Protein oxidation and aging

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Abstract
Organisms are constantly exposed to various forms of reactive oxygen species (ROS) that lead to oxidation of proteins, nucleic acids, and lipids. Protein oxidation can involve cleavage of the polypeptide chain, modification of amino acid side chains, and conversion of the protein to derivatives that are highly sensitive to proteolytic degradation. Unlike other types of modification (except cysteine oxidation), oxidation of methionine residues to methionine sulfoxide is reversible; thus, cyclic oxidation and reduction of methionine residues leads to consumption of ROS and thereby increases the resistance of proteins to oxidation. The importance of protein oxidation in aging is supported by the observation that levels of oxidized proteins increase with animal age. The age-related accumulation of oxidized proteins may reflect age-related increases in rates of ROS generation, decreases in antioxidant activities, or losses in the capacity to degrade oxidized proteins.

Oxidative stress
Organisms are constantly exposed to a multiplicity of systems that generate reactive oxygen species (ROS) that can oxidize intracellular proteins, lipids, and nucleic acids. These include various radical species (OH, O₂⁻, CO₂⁻ and NO⁻), a number of non-radical compounds (H₂O₂, ONO₂⁻, HOCl, O³, ONOCO₂⁻, CO, N₂O₂, NO₂ and O₂), and free radicals produced in the course of ROS interactions with proteins, nucleic acids, and lipids (C, RS, RSO, RSSR⁻, R⁻, RO and ROO⁻). Moreover, proteins can be modified by aldehydes and ketones produced during reactions of ROS with lipids [1–3] and glycated proteins [4,5]. For reviews, see Refs. [6,7].

Basic mechanisms involved in the oxidation of proteins were elucidated by the pioneering studies of Swallow [8], Garrison et al. [9,10], Garrison [11], Schuessler and Schilling [12], and Kopaldova and Liebster [13], who exposed proteins, peptides and amino acids to ionizing radiation under conditions where only OH and O₂⁻ were formed. Results of these studies showed that radical-mediated oxidation of proteins leads to fragmentation of the polypeptide chain, oxidation of amino acid side chains, and generation of protein–protein cross linkages. It was subsequently established that all of these protein modifications can also be mediated by metal-catalyzed oxidation (MCO) systems [14,15]. For reviews, see Refs. [16–18].

Oxidative cleavage of the polypeptide backbone
Based on the studies of Garrison et al. [9–11], the oxidative cleavage of the polypeptide occurs as illustrated in Figure 1. Oxidation is initiated by the OH-dependent abstraction of an α-hydrogen atom from the polypeptide chain to form H₂O and a carbon-centered radical derivative of the protein (reactions a, b) that undergoes rapid reaction with O₂ to form a peroxy-radical (reaction c). This derivative is readily converted to the protein peroxide...
by reactions with $\text{HO}_2$ or $\text{Fe}^{2+}$ and $\text{H}^+$ (reactions d, e). This peroxide can react further with $\text{HO}_2$ or $\text{Fe}^{2+}$ (reactions f, g) or by dismutation (reaction o) to form the alkoxyl derivative. The alkoxyl derivative can undergo peptide bond cleavage by either of two different mechanisms. One involves conversion to a hydroxy derivative (reactions i, j) followed by generation of an unsaturated derivative (reaction k) that undergoes peptide bond cleavage by the $\alpha$-amidation pathway (reaction l) to form an amide derivative of the N-terminal portion of the protein (compound I) and an N-alkyl carbonyl derivative of the carboxyl end of the protein (compound II). Significantly, this provides a mechanism for the
generation of a peptide carbonyl derivative. Alternatively, the alkoxyl derivative may undergo cleavage by the diamide pathway (reactions h and m), which leads to formation of a diamide derivative of the N-terminal cleavage product (compound III) and an isocyanate derivative of the carboxyl end of the protein (compound IV). As shown in Figure 2, peptide bond cleavage can also occur by direct attack of a glutamyl [11] or prolyl residues [19] by OH.

β-Scission

Dean et al. [20–22] have shown that, in addition to the reactions summarized in Figures 1 and 2, exposure of proteins to ionizing radiation leads to β-scission of amino acid side chains. For example, β-scission of alanine, valine, leucine, and aspartic acid residues leads to the generation of free formaldehyde, acetone, isobutyraldehyde, and glyoxylic acid, respectively. In each case, cleavage of the side chain leads to the formation of a carbon-centered radical (\( \sim \) NH \( \sim \) CHCO \( \sim \)) in the polypeptide chain, as occurs when glycine residues undergo OH-dependent α-hydrogen abstraction.

Oxidation of amino acid residue side chains of proteins

Side chains of amino acid residues that are most susceptible to oxidation by ROS are shown in Table I, together with the products formed.

![Figure 2](image-url) Cleavage of the protein backbone by oxidation of proline and glutamic acid side chains.

Oxidation of methionine residues

Methionine (Met) residues of proteins are readily oxidized by ROS to a mixture of S- and R-stereoisomers of methionine sulfoxide (MetO) [31,32,61]. The S-isomer is often referred to as MetA and the R-isomer is sometimes referred to as MetB. However, unlike oxidation of other amino acid residues (except cysteine), oxidation of Met to MetO is reversible. MetO can be reduced back to Met by the action of reductases (MsrA and MsrB) that can catalyze the reduction of the S- and R-isomers of MetO, respectively, back to Met. Both enzymes utilize thioredoxin \( \{\text{Th(SH)}_2\} \) as a source of reducing equivalents (reaction 2). MsrA has a cysteine at the catalytic site and, in most organisms, MsrB has a selenocysteine at the catalytic site [62–66]. As shown in reaction 1, the ROS-mediated oxidation of Met residues leads to the formation of an inactive form of the ROS (IRS). Moreover, in the presence of NADPH, the oxidized form of thioredoxin (ThS-S) can be converted back to its reduced form by the enzyme thioredoxin reductase (reaction 3). As noted by Levine et al. [67], the coupling of reactions 1–3 leads to the overall reaction 4 and thus provides a mechanism for conversion of ROS to IRS, i.e., for the scavenging of ROS species.

\[
\text{Met} + \text{ROS} \rightarrow \text{MetO} + \text{IRS} \quad (1)
\]

\[
\text{MetO} + \text{Th(SH)}_2 \rightarrow \text{Met} + \text{ThS-S} \quad (2)
\]

\[
\text{ThS-S} + \text{NADPH} + \text{H}^+ \rightarrow \text{Th(SH)}_2 + \text{NADP}^+ \quad (3)
\]
ROS + NADPH + H⁺ → IRS + NADP⁺  

This concept is supported by studies showing that mutations leading to a decrease in Msr activities are associated with a decrease in resistance to oxidative stress and to a shortening of the maximal lifespan, whereas mutations leading to overproduction of Msr activities lead to an increase in resistance to oxidative stress and large increases in lifespan [68–73].

### Generation of protein carbonyl derivatives

Early studies by Garrison et al. [9] showed that oxidation of proteins can lead to the generation of carbonyl derivatives. Unaware of these earlier findings, Levine et al. [74,75] rediscovered the fact that carbonyl derivatives are products of protein oxidation. Subsequently, it was shown that MCO systems catalyze the conversion of lysine residues to α-aminoadipic semialdehyde and that arginine and proline residues are oxidized to glutamic semialdehyde [23,76], and that these oxidations account for 40–100% of the carbonyl derivatives formed by MCO oxidation of purified proteins [29]. In the meantime, a number of highly sensitive procedures have been developed for the measurement of protein carbonyl derivatives [77–79]. In view of the fact that the generation of carbonyl derivatives is orders of magnitude greater than other kinds of protein oxidation [20], the carbonyl content of proteins has become the most generally used method for estimation of oxidative-stress-mediated protein oxidation. For reviews, see Refs. [16–18, 80].

### Table I. Oxidation of amino acid side chains.

| Amino acid | Products | References |
|------------|----------|------------|
| Arginine   | Glutamic semialdehyde | [23] |
| Cysteine   | CyS–SCy; CyS–SG; CySOH; CySOOH; CysO₂H | [8,11,24–26] |
| Glutamic acid | Oxalic acid; pyruvate adducts | [11] |
| Histidine  | 2-Oxohistidine; 4-OH-glutamate | [11,13,27] |
| Leucine    | 3-OH-leucine; 4-OH-leucine; 5-OH-leucine | [11] |
| Lysine     | α-aminoacidoplasticsemialdehyde; N₆-(carboxymethyl)lysine | [23,28,29] |
| Methionine | Methionine sulfoxide; methionine sulfone | [9,30–32] |
| Phenylalanine | 2-, 3-, and 4-Hydroxyphenylalanine; 2,3-dihydroxyphenylalanine | [33–39] |
| Proline    | Glutamysemialdehyde; 2-pyrrolidone, 4- and 5-OH-proline; pyroglutamic acid | [9,19,23,40–42] |
| Threonine  | 2-Amino-3-keto-butyric acid | [43] |
| Tryptophan | 2-, 4-, 5-, 6-, 7-Hydroxy tryptophan; formylkynurenine; 3-OH-kynurenine; nitrotryptophan | [44–48] |
| Tyrosine   | 3,4-Dihydroxyphenylalanine; tyr–tyr crosslinks; 3-nitrotyrosine; 3-chlorotyrosine; 3,5-dichlorotyroxine | [33,36,38,49–60] |

### Relationship between aging and protein oxidation

**Historical note**

The demonstration that oxidation of proteins is implicated in aging and age-related diseases was an outgrowth of studies designed to determine basic mechanisms involved in the turnover of proteins. Earlier studies showed that the rates of turnover of various enzymes was dictated by nutritional factors, but the mechanisms involved were not established [88–89]. This prompted studies to examine the effects of nitrogen or carbon starvation on the levels of...
of specific enzymes in bacteria. It was found that starvation led to a decrease in activities of a number of enzymes, the identity of which depended on the kind of starvation used [90]. Subsequently, it was found that addition of purified glutamine synthetase to cell-free extracts of starved cells led to its oxidation by a classical MCO system composed of Fe(II) or Cu(I) plus O₂ and an electron donor (NADH or NADPH) [90], or by incubation with ascorbate, Fe(III) or Cu(II), and H₂O₂ [75]. Further studies showed that oxidation of proteins by these MCO systems converted them to forms that are highly sensitive to degradation by proteases present in cell-free extracts of mammalian tissues and bacterial extracts [92–102]. Subsequently, it was noted that workers in the field of aging had demonstrated that aging is associated with a decrease in the activities of a number of enzymes and that some enzymes in old animals are more sensitive to heat denaturation than enzymes from young animals [103–108]. These observations prompted investigations to determine the effect of MCO of enzymes.

**Figure 3.** Generation of protein–protein cross-linkages. Reactions a, b, c, d and e refer to formation of cross-linked derivatives as described in the text. p¹ and p² refer to two different proteins. PUFA, polyunsaturated fatty acids.

**Figure 4.** Glycation/glycoxidation-mediated generation of protein–protein cross-linked derivatives. p¹-LysNH₂ and p²-LysNH₂ refer to epsilon amino groups of two different proteins (p¹ and p²).
on their heat stability patterns. Results summarized in Figure 5 show that prior to MCO treatment a purified preparation of glucose-6-phosphate dehydrogenase (G-6-PDH) undergoes a linear decrease in activity upon incubation at 51°C, as is characteristic of this enzyme from young animals [104,105]. However, treatment of the enzyme with the ascorbate/H2O2-/Fe(III) MFO system led to a 50% loss of activity, and the remaining activity exhibited a bi-phasic response pattern [79] as is characteristic of enzymes isolated from old cells [103,104]. This possibility was verified by the demonstration that the carbonyl content of cultured dermal fibroblasts from normal human individuals of different ages (10–80 years) increased exponentially as a function of the donor age (Figure 6) [99]. This was confirmed in subsequent studies showing that a similar age-related increase in carbonyl content occurred in the occipital lobe of human brain tissue [109], the human eye lens cortex [110], in rat liver hepatocytes [111], Mongolian gerbils [112], and house flies [113].

Why do oxidized proteins accumulate during aging?

It is well established that the level of oxidized proteins increases during aging in many animals. However, accumulation of oxidized proteins is a complex function of the rates and kinds of ROS formed, the levels of numerous antioxidant systems, and the rates of degradation of oxidized proteins by a multiplicity of proteases that have been shown to decline during aging. Because the cellular levels of oxidized proteins are dependent upon so many variables, the mechanisms responsible for accumulation of oxidatively modified proteins in one individual may be very different from those involved in another individual. Since these activities are subjects of other reviews in this series of articles, they will not be discussed here.

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