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CHEMICAL MODIFICATION OF PROTEINS BY REACTIVE OXYGEN SPECIES

EARL R. STADTMAN AND RODNEY L. LEVINE

1.1 INTRODUCTION

Proteins are highly sensitive to oxidative modifications by reactive oxygen species (ROS) and reactive nitrogen species (RNS). These include a number of primary radical species ($^{\bullet}OH$, $O_2^{\bullet-}$, $CO_2^{\bullet-}$, $NO^{\bullet}$), several nonradical species (H_2O_2, HOCl, O^3, ONO_2^-, $ONOCO_2^-$, CO, N_2O_2, NO_2, $^1O_2$), and also free radicals ($^{\bullet}C$, $RS^{\bullet}$, $RSO^{\bullet}$, $RSOO^{\bullet}$, $RSSR^{\bullet-}$, $R^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$) produced in secondary reactions of these reactive oxygen species with proteins, lipids, and nucleic acids. In addition native proteins can be modified by highly reactive aldehydes and ketones produced during ROS-mediated oxidation of lipids (Schuenstein and Esterbauer, 1979; Esterbauer et al., 1991; Uchida and Stadtman, 1993) and glycated proteins (Monnier, 1990; Monnier et al., 1995). (For reviews, see Baynes, 1991; Kristal and Yu, 1992.) Basic chemical mechanisms involved in free radical-mediated oxidation of proteins were elucidated by pioneering studies of Swallow (1960), Garrison et al. (1962), Garrison (1987), and Schuessler and Schilling (1984), and Kopoldova and Liebsier (1963), who exposed aqueous solutions of proteins to ionizing radiation (X rays, gamma rays) under conditions where only $^{\bullet}OH$ and/or $O_2^{\bullet-}$ was formed. They demonstrated that these conditions lead to oxidation of amino acid residue side chains, fragmentation of the polypeptide chain, and the formation of protein–protein cross-linked aggregates. Although most proteins are not normally subjected to ionizing

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radiation, basic principles established in these pioneering studies apply also under more physiological situations where metal-ion-catalyzed reactions mimic the effects of ionizing radiation (Garrison et al., 1970; Borg and Schaich, 1988). (For reviews, see Stadtman and Berlett, 1997; Stadtman, 1998a; Butterfield and Stadtman, 1997.)

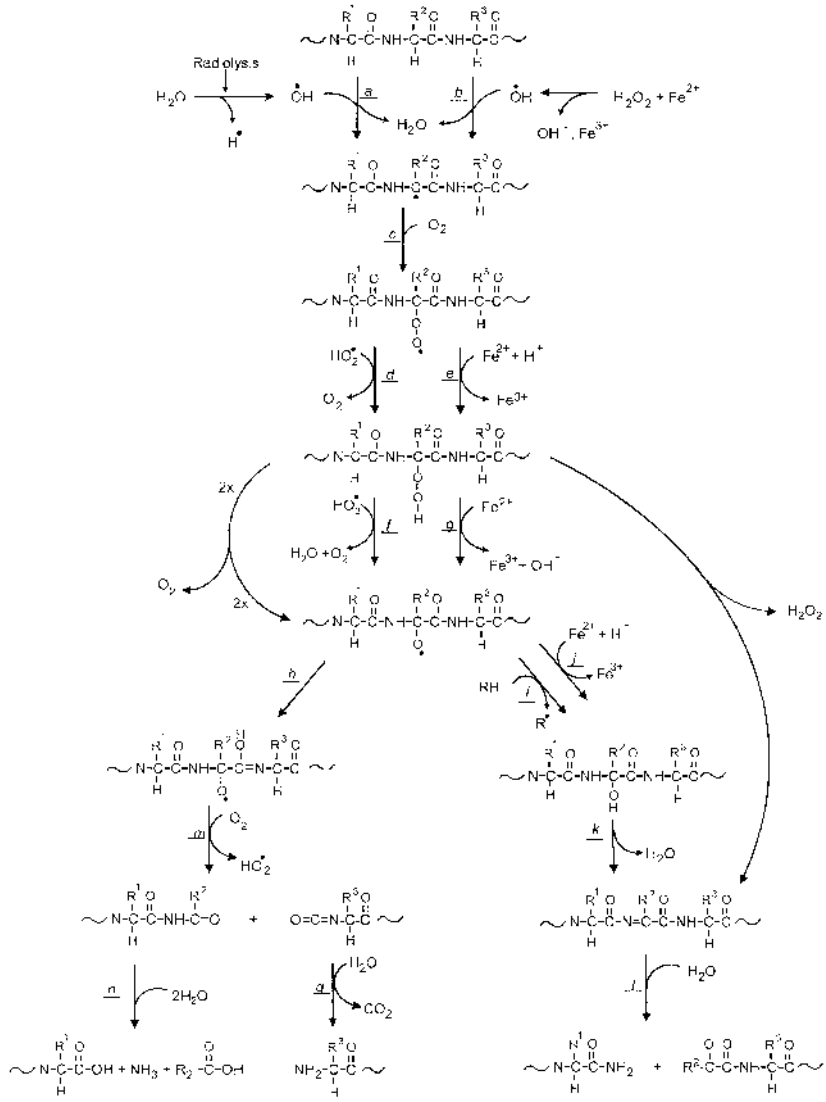

FIGURE 1.1 Role of reactive oxygen species in oxidation and cleavage of the protein backbone.

1.2 PEPTIDE BOND CLEAVAGE

Hydroxyl radicals (•OH) formed during exposure to ionizing radiation, or by ferrous ion-catalyzed cleavage of H_2O_2, are able to abstract the α-hydrogen atom of any amino acid residue of a protein, leading to formation of a carbon-centered radical (Fig. 1.1, reaction *a* or *b*). These carbon-centered radicals undergo rapid addition of O_2 to form peroxy radical derivatives (Fig. 1.1, reaction *c*) that are readily converted to the peroxide and subsequently to the alkoxyl derivatives by reaction with either $HO_2^{\bullet}$ or Fe^{2+} (Fig. 1.1, reactions *d*, *e* and *f*, *g*). This sets the stage for peptide bond cleavage by either of two different pathways, namely by the *diamide and* α*-amidation pathways* (Garrison, 1987). In the *diamide* pathway (Fig. 1.1, reactions *h*, *m*), the *C*-terminal amino acid of the peptide fragment derived from the *N*-terminal portion of the protein is present as a diamide derivative, and the *N*-terminal amino acid of the fragment derived from the *C*-terminal portion of the protein is present as an isocyanate derivative. In the α*-amidation* pathway (Fig. 1.1, reactions *j*, *i*, *k*), the *C*-terminal amino acid residue of the fragment derived from the *N*-terminal portion of the protein exists as an amide, and the *N*-terminal amino acid of the fragment derived from the *C*-terminal portion of the protein exists as an α-ketoacyl derivative. Significantly, cleavage by the α*-amidation* pathway provides a mechanism for the introduction of a carbonyl group into a peptide.

In addition to the peptide bond cleavage by the pathways illustrated in Figure 1.1, cleavage can occur also as a consequence of the •OH-dependent

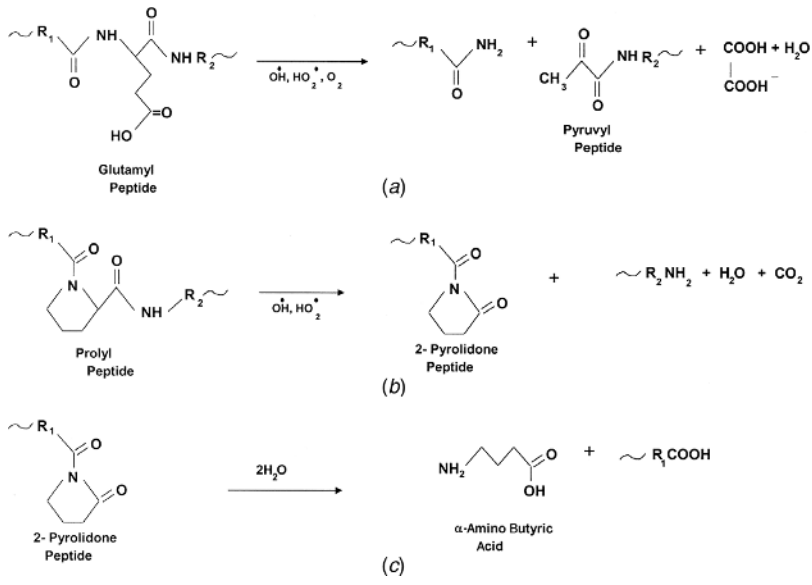

FIGURE 1.2 Cleavage of the protein backbone by oxidation of proline and glutamic acid side chains.

abstraction of a hydrogen atom from the side chain of glutamyl residues (Garrison, 1987) and also of prolyl residues (Uchida et al., 1990) of proteins according to the overall reactions *a* and *b*, respectively (Fig. 1.2).

Moreover, upon acid hydrolysis, the 2-pyrrolidone formed in reaction b is converted to 4-aminobutyric acid (reaction *c*, Fig. 1.2). The presence of 4-aminobutyric acid in acid hydrolysates of proteins is therefore presumptive evidence for peptide cleavage by the 2-pyrrolidone pathway.

1.3 β-SCISSION

In addition to the reactions illustrated in Figures 1.1 and 1.2, it was established that exposure of proteins to ionizing radiation leads to β-scission of amino acid

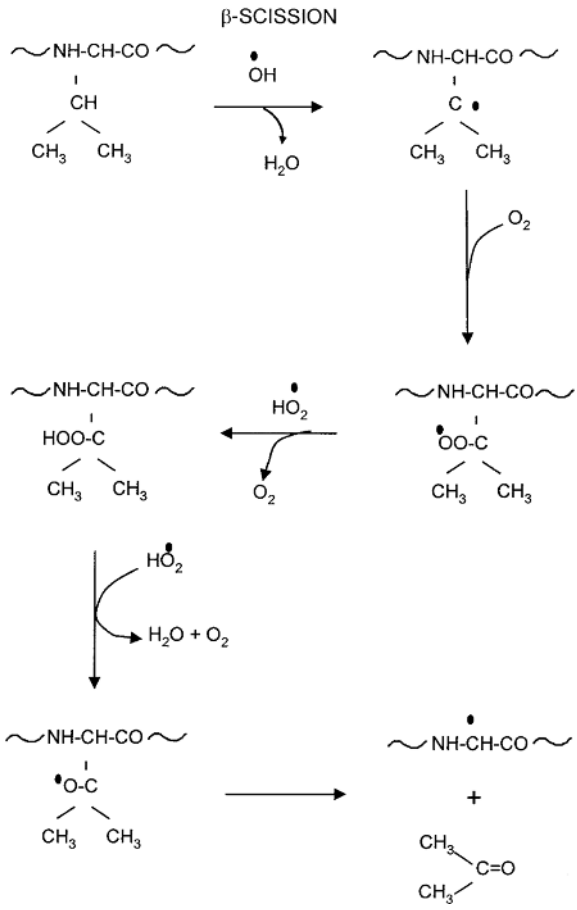

FIGURE 1.3 Beta-scission of protein amino acid side chains.

side chains (Dean et al., 1997; Headlam et al., 2000; Headlam and Davies, 2002). Thus β-scission of alanine, valine, leucine, and aspartic acid protein side chains leads to the formation of low molecular carbonyl compounds, including free formaldehyde, acetone, isobutyraldehyde, and glyoxylic acid, respectively, and in each case the side chain cleavage leads to the generation of a carbon-centered radical (∼NH$^{\bullet}$CHCO∼) in the polypeptide chain, as occurs when glycine residues undergo $^{\bullet}$OH-dependent α-hydrogen abstraction. The mechanism involved in all of these reactions is like that observed for the β-scission of a valine residue that, as shown in Figure 1.3, leads to the formation of acetone (Headlam and Davies, 2002).

1.4 OXIDATION OF AMINO ACID RESIDUE SIDE CHAINS

1.4.1 Oxidation of Aromatic and Heterocyclic Amino Acid Residues

The aromatic amino acids are very susceptible to oxidation by various forms of ROS (reviewed by Stadtman, 1998b; Davies et al., 1999; Dean et al., 1997). As is illustrated in Table 1.1, oxidation of phenylalanine residues yields 2-, 3-, and 4-mono-hydroxy derivatives and the 2,3-dihydroxy derivative. Hydroxy radical-mediated oxidation of tyrosine residues gives rise to dityrosine (2,2′-biphenyl-derivatives) DOPA. Reactions with RNS lead to formation of 3-nitrotyrosine, and reactions with HOCl lead to the generation of 3-chlorotyrosine and 3,5-dichlorotyrosine derivatives. Oxidation of tryptophan residues leads to *N*-formyl-kynurenine, 3-hydroxy-kynurenine, kynurenine, and 2-, 4-, 5-, 6-, or 7-hydroxy-tryptophans. Histidine residues of proteins are major targets of oxidation by metal-catalyzed oxidation systems (Creeth et al., 1983). Upon oxidation, histidine residues are converted to 2-oxo-histidine, 4-hydroxy glutamate, asparagine, and aspartate. As noted in Table 1.1, proline residues are oxidized to glutamic semialdehye, pyroglutamic acid, 2-pyrrolidone, and 4- and 5-hydroxy derivatives, and lysine residues are oxidized to α-aminoadiphylsemialdehyde and N^{ε}-(carboxymethyl)lysine derivatives.

1.4.2 Methionine Oxidation

Methionine (Met) residues of proteins are readily oxidized to methionine sulfoxide (MetO) by many different forms of ROS/RNS (Brot and Weissbach, 1983; Pryor et al., 1994; Pryor and Uppu, 1993; Vogt, 1995), as illustrated by reaction (1) below in which H_2O_2 represents the ROS. However, in contrast to the oxidation of other amino acid residues (except cysteine residues), the oxidation of Met residues to MetO is reversible. Most organisms contain MetO reductases (Msr's) that catalyze the thioredoxin [$Th(SH)_2$]-dependent reduction of MetO back to methionine (reaction 2). Moreover cells contain thioredoxin reductases that catalyze the NADPH-dependent reduction of oxidized thioredoxin [Th(SS)] back to $Th(SH)_2$ (reaction 3). Accordingly the coupling of reactions (1), (2), and (3) is described by reaction (4), and coupling provides a biological mechanism

TABLE 1.1 Oxidation of Amino Acid Residue Side Chains

Amino Acid	Products	References
Arginine	Glutamic semialdehyde	Amici et al., 1989
Cysteine	CyS-SCy; CyS-SG; CySOH; CySOOH; $CySO_2H$	Garrison, 1987; Swallow, 1960; Brodie and Reed, 1990; Takahashi and Goto, 1990; Zhou and Gafni, 1991
Glutamic acid	Oxalic acid; pyruvate adducts	Garrison, 1987
Histidine	2-Oxohistidine; 4-OH-glutamate; aspartic acid; asparagine	Garrison, 1987; Kopoldova and Liebsier, 1963; Uchida and Kawakishi, 1993
Leucine	3-OH-leucine; 4-OH leucine; 5-OH-leucine	Garrison, 1987
Lysine	α-aminoadipylsemialdehyde; N^{ε}-(carboxymethyl)lysine	Amici et al., 1989; Reddy et al., 1995; Daneshvar et al., 1997; Requena et al., 2001; Pietzsch and Bergmann, 2004
Methionine	Methionine sulfoxide; methionine sulfone	Garrison et al., 1962; Pryor et al., 1994; Vogt, 1995; Berlett and Stadtman, 1996; Berlett et al., 1996, 1998
Phenylalanine	2-, 3-, and 4-Hydroxyphenylalanine; 2,3-dihydroxyphenylalanine	Fletcher and Okada, 1961; Davies et al., 1987; Solar, 1985; Maskos et al., 1992a, b; Beckman et al., 1992; Gieseg et al., 1993
Proline	Glutamylsemialdehyde; 2-pyrrolidone, 4- and 5-OH-proline; pyroglutamic acid	Creeth et al., 1983; Poston, 1988; Amici et al., 1989; Uchida et al., 1990; Kato et al., 1992
Tryptophan	2-, 4-, 5-, 6-, 7-Hydroxytryptophan; formylkynurenine; 3-OH-kynurenine; nitrotryptophan	Armstrong and Swallow, 1969; Winchester and Lynn, 1970; Maskos et al., 1992a; Guptasarma et al., 1992; Pryor and Uppu, 1993; Kikugawa et al., 1994
Tyrosine	3,4-Dihydroxyphenylalanine; tyr-tyr cross-links; 3-nitrotyrosine; 3-chlorotyrosine; 3,5-dichlorotyrosine	Fletcher and Okada, 1961; Maskos et al., 1992a; Beckman et al., 1992; Giulivi and Davies, 1993; Heinecke et al., 1993; Dean et al., 1993; Huggins et al., 1993, van der Vliet et al., 1995; Ischiropoulos and Al-Medi, 1995; Domigan et al., 1995; Kettle, 1996; Berlett et al., 1996, 1998; Berlett and Stadtman, 1996; Fu et al., 2000; Buss et al., 2003

for the scavenging of H_2O_2 and other forms of ROS/RNS when they replace H_2O_2 in reaction (1).

$$\text{Met} + H_2O_2 \rightarrow \text{MetO} + H_2O \quad (1)$$

$$\text{MetO} + \text{Th(SH)}_2 \rightarrow \text{Met} + \text{Th(SS)} + H_2O \quad (2)$$

$$\text{NADPH} + H^+ + \text{Th(SS)} \rightarrow \text{NADP}^+ + \text{Th(SH)}_2 \quad (3)$$

$$\text{Sum:} \quad \text{NADPH} + H^+ + H_2O_2 \rightarrow \text{NADP}^+ + 2H_2O \quad (4)$$

Significantly the oxidation of methionine leads to a mixture of the R- and S-isomers of methionine sulfoxide (Schoneich et al., 1993). One of these enzymes (referred to as MsrA) contains a cysteine residue at the catalytic site and is stereo-specific for reduction of the S-isomer of MetO, whereas in most organisms, including mammals, the other reductase (MsrB) contains a selenocysteine at the catalytic site and is specific for reduction of the R-isomer of MetO (Krukov et al., 2002; Moskovitz et al., 1996, 2000, 2001, 2002; Kumar et al., 2002). Substitution of serine for cysteine in MsrA leads to loss of activity (Moskovitz et al., 2000), and substitution of cysteine for the selenocysteine residue in MsrB leads to a considerable decrease in its activity (Bar-Noy and Moskovitz, 2002).

Based on the consideration that cyclic oxidation-reduction of Met residues of proteins leads to consumption of ROS (reaction 4), it was proposed that this process may serve an important antioxidant function to protect cells from oxidative damage (Levine et al., 1996). This concept is supported by results of studies showing that mutations leading to a decrease in Msr activities in bacteria, yeast, and mice are associated with decreases in the resistance to oxidative stress and to elevations in the levels of oxidized proteins (Moskovitz et al., 1995, 1997, 1998, 2001; St. John et al., 2001), whereas overexpression of Msr in yeast, bacteria, and *Drosophila* leads to increases in their resistance to oxidative stress (Moskovitz et al., 1995, 1997, 1998, 2001; St. John et al., 2001; Ruan et al., 2002). Interestingly mutations in mice leading to a loss in the MsrA level lead also to a 40% decrease in the maximal life span (Moskovitz et al., 2001), and overexpression of MsrA in *Drosophila* leads to a nearly twofold increase in the maximal life span (Ruan et al., 2002). Oxidation of cysteine residues is the subject of another chapter in this book and will not be discussed here.

1.4.3 Protein Carbonylation

Whereas all amino acid residues of proteins are subject to oxidation by ROS (Dean et al., 1997; Davies et al., 1999), radiation-induced oxidation (Garrison et al., 1962) and metal ion-catalyzed oxidation (Levine, 1983; Levine et al., 1994) of some residues lead to the formation of protein carbonyl derivatives. As noted above, oxidation of glutamyl residues by the α-amidation pathway leads to peptide bond cleavage and formation of a peptide that is *N*-acylated by a pyruvyl group (Fig. 1.1, reaction *l*). Moreover metal-catalyzed oxidation of proline and arginine residues of proteins leads to formation of

glutamic semialdehyde derivatives of the protein, and oxidation of lysine residues leads to the formation of adipic semialdehyde derivatives (Oliver et al., 1984, 1985; Amici et al., 1989; Daneshvar et al., 1997; Requena et al., 2001). Threonine residues are oxidized to 2-amino-3-keto-butyric acid derivatives (Taborsky, 1973). In view of the fact that the formation of protein carbonyl groups is orders of magnitude greater than other oxidative modifications, the level of protein carbonyl groups has become the most widely used marker of protein oxidation during oxidative stress, aging, and diseases. (For reviews, see Stadtman, 1988, 1998b; Stadtman and Berlett, 1997; Butterfield and Stadtman, 1997; Dean et al., 1997, 1999; Levine and Stadtman, 2001.)

1.4.4 Protein–Protein Cross-Linkage

ROS-mediated oxidation reactions can lead to formation of protein-protein cross-linkages by several different mechanisms, illustrated in Figure 1.4. (1) Reaction of a carbonyl group in one protein with the N^{ε}-amino group of a lysine residue in another protein leads to formation of a Schiff-base cross-link (Fig. 1.4, reaction *a*). (2) Oxidation of cysteine residues in two different proteins can lead to formation of inter-molecular cross-linked disulfide derivatives (Swallow, 1960; Garrison, 1987) (Fig. 1.4, reaction *b*). (3) Interaction of carbon-centered radicals in two different proteins will lead to carbon–carbon cross-linked derivatives (Garrison, 1987) (Fig. 1.4, reaction *c*). (4) Michael addition of either a histidine, lysine, or cysteine residue of one protein with an α-β-unsaturated aldehyde, such as 4-hydroxynonenal (HNE), formed during the oxidation of poly-unsaturated fatty acids, gives rise to an active aldehyde derivative that can interact with the N^{ε}-lysine amino group of another protein to form a Schiff-base cross-linked derivative (Schuenstein and Esterbauer, 1979; Uchida and Stadtman, 1993; Friguet et al., 1994a) (Fig. 1.4, reaction *d*). (5) Protein–protein cross linkages are also formed by interaction of N^{ε}-lysine residues of two different proteins with the carbonyl groups of malondialdehyde generated in the oxidation of polyunsaturated fatty acids (Burcham and Kuhan, 1996) (Fig. 1.4, reaction *e*). (6) Cross-linkages are also formed by combination of two tyrosine residues of proteins following one electron oxidation of the aromatic rings (Heinecke et al., 1993; Huggins et al., 1993; Giulivi and Davies, 1993; Dean et al., 1993). Cross-links are also formed by interactions of lysine or arginine residues with Schiff-base or Amadori products generated during glycation-glycoxidation of proteins (Monnier, 1990; Wells-Knecht et al., 1993; Cerami et al., 1987) (Fig. 1.5, reactions *a* and *b*). For reactions described in Figures 1.4 and 1.5, P^1 and P^2 refer to different proteins leading to intermolecular cross-links; however, similar reactions can occur within the same protein to form intramolecular cross-links.

1.4.5 Protein Modification by Reactive Nitrogen Species

Nitric oxide ($NO^{\bullet}$), produced in the metabolism of arginine, plays an important role in a number of cellular processes, including smooth muscle relaxation, neurotransmission, autoimmune inflammatory states, and demyelination (reviewed

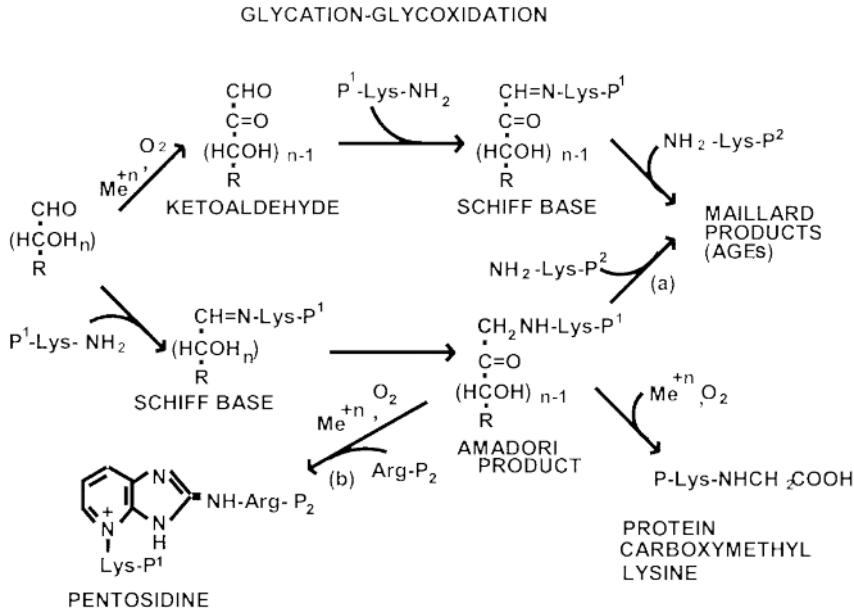

FIGURE 1.4 Glycation/glycoxidation-mediated generation of protein-protein cross-linked derivatives. P^1-LysNH$_2$ and P^2-Lys-NH$_2$ refer to epsilon amino groups of two different proteins (P^1 and P^2).

by Weinberg et al., 1998; Smith et al., 1999). However, NO$^\bullet$ reacts rapidly with $O_2^{\bullet -}$ to form the highly reactive peroxynitrite (PN) that is able to nitrate tyrosine residues (Beckman et al., 1992; Ischiropoulos et al., 1992; Ischiropoulos and Al-Medi, 1995) and to oxidize methionine residues (Pryor et al., 1994; Pryor and Squadrito, 1995; Berlett et al., 1998) and cysteine residues (Gatti et al., 1994) of proteins. The ability of PN to nitrate tyrosine residues and oxidize methionine residues is strongly affected by the presence of CO_2. Carbon dioxide stimulates the PN-dependent nitration of tyrosine residues (Lymar and Hurst, 1995; Denicola et al., 1996; Uppu et al., 1996) but inhibits the PN-dependent oxidation of methionine residues (Berlett and Stadtman, 1996; Denicola et al., 1996; Tien et al., 1999).

1.4.6 Chlorination Reactions

Activation of neutrophils leads to the release of myeloperoxidase and generation of hydrogen peroxide, which, in the presence of chloride ions, facilitates the generation of hypochlorous acid (reaction 5):

$$H_2O_2 + Cl^- \rightarrow HOCl + OH^- \tag{5}$$

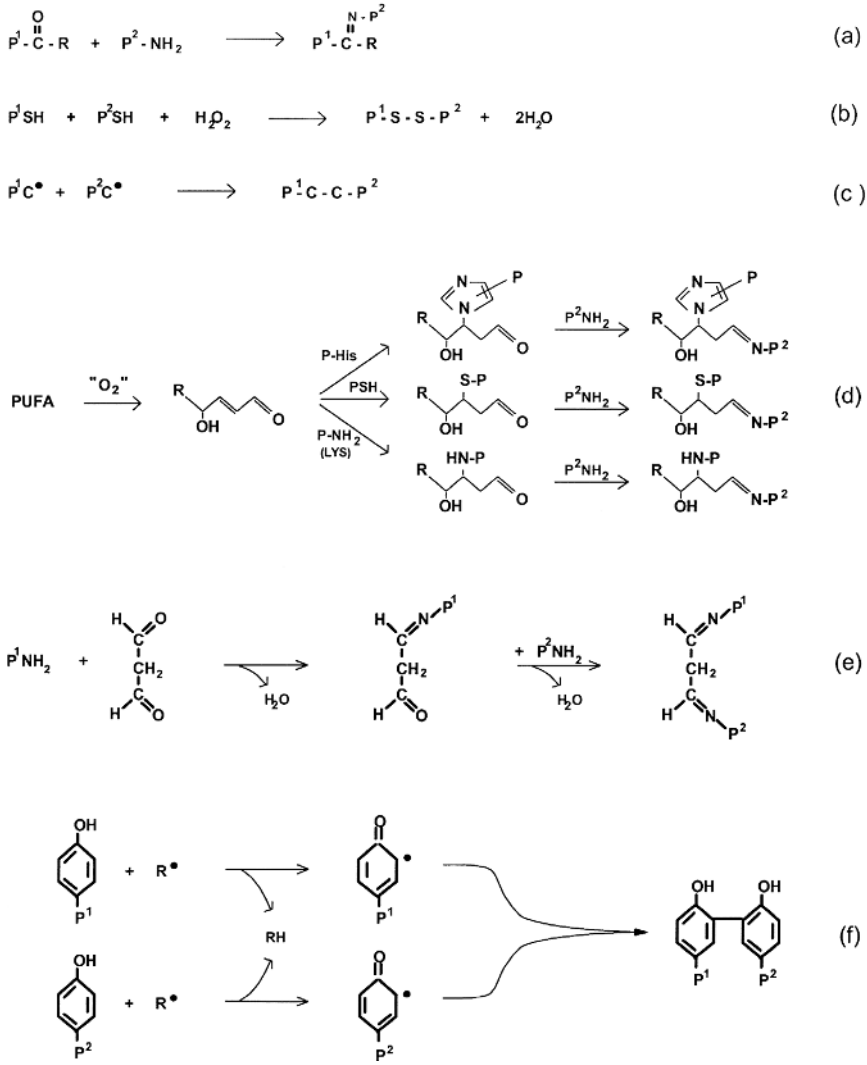

FIGURE 1.5 Generation of protein-protein cross-linkages. Reactions *a*, *b*, *c*, *d*, *e*, and refer to formation of cross-linked derivatives as described in the test. P^1 and P^2 refer to two different proteins. PUFA, polyunsaturated fatty acids.

Hypochlorous acid is a highly reactive compound that undergoes reactions with sulfur-containing amino acid residues (Folkes et al., 1995; Peskin and Winterbourn, 2001; Armesto et al., 2000). Reaction with cysteine residues leads to formation of–SCl derivatives, which may react with other cysteine residues to

form disulfides or undergo further reactions to form sulfenic, sulfinic, and sulfonic acid derivatives. The reaction with methionine residues leads to formation of methionine sulfoxide. Hypochlorous acid reacts with superoxide anion to form $^{\bullet}OH$ (reaction 6) (Folkes et al., 1995; Candias et al., 1993), and with *N*-terminal amino acid residues and the epsilon amino group of lysine residues to form the chloramine derivatives (Hawkins and Davies, 1998, 1999; Hawkins et al., 2002; Chapman et al., 2003) that upon decomposition can give rise to nitrogen-centered radicals, protein aggregation, and protein fragmentation by as yet ill-defined mechanisms. Interestingly treatment of cultured human Burkett's lymphoma cells with HOCl induces apoptosis by a choloramine-mediated pathway, whereas exposure to high concentrations of H_2O_2 leads to death by pyknosis/necrosis (Englert and Shacter, 2002):

$$HOCl + O^{\bullet -} \rightarrow O_2 + Cl^- + {}^{\bullet}OH \qquad (6)$$

Furthermore HOCl reacts with tyrosine residues of proteins to form 3-chlorotyrosine (Kettle, 1996; Domigan et al., 1995; Buss et al., 2003) and also 3,5-dichlorotyrosine (Fu et al., 2000). In the latter study it was shown that oxidation of free tyrosine by HOCl gives rise to 3-chloro- and 3,5-dichloro-4-hydroxyphenylaldehydes. Other studies by Eiserich et al. (1996) showed that reaction of nitrite with HOCl yields reactive oxygen intermediates that can nitrate and chlorinate tyrosine residues.

1.4.7 Accumulation of Oxidized Proteins

It is well established that the level of oxidized proteins increases with animal age and in the development of a number of diseases (for reviews, see Oliver et al., 1984; Takahashi and Goto, 1990; Levine and Stadtman, 1992; Stadtman, 1992, 1998b, 2002; Agarwal and Sohal, 1994; Butterfield and Stadtman, 1997; Stadtman and Berlett, 1997, 1998; Smith et al., 1999; Halliwell and Gutteridge, 1999; Levine, 2002). However, because the cellular levels of oxidized proteins are dependent on many variables, the mechanisms responsible for accumulation of oxidatively modified proteins under one condition may be very different from those in another condition. Thus ROS and RNS generation varies, depending on the kind and length of exposure to a multiplicity of oxidative stress conditions, including irradiation by X, gamma, or UV rays, inflammation initiated by activation of neutrophils and/or macrophages, alteration of regulatory pathways involved in the conversion of arginine to nitric oxide, auto-oxidation of electron transport carriers, variations in the levels of toxic atmospheric pollutants, activation of oxidases, and mobilization of metal ions that are involved in metal-catalyzed oxidation processes. However, the cellular levels of these ROS/RNS are subject to control by a multiplicity of antioxidant enzymes/proteins (superoxide dismutases, catalases, peroxidases, glutathione transferases, Msr's, glutaredoxin

reductase, thioredoxin/thioredoxin reductase, ceruloplasmin, ferritins), metabolites (bilirubin, uric acid, NADPH/NADP, GSH/GSSG), and vitamins (vitamins C, E, and A) that are able, directly or indirectly, to neutralize their deleterious effects. Moreover oxidation of proteins predisposes them to proteolytic degradation (Oliver et al., 1980, 1982; Levine et al., 1981; Rivett, 1985a, b, c; Davies, 1986; Wolf et al., 1986; Davies and Goldberg, 1987; Davies et al., 1987; Davies and Delsignore, 1987; Roseman and Levine, 1987; Grune et al., 1995, 1996, 1997). (For reviews, see Davies, 1988a, b; Rivett, 1986; Rivett et al., 1985; Davies et al., 1990; Pacifici and Davies, 1990; Dean et al., 1994; Szweda et al., 2002; Shringarpure and Davies, 2002; Dunlop et al., 2002; Drake et al., 2002.) Accordingly the cellular levels of oxidized proteins are dependent on several protease (lysosomal, cathepsin, 20S, and 26S proteasome) activities. In view of the multitude of factors that govern the generation of ROS/RNS and the antioxidant systems that neutralize their effects, and also the fact that oxidized proteins are targets for proteolytic degradation, it is obvious that the intracellular accumulation of oxidized proteins reflects the balance between all of these processes. Therefore the accumulation of oxidized proteins as occurs during aging and in some diseases may reflect an increase in the rates of ROS/RNS formation, a loss in antioxidant capacity, or a decrease in the ability of proteases to degrade oxidized proteins. With regard to the latter it has been shown that the age-dependent increase in the level of oxidized protein is associated with an age-dependent loss of the 20S proteasome, which represents a major enzyme for the degradation of oxidized proteins (Starke-Reed and Oliver, 1989; Berlett and Stadtman, 1997; Carney et al., 1991; Petropoulos et al., 2000; Davies, 2001; Grune et al., 2001; Bulteau et al., 2002; Dunlop et al., 2002; Shringarpure and Davies, 2002; Szweda et al., 2002). Some workers failed to observe an age-related decline in the level of the alkaline proteasome but did observe an age-associated decline in the lysosomal proteolytic system (Vittorini et al., 1999). Results of recent studies have shown that whereas mild oxidation of some proteins facilitates their degradation by the 20S proteasome, prolonged oxidation converts them to forms that are not only resistant to degradation but are able to inhibit the ability of the proteasome to degrade other oxidized proteins (Rivett, 1986; Sitte et al., 2000; Davies, 2001). Moreover cross-linked derivatives of proteins formed by interactions with HNE (Friguet et al., 1994a, 1994b) and lipofuscin/ceroid (Sitte et al., 2000) inhibit proteolytic degradation of oxidized proteins. It is therefore evident that the inhibition of protease activities by overoxidation of proteins has a role in the accumulation of oxidized protein during aging and in some diseases.

LIST OF ABBREVIATIONS

Met(O), methionine sulfoxide
Msr's, methionine sulfoxide reductase
ROS, reactive oxygen species
RNS, reactive nitrogen species

REFERENCES

Agarwal S., Sohal R.S. (1994). Aging and protein damage. *Mech Aging Dev* 75: 11–19.

Amici A., Levine R.L., Tsai L., Stadtman E.R. (1989). Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed reactions. *J Biol Chem* 264: 3341–3346.

Armesto X.L., Canle L.M., Fernandez M.I., Garcia M.V., Santaballa J.A. (2000). First steps in the oxidation of sulfur-containing amino acids by hypohalogenation: Very fast generation of intermediate sulfenyl halides and halosulfonium cations. *Tetrahedron* 56: 1103–1109.

Armstrong R.C., Swallow A.J. (1969). Pulse- and gamma-radiolysis of aqueous solutions of tryptophan. *Radiat Res* 41: 563–579.

Bar-Noy S., Moskovitz J. (2002). Mouse methionine mulfoxide reductase B: Effect of selenocysteine incorporation on its activity and expression of the seleno-containing enzyme in bacterial and mammalian cells. *Biochem Biophys Res Commun* 297: 956–961.

Baynes J.W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes* 40: 405–412.

Beckman J.S., Ischiropoulos H., Zhu L., van der Woerd M., Smith C., Chen J., Harrison J., Martin J.C., Tsai M. (1992). Kinetics of superoxide dismutase- and iron-catalyzed nitration of phenolics by peroxynitrite. *Arch Biochem Biophys* 298: 438–445.

Berlett B.S., Levine R.L., Stadtman E.R. (1996). Comparison of the effects of ozone on the modification of amino acid residues in glutamine synthetase and bovine serum albumin. *J Biol Chem* 271: 4177–4182.

Berlett B.S., Levine R.L., Stadtman E.R. (1998). Carbon dioxide stimulates peroxynitrite-mediated nitration of tyrosine residues and inhibits oxidation of methionine residues of glutamine synthetase: Both modifications mimic effects of adenylylation. *Proc Natl Acad Sci USA* 95: 2784–2789.

Berlett B.S., Stadtman E.R. (1996). Carbon dioxide stimulates nitration of tyrosine residues and inhibits oxidation of methionine residues in *Escherichia coli* by peroxynitrite. *FASEB J* 10: Abstract 585.

Berlett B.S., Stadtman E.R. (1997). Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem* 272: 20313–20316.

Borg D.C., Schaich K.M. (1988). Iron and iron-derived radicals. In: Halliwell B. (ed.), *Proceedings of an Upjohn Symposium, Federation of American Societies for Experimental Biology*, Bethesda, MD, pp. 20–26.

Brot N., Weissbach H. (1983). Biochemistry and physiological role of methionine sulfoxide reductase in proteins. *Arch Biochem Biophys* 233: 271–288.

Bulteau A.-L., Szweda L.I., Friguet B. (2002). Age-dependent declines in proteasome activity in the heart. *Arch Biochem Biophys* 397: 298–304.

Burcham P.C., Kuhan Y.T. (1996). Introduction of carbonyl groups into proteins by the lipid peroxidation product, malondialdehye. *Biochem Biophys Res Commun* 220: 996–1001.

Buss I.H., Senthilmohan R., Darlow B.A., Mogridge N., Kettle A.J., Winterbourn C.C. (2003). 3-Chlorotyrosine as a marker of protein damage by myeloperoxidase in tracheal aspirates from preterm infants: Association with adverse respiratory outcome. *Pediatric Res* 53: 455–462.

Butterfield D.A., Stadtman E.R. (1997). Protein oxidation processes in aging brain. In: Timiras P.S., Bittar E.E. (eds.), *Advances in Cell Aging and Gerontology*, Vol. 2, JAI Press, pp. 161–191.

Candias L.P., Patel K.B., Stratford M.R.L., Wardman P. (1993). Free hydroxyl radicals are formed on reaction between the neutrophil-derived species superoxide anion and hypochlorous acid. *FEBS Lett* 333: 151–153.

Carney J.M., Starke-Reed P.E., Oliver C.N., Landum M.S., Cheng. M.S., Wu J.F. (1991). Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound *N-tert*-butyl-α-phenylalanine. *Proc Natl Acad Sci USA* 88: 3633–3636.

Cerami A., Vlassara H., Brownlee M. (1987). Glucose and aging. *Sci Am* 256: 90–96.

Chapman A., Winterbourn C.C., Brennan S.O., Jordan T.W., Kettle A.J. (2003). Characterization of non-covalent oligomers of proteins treated with hypochlorous acid. *Biochem J* 375: 33–40.

Creeth J.M., Cooper B., Donald A.S.R., Clamp J.R. (1983). Studies of the limited degradation of mucous glycoproteins. *Biochem J* 211: 323–332.

Daneshvar B., Frandsen H., Autrup H., Dragsted L.O. (1997). γ-Glutamyl semialdehyde and 2-amino-adipic semialdehyde: Biomarkers of oxidative damage to proteins. *Biomarkers* 2: 117–123.

Davies K.J.A. (1986). Intramolecular proteolytic systems may function as secondary defenses: A Hypothesis. *Free Radic Biol Med* 2: 155–173.

Davies K.J.A. (1988a). Proteolytic systems as secondary antioxidant defenses. In: Chow C.K. (ed.), *Cellular Antioxidant Defense Mechanisms*, Vol. 2, CRC Press, Boca Raton, FL, pp. 25–67.

Davies K.J.A. (1988b). Oxidative stress causes protein Degradation and lipid peroxidation by different mechanisms in red blood cells. In: Sevanian A. (ed.), *Lipid Peroxidation in Biological Systems*, American Oil Chemists' Society, Champaign, IL, pp. 100–116.

Davies K.J.A. (2001). Degradation of oxidized proteins by the 20S proteasome. *Biochimie* 83: 301–310.

Davies K.J.A., Delsignore M.E. (1987). Protein damage and degradation by oxygen radicals. III. Modification of secondary and tertiary Structure. *J Biol Chem* 262: 9908–9913.

Davies K.J.A., Delsignore M.E., Lin S.W. (1987). Protein damage by oxygen radicals. II. Modification of amino acids. *J Biol Chem* 262: 9902–9907.

Davies K.J.A., Goldber A.L. (1987). Proteins damaged by oxygen radicals are rapidly degraded in extracts of red blood cells. *J Biol Chem* 262: 8227–8234.

Davies K.J.A., Wiese A.G., Sevanian A., Kim E.H. (1990). Repair systems in oxidative stress. In: Finch C.E., Johnson T.E. (eds.), *Molecular Biology of Aging*, Wiley-Liss, New York, pp. 123–141.

Davies M.J., Fu S., Wang H., Dean R.T. (1999). Stable markers of oxidant damage to proteins and their application in the study of human disease. *Free Radic Biol Med* 27: 1151–1163.

Dean R.T., Armstrong S., Fu S., Jessup W. (1994). Oxidized proteins and their enzymatic proteolysis in eucaryotic cells: A critical appraisal. In: Nohl H., Esterbauer H., Rice-Evans C. (eds.), *Free Radicals in the Environment, Medicine, and Toxicology*, Richelieu Press, London, pp. 47–79.

Dean R.T., Fu S., Stocker R., Davies M.J. (1997). Biochemistry and pathology of radical-mediated protein oxidation. *Biochem J* 324: 1–18.

Dean R.T., Gieseg S., Davies M.J. (1993). Reactive species and their accumulation on radical-damaged proteins. *Trends Biochem Sci* 18: 437–441.

Denicola A., Freeman B.A., Trujillo M., Radi R. (1996). Peroxynitrite reaction with carbon dioxide/bicarbonate: Kinetics and influence on peroxynitrite-mediated oxidations. *Arch Biochem Biophys* 333: 49–58.

Domigan N.M., Charlton T.S., Duncan M.W., Winterbourn C.C. (1995). Chlorination of tyrosyl residues in peptides by myeloperoxidase and human neutrophils. *J Biol Chem* 270: 16542–16548.

Drake S.K., Bourdon E., Wehr N.B., Levine RL., Backlund P.S., Yergey A.L., Rouault T.A. (2002). Numerous proteins in mammalian cells are prone to iron-dependent oxidation and proteasomal degradation. *Dev Neurosci* 24: 114–124.

Dunlop R.A., Rodgers K.J., Dean R.T. (2002). Recent developments in the intracellular degradation of oxidized proteins. *Free Radic Biol Med* 33: 894–906.

Eiserich J.P., Cross C.E., Jones D., Halliwell B. (1996). Formation of nitrating and chlorinating species by reaction of nitrite with hypochlorous acid. *J Biol Chem* 271: 19199–19206.

Englert R.P., Shacter E. (2002). Distinct modes of cell death induced by different reactive oxygen species. *J Biol Chem* 277: 20518–20526.

Esterbauer H., Schaur R.J., Zoller H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde, and related aldehydes. *Free Radic Biol Med* 11: 81–128.

Fletcher G.L., Okada S. (1961). Radiation-induced formation of dihydroxy phenylalanine from tyrosine and tyrosine-containing peptides in aqueous solution. *Radiat Res* 15: 349–351.

Folkes L.K., Candias L.P., Wardman P. (1995). Kinetics and mechanisms of hypochlorous acid reactions. *Arch Biochem Biophys* 323: 120–126.

Friguet B., Stadtman E.R., Szweda L.I. (1994a). Modification of glucose-6-phosphate dehydrogenase by 4-hydroxy-2-nonenal. *J Biol Chem* 269: 21639–21643.

Friguet B., Szweda L.I., Stadtman E.R. (1994b). Susceptibility of glucose-6-phosphate dehydrogenease modified by 4-hydroxy-2-nonenal and metal-catalyzed oxidation to proteolysis by the multicatalytic protease. *Arch Biochem Biophys* 311: 168–173.

Fu S., Wang H., Davies M., Dean R. (2000). Reactions of hypochlorous acid with tyrosine and peptidyl-tyrosyl residues give dichlorinated and aldehydic products in addition to 3-clorotyrosine. *J Biol Chem* 275: 10851–10858.

Garrison W.M. (1987). Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chem Rev* 87: 381–398.

Garrison W.M., Jayko M.E., Bennett W. (1962). Radiation-induced oxidation of protein in aqueous solution. *Radiation Res* 16: 483–502.

Garrison W.M., Kland-English M., Sokol H.A., Jayko M.E. (1970). Radiolytic degradation of peptide main chain in dilute aqueous solution containing oxygen. *J Physiol Chem* 74: 4506–4509.

Gatti R.M., Radi R., Agusto O. (1994). Peroxynitrite-mediated oxidation of albumin to the protein-thiyl free radical. *FEBS Lett* 348: 287–290.

Gieseg S.P., Simpson J.A., Charlton T.S., Duncan M.W., Dean R.T. (1993). Protein-bound 3,4-dihydroxyphenylalanine is a major product formed during hydroxyl radical damage to proteins. *Biochemistry* 32: 4780–4786.

Giulivi C., Davies K.J.A. (1993). Dityrosine and tyrosine oxidation products are endogenous markers for the selective proteolysis of oxidatively modified red blood cell hemoglobin by the 19S proteasome. *J Biol Chem* 268: 8752–8759.

Grune T., Reinheckel T., Davies K.J.A. (1996). Degradation of oxidized proteins in K562 human hematopoietic cells by proteasome. *J Biol Chem* 271: 15504–15509.

Grune T., Reinheckel T., Davies K.J.A. (1997). Degradation of oxidized proteins in mammalian cells. *FASEB J* 11: 526–534.

Grune T., Reinheckel T., Joshi M., Davies K.J.A. (1995). Protein degradation in cultured liver cells during oxidative stress. *J Biol Chem* 270: 2344–2351.

Grune T., Shringarpure R., Sitte N., Davies K. (2001). Age-related changes in protein oxidation and proteolysis in mammalian cells. *J Gerontol* 56: B459–B467.

Guptasarma P., Balasubramanian D., Matsugo S., Sito I. (1992). Hydroxyl radical-mediated damage to proteins, with special reference to the crystallins. *Biochemistry* 31: 4296–4302.

Halliwell B., Gutteridge J.M.C. (1999). Aging, nutrition, disease, and therapy: A role for antioxidants. In: Halliwell B., Gutteridge J.M.C. (eds.), *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, pp. 784–859.

Hawkins C., Davies M.J. (1998). Hypochlorite-induced damage to proteins: Formation of nitrogen-centered radicals from lysine residues and their role in protein fragmentation. *Biochem J* 332: 617–625.

Hawkins C.L., Davies M.J. (1999). Hypochlorite-induced oxidation of proteins in plasma: Formation of chloramines and nitrogen-centered radicals and their role in protein fragmentation. *Biochem J* 340: 539–548.

Hawkins C.L., Pattison D.I., Davies M.J. (2002). Reaction of protein chloramines with DNA and nucleosides: Evidence for formation of radicals, proteinвDNA cross-links, and DNA fragmentation. *Biochem J* 365: 605–615.

Headlam H.A., Davies M.J. (2002). Beta-scission of side-chain alkoxyl radicals on peptides and proteins results in the loss of side-chains as aldehydes and ketones. *Free Radic Biol Med* 32: 1171–1184.

Headlam H.A., Mortimer A., Easton C.J., Davies M.J. (2000). Beta-Scission of C-3 (beta-carbon) alkoxyl radicals on peptides and proteins: a novel pathway which results in the formation of alpha-carbon radicals and the loss of amino acid side chains. *Chem Res Toxicol* 13: 1087–1095.

Heinecke J.W., Li W., Daehnke III H.L., Goldstein J.A. (1993). Dityrosine, a specific marker of oxidation is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages. *J Biol Chem* 268: 4069–4077.

Huggins T.G., Wells-Knecht M.C., Detorie N.A., Baynes J.W., Thorpe S.R. (1993). Formation of O-tyrosine and dityrosine in proteins during radiolytic and metal-catalyzed oxidation. *J Biol Chem* 268: 12341–12347.

Ischiropoulos H., Al-Medi A.B. (1995). Peroxynitrite-mediated oxidative protein modifications. *FEBS Lett* 364: 279–282.

Ischiropoulos H., Zhu L., Beckman J.S. (1992). Peroxynitrite formation from macrophage-derived nitric oxide. *Arch Biochem Biophys* 298: 446–451.

Kato Y., Uchida K., Kawakishi S. (1992). Oxidative fragmentation of collagen and prolyl peptide by Cu(II)/H_2O_2 conversion of proline residue to 2- pyrrolidone. *J Biol Chem* 267: 23646–23651.

Kettle A.J. (1996). Neutrophils convert tyrosyl residues in albumin to chlorotyrosine. *FEBS Lett* 379: 103–106.

Kikugawa K., Kato T., Okamoto Y. (1994). Damage of amino acids and proteins induced by nitrogen dioxide, free radical toxin in air. *Free Radic Biol Med* 16: 373–382.

Kopoldova J., Liebsier J. (1963). The mechanism of radiation chemical degradation of amino acids. V. *Intern J Appl Radiation Isotopes* 14: 493–498.

Kristal B.S, Yu B.P. (1992). An emerging hypothesis: Synergistic induction of aging by free radicals and Maillard reactions. *J Gerontol* 47: B104–B107.

Krukov G.V., Kumar R.A., Koc A., Sun Z., Gladyshev V.N. (2002). Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase. *Proc Natl Acad Sci USA* 99: 4245–4250.

Kumar R.A., Koc A, Cerny R.L., Gladyshev V.N. (2002). Reaction mechanism, evolutionary analysis, and role of zinc in *Drosophila* methionine-R-sulfoxide reductase. *J Biol Chem* 277: 37527–37535.

Levine R.L. (1983). Oxidative modifications of glutamine synthetase. *J Biol Chem* 258: 11823–11827.

Levine R.L. (2002). Carbonyl-modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med* 32: 790–796.

Levine R.L. Mosoni L., Berlett B.S., Stadtman E.R. (1996). Methionine residues as endogenous antioxidants in protein. *Proc Natl Acad Sci USA* 93: 15036–15040.

Levine R.L., Oliver C.N., Fulks R.M., Stadtman E.R. (1981). Turnover of bacterial glutamine synthetase: Oxidative inactivation precedes proteolysis. *Proc Natl Acad Sci USA* 78: 2120–2124.

Levine R.L., Stadtman E.R. (1992). Oxidation of proteins during aging. *Generations* 14: 39–42.

Levine R.L., Stadtman E.R. (2001). Oxidative modification of proteins during aging. *Exp Gerontol* 36: 1495–1502.

Levine R.L., Williams J.A., Stadtman E.R., Shacter E. (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol* 233: 346–357.

Lymar S.V., Hurst J.K. (1995). Rapid reaction between peroxynitrite ion and carbon dioxide: Implications for biological activity. *J Am Chem Soc* 117: 8867–8868.

Maskos Z., Rush J.C., Koppenol W.H. (1992a). The hydroxylation of phenylalanine and tyrosine: A comparison with salicylate and tryptophan. *Arch Biochem Biophys* 292: 521–529.

Maskos Z., Rush J.D., Koppenol W.H. (1992b). The hydroxylation of tryptophan. *Arch Biochem Biophys* 296: 514–520.

Monnier V. (1990). Nonenzymatic glycosylation, the Maillard reaction and the aging process. *J Gerontol* 45: B106–B111.

Monnier V., Gerhardinger C., Marion M.S., Tanaka S. (1995). Novel approaches toward inhibition of the Maillard reaction in vivo: Search, isolation, and characterization of prokaryotic enzymes which degrade glycated substrates. In: Cutler R.G., Packer L., Bertram J., Mori A., (eds.), *Oxidative Stress and Aging*, Berkhauser Verlag, Basel, pp. 141–149.

Moskovitz J., Bar-Noy S., Williams W.M., Requena J., Berlett B.S., Stadtman E.R. (2001). Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci USA* 98: 12920–12925.

Moskovitz J., Berlett B.S., Poston J.M., Stadtman E.R. (1997). The yeast peptide-methionine sulfoxide reductase functions as an antioxidant in vitro. *Proc Natl Acad Sci USA* 94: 9585–9589.

Moskovitz J., Flescher E., Berlett B.S., Azare J., Poston J.M., Stadtman E.R. (1998). Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyes cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proc Natl Acad Sci USA* 95: 14071–14075.

Moskovitz J., Poston J.M., Berlett B.S., Nosworthy N.J., Szczepanowski R., Stadtman E.R. (2000). Identification and characterization of a putative active site for peptide methionine sulfoxide reductase (MsrA) and its substrate stereospecificity. *J Biol Chem* 275: 14167–14172.

Moskovitz J., Rahman M.A., Strassman J., Yancey S.O., Kushner S.R., Brot N., Weissbach H. (1995). *Escherichia coli* peptide methionine sulfoxide reductase gene: regulation of expression and role in protecting against oxidative damage. *J Bacteriol* 177: 502–507.

Moskovitz J., Singh F.K., Requena J., Wilkinson B.J., Jayaswal R.K., Stadtman E.R. (2002). Purification and characterization of methionine sulfoxide reductases from mouse and *Staphylococcus aureus* and their substrate stereospecificity. *Biochem Biophys Res Commun* 290: 62–65.

Moskovitz J., Weissbach H., Brot N. (1996). Cloning and expression of a mammalian gene that is involved in methionine sulfoxide reduction in proteins. *Proc Natl Acad Sci USA* 93: 2095–2099.

Oliver C.N., Ahn B., Wittenberger M.E., Levine R.L., Stadtman E.R. (1985). Age-related alterations of enzymes may involve mixed-function oxidation reactions. In: Adleman R.C., Dekker E.E. (eds.), *Modifications of Proteins during Aging*, Alan R. Liss., pp. 39–52.

Oliver C.N., Fulks R., Levine R.L., Fucci L., Rivett A.J., Roseman J.E., Stadtman E.R. (1984). Oxidative inactivation of key metabolic during aging. In: Roy A.K., Chatterjee B. (eds.), *Molecular Biology of Aging*, Academic Press, New York, pp. 235–260.

Oliver C.N., Levine R.L., Stadtman E.R. (1980). Regulation of glutamine synthetase degradation. In: Holzer H. (ed.), *Metabolic Interconversion of Enzymes*, Springer-Verlag, Berlin, pp. 259–267.

Oliver C.N., Levine R.L., Stadtman E.R. (1982). Regulation of glutamine synthetase degradation. In: Ornston L.N., Sligar S.G. (eds.), *Experiences in Biochemical Perception*, Academic Press, New York, pp. 233–249.

Pacifici R.E., Davies K.J.A. (1990). Protein degradation as an index of oxidative stress. *Methods Enzymol* 1886: 485–502.

Pietzsch J., Bergmann R. (2004). Analysis of 6-hydroxy-2-aminocaproic acid (HACA) as a specific marker of protein oxidation: The use of *N(O,S)*-ethoxycarbonyl trifluoroethyl ester derivatives and gas chromatography/mass spectrometry. *Amino Acids* 26: 45–51.

Peskin A.V., Winterbourn C.C. (2001). Kinetics of the reactions of hypochlorous acid and amino acid chloramines with thiols, methionine, and ascorbate. *Free Radic Biol Med* 30: 572–579.

Petropoulos I., Conconi M., Wang X., Hoenel B., Brégégère F., Milner Y., Friguet B. (2000). Increase of oxidatively modified protein is associated with a decrease of proteasome activity and content in aging epidermal cells. *J Gerontol Biol Sci* 55A: B220–B227.

Poston J.M. (1988). Detection of oxidized amino acid residues, using P-aminobenzoic acid adducts. *Fed Proc* (Abstract) 46: 1979.

Pryor W.A., Jen X., Squadrito G.L. (1994). One- and two-electron oxidations of methionine by peroxynitrite. *Proc Natl Acad Sci USA* 91: 11173–11177.

Pryor W.A., Squadrito G.L. (1995). The chemistry of peroxynitrite: A product from the reaction of nitric oxide with superoxide. *Am J Physiol* 268: L699–L722.

Pryor W.A., Uppu R.M. (1993). A kinetic model for the competitive reactions of ozone with amino acid residues in proteins in reverse micelles. *J Biol Chem* 268: 3120–3126.

Reddy S., Bichler J., Wells-Knecht K.J., Thorpe S.R., Baynes J.W. (1995). N^{ε}-(carboxymethyl) lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. *Biochemistry* 34(34): 10872–10878.

Requena J., Chao C.C., Stadtman E.R. (2001). Glutamic and aminoadipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins. *Proc Natl Acad Sci USA* 98: 69–64.

Rivett A.J. (1985a). The effect of mixed-function oxidation of enzymes on their susceptibility to degradation by the nonlysosomal cysteine protease. *Arch Biochem Biophys* 247: 624–632.

Rivett A.J. (1985b). Preferential degradation of the oxidatively modified form of glutamine synthetase by intracellular mammalian proteases. *J Biol Chem* 260: 300–305.

Rivett A.J. (1985c). Purification of a liver alkaline protease which degrades oxidatively modified glutamine synthetase: Characterization as a high molecular weight cysteine protease. *J Biol Chem* 260: 12600–12606.

Rivett A.J. (1986). Regulation of intracellular protein turnover: Covalent modification as a mechanism of marking proteins for degradation. *Curr Top Cell Reg* 28: 291–337.

Rivett A.J., Roseman J.E., Oliver C.N., Levine R.L., Stadtman E.R. (1985). Covalent modification of proteins by mixed-function oxidation: Recognition by intracellular proteases. In: Khairallah E.A., Bond J.S. (eds.), *Intracellular Protein Catabolism*, Alan R. Liss, New York, pp. 317–328.

Roseman J.E., Levine R.L. (1987). Purification of a protease from *Escherichia coli* with specificity for oxidized glutamine synthetase. *J Biol Chem* 262: 2101–2110.

Ruan H., Tang X.D., Chen M.-L., Joiner M.A., Sun G., Brot N., Weissbach H., Heineman S.H., Iverson L., Wu C.-F., Hoshi T. (2002). High quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc Natl Acad Sci USA* 99: 2748–2753.

Schoneich C., Zhao F., Wilson G.S., Borchardt R.T. (1993). Iron-thiolate induced oxidation of methionine to methionine sulfoxide in small model peptides. Intramolecular catalysis by histidine. *Biochem Biophys Acta* 1158: 307–322.

Schuenstein E., Esterbauer H. (1979). Formation and properties of reactive aldehydes. In: *Submolecular Biology of Cancer, CIBA Foundation Series 67, Excerpta Medica*, Elsevier, Amsterdam, pp. 225–244.

Schuessler H., Schilling K. (1984). Oxygen effect in radiolysis of proteins. Part 2. Bovine serum albumin. *Int J Radiat Biol* 45: 267–281.

Shringarpure R., Davies K.J.A. (2002). Protein turnover by the proteasome in aging and disease. *Free Radic Biol Med* 32: 1084–1089.

Sitte N., Huber M., Grune T., Ladhoff A. Doecke W-D., Zglinicki T., Davies K.J.A. (2000). Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J* 14: 14490–14498.

Smith C.D., Carney J.M., Starke-Reed P.E., Oliver C.N., Stadtman E.R., Floyd R.A. (1991). Excess brain protein oxidation and enzyme dysfunction in normal and Alzheimer's disease. *Proc Natl Acad Sci USA* 88: 10540–10543.

Smith K.J., Kapoor R., Felts P.A. (1999). Demyelination: The role of reactive oxygen and nitrogen species. *Brain Pathol* 9: 69–92.

Solar S. (1985). Reactions of OH with phenylalanine in neutral aqueous solution. *Radiat Phys Chem* 26: 103–108.

St. John G., Brot N., Ruan J., Erdjument-Bromage H., Tempst R., Weissbach H., Nathan C. (2001). Peptide methionine sulfoxide reductase from *Escherichia coli* and *Mycobacterium tuberculosis* protects bacteria against oxidative damage from reactive nitrogen intermediates. *Proc Natl Acad Sci USA* 98: 9901–9906.

Stadtman E.R. (1988). Biomarkers of Aging. *Exp Gerontol* 10: 327–347.

Stadtman E.R. (1992). Protein oxidation and aging. *Science* 257: 1220–1224.

Stadtman E.R. (1998a). Free radical-mediated oxidation of proteins. In: Ozben T. (ed.), *Free Radicals, Oxidative Stress, and Antioxidants: Pathological and Physiological Significance*, NATO ASI Series, Series A: Life Sciences, Vol. 296, Plenum Press, New York, pp. 51–143.

Stadtman E.R. (1998b). Free radical-mediated oxidation of proteins. In: Ozben T. (ed.), *Free Radicals, Oxidative Stress, and Antioxidants: Pathological and Physiological Significance*, NATO ASI Series, Series A: Life Sciences,. Vol. 296, Plenum Press, New York, pp. 161–191.

Stadtman E.R. (2002). Importance of individuality in oxidative stress and aging. *Free Radic Biol Med* 33: 597–604.

Stadtman E.R., Berlett B.S. (1997). Free radical-mediated modification of proteins. In: Wallace K.B. (ed.), *Free Radical Toxicology*, Taylor and Francis, Washington, D.C., pp 71–87.

Stadtman E.R., Berlett B.S. (1998). Reactive oxygen-mediated protein oxidation in aging and disease. *Drug Metab Rev* 30: 225–243.

Starke-Reed P.E., Oliver C.N. (1989). Protein oxidation and proteolysis during aging and oxidative stress. *Arch Biochem Biophys* 275: 559–567.

Swallow A.J. (1960). *Effect of ionizing radiation on proteins, RCO groups, peptide bond cleavage Inactivation, –SH Oxidation*. In: Swallow A.J. (ed.), Pergamon Press, New York, pp. 211–224.

Szweda P.A., Friguet B., Szweda L.I. (2002). Proteolysis, free radicals, and aging. *Free Radic Biol Med* 33: 29–36.

Taborsky G. (1973). Oxidative modification of proteins in the presence of ferrous iron and air. Effect of ionic constituents of the reaction medium on the nature of the oxidation products. *Biochemistry* 12: 1341–1348.

Takahashi R., Goto S. (1990). Alteration of aminoacyl-tRNA synthetase with age: Heat labilization of the enzyme by oxidative damage. *Arch Biochem Biophys* 277: 228–233.

Tien M., Berlett B.S., Levine R.L., Chock P.B., Stadtman E.R (1999). Peroxynitrite-mediated modification of proteins at physiological carbon dioxide concentration: pH dependence of carbonyl formation, tyrosine nitration, and methionine oxidation. *Proc Natl Acad Sci USA* 96: 7809–7814.

Uchida K., Kato Y., Kawakishi S. (1990). A novel mechanism for oxidative damage of prolyl peptides induced by hydroxyl radicals. *Biochem Biophys Res Commun* 169: 265–271.

Uchida K., Kawakishi S. (1993). 2-Oxohistidine as a novel biomarker for oxidative modification of proteins. *FEBS Lett* 332: 208–210.

Uchida K., Stadtman E.R. (1993). Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase: A possible involvement of intra- and intermolecular cross-linking reaction. *J Biol Chem* 268: 6388–6393.

Uppu R.M., Squadrito G.L., Pryor W.A. (1996). Acceleration of peroxynitrite oxidations by carbon dioxide. *Arch Biochem Biophys* 327: 335–342.

van der Vliet A., Eiserich J.P., O'Neill C.A., Halliwell B., Cross C.E. (1995). Tyrosine modification by reactive nitrogen species: A closer look. *Arch Biochem Biophys* 319: 341–349.

Vittorini S., Paradiso C, Donati A., Cavallini G., Masini M. Gori Z., Pollera M., Bergamini E. (1999). The age-related accumulation of protein carbonyl in rat liver correlates with the age-related decline in liver proteolytic activities. *J Gerontol A Biol Sci Med Sci* 54: B318–B323.

Vogt W. (1995). Oxidation of methionine residues in proteins: Tools, targets, and reversal. *Free Radic Biol Med* 18: 93–105.

Weinberg J.B., Gilkeson G.S., Mason R.P., Chamulitrat W. (1998). Nitrosylation of blood hemoglobin and renal nonheme proteins in autoimmune MRL-*lpr/lpr* mice. *Free Radic Biol Med* 24: 191–196.

Wells-Knecht M.C., Huggins T.G., Dyer G., Thorpe S.R., Baynes J.W. (1993). Oxidized amino acids in lens protein with age. *J Biol Chem* 268: 12348–12352.

Winchester R.V., Lynn K.R. (1970). X- and γ-radiolysis of some tryptophan dipeptides. *Int J Radiat Biol* 17: 541–549.

Wolf S.P., Garner A., Dean R.T. (1986). Free radicals, lipids, and protein degradation. *Trends Biochem Sci* 11: 27–31.

Zhou J.Q., Gafni A. (1991). Exposure of rat muscle phosphoglycerate kinase to a nonenzymatic MFO system generates the old form of enzyme. *J Gerontol* 46: B217–B222.