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Protein methionine content and MDA-lysine adducts are inversely related to maximum life span in the heart of mammals

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Abstract

Aging affects all organisms and its basic mechanisms are expected to be conserved across species. Oxidation of proteins has been proposed to be one of the basic mechanisms linking oxygen radicals with the basic aging process. If oxidative damage to proteins is involved in aging, long-lived animals (which age slowly) should show lower levels of markers of this kind of damage than short-lived ones. However, this possibility has not been investigated yet. In this study, steady-state levels of markers of different kinds of protein damage – oxidation (glutamic and aminoadipic semialdehydes), mixed glyco- and lipoxidation (carboxymethyl- and carboxyethyllysine), lipoxidation (malondialdehydelysine) and amino acid composition – were measured in the heart of eight mammalian species ranging in maximum life span (MLSP) from 3.5 to 46 years. Oxidation markers were directly correlated with MLSP across species. Mixed glyco- and lipoxidation markers did not correlate with MLSP. However, the lipoxidation marker malondialdehydelysine was inversely correlated with MLSP ($r^2 = 0.85$; $P < 0.001$). The amino acid compositional analysis revealed that methionine is the only amino acid strongly correlated MLSP and that such correlation is negative ($r^2 = 0.93$; $P < 0.001$). This trait may contribute to lower steady-state levels of oxidized methionine residues in cellular proteins. These results reinforce the notion that high longevity in homeothermic vertebrates is achieved in part by constitutively decreasing the sensitivity of both tissue proteins and lipids to oxidative damage. This is obtained by modifying the constituent structural components of proteins and lipids, selecting those less sensitive to oxidative modifications.

Keywords: Aminoadipic semialdehyde; N^{ε}-(Carboxyethyl)lysine; N^{ε}-(Carboxymethyl)lysine; Glutamic semialdehyde; Maillard reaction; N^{ε}-(Malondialdehyde)lysine; Maximum life span; Peroxidizability index; Protein oxidation

1. Introduction

What are the mechanisms determining the rate of animal aging? Aging affects all organisms and its basic mechanisms are expected to be conserved across species. The idea that reactive oxygen species (ROS), especially those of mitochondrial origin (Harman, 1972), are causally related to the basic aging process is increasingly receiving support from scientific studies (reviewed in Barja, 2004a,b) including, most importantly, comparisons between animal species with different aging rates.

Strong support for this theory comes from observations that mitochondria isolated from longer-lived organisms

Abbreviations: AASA, aminoadipic semialdehyde; CEL, N^{ε}-(Carboxyethyl)lysine; CML, N^{ε}-(Carboxymethyl)lysine; GC/MS, gas chromatography/mass spectrometry; GSA, glutamic semialdehyde; MDAL, N^{ε}-(Malondialdehyde)lysine; MLSP, maximum life span; PI, peroxidizability index; ROS, reactive oxygen species

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produce ROS in vitro at lower rates than those from shorter-lived organisms across a range of mammalian (Ku et al., 1993) and avian species (Barja, 2004a). This correlation is also found in comparisons between members of the same species in which life span is altered by experimental manipulation, such as by diet. Dietary restriction is the only treatment that extends mean and maximum life span in a variety of laboratory animals. In rodents, extension of life span by dietary restriction is accompanied by lower rates of ROS production by isolated mitochondria and lower levels of DNA and protein damage (Sohal et al., 1994; Gredilla et al., 2001; Pamplona et al., 2002a,b).

Oxidation of proteins has been proposed to be one of the basic mechanisms linking oxygen radicals with the basic aging process (Berlett and Stadtman, 1997). According to free radical hypothesis of aging, a high oxidative state should contribute to accelerated tissue damage caused by deleterious by-products of oxidative metabolism (Beckman and Ames, 1998) and the Maillard reaction or carbonyl-amine reaction including glycoxidation and lipoxidation reactions (Baynes, 2002).

In order to examine oxidative stress-related molecular traits associated with the aging rate of mammals we used GC/MS methods to define the steady-state levels of protein damage. We measured different kinds of protein oxidative markers in the heart of eight mammalian species showing more than one order of magnitude of difference in maximum life span (MLSP)—from 3.5 years in mice to 46 years in horses. Our choice of tissue was guided by the fact that the heart is a vital organ critical for aging mainly composed of high glucose- and oxygen-consuming post-mitotic cells. Oxidative damage can directly affect a number of protein amino acid residues, resulting in the characteristic introduction of carbonyl groups that can be assayed using the classic protein carbonyl method (Stadtman, 1992). Recently, GC/MS-based methods allowing measurement of the specific protein carbonyl-containing residues glutamic semialdehyde (GSA) and aminoadipic semialdehyde (AASA) have been developed (Requena et al., 2001). Oxidative damage can also be indirectly inflicted to proteins by reaction of these with glyco- or lipoxidation-derived products (Thorpe and Baynes, 2003), in some cases also leading to carbonylation. To better characterise types of protein damage, we measured three specific markers: glutamic semialdehyde and aminoadipic semialdehyde as indicators of oxidation; N^{ε}-(carboxyethyl)lysine (CEL) and N^{ε}-(carboxymethyl)lysine (CML) as indicators of mixed glycoxidation and lipoxidation; and N^{ε}-(malondialdehyde)lysine (MDAL) as indicator of lipoxidation. In addition, to evaluate the role of structural components that can act as substrate targets determining the degree of protein damage, amino acid profiles were determined. We also measured the fatty acid unsaturation index because it can affect levels of protein damage and life span. The fatty acid composition of membrane phospholipids is suggested to be highly relevant for aging, because it modulates the susceptibility to oxidative and lipoxidative macromolecular damage (Pamplona et al., 2002b, 2004). A high membrane fatty acid unsaturation is associated with high levels of lipoxidative damage (Herrero et al., 2001; Pamplona et al., 2004) and the degree of fatty acid unsaturation negatively correlates with maximum life span across mammalian species (Pamplona and Barja, 2003).

2. Material and methods

2.1. Chemicals

Reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise specified. [$^2H_8$]-lysine was purchased from CDN Isotopes (CDN Isotopes Inc., Canada). CML and [$^2H_4$]-CML, CEL and [$^2H_4$]-CEL, and MDAL and [$^2H_8$]-MDAL were prepared as previously described (Knecht et al., 1991; Ahmed et al., 1997; Requena et al., 1997). GSA and [$^2H_5$]-GSA, and AASA and [$^2H_4$]-AASA were prepared as previously described (Requena et al., 2001).

2.2. Animals

Male mice, rats, guinea pigs and rabbits were killed at the laboratory by decapitation. Male sheep, pigs, cows and horses were killed at the abattoir. The mean age of the animals was 8 months (mice), 11 months (rat), 1.4 years (guinea pig), 1.5 years (rabbit), 1 year (pig) and 1.5–2.5 years (sheep, cow, horse). The selection of these ages allows a comparison among young adults of all the species while maintaining a compromise between the choices of similar chronological or biological ages for interspecies comparison. The maximum longevities of the selected species are well known (Altman and Dittmer, 1972) and vary progressively from 3.5 to 46 years: mouse (*Mus musculus*, MLSP = 3.5 years, $n = 7$), rat (*Rattus norvegicus*, MLSP = 4 years, $n = 7$), guinea pig (*Cavia porcellus*, MLSP = 8 years, $n = 7$), rabbit (*Oryctolagus caniculus*, MLSP = 13 years, $n = 7$), sheep (*Ovis aries*, MLSP = 20 years, $n = 7$), pig (*Sus scrofa*, MLSP = 27 years, $n = 7$), cow (*Bos taurus*, MLSP = 30 years, $n = 7$) and horse (*Equus caballus*, MLSP = 46 years, $n = 7$). All the animals were in good health according to routine veterinary controls at the abattoir. Heart samples were taken from ventricles. The samples were obtained in all animals at the same time after death (5 min), they were cut in small pieces, immediately frozen in liquid nitrogen, and transferred before 2 h to a 280 °C freezer for storage to be used later for analyses.

2.3. Preparation of the heart samples

Heart tissue was immersed and rinsed in cold homogenization buffer (10 mM Hepes, 1 μM 2,6-di-*tert*-butyl-4-methylphenol (BHT), 1 mM diethylenetriaminepentaacetic acid (DETAPAC), 1 mM phytic acid, 0.5 μg/m pepstatin, 0.5 μg/m aprotinin, 0.7 μg/ml pepstatin, 40 μg/ml phenylmethanesulfonyl fluoride (PMSF) and 1.1 mM EDTA, adjusted to pH 7.4). This was followed by homogenization