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Mini Review

Nonenzymatic posttranslational protein modifications in ageing

Vukić Šoškić, Karlfried Groebe, André Schrattenholz *

ProteoSys AG, Carl Zeiss Strasse 51, 55129 Mainz, Germany

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Abstract

One of the most fundamental molecular aspects of aging is accumulating oxidative damage caused by reactive oxygen species (ROS) as proposed by the free radical theory of aging. These unwanted chemical side products of normal metabolism lead to the formation of altered, less active and potentially toxic species of DNA, RNA, proteins, lipids, and small molecules.

Due to gradually accumulating small contributions of irreversible reactions during ageing, uncatalyzed chemical side reactions occur with increasing frequencies and repair functions decline. Eventually key biochemical pathways are impaired by increasingly less efficient cellular stress management. In this review, we describe the chemical nature of nonenzymatic age-related modifications of proteins and provide an overview of related analytical challenges and approaches, with a focus on mass spectrometry. We include the description of a strategy to rapidly exploit the wealth of mass spectrometric information from standard MALDI-TOF peptide fingerprints for the characterisation of age-related oxidative amino acid modifications.

Keywords: Ageing; Oxidation; Reactive oxygen species; Mass spectroscopy; Posttranslational modification; Carbonylation; Nitrosylation; Proteomics; Advanced glycation; AGE

1. Introduction

Age-related chemical side reactions that can occur on proteins include: racemisation (McCudden and Kraus, 2006), deamidation (Robinson and Robinson, 2001), oxidation of amino acids (Stadtman, 2004, 2006; Stadtman et al., 2005), formation of adducts involving reactive nitrogen and chlorine species (van der Vliet et al., 1995), chemical modification of proteins by products of lipid peroxidation reactions (lipoxidation) and Maillard reaction products (Baynes, 2000, 2001, 2002); Table 1 shows a summary.

2. Oxidative modifications

It is now beyond doubt that reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) generated in vivo, play a role in aging, as already proposed in 1956 (Harman, 1956; Beckman and Ames, 1998). Since reactive by-products of normal metabolism also lead to damage (Hayflick, 2007), this theory has recently been extended to the oxidative "garbage catastrophe theory" where ROS or reactive oxygen intermediates are responsible for the accumulation of age-related cellular damage of biomolecules (Stadtman, 2004, 2006; Stadtman et al., 2005). Studies on oxidatively modified proteins have revealed an

Abbreviations: 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; AGE, advanced glycation end-product; ALA, advanced lipoxidation end-product; Asn, asparagine; Asp, aspartic acid; Trp, tryptophan; Tyr, tyrosine; CEL, N^{ε}-(carboxyethyl)lysine; CML, N^{ε}-(carboxymethyl)lysine; DNPH, 2,4 dinitrophenylhydrazine; DNP-hydrazone, 2,4-dinitrophenylhydrazone; DOGDIC, 3-deoxyglucosone-derived imidazoline cross-link; GODIC, glyoxal-derived imidazoline cross-link; GOLD, glyoxal-lysine dimer; LC-MS/MS, liquid chromatography tandem mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization; MetSOx, methionine sulfoxide and methionine sulfone; MOLD, methylglyoxal-lysine dimer; MODIC, methylglyoxal-derived imidazoline cross-link; MS/MS, tandem mass spectrometry; PMF, peptide mass fingerprinting; Q-TOF, quadrupole time of flight mass spectrometry; ROS, reactive oxygen species; RNS, reactive nitrogen species.

* Corresponding author. Tel.: +49 6131 5019215; fax: +49 6131 5019211.
E-mail address: andre.schrattenholz@proteosys.com (A. Schrattenholz).

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Table 1
Age-related amino acid residue modifications

Amino acid	Reaction type/product formed			
	Oxidation	Maillard reaction	Isomerisation	Deamidation
Arg	Glutamic semialdehyde	Advanced glycation end-product (AGE) and advanced lipoxidation end-product (ALE)	na	na
Asn	na	Na	na	Asp
Asp	na	na	L-Isoaspartate D-Aspartate	na
Cys	Sulfinic acids and Cysteic acid	na	na	na
Gln	na	na	na	Glu
Glu	4-Hydroxy-glutamate	na	L-Isoglutamate D-Glutamate	na
His	2-Oxo-histidine	na	na	na
Leu	5- Hydroxyl-leucine	na	na	na
Lys	2-Aminoadipic-semialdehyde, 3-, 4- and 5-hydroxy lysine	Advanced glycation end-product (AGE) and advanced lipoxidation end-product (ALE)	na	na
Met	MetSOx	na	na	na
Phe	2-, 3-phenylanine and Tyr	na	na	na
Pro	glutamic semialdehyde, pyroglutamic acid, 2-pyrrolidone, 4-hydroxy-proline	na	*cis*/*trans* isomerisation	na
Thr	2-Amino-3-keto-butyric acid	na	na	na
Trp	*N*-formyl-kynurenine, kynurenine, 2-,4-,5-, 6- and 7-hydroxy-tryptophan	na	na	na
Tyr	3,4-Dihydroxy phenylanine Tyr–Tyr cross-linked proteins, 3-nitro-tyrosine, 3,5-dichloro-tyrosine	na	na	na
Val	3- and 4-hydroxy valine	na	na	na

age-related increase in the level of protein carbonylation (Levine, 2002), oxidized methionine (Wells-Knecht et al., 1997), cross-linked (Squier and Bigelow, 2000) and glycated proteins (Baynes, 2001), as well as the accumulation of catalytically less active enzymes (Rothstein, 1985) that are more susceptible to heat inactivation and to proteolytic degradation (Stadtman, 2001). One of the best-known markers of age-related protein oxidation is the carbonyl group.

The carbonyl content of proteins has been observed to increase with age (Levine, 2002). The main carbonyl products of metal-catalyzed oxidation of proteins *in vitro* have been shown to be glutamic and aminoadipic semialdehydes (Requen2001; Pamplona et al., 2005). Other markers may be derived from ROS-induced protein oxidation, but may be susceptible to further reactions.

Tyrosine residues may be oxidized by hypochlorite, peroxynitrite or by radicals formed in transition metal ion-catalyzed Fenton and Haber-Weiss reactions (e.g. hydrogen peroxide/Fe^{2+}). The ensuing tyrosyl radicals may subsequently form intra- or intermolecular Tyr–Tyr bonds (Balasubramanian and Kanwar, 2002; van der Vliet et al., 1995). Several other oxidized residues, like hydroperoxides of amino acid side chains are highly unstable. *N*-formylkynurenine (an oxidation product of Trp) can be generated enzymatically and non-enzymatically (Korlimbinis and Truscott, 2006). MetSOx and disulfides may be enzymatically reduced (Chao et al., 1997). Intermolecular cross-links are the most important age-related chemical alterations in collagen and elastin. These cross-links are initially formed (through lysyl oxidase) to provide optimal function during development and maturation, but can subsequently overstiffen and compromise the structure and function of the fibers throughout the body when present in excess (Bailey, 2001).

3. Spontaneous deamidation, isomerization, and racemization of aspartyl and asparaginyl residues

Asparagine and aspartyl residues represent hot spots for spontaneous protein degradation under physiological conditions (Clarke, 2003). For both types of residues, the nucleophilic attack of the peptide-bond nitrogen atom of the following residue on the side chain carbonyl group results in the formation of a five-membered succinimide ring intermediate as shown in Fig. 1 (Dehart and Anderson, 2007).

The succinimidyl residue is hydrolyzing with half-times of hours under cellular conditions to give a mixture of aspartyl and isoaspartyl forms. The latter residues induce kinks in polypeptide chains. The succinimide is also racemization-prone (Radkiewicz et al., 2001) and generates the D-succinimidyl, D-aspartyl and D-isoaspartyl forms. Thus, from the original L-aspartyl and L-asparaginyl residues encoded by protein biosynthesis reactions, spontaneous aging results in the formation of at least five altered forms, i.e. D-aspartyl-, D- and L-isoaspartyl-, and D- and L-succinimidyl isoforms. Of these, the L-isoaspartyl form is the most frequently found.

Spontaneous direct hydrolysis of asparagine residues by water attack on the side chain amide group can also result in aspartyl residue formation (Robinson and Robinson, 2001; Robinson, 2002). However, at neutral pH, the rate