JOURNAL OF PROTEOMICS 92 (2013) 63–70

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging☆

*Martin A. Baraibar, Romain Ladouce, Bertrand Friguet**

Laboratoire de Biologie Cellulaire du Vieillissement, UR4-IFR83, Université Pierre et Marie Curie–Paris 6, 4 place Jussieu, 75252 Paris Cedex 05, France

ARTICLE INFO

Available online 18 May 2013

Keywords:
Protein carbonyls
Protein damage
Aging
Oxidative stress
Fluorescent-based proteomics approaches
Difference gel electrophoresis

ABSTRACT

Increased protein carbonyl content is a hallmark of cellular and organismal aging. Protein damage leading to the formation of carbonyl groups derives from direct oxidation of several amino acid side chains but can also derive through protein adducts formation with lipid peroxidation products and dicarbonyl glycating compounds. All these modifications have been implicated during oxidative stress, aging and age-related diseases. However, in most cases, the proteins targeted by these deleterious modifications as well as their consequences have not yet been clearly identified. Indeed, this is essential to determine whether and how these modified proteins are impacting on cellular function, on the development of the senescent phenotype and the pathogenesis of age-related diseases. In this context, protein modifications occurring during aging and upon oxidative stress as well as main proteomic methods for detecting, quantifying and identifying oxidized proteins are described. Relevant proteomics studies aimed at monitoring the extent of protein carbonylation and identifying the targeted proteins in the context of aging and oxidative stress are also presented. Proteomics approaches, i.e. fluorescent based 2D-gel electrophoresis and mass spectrometry methods, represent powerful tools for monitoring at the proteome level the extent of protein oxidative and related modifications and for identifying the targeted proteins.

Biological significance

Accumulation of damaged macromolecules, including oxidatively damaged (carbonylated) proteins, is a hallmark of cellular and organismal aging. Since protein carbonyls are the most commonly used markers of protein oxidation, different methods have been developed for the detection and quantification of carbonylated proteins. The identification of these protein targets is of valuable interest in order to understand the mechanisms by which damaged proteins accumulate and potentially affect cellular functions during oxidative stress, cellular senescence and/or aging in vivo. The specificity of hydrazide derivatives to carbonyl groups and the presence of a wide range of functional groups coupled to the hydrazide, allowed the design of novel strategies for the detection and quantification of carbonylated proteins. Of note is the importance of fluorescent probes for monitoring carbonylated proteins. Proteomics approaches, i.e. fluorescent based 2D-gel electrophoresis and mass spectrometry methods, represent powerful tools for monitoring at the proteome level the extent of protein oxidative and related modifications and for identifying the targeted proteins.

This article is part of a Special Issue entitled: Posttranslational Protein modifications in biology and Medicine.

☆ This article is part of a Special Issue entitled: Posttranslational Protein modifications in biology and Medicine.

* *Corresponding author*. Tel.: +33 1 44 27 32 05; fax: +33 1 44 27 51 40.
E-mail address: bertrand.friguet@upmc.fr (B. Friguet).

1874-3919/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jprot.2013.05.008

1. Introduction

Proteins are targets of various posttranslational deleterious modifications (oxidation, glycation, conjugation with products of lipid peroxidation in particular), which are altering their biological functions. Reactive oxygen species (ROS) and nitrogen species (RNS), originating from oxidative metabolism as well as other toxic compounds such as dicarbonyl and reactive aldehydes, are mainly responsible for these damages [1]. The impact of these modifications increases upon oxidative stress and during aging, suggesting that they may be causally involved in the aging process that corresponds to the progressive and irreversible decline of various physiological functions of the organism in the latter part of its life [2]. In fact, aging is a complex process controlled by genetic factors but also due to influences from the external environment. The role of certain genes involved in the response against stress (including oxidative stress), the over expression of which leads to increased longevity, coupled with observations that increasing the concentration of damaged cellular components is clearly associated with aging shows the importance of maintenance systems in the aging process [3]. In addition, these maintenance (degradation and repair) systems are often themselves sensitive to oxidative stress and affected during aging, losing effectiveness over time [4,5]. Thus, the accumulation of altered proteins in conditions of increased oxidative stress and during aging is due to both increased production of modified proteins and a decrease in the efficacy of the mechanisms responsible for their removal. In this context, proteins play an important role since they are both targets and directly involved in damage recognition, repair and disposal of damaged entities.

Although it is recognized that cellular aging causes changes in the proteome, the nature and targets of these changes and their consequences have not yet been clearly identified. This is essential to understand the mechanisms by which damaged proteins accumulate and potentially affect cellular functions during oxidative stress and/or aging *in vivo* [6]. In addition, their identification would reveal the structural and functional characteristics of these proteins that are preferential targets by these modifications. Moreover, it would be possible to have a global view on the metabolic pathways altered during cellular and organismal aging. In this context, proteomics approaches, including 2D-gel electrophoresis based methods, represent powerful tools to address these questions by monitoring at the proteome level the extent of proteins oxidative and related modifications and by identifying the targeted proteins [7–9]. In this mini-review, protein modifications occurring during aging and upon oxidative stress as well as main proteomic methods for detecting, quantifying and identifying oxidized proteins are first described. Relevant proteomics studies aimed at monitoring the extent of protein carbonylation and identifying the targeted proteins in the context of aging and oxidative stress are then reported.

2. Protein modification by oxidation and related pathways

Three types of alterations have been typically described to occur in proteins during aging: oxidation, glycation/glycoxidation and conjugation with products of lipid peroxidation. Indeed, proteins are very sensitive to the action of ROS [1]. The degree of alteration of a protein by oxidation depends on several factors including the nature of the oxidant, the proximity of a radical and the transition metal with respect to the target protein, the protein composition and structure. Among other reactive oxygen species such as the superoxide ion and hydrogen peroxide, the most oxidizing reactive oxygen species is by far the hydroxyl radical (OH.) which is formed either by radiolysis of water molecules or through the Fenton reaction that results in metal catalyzed oxidation of proteins [10].

ROS can readily react directly with proteins leading to the following scenario:

i. Oxidation of the side chains of cysteine and methionine which are particularly sensitive to oxidation and which leads to the formation of disulfides, sulfenic acid and methionine sulfoxides that correspond to reversible oxidative modifications. Aromatic amino acids (tryptophan, tyrosine, phenylalanine) represent also sensitive targets of ROS. Incidentally, methionine, cysteine, tyrosine and tryptophan are targets of the RNS peroxinitrite.
ii. Oxidation of the carbon skeleton of the protein, which leads to fragmentation of polypeptide chains and/or the formation of intra or inter-molecular cross-links.
iii. Formation of carbonyl groups on the protein either by direct oxidation of sensitive amino acid side chains (lysine, arginine, proline, threonine) or due to the fragmentation of the carbon chain by oxidation of a glutamyl residue or by α-amidation.

Introduction of carbonyl groups on proteins can also occur through the reaction of aldehydic products of lipid peroxidation and of dicarbonyl compounds upon glycation and glycoxidation. Indeed, proteins can be modified through the reaction between arginine and lysine amino groups with reducing sugars or reactive aldehydes, such as glyoxal and methylglyoxal, based on the Maillard reaction. This reaction is named glycation or non-enzymatic glycosylation. Glycation, which leads to the formation of early stage glycation adducts and then advanced glycation end products (AGE), is considered as one of the major cause of spontaneous damage to cellular and extracellular proteins [11]. Formation of AGEs on proteins is found in many tissues and is thought to contribute to a variety of age-associated diseases, such as Alzheimer's and Parkinson's [12,13]. Although glycation is not an oxidative phenomenon, it can be amplified by oxidative stress and hence referred as to glycoxidation [14]. Thus, under conditions of oxidative stress, reducing sugars (glucose, fructose) and ascorbic acid can be self-oxidizing generating highly reactive dicarbonyl compounds, which can in turn react with proteins to form AGEs. Finally, among the most important glycating agents are the dicarbonyl compounds glyoxal and methylglyoxal that are derived from glucose auto-oxidation and glycolytic intermediates. The reactivity of these dicarbonyls is much higher than that of glucose, so they represent important precursors of AGEs in physiological systems. Interestingly, carbonylated adducts are being formed upon conjugation with these by dicarbonyl compounds.

In addition, ROS can oxidize membrane lipids generating lipid hydroperoxides and many aldehydes such as acrolein,

malonaldehyde, 4-hydroxy-2-nonenal (HNE) and other hydroxyalkenals. These compounds have a longer life span than reactive oxygen species and can diffuse into the cell, acting as a "toxic secondary messenger" amplifying the damage of free radicals [15]. Upon reaction with nucleophile amine, thiol or imidazole groups, these lipid peroxidation products can form adducts with lysine, histidine and cysteine but also with phospholipids, nucleic acids and small molecules such as glutathione or coenzyme A. Among aldehydes, HNE is one of the most reactive [16]. Because it can attack the proteins by two different mechanisms, one molecule of HNE can react with residues belonging to the same protein or two different proteins and cause of intra or intermolecular crosslinking [17]. Finally, the reaction of these lipid peroxidation products with proteins tends to add carbonyl groups on proteins and lead to the formation of complex protein structures either cyclic, rich in double bonds and crosslinks, yellow/brown colored, and fluorescent [18] reminiscent of the age pigment called "lipofuscin", a well-known marker of oxidative stress which increases with aging [19,20].

Protein oxidative modification most often results in decreased enzymatic activity, decreased stability to heat, increased hydrophobicity and increase susceptibility to proteolytic degradation [10]. The involvement of protein oxidation upon oxidative stress and during aging has been suggested in many studies [21–25]. Indeed, the increase in protein carbonyl content as a function of age has been described in several tissues [26], and the carbonyl content of proteins in cultured human fibroblasts increases with donor age [27] and replicative senescence [28]. Thus, the increase in carbonylated proteins represents a hallmark of aged cells, organs and organisms and is believed to be causally involved the functional decline and increased vulnerability that occurs during the aging process [6].

3. Detection, quantification and identification of carbonylated proteins

Since protein carbonyls are the most commonly used marker of protein oxidation, different methods have been developed for the detection and quantification of carbonylated proteins [29,30]. Because protein carbonyls have no distinguishing UV or visible spectrophotometric absorbance/fluorescence properties, it is not possible to detect them directly. Therefore, their detection and quantification require the use of specific chemical probes that serve as handles for determination. Several probes, such as 2,4-dinitrophenylhydrazine (DNPH) [31], tritiated sodium borohydride [32], biotin-containing probes (biotin-hydrazide and N'- aminooxymethylcarbonylhydrazino-D-biotin (ARP)) [33], and fluorescence probes have been used [34]. Except for tritiated sodium borohydride, a common feature of all probes is a hydrazine-like moiety that can react specifically with carbonyl groups. Among them, DNPH is the best known and mostly used, which binds covalently to the carbonyl groups (through a Schiff base) to allow formation of stable hydrazones. Immunochemical and/or spectrophotometric assays of protein carbonyls previously derivatized with 2-4-dinitrophenylhydrazine (DNPH) to form 2-4-dinitrophenylhydrazone (DNP) proteins adducts are available [35]. Total carbonyls groups on a specific protein or in a mixture of proteins can be detected and quantified spectrophotometrically due to the DNP characteristic absorption spectrum with a maximum at 365–375 nm. An important caveat to be considered when DNPH is used for spectrophotometric determination of protein carbonyl content, is that some proteins (e.g. cytochrome c and hemoglobin, among others) have absorbance wavelengths similar to DNPH and may interfere with its measurement, leading to inaccurate estimation of carbonyls content [36].

Given the commercial availability of antibodies against DNP, the detection of total carbonyl groups can be performed also by western blot, dot blot or ELISA (after derivatization of carbonyl groups to DNP adducts). Although an increased load (amount) of carbonylated proteins has been clearly associated with aging, in most cases the target proteins have not been identified and are one of the main limitations of the above-mentioned techniques. Identification of these proteins would be expected to give some insights into the mechanisms by which these damaged proteins could affect cellular function. Global approaches, such as proteomics, remain advantageous because they provide comparative information on the constant changing of the cell proteome through its biochemical interactions with the genome and environment. Thereby, proteomics is useful for identification of carbonylated proteins in a complex mixture. Moreover, identification of such modified proteins may also help to unrevealing how these damaged proteins are building up during the aging process or disease condition. For this purpose it is necessary to separate proteins by 2D electrophoresis or to enrich carbonylated peptides (i.e. by affinity chromatography) before shot gun mass spectrometry analyses (Fig. 1). Despite the limitations of 2D gel electrophoresis, it is still considered as one of the best technique for separation of a complex mixture of soluble proteins before carbonyls detection [37]. The use of two-dimensional gels has proved very useful for the detection of specific carbonylated protein spots during oxidative stress and replicative senescence [7,8]. Selected spots can then be identified by peptide sequencing using liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques after trypsin digestion and HPLC separation of the tryptic peptides. Different studies adopting this 2-D gel based proteomics approaches led to the finding that not all proteins in a given proteome were subject to equivalent oxidative attacks, supporting the current view that the accumulation of carbonylated proteins upon oxidative stress, aging and disease is a selective rather than a random process [6,38]. The main caveat of immunodetection of carbonylated-derivatized proteins after 2D electrophoretic separation is that different gels need to be used per sample: at least one for total protein staining and other for electrotransfer to western blot membranes for immunodetection of carbonylated proteins. In addition, several technical limitations exist. Because of subtle changes in experimental conditions, the protein carbonylation patterns on a single Western blot after 2D-PAGE usually cannot be fully duplicated, which makes it difficult to find the carbonylated proteins changed between samples and gels in order to quantify their changes. Although a comparison of protein carbonylation profiles from Western blot after 2D-PAGE can be carried out with the assistance of various software programs, it typically requires some computerized justification of two-dimensional Western blot images so that two images can be superimposed and compared. These difficulties limit the speed and accuracy of quantitation of protein carbonylation after two-dimensional Western blot. Finally, the method is time

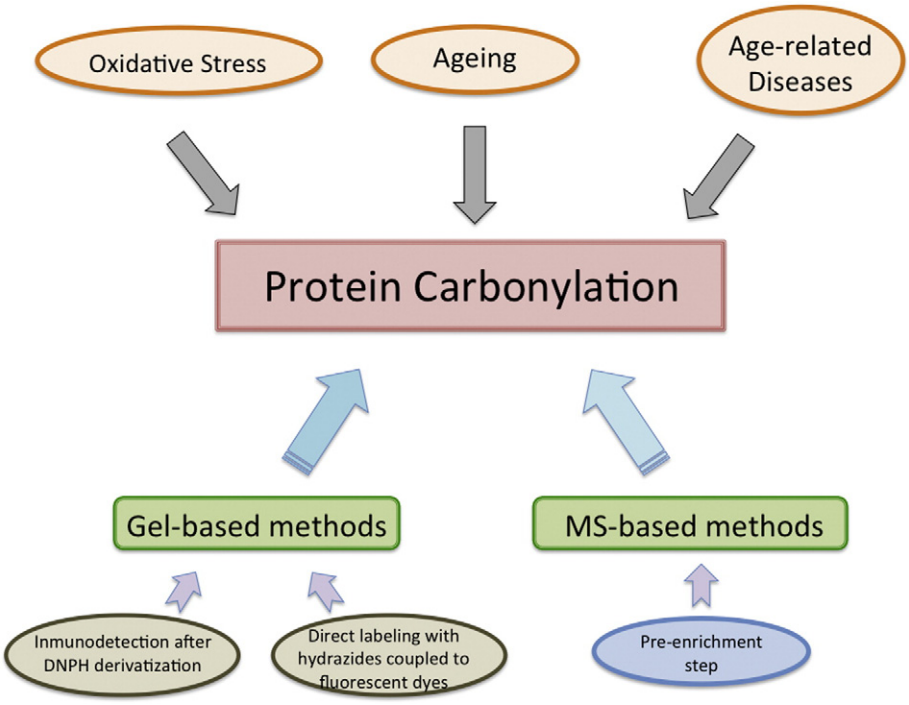

Fig. 1 – Proteomics approaches for the detection, quantification and identification of carbonylated proteins.

consuming and employs several reagents. Importantly, and there is no possibility of multiplexing and the quantification is only relative.

Several authors have also explored gel-free methods using mass spectrometry approaches [39,40]. For this purpose, previous to the analysis, carbonylated proteins are enriched with hydrazides-containing probes, followed by trypsin digestion, affinity capture of the carbonylated/tryptic peptides and mass spectrometric identification of the carbonylated proteins. Mass spectrometry is currently the most versatile technology in proteomics for the identification of proteins and for the identification of carbonylated residues. However, the disadvantage of these approaches is that they do not allow quantitative analysis of the degree of carbonylation of the identified proteins, which limits their usefulness for studying changes in the pattern of protein oxidation under different biological conditions.

Due to the limitations mentioned above, alternatives for carbonylated protein detection and quantification are emerging. The specificity of hydrazide derivatives to carbonyl groups and the presence of a wide range of functional groups coupled to the hydrazide, allow the design of novel strategies for the detection and quantification of carbonylated proteins. Of note is the importance of fluorescent probes for monitoring carbonylated proteins. In fact, the use of fluorescence probes such as fluorescein-5-thio-semicarbazide for protein carbonyl detection has been proposed more than two decades ago [41] and successfully applied, for instance, to the proteomic detection of protein carbonyl in aging liver tissues [42]. A fluorescence probe offers at least two advantages when used in conjunction with gel-based analysis of carbonylated proteins. First, there are no Western blot experiments to be carried out, so the whole process, from gel running to image documenting, can be completed in a much shorter time and without addition of unspecific signal. Second, the same 2D (or 1D) gel can be used through the multiplexing technology for both total protein staining and protein carbonyl imaging, which reduces error associated with gel spot (localization) and excision for subsequent mass spectrometric analysis. This is in contrast to that of DNPH- or biotin-based 2D gel analysis of carbonylated proteins, whereby gels are used for total protein staining and Western blot membranes are used for imaging of the carbonylated proteins, being the probability of mismatch very high. In addition, labeling of proteins with fluorescent dyes has been extensively applied in proteomic investigations, such as the case of the fluorescence difference gel electrophoresis (DIGE) for expression proteomics [43]. 2D-DIGE is the proteomic approach that stands out for its potential to directly compare proteins from (at least) two different groups of samples. This advanced gel electrophoretic method represents one of the most powerful analytical tools for conducting comparative protein biochemical investigations. The multiplexing possibilities offer by the DIGE greatly reduces gel-to-gel variations and thereby greatly improves the evaluation of trends in changed protein expression pattern. Analytical DIGE systems can be employed with two-dye or three-dye systems, depending on specific applications. Advanced DIGE, using an internal pooled standard, is a highly accurate quantitative method that enables multiple samples comparison [44]. We have recently developed a novel application of the DIGE approach, but for the detection and quantification of carbonylated proteins, referred as to Oxi-DIGE [45]. In Oxi-DIGE, protein carbonyls derived from any biological sample are labeled with two spectrally resolvable fluorescent hydrazide probes that bind specifically to carbonyl groups in proteins. The matched dyes have the same ionic and pH characteristics but absorb and/or emit light at different wavelengths, producing different color fluorescence. Recent studies by the group of Joaquim Ros and Elisa Cabiscol have also demonstrated the

usefulness of fluorescent hydrazides for analyzing protein carbonylation caused by oxidative stress and chronological aging in yeast [46]. A central advantage of the use of sensitive fluorescent probes is the detection of lower abundance carbonylated proteins. For spot excision from the gel to establish its identification, either total protein stain by NHS-ester cyanines or BodipyFL-Hz would be the method of choice [46]. Moreover, in carrying out Oxi-DIGE, labeled carbonylated proteins from different groups of samples are co-resolved on a single 2D gel for direct quantification, Finally, the Oxi-DIGE method provides a significant improvement in terms of reproducibility and statistical support of the data for proteomic analysis of carbonylated proteins, which is essential for the robust identification of such a scarce modification, and can be applied to the identification of carbonylated proteins in any biological sample.

4. Proteomic assessment of protein carbonylation upon oxidative stress and during aging

There is now convincing evidence that ROS and oxidative stress play an important role in physiological processes as well the aetiology and/or progression of a number of human diseases. During the last years, protein carbonyls have been used as biomarkers of oxidative stress since they have advantages in comparison with the measurement of other oxidation products due to their relative early formation and the stability of carbonylated proteins. Increased protein carbonylation in different tissues and organ systems has been used as a link between oxidative stress and several diseases as well as pathological conditions (Alzheimer's disease, chronic lung disease, diabetes, sepsis, ischemia-reperfusion, cystic fibrosis, rheumatoid arthriris, etc.) [29].

The effects of oxidative stress on human stem cells and the consequences on their differentiation capacity is a topic of increased interest. We have recently characterized the proteome changes of adult human muscle stem cells in response to oxidative stress [8]. Using a dual proteomic approach, we intended to unravel the mechanism involved in human myoblast dysfunction upon oxidative stress. Selective proteins either modulated at the expression level or those targeted by oxidation (carbonylated) were identified after a sub-toxic insult of hydrogen peroxide, which induced intermediate cellular damage without suppressing the myoblasts anti-oxidant response mechanisms. Twenty-one protein spots were evidenced, as increasingly carbonylated upon oxidative stress, indicating that only a restricted set of proteins is prone to accumulation upon oxidative stress. Major functional categories include energy metabolism, cellular assembly, protein synthesis, cell morphology and protein degradation. Consistently, our results indicate that proteins involved in several cellular pathways are affected upon oxidative stress and that the impairment of these pathways may be implicated in oxidative stress-induced cellular dysfunction [8].

Oxidative stress and protein carbonylation in skeletal muscle has been addressed in particular during rest-contraction cycles. Particular attention has been devoted to study the influence of acute and regular exercise in protein homeostasis. Mounting evidence show that aerobic exercise decreases the incidence of oxidative stress-associated diseases [47,48]. This beneficial effect seems to be related to the fact that exercise-induced ROS production is necessary for oxidative stress-related adaptations. The effects of exercise on the proteasome system and carbonyl accumulation could be dependent on whether the exercise is acute or chronic and also the type of exercise (resistance or endurance). An acute bout of strenuous squatting and sprinting performed by resistance trained men results in elevated protein carbonyls, while having little impact on 8-hydroxydeoxyguanosine or malondialdehyde during the immediate postexercise period [49]. On the other hand, regular exercise has the capability to develop compensation to oxidative stress, resulting in overcompensation against the increased level of ROS production and oxidative damage to proteins [50]. In addition, regular exercise training-associated adaptation is a precondition against treatment with hydrogen peroxide, which causes a significant degree of damage for untrained subjects [51]. A great deal of evidence exists that suggests that regular exercise-induced adaptations to ROS handling, through redox signaling, including antioxidant and oxidative damage repair systems, signify cantly contribute to the health-promoting effects of regular exercise.

Previous studies highlighted the importance of protein carbonylation in the early stages of apoptosis upon oxidative stress. Carbonylation of critical glycolytic enzymes in etopside-treated HL60 cells seems to decrease glucose utilization and cause cell death [52], while oxidation of several chaperones has been associated with apoptosis of irradiated HL60 human leukaemia cells [53]. For instance, the occurrence of carbonyls in glyceraldehyde-3-phosphate dehydrogenase, adenine nucleotide translocator and Bcl-2 has been shown to play a role in nitric oxide-induced apoptosis in insulin-producing RINm5F cells [54]. More recently, Dasgupta and colleagues showed that a moderate and transient depletion of glutathione in neuronal PC12 cells leads to increased accumulation of carbonylated proteins and cell death [55]. The mechanism linking GSH depletion and increased production of ROS by mitochondria has been characterized [56]. Both the amount of protein carbonyls and cellular toxicity diminish as cellular glutathione is biosynthetically replenished. However, cell death persists if oxidized proteins are not removed by the proteasome. These results suggest that the build-up of carbonylated proteins is responsible for the loss of cell viability in this system. Furthermore, the effectiveness of several ROS scavengers at preventing cell death suggests that protein carbonyls are indeed the toxic species. Experiments using cyclosporin place protein carbonylation downstream of the MPTP opening and upstream of caspase activation [55].

Aging is a progressive collapse of defense and repair functions that leads to physiological frailty, loss of homeostasis and eventually to death. This failure of the repair processes has been shown to be associated with damage accumulation in all biomolecules (proteins, lipids and nucleic acids) that are caused through spontaneous chemical reactions by numerous endogenous and exogenous reactive agents. Even though macromolecular damage is among the oldest hypotheses regarding the causes of aging, identifying which types of damage have greater impact on homeostasis failure and determining whether such damage can be prevented and/or reversed, is still a major challenge [57,58]. Proteins are constantly attacked by exogenous (e.g. UV light,

chemicals) and endogenous agents (e.g. ROS, aldehydes and sugar metabolites) with a potential for damage. Thus, it has been proposed that proteins would represent almost 70% of ROS targeted entities [59]. In fact, proteins are damaged through different pathways, the most represented being oxidation, glycation, their combination also referred as glycoxidation and conjugation with lipid peroxidation products [60]. The age-related accumulation of damaged proteins is believed to result from both the increased occurrence of damage which is due, at least in part, to alterations in the detoxification of the damaging agents and the decreased efficiency of the different systems involved in the elimination of damaged proteins.

Although the accumulation of oxidized and/or carbonylated proteins is widely recognized as a hallmark of mammalian cellular and tissular aging, in most cases the target proteins have not been identified and only recently the identity of those proteins that are preferentially accumulating with age as carbonylated have been addressed by using oxidative modification proteomic methods (please see reference [6] for review). Interestingly, identification of oxidized proteins in humans and rat and mouse model organisms has been achieved mainly for age-related neurodegenerative diseases. As a consequence, most of the proteins targeted by oxidation and identified in these studies are originating from the brain. For the most part, these studies were achieved in order to address the importance of protein carbonylation in the pathogenesis of such diseases as amyotrophic lateral sclerosis, Alzheimer's, Parkinson's and Huntington's, rather than aging *per se*. This is particularly relevant for studies with human for which cerebrospinal fluid, plasma and brain proteins have been identified only in the case of neurodegenerative diseases and not for normal aging [61]. However, a subset of carbonylated proteins that are preferentially accumulating with age have been identified in skeletal muscle, liver, brain and bronchoalveolar fluids of rats and/or mice including models senescence-accelerated strains [62–67]. Among identified proteins, several of them were related to important metabolic and cellular pathways and the most significant were: *glycolysis/gluconeogenesis*, *pyruvate metabolism*, *amino acids degradation*, *nrf-2 oxidative stress response and cellular function and maintenance*.

5. Concluding remarks

Accumulation of oxidized (carbonylated) proteins is a widely used biomarker of oxidative stress; a hallmark of cellular and organismal aging and it has been also evidenced in several age-related diseases. However, the key question whether protein oxidation is mechanistically involved in ageing and age-related diseases, remains unanswered. In this context, reliable proteomic approaches for evidencing, quantifying and identifying the specific proteins targeted by oxidation during oxidative stress, aging are age-related diseases are of key value. Recent studies have shown that only a restricted set of proteins is targeted by oxidation, including proteins critically involved in key cellular functions, hence indicating that some proteins are more prone than others to accumulate in an oxidized form. This "Oxi-proteome" (i.e. the restricted set of proteins targeted by oxidation) constitutes also a potential molecular substratum for many cellular dysfunctions described during the aging process and age related diseases. Progress in the identification of carbonylated proteins should provide new biomarkers for disease diagnosis (possibly pre-symptomatic), biomarkers for oxidative damage, as well as basic information to aid the establishment an efficacious antioxidant therapy and also enhances the opportunities for individual disease treatment.

Disclosure statement

The Université Pierre et Marie Curie has filed a Patent Application (B. Friguet, M. Baraibar, R. Ladouce. A method for detecting and/or quantifying carbonylated proteins. WO/2012/175519) referring to the technique of protein detection and quantification of carbonylated proteins quoted in this paper. The authors share a partial financial interest in this patent application.

Aknowledgements

The cited work from our laboratory has been funded by the EU FP6 and FP7 EU grants Proteomage (#518530), MyoAge (#223576) and Mark-Age (#200880) as well as by national grants. The authors are also very thankful to the COST Action CM 1001. M.A.B is currently the recipient of a post-doctoral fellowship from the Association Française contre les Myopathies (AFM) and R. L. is currently the recipient of a stipend from the SATT Lutech.

REFERENCES

[1] Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 1997;272:20313–6.
[2] Levine RL, Stadtman ER. Oxidative modification of proteins during aging. Exp Gerontol 2001;36:1495–502.
[3] Friguet B. Oxidized protein degradation and repair in ageing and oxidative stress. FEBS Lett 2006;580:2910–6.
[4] Petropoulos I, Friguet B. Protein maintenance in aging and replicative senescence: a role for the peptide methionine sulfoxide reductases. Biochim Biophys Acta 2005;1703:261–6.
[5] Baraibar MA, Friguet B. Changes of the proteasomal system during the aging process. Prog Mol Biol Transl Sci 2012;109:249–75.
[6] Baraibar MA, Liu L, Ahmed EK, Friguet B. Protein oxidative damage at the crossroads of cellular senescence, aging, and age-related diseases. Oxid Med Cell Longev 2012;2012:919832.
[7] Ahmed EK, Rogowska-Wrzesinska A, Roepstorff P, Bulteau AL, Friguet B. Protein modification and replicative senescence of WI-38 human embryonic fibroblasts. Aging Cell 2010;9:252–72.
[8] Baraibar MA, Hyzewicz J, Rogowska-Wrzesinska A, Ladouce R, Roepstorff P, Mouly V, et al. Oxidative stress-induced proteome alterations target different cellular pathways in human myoblasts. Free Radic Biol Med 2011;51:1522–32.
[9] Bayot A, Gareil M, Rogowska-Wrzesinska A, Roepstorff P, Friguet B, Bulteau AL. Identification of novel oxidized protein substrates and physiological partners of the mitochondrial ATP-dependent Lon-like protease Pim1. J Biol Chem 2010;285:11445–57.
[10] Stadtman ER, Levine RL. Protein oxidation. Ann N Y Acad Sci 2000;899:191–208.

[11] Thornalley PJ, Battah S, Ahmed N, Karachalias N, Agalou S, Babaei-Jadidi R, et al. Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry. Biochem J 2003;375:581–92.
[12] Reddy VP, Beyaz A. Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. Drug Discov Today 2006;11:646–54.
[13] Grillo MA, Colombatto S. Advanced glycation end-products (AGEs): involvement in aging and in neurodegenerative diseases. Amino Acids 2008;35:29–36.
[14] Baynes JW. The role of AGEs in aging: causation or correlation. Exp Gerontol 2001;36:1527–37.
[15] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.
[16] Carini M, Aldini G, Facino RM. Mass spectrometry for detection of 4-hydroxy-trans-2-nonenal (HNE) adducts with peptides and proteins. Mass Spectrom Rev 2004;23:281–305.
[17] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res 2003;42:318–43.
[18] Tsai L, Szweda PA, Vinogradova O, Szweda LI. Structural characterization and immunochemical detection of a fluorophore derived from 4-hydroxy-2-nonenal and lysine. Proc Natl Acad Sci U S A 1998;95:7975–80.
[19] Jung T, Bader N, Grune T. Lipofuscin: formation, distribution, and metabolic consequences. Ann N Y Acad Sci 2007;1119: 97–111.
[20] Itakura K, Oya-Ito T, Osawa T, Yamada S, Toyokuni S, Shibata N, et al. Detection of lipofuscin-like fluorophore in oxidized human low-density lipoprotein. 4-hydroxy-2-nonenal as a potential source of fluorescent chromophore. FEBS Lett 2000;473:249–53.
[21] Baraibar MA, Friguet B. Oxidative proteome modifications target specific cellular pathways during oxidative stress, cellular senescence and aging.Exp Gerontol 2013 [in press].
[22] Cabiscol E, Piulats E, Echave P, Herrero E, Ros J. Oxidative stress promotes specific protein damage in Saccharomyces cerevisiae. J Biol Chem 2000;275:27393–8.
[23] England K, O'Driscoll C, Cotter TG. Carbonylation of glycolytic proteins is a key response to drug-induced oxidative stress and apoptosis. Cell Death Differ 2004;11:252–60.
[24] Nystrom T. Role of oxidative carbonylation in protein quality control and senescence. EMBO J 2005;24:1311–7.
[25] Mirzaei H, Regnier F. Creation of allotypic active sites during oxidative stress. J Proteome Res 2006;5:2159–68.
[26] Levine RL, Stadtman ER. Oxidative modification of proteins during aging. Exp Gerontol 2001;36:1495–502.
[27] Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Stadtman ER. Age-related changes in oxidized proteins. J Biol Chem 1987;262:5488–91.
[28] Ahmed EK, Picot CR, Bulteau AL, Friguet B. Protein oxidative modifications and replicative senescence of WI-38 human embryonic fibroblasts. Ann N Y Acad Sci 2007;1119:88–96.
[29] Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 2003;329:23–38.
[30] Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. J Biol Chem 2008;283:21837–41.
[31] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–78.
[32] Lenz AG, Costabel U, Shaltiel S, Levine RL. Determination of carbonyl groups in oxidatively modified proteins by reduction with tritiated sodium borohydride. Anal Biochem 1989;177: 419–25.
[33] Chung WG, Miranda CL, Maier CS. Detection of carbonyl-modified proteins in interfibrillar rat mitochondria using N′-aminooxymethylcarbonylhydrazino-D-biotin as an aldehyde/keto-reactive probe in combination with Western blot analysis and tandem mass spectrometry. Electrophoresis 2008;29:1317–24.
[34] Yan LJ, Forster MJ. Chemical probes for analysis of carbonylated proteins: a review. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:1308–15.
[35] Levine RL, Wehr N, Williams JA, Stadtman ER, Shacter E. Determination of carbonyl groups in oxidized proteins. Methods Mol Biol 2000;99:15–24.
[36] Yan LJ. Analysis of oxidative modification of proteins.Curr Protoc Protein Sci 2013 [Chapter 14:Unit14 4]. http://dx.doi.org/10.1002/0471140864.ps1404s56 [in press] [pii: S0531-5565(12)00294-X].
[37] Rogowska-Wrzesinska A, Le Bihan MC, Thaysen-Andersen M, Roepstorff P. 2D gels still have a niche in proteomics. J Proteomics 2013;88:4–13.
[38] Baraibar MA, Barbeito AG, Muhoberac BB, Vidal R. A mutant light-chain ferritin that causes neurodegeneration has enhanced propensity toward oxidative damage. Free Radic Biol Med 2012;52:1692–7.
[39] Madian AG, Regnier FE. Proteomic identification of carbonylated proteins and their oxidation sites. J Proteome Res 2010;9:3766–80.
[40] Bollineni R, Fedorova M, Hoffmann R. Identification of carbonylated peptides by tandem mass spectrometry using a precursor ion-like scan in negative ion mode. J Proteomics 2011;74:2351–9.
[41] Ahn B, Rhee SG, Stadtman ER. Use of fluorescein hydrazide and fluorescein thiosemicarbazide reagents for the fluorometric determination of protein carbonyl groups and for the detection of oxidized protein on polyacrylamide gels. Anal Biochem 1987;161:245–57.
[42] Chaudhuri AR, de Waal EM, Pierce A, Van Remmen H, Ward WF, Richardson A. Detection of protein carbonyls in aging liver tissue: a fluorescence-based proteomic approach. Mech Ageing Dev 2006;127:849–61.
[43] Unlu M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. Electrophoresis 1997;18:2071–7.
[44] Alban A, David SO, Bjorkesten L, Andersson C, Sloge E, Lewis S, et al. A novel experimental design for comparative two-dimensional gel analysis: two-dimensional difference gel electrophoresis incorporating a pooled internal standard. Proteomics 2003;3:36–44.
[45] Baraibar MA, Ladouce R, Friguet B. A method for detecting and/or quantifying carbonylated proteins; 2012 [WO/2012/175519].
[46] Tamarit J, de Hoogh A, Obis E, Alsina D, Cabiscol E, Ros J. Analysis of oxidative stress-induced protein carbonylation using fluorescent hydrazides. J Proteomics 2012;75: 3778–88.
[47] Katzmarzyk PT, Janssen I. The economic costs associated with physical inactivity and obesity in Canada: an update. Canadian journal of applied physiology. Can J Appl Physiol 2004;29:90–115.
[48] Tremblay MS, Shephard RJ, Brawley LR, Cameron C, Craig CL, Duggan M, et al. Physical activity guidelines and guides for Canadians: facts and future. Can J Public Health 2007;98(Suppl. 2):S218–24.
[49] Bloomer RJ, Fry AC, Falvo MJ, Moore CA. Protein carbonyls are acutely elevated following single set anaerobic exercise in resistance trained men. J Sci Med Sport 2007;10:411–7.
[50] Radak Z, Taylor AW, Ohno H, Goto S. Adaptation to exercise-induced oxidative stress: from muscle to brain. Exerc Immunol Rev 2001;7:90–107.
[51] Radak Z, Sasvari M, Nyakas C, Pucsok J, Nakamoto H, Goto S. Exercise preconditioning against hydrogen peroxide-induced oxidative damage in proteins of rat myocardium. Arch Biochem Biophys 2000;376:248–51.

[52] England K, O'Driscoll C, Cotter TG. Carbonylation of glycolytic proteins is a key response to drug-induced oxidative stress and apoptosis. Cell Death Differ 2004;11:252–60.
[53] Magi B, Ettorre A, Liberatori S, Bini L, Andreassi M, Frosali S, et al. Selectivity of protein carbonylation in the apoptotic response to oxidative stress associated with photodynamic therapy: a cell biochemical and proteomic investigation. Cell Death Differ 2004;11:842–52.
[54] Cahuana GM, Tejedo JR, Jimenez J, Ramirez R, Sobrino F, Bedoya FJ. Nitric oxide-induced carbonylation of Bcl-2, GAPDH and ANT precedes apoptotic events in insulin-secreting RINm5F cells. Exp Cell Res 2004;293:22–30.
[55] Dasgupta A, Zheng J, Bizzozero OA. Protein carbonylation and aggregation precede neuronal apoptosis induced by partial glutathione depletion. ASN Neuro 2012;4.
[56] Armstrong JS, Steinauer KK, Hornung B, Irish JM, Lecane P, Birrell GW, et al. Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. Cell Death Differ 2002;9:252–63.
[57] Rattan SI, Clark BF. Understanding and modulating ageing. IUBMB Life 2005;57:297–304.
[58] Vijg J, Campisi J. Puzzles, promises and a cure for ageing. Nature 2008;454:1065–71.
[59] Davies D. A quiet life with proteins. Annu Rev Biophys Biomol Struct 2005;34:1–20.
[60] Stadtman ER. Protein oxidation and aging. Free Radic Res 2006;40:1250–8.
[61] Butterfield DA. Oxidative stress in neurodegenerative disorders. Antioxid Redox Signal 2006;8:1971–3.
[62] Meany DL, Xie H, Thompson LV, Arriaga EA, Griffin TJ. Identification of carbonylated proteins from enriched rat skeletal muscle mitochondria using affinity chromatography-stable isotope labeling and tandem mass spectrometry. Proteomics 2007;7:1150–63.
[63] Hamelin M, Mary J, Vostry M, Friguet B, Bakala H. Glycation damage targets glutamate dehydrogenase in the rat liver mitochondrial matrix during aging. FEBS J 2007;274:5949–61.
[64] Rabek JP, Boylston III WH, Papaconstantinou J. Carbonylation of ER chaperone proteins in aged mouse liver. Biochem Biophys Res Commun 2003;305:566–72.
[65] Nabeshi H, Oikawa S, Inoue S, Nishino K, Kawanishi S. Proteomic analysis for protein carbonyl as an indicator of oxidative damage in senescence-accelerated mice. Free Radic Res 2006;40:1173–81.
[66] Wang P, Powell SR. Decreased sensitivity associated with an altered formulation of a commercially available kit for detection of protein carbonyls. Free Radic Biol Med 2010;49:119–21.
[67] Umstead TM, Freeman WM, Chinchilli VM, Phelps DS. Age-related changes in the expression and oxidation of bronchoalveolar lavage proteins in the rat. Am J Physiol Lung Cell Mol Physiol 2009;296:L14–29.