keto reductase superfamily, aldehyde dehydrogenases, dihydrodioldehydrogenases and glyoxalases) contribute to the detoxification of α-oxoaldehydes. In mammals, phosphorylation of the Amadori product seems implicated in the reversal of the early glycation step. No proteolytic pathways are known to remove the cytosolic AGE especially when they are implicated in the crosslinking of proteins, but one could postulate the involvement of the lysosome where these products tend to accumulate (Grune et al., 1997).

There is only a weak correlation between glycemia and species longevity, but AGE are directly implicated in many age-related diseases such as nephropathies, atherosclerosis, senile cataract, stroke, Alzhemier's Pick's and Parkinson's diseases (Lee and Cerami, 1992). Pentosidine levels increase during aging of cultured fibroblasts (Sell et al., 1998). Several intracellular proteins have been shown to be glycated and inactivated in age-related pathologies (Furth, 1997). Glycation inhibits the transcription of glycokinase (Kajimoto et al., 1999). There is an age-related accumulation of crosslinked proteins and aggregated material called lipofuscin, ceroid or AGE-pigment-like fluorophores, in lysosomes (Rattan, 1995, 1996). A defect of the lysosomal regulation (macroautophagy, protease expression) could be due in part to AGE (Terman and Brunk, 1998; Kasper et al., 1999) and by a vicious cycle it can promote the formation of lipofuscin. Also, a decreased efficiency of the α-oxoaldehydes detoxification pathways may accelerate the appearance of AGE-related pathologies.

4. Heat shock response

Heat shock response and increased expression of HSP is one of the most powerful means of cytoprotection against protein misfolding and aggregation (Feder and Hofmann, 1999). HSP serves as a sensor in recognition of reversibly and irreversibly damaged proteins by repairing moderated protein misfolding and by preventing inter- or intramolecular aggregation. Families of Hsp110, Hsp90 and especially Hsp70 (Hsp72, Hsc70) working with cochaperones Hsp40, Hip, Hop, BAG1) are implicated in the

processes of refolding, prevention of aggregation and proteolysis. HSP are present both under normal conditions as sensors of cellular redox status and under stress when they are overexpressed to cope with an increased concentration of unfolded proteins.

The mechanism for the induction of HSP expression is through the so-called HS transcription factors (HSF). In unstressed cells, HSF1, which is considered to be the main HSF, is maintained as a non-DNA-binding complex by stoichiometric interaction with Hsp72 or as a complex of chaperones. When a cell is exposed to stress, HSF1 is released from the quenching chaperone(s), gets trimerized and phosphorylated followed by its translocation to the nucleus and binding to the promoter of stress-inducible HSP genes to induce their transcription. The release of HSF1 from the quenching chaperone(s) is thought to result from either a direct change in HSF conformation or as a consequence of the release of the HSP to bind to denatured proteins promoted by stress. Another pathway allows the pre-existing Hsp33 to respond more quickly to the changes in the cellular redox status (Jakob et al., 1999). It has been reported that protein glycation as well as oxidation could induce stress response, since introduction of AGE-like proteins into *Xenopus laevis* oocytes elicited a specific stress response (Mifflin and Cohen, 1994). It has also been reported that HSP can induce ubiquitination (Benjamin and McMillan, 1998), protect the proteasome against oxidation (Conconi et al., 1998), could have their own protease activity (Faccio et al., 2000) and play a role in cell survival (Liao et al., 2000).

During aging, the stress response and the HSP production are decreased (Feder and Hofmann, 1999). The mechanism underlying this decrease is not well defined and could be due to a low level of HSF, to a defect of trimerization or phosphorylation, to a decrease of the DNA-binding activity or to a defect of mRNA maturation of HSP. Also, the fact that the chaperone-system is often due to a bichaperone system, may present some problem during aging. For example, if one of the chaperones is present in large excess over the other, it may in fact increase protein aggregation.

5. Beneficial effects of mild stress

Cellular resistance of a variety of stresses is directly correlated with longevity, supporting the idea that the genetic network regulating cellular stress response is functionally important in aging. Therefore, it has been suggested that repeated mild stress-induced upregulation of HSP expression and synthesis could have beneficial effects on aging and longevity (Lithgow et al., 1995; Minois, 2000; Rattan, 2000). This is because stress-response to mild stress allows cells to enhance their cellular defense processes, to adapt to gradual changes in their environment and survive in otherwise lethal conditions.

Transient overexpression of HSP has been shown to increase the stress resistance and prolong the lifespan of transgenic *Drosophila melanogaster* (Tatar et al., 1997) and *Caenorhabditis elegans* (Lithgow et al., 1995). Mild heat shock could increase the age-specific survival by either increasing the expression of HSP that may regularly renature, assemble and disassemble non-HSP, or by activating other stress-response mechanisms such as SOD and glutathione reductase. Increase of lifespan in long-term calorie-restricted rodents reveal a relationship between the ability to mount both a stress response and UPP-response (Scrofano et al., 1998), and the reduction of oxidative stress (Hall et al., 2000)

and glycoxidative damages (Cefalu et al., 1995). In the case of human cells undergoing aging in vitro, it has been reported that mild repeated heat shock prevents the onset of several age-related changes (Rattan, 1998). Such anti-aging effects of mild repeated stress have been interpreted as hormetic effects (Rattan, 2000).

Physiologically, mild HS could act by initiating two processes. First, slow and cumulative induction of Hsp72 suppresses apoptosis by inhibiting the JNK pathway (Gabai et al., 1998). Second, it may reduce protein aggregation by activating internal repair and degradation processes. Our studies are aimed at showing if there is a relationship between MRHS treatment, and maintenance of the HS response, decrease of cytosolic damaged proteins and the delay of senescence in human cells. Recently, we have observed that experimentally induced formation of AGE in cultured fibroblasts can be significantly prevented by mild HS treatment (unpublished data). Since the chaperone-system governs the reparation of early damages, especially protein oxidation, it is probably one of the key steps in preventing the accumulation of highly damaged proteins during aging. Exposing cells to repeated mild stress appears to have hormetic effects in maintaining the activity of defense pathways and in slowing down the accumulation of abnormal proteins during aging.

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