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Free radical-mediated oxidation of free amino acids and amino acid residues in proteins

Opening Review Article

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Summary. We summarize here results of studies designed to elucidate basic mechanisms of reactive oxygen (ROS)-mediated oxidation of proteins and free amino acids. These studies have shown that oxidation of proteins can lead to hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and to conversion of some amino acid residues to carbonyl derivatives. Oxidation can lead also to cleavage of the polypeptide chain and to formation of cross-linked protein aggregates. Furthermore, functional groups of proteins can react with oxidation products of polyunsaturated fatty acids and with carbohydrate derivatives (glycation/glycoxidation) to produce inactive derivatives. Highly specific methods have been developed for the detection and assay of the various kinds of protein modifications. Because the generation of carbonyl derivatives occurs by many different mechanisms, the level of carbonyl groups in proteins is widely used as a marker of oxidative protein damage. The level of oxidized proteins increases with aging and in a number of age-related diseases. However, the accumulation of oxidized protein is a complex function of the rates of ROS formation, antioxidant levels, and the ability to proteolytically eliminate oxidized forms of proteins. Thus, the accumulation of oxidized proteins is also dependent upon genetic factors and individual life styles. It is noteworthy that surface-exposed methionine and cysteine residues of proteins are particularly sensitive to oxidation by almost all forms of ROS; however, unlike other kinds of oxidation the oxidation of these sulfur-containing amino acid residues is reversible. It is thus evident that the cyclic oxidation and reduction of the sulfur-containing amino acids may serve as an important antioxidant mechanism, and also that these reversible oxidations may provide an important mechanism for the regulation of some enzyme functions.

Keywords: Protein carbonyls – Oxygen free radicals – Methionine oxidation/reduction – Oxidized protein proteolysis

Introduction

Free amino acids and amino acid residues in proteins are highly susceptible to oxidation by one or more reactive species (ROS) that: (a) are present as pollutants in the atmosphere; (b) are generated as by-products of normal metabolic processes; and (c) are formed during exposure to X-, λ-, or U.V.-irradiation. The mechanisms of these ROS-mediated oxidations reactions were elucidated by Garrison et al. (1962), Garrison (1987), Swallow (1960), and Shuessler and Schilling (1984) who exposed solutions of amino acids, peptides, and proteins to ionizing radiation under conditions where $^{\bullet}OH$ or a mixture of $^{\bullet}OH$ and $O_2^{\bullet-}$ are formed. Results of these studies demonstrated that the $^{\bullet}OH$-dependent abstraction of a hydrogen atom from the α-carbon of amino acids and the protein polypeptide backbone and also from the aliphatic side chains of hydrophobic amino acid residues of proteins are initial sites of attack. As illustrated in Fig. 1, abstraction of the hydrogen atom leads to formation of a carbon-centered radical (reaction *c*), which in the presence of oxygen is rapidly converted to the peroxyl radical (reaction *d*). This peroxyl radical is readily converted to the alkyl peroxide by reaction with the protonated form of the superoxide radical (reaction *e*) or by abstraction of a hydrogen atom from another molecule (reaction *f*). Further reactions with $HO_2^{\bullet}$ can lead to formation of the alkoxyl radical (reaction *h*) and its conversion to the hydroxy derivative (reaction *j*). Although the reaction sequence illustrated in Fig. 1 was established by using ionizing radiation for the generation of $^{\bullet}OH$ and $HO_2^{\bullet}$, it is likely that cleavage of H_2O_2 by iron or copper (reaction *b*, Fig. 1) is a major source of $^{\bullet}OH$ under physiological conditions. Furthermore, as shown in Fig. 1, Fe(II) is also able to replace

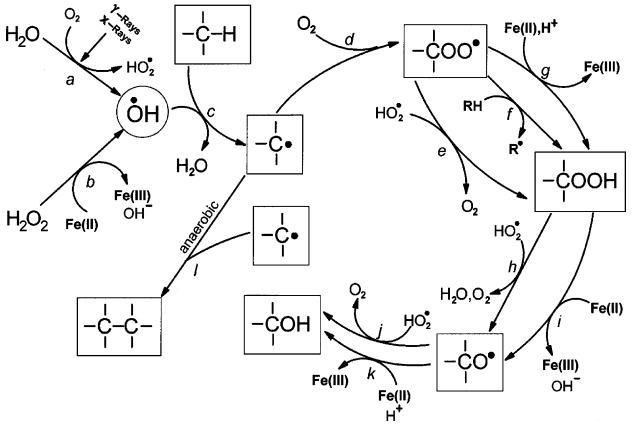

Fig. 1. Free radical-mediated oxidation of the protein polypeptide backbone

$HO_2^\bullet$ in the reactions leading to formation of the alkyl peroxide, alkoxyl radical, and the hydroxy derivative (reactions *g*, *i*, and *k*). Significantly, all reactions depicted in Fig. 1, following the hydrogen abstraction by $^\bullet$OH, are dependent upon the addition of O_2 to the carbon-centered radical (reaction *d*). In the absence of O_2, two carbon-centered radicals can react with one another to produce carbon–carbon cross-linked derivatives (reaction *l*, Fig. 1).

Peptide bond cleavage

In addition to the reactions illustrated in Fig. 1, the oxidation of proteins by ROS can lead also to the cleavage of peptide bonds. As discussed by Garrison (1987), the alkoxyl radicals and alkylperoxide derivatives of proteins can undergo cleavage by either the α-amidation or diamide pathways (Fig. 2). In the α-amidation pathway, the C-terminal amino acid of the fragment derived from the N-terminal region of the protein will exist as the amide derivative and the N-terminal amino acid of the fragment derived from the C-terminal portion of the protein will exist as an α-keto-acyl derivative. In contrast, the C-terminal amino acid of the fragment derived from the N-terminal portion of the protein via the diamide pathway will exist as the diamide derivative and the N-terminal amino acid residue of the peptide fragment derived from the C-terminal region of the protein will exist as the isocyanate derivative.

In other studies reviewed by Garrison (1987), it was demonstrated that the oxidation of glutamyl and aspartyl residues of proteins can also lead to peptide bond cleavage in which the N-terminal amino acid of the C-terminal fragment will exist as the N-pyruvyl derivative (reactions C and D, Fig. 2). In addition, based on the finding that the number of peptide fragments formed in the radiolysis of proteins is approximately equal to the number of prolyl residues, Schuessler and Schilling (1984) proposed that oxidation of prolyl residues might lead to protein fragmentation. This was subsequently verified by the studies of Uchida et al. (1990) showing that the oxidation of proline residues of proteins can lead to peptide bond cleavage by a mechanism that involves oxidation of the proline residues to the 2-pyrrolidone derivative (reaction B, Fig. 2). It is noteworthy that acid hydrolysis of 2-pyrrolidone yields 4-aminobutyric acid. Thus, the presence of 4-aminobutyric acid in protein hydrolysates might be a measure of cleavage by the prolyl oxidation pathway.

Site-specific metal-catalyzed oxidation

In addition to the reactions summarized in Figs. 1 and 2, the side-chains of amino acid residues of some proteins are readily oxidized by metal ion-catalyzed oxidation (MCO) systems (Levine, 1983; Rivett et al., 1985; Fucci et al., 1983; Stadtman, 1990; Amici et al., 1989). Oxidation of the side-chains of lysine, arginine, proline, and threonine residues has been shown to yield carbonyl derivatives and histidine residues are converted to 2-oxo-histidine (Table 1). In studies with *E. coli* glutamine synthetase, it was found that amino acid residues situated at metal binding sites on the enzyme are uniquely sensitive to metal-catalyzed oxidation by a site-specific mechanism (Farber and Levine, 1986; Climent and Levine, 1991; Sahakian et al., 1991). The case in which a lysine residue is assumed to be the target for Fe(II)-catalyzed oxidation is illustrated in Fig. 3. According to this mechanism, the chelate complex formed by the binding of Fe(II) to the amino group of lysine can react with hydrogen peroxide to generate a hydroxyl radical that will preferentially attack the lysine moiety leading to its conversion to a 2-amino-adipic-semialdehyde residue. Similar reactions of the Fe(II) with other amino acid targets lead to the generation of carbonyl derivatives (Table 1). This site-specific mechanism is supported by the demonstration that the metal-catalyzed reactions are inhibited by catalase but not by $^\bullet$OH scavengers, presumably because the scavengers cannot compete with the ''caged'' reaction of $^\bullet$OH with amino acids at the metal binding site. The importance of this site-specific mechanism of amino acid residue oxidation is supported by recent studies showing that the oxidation of proline, lysine, and arginine residues to aldehyde derivatives accounts for all of the protein carbonyl groups generated in the oxidation of glutamine

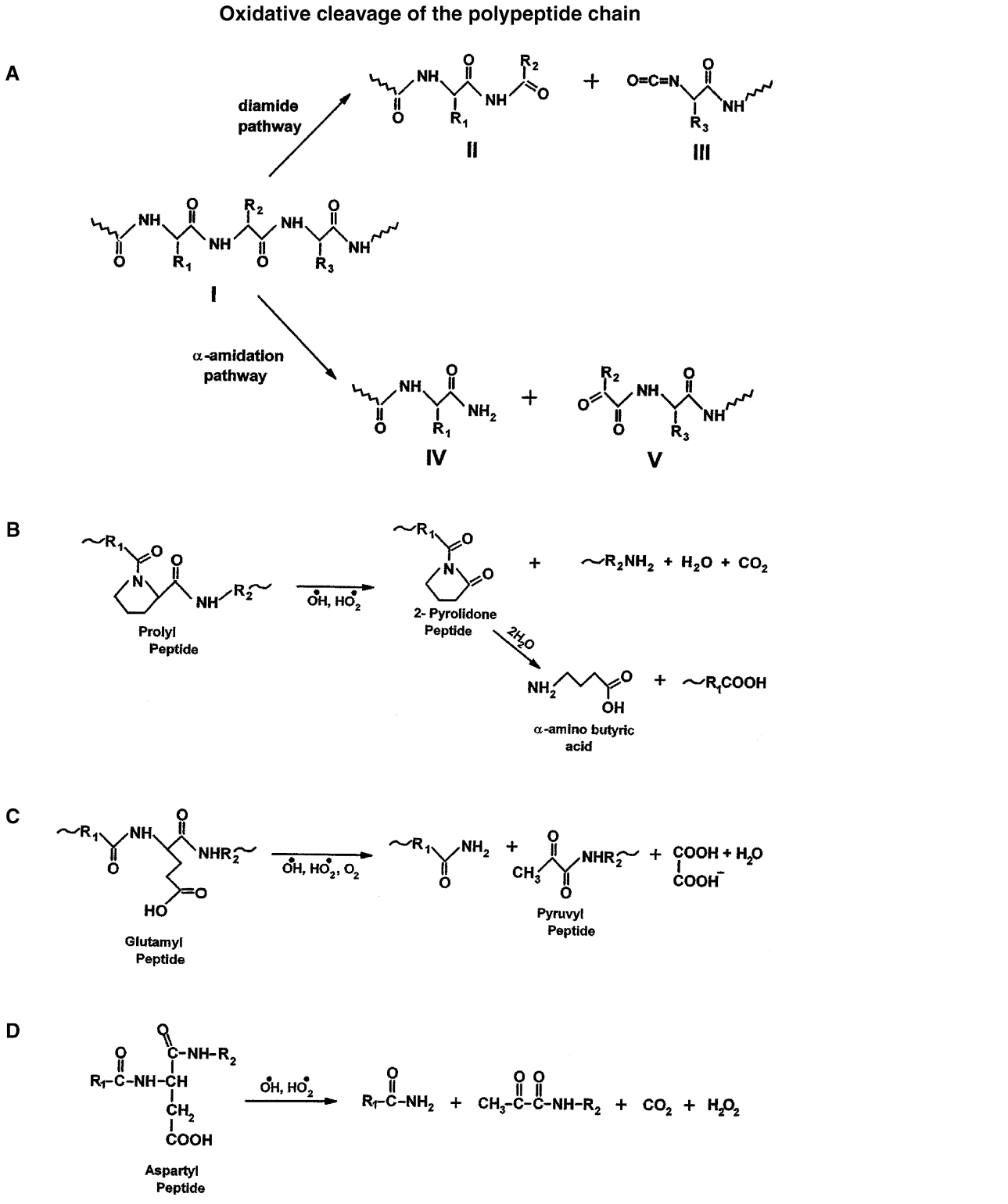

Fig. 2. Free radical-mediated cleavage of the polypeptide chain. **A** Cleavage by the diamide and α-amidation pathways. **B** Cleavage by oxidation of prolyl residues. **C** Cleavage by oxidation of glutamyl residues. **D** Cleavage by oxidation of aspartyl residue

Table 1. Oxygen free radical-mediated oxidation of protein amino acid residue side chains

Amino acid	Products	Reference
Arginine	Glutamic-semialdehyde	Amici et al. (1989); Requena et al. (2001)
Lysine	2-Amino-adipic-semialdehyde	Amici et al. (1989); Berlett et al.[a]; Requena et al. (2001)
Proline	Glutamic-semialdehyde	Amici et al. (1989)
	2-pyrrolidone	Uchida et al. (1990)
	4-,5-hydroxyproline	Poston (1988); Creeth (1983)
	pyroglutamic acid	
Cysteine	Cysteine disulfides, Sulfenic acid	Garrison (1987); Swallow (1960)
Threonine	2-amino-3-keto butyric acid	Taborsky (1973)
Leucine	3-,4-,5-hydroxyleucine	Garrison (1987); Kopoldova and Liebster (1963)
Histidine	2-oxo-histidine	Uchida and Kawakishi (1993)

[a] Berlett, BS, Miller DG, Szweda L, Stadtman ER, unpublished data

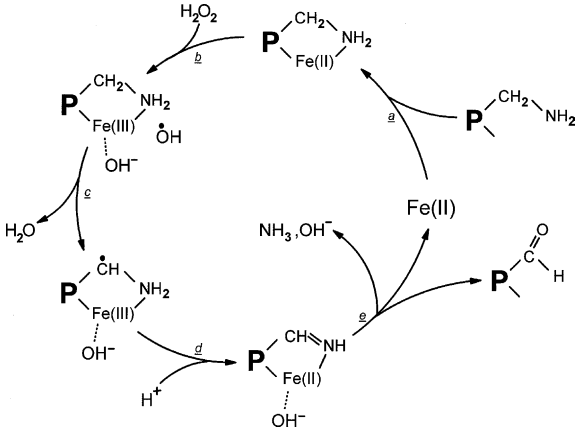

Fig. 3. Site-specific metal-catalyzed oxidation of protein amino acid residues

synthetase by MCO systems *in vitro* and for 50–60 percent of the reactive carbonyl groups detected in proteins present in rat liver extracts (Requena et al., 2001, and this volume).

Protein carbonylation

As illustrated in Figs. 2 and 3 and in Table 1, direct reaction of proteins with ROS can lead to formation of protein derivatives or peptide fragments possessing highly reactive carbonyl groups (ketones, aldehydes). But, as illustrated in Fig. 4, proteins containing reactive carbonyl groups can also be generated by secondary reactions of primary amino groups of lysine residues of proteins with reducing sugars or their oxidation products (glycation/glycoxidation reactions) (Cerami, 1987; Lee and Cerami, 1990; Wolf and Dean, 1987; Monnier et al., 1990 and 1995; Grandhee and Monnier, 1991; Kristal and Yu, 1992; Mullarkey et al., 1990; Verzijl et al., 2000; Wells-Knecht et al., 1995) and also by Michael- addition reactions of lysine, cysteine, or histidine residues with α, β-unsaturated aldehydes formed during the peroxidation of poly-unsaturated fatty acids (Schuenstein and Esterbauer, 1979; Uchida and Stadtman, 1993; Friguet et al., 1994; Nadkarni and Sayre, 1995; Sayre et al., 1993; Bruenner et al., 1994 and 1995). Because the presence of protein carbonyl derivatives in cells reflects damage induced by multiple forms of ROS, a number of analytical procedures have been developed to determine the protein carbonyl content of biological materials (Lenz et al., 1989; Levine

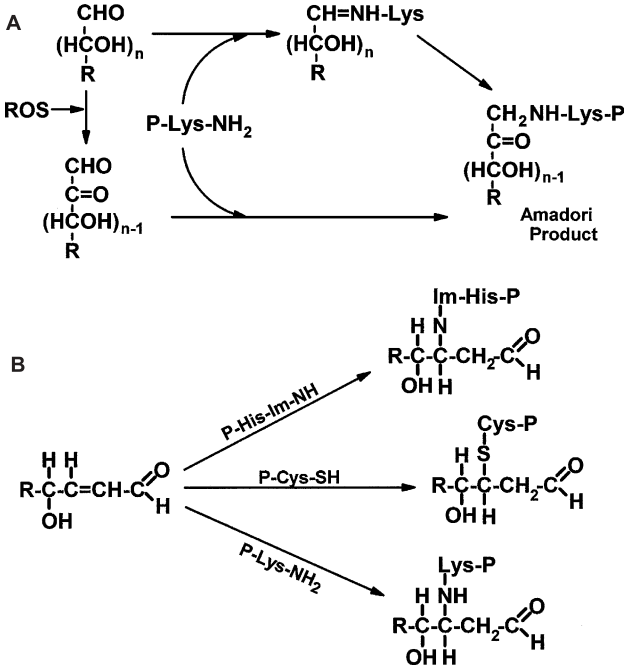

Fig. 4. Generation of carbonyl derivatives of proteins. **A** By glycation/glycoxidation of lysine amino groups. **B** By reactions of α-β-unsaturated aldehydes with lysine, cysteine, or histidine residues of proteins

et al., 1990 and 2000; Shacter et al., 1994; Keller et al., 1993; Buss et al., 1997; Smith et al., 1998; Robinson et al., 1999). These methods have become widely used measures of oxidative stress-induced cellular damage. Based on such measurements, it is well established that the accumulation of oxidized proteins is associated with aging, ischemia-reperfusion injury, and a number of age-related diseases including diabetes, Alzheimer's disease, amyotrophic lateral sclerosis, cataractogenesis, atherosclerosis, and many others. For reviews, see Stadtman and Berlett (1997 and 1998) and Levine (Levine, 2002).

Because protein carbonyl levels are appreciably greater than other oxidative modifications and because some workers (Cao and Cutler,1995) have had difficulties with protein carbonyl assays, it was argued (Dean et al., 1993) that other kinds of amino acid modifications are better markers of protein oxidative damage. Difficulties encountered in the assay of protein carbonyl levels may reflect lack of attention to experimental details (Levine, 2002). However, variations in the levels of protein carbonyl contents among different species or among individuals of the same species may reflect differences in their resistance to

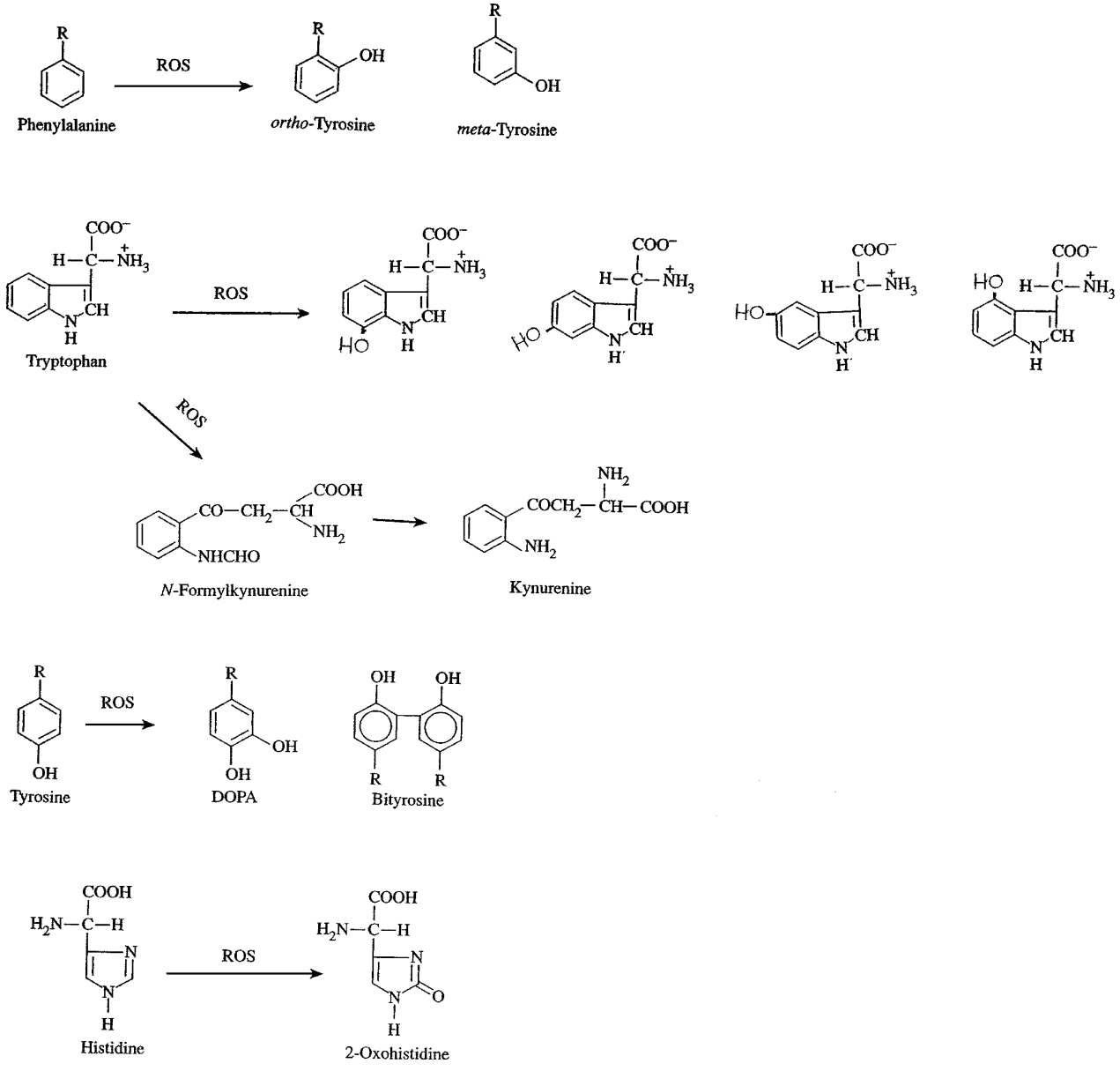

Fig. 5. Oxidation of aromatic amino acid residues

oxidative stress. Such variation is highlighted by results of studies in this laboratory showing that there was an age-dependent increase in the protein carbonyl content of liver proteins of one batch of rats, but not in a second batch of the same strain of rats obtained 10 years later from the same breeder, who maintained that the dietary and housing conditions were unchanged. The observed differences were not due to differences in analytical procedures since re-assay of samples from the first batch of animals that had been kept at $-80°C$ conditions yielded results similar to those originally observed. The obvious conclusion is that during the 10-year interval between the first and the second study, the rats had either adapted to an abnormal environment or a strain of rats had been inadvertently selected for that was more resistant to the stresses responsible for the age-related changes observed in the earlier study. Significantly, according to the breeder, the life-span of the second batch of animals was 30% greater than that of the first batch. In any case, the results of these studies emphasize the need for caution in the interpretations of apparently conflicting results obtained in two different laboratories, or even two different studies within the same laboratory.

Aromatic amino acid oxidation

The aromatic amino acid residues of proteins are prime targets for oxidation by various forms of ROS. As illustrated in Fig. 5, phenylalanine residues are oxidized to *ortho*- and *meta*-tyrosine derivatives (Maskos et al., 1992; Wells-Knecht et al., 1993; Balakrishnan and Reddy, 1970); tyrosine residues are converted to the 3,4-dihydroxy (dopa) derivative (Fletcher and Okada, 1961; Davies et al., 1987; Maskos et al., 1992; Dean et al., 1993), and also to bi-tyrosine cross-linked derivatives (Boguta and Dancewiez, 1981; Guilivi and Davies, 1993; Wells-Knecht et al., 1993; Huggins et al., 1993; Heinecke et al., 1993; Jacob et al., 1996; Leuwenburgh et al., 1977). Tryptophan residues are converted to either the 2-, 4-, 5-, 6-, or 7-hydroxy derivatives, and also to N-formylkynurenine and kynurenine (Armstrong and Swallow, 1969; Winchester and Lynn, 1970; Kikugawa et al., 1994, Maskos et al., 1992b).

Methionine oxidation

The roles of methionine residues in proteins have not been well defined, but consideration of available studies leads to the conclusion that methionine, like cysteine, can function as an antioxidant and as a key component of a system for regulation of cellular metabolism (Levine et al., 2000). Methionine is readily oxidized to methionine sulfoxide by many different reactive oxygen and nitrogen species (Lavine, 1947; Vogt, 1995). The oxidation of surface-exposed methionines can thus serve to protect other functional essential residues from oxidative damage (Reddy et al., 1994; Levine et al., 1996). Methionine sulfoxide reductases have the potential to reduce the residue back to methionine, increasing the scavenging efficiency of the system. The anti-oxidant function of methionines in proteins need not be limited to the protein itself. An elegant example comes from Stocker and colleagues who established that high density lipoproteins reduce cholesteryl ester hydroperoxides to alcohols, with the concomitant oxidation of two methionine residues to the sulfoxides (Garner et al., 1998). Since the oxidized apolipoprotein was reduced by methionine sulfoxide reductase (Sigalov and Stern, 1998), the apolipoprotein could function catalytically in the reduction of the hydroperoxides.

Further, the cyclic oxidation and reduction of methionine is a reversible covalent modification. Such reversible modifications have long been recognized to provide the mechanistic basis for most systems of cellular regulation, with phosphorylation–dephosphorylation being especially pervasive. Thus, interconversion of methionine and methionine sulfoxide can also function to regulate the biological activity of proteins, through alteration in catalytic efficiency and through modulation of the surface hydrophobicity of the protein (Ciorba et al., 1997; Levine et al., 1996).

Protein–protein cross linkage

As illustrated in Fig. 6, oxidative modification of proteins can also give rise to intra- or inter-protein cross-linked derivatives by several different mechanisms, including: (a) direct interaction of two carbon-centered radicals (Garrison, 1987); (b) interaction of two tyrosine radicals (Fig. 5); (c) oxidation of cysteine sulfhydryl groups (Garrison, 1987; Zhou and Gafni, 1991; Brodie and Reed, 1990; Takahashi and Goto, 1990); (d) interactions of the carbonyl groups of oxidized proteins with the primary amino groups of lysine residues in the same or a different protein; (e) reactions of both aldehyde groups of malondialdehyde with two different lysine residues in the same or two different protein molecules; (f) interactions of glycation/glycoxidation derived protein carbonyls with either a lysine or an arginine residue of the same or a different protein molecule (Grandhee and Monnier, 1991; Verzyl et al., 2000); Wells-Knecht et al., 1995);

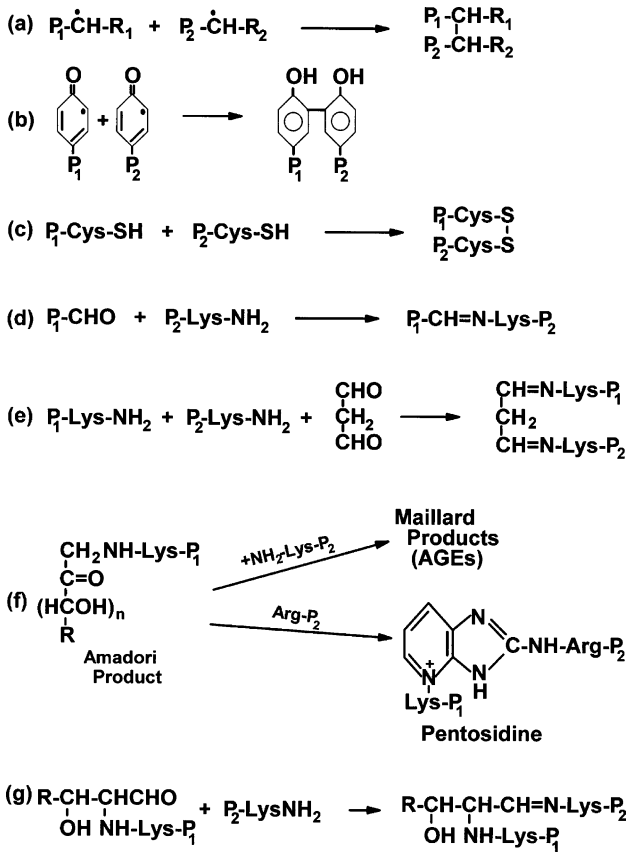

Fig. 6. Formation of protein–protein cross-linked derivatives

(g) interaction of a primary amino group of a lysine residue with protein aldehydes obtained via Michael-addition reactions with the lipid peroxidation products (*viz.* 4-hydroxy-2-nonenal) (Uchida and Stadtman, 1993; Friguet et al., 1994).

Protein modification by reactive nitrogen species

Nitric oxide ($NO^{\bullet}$) generated from arginine by the action of nitric oxide synthetases plays an important role in the regulation of various physiological functions (Ignarro et al., 1989; Moncada et al., 1991; Culotta and Koshland, 1992; Wink and Mitchell, 1998). But it also reacts with $O_2^{\bullet -}$ to form peroxynitrite ($ONOO^-$) (Pyror and Squadrito, 1995; Beckman et al., 1990; Radi et al., 1991), which under physiological conditions can react with CO_2 to form nitrosoperoxocarboxylate [(NPC), $ONOOCO_2^-$] (Lymar and Hurst, 1995 and 1996). Much of our knowledge of $NO^{\bullet}$ chemistry comes from studies carried out in a number of different laboratories. For review, see Radi et al. (2001) and Squadrito and Pryor (1998). Based on these studies, it has been established that aromatic amino acid, cysteine, and methionine residues of proteins are particularly sensitive to modification by one or another forms of reactive nitrogen species (RNS). Their ability to do so is strongly dependent upon pH and the availability of CO_2 (Tien et al., 1999). Results of *in vitro* experiments have shown that NPC is primarily responsible for the nitration of tyrosine residues of proteins (Tien et al., 1999; Gow et al., 1996), whereas peroxynitrite is largely responsible for the oxidation of methionine residues to methionine sulfoxide (Tien et al., 1999) and the nitrosation of protein sulfhydryl groups to form RSNO derivatives (Viner et al., 1999; Stubauer et al., 1999; Rubbo et al., 1994). There is still uncertainty about detailed mechanisms of these reactions. For discussions, see Radi et al. (2001) and Wink and Mitchell (1998).

The cyclic phosphorylation/dephosphorylation or adenylylation/deadenylylation of the hydroxyl group of unique tyrosine residues of some enzymes and cell signaling molecules represents an important mechanism for cellular regulation of their activities. Therefore, the effect of tyrosine nitration on the activity of such proteins has received considerable attention. In the case of *E. coli* glutamine synthetase, it was shown that nitration of either one of two different tyrosine residues inhibited the ability of the enzyme to be adenylylated; moreover, nitration converted the enzyme to a form with regulatory characteristics that were similar to those obtained by adenylylation of the enzyme (Berlett et al., 1996). In a similar study, it was demonstrated that nitration of the tyrosine residue of the tyrosine residues of a penta-decameric peptide corresponding to the active site of a substrate for the lck-kinase inhibited the ability of the kinase to phosphorylate the peptide (Kong et al., 1996). In view of the fact that the nitration reactions are non-reversible, these studies demonstrate that nitration of tyrosine residues of regulatory proteins may seriously compromise cyclic cascades involved in cell signaling and in the regulation of metabolic enzyme activities. To the contrary, peroxynitrite-dependent nitrosation of sulfhydryl groups of cell signaling proteins has been shown to facilitate their biological functions (Mohr et al., 1987; Li et al., 1997; Stamier et al., 1998).

Chlorination reactions

When stimulated to undergo oxidative burst, neutrophils produce $O_2^{\bullet -}$ and also release myeloperoxidase, which catalyzes conversion of H_2O_2 and Cl^-, the highly reactive

	Reference
Methionine + HOCl —> Met(O) + HCl	Maier et al. (1989); Peskin and Winterbourn (2001)
Tyrosine + HOCl —> 3-chlorotyrosine + HCl	Kettle (1996)
RCH_2NH_2 + HOCl —> $RCH_2NHCl + H_2O$	Hawkins and Davies (1999)
RCH_2NH_2 + HOCl —> RCHO + NH_3 +HCl	Hawkins and Davies (1999)
RSH + HOCl —> RSOH + HCl	Peskin and Winterbourn (2001)
$O_2^{\cdot-}$ + HOCl —> $^{\cdot}OH + O_2$ + HCl	Candeias et al. (1993)
Fe(II) + HOCl —> $^{\cdot}OH$ + Fe(III) + HCl	Candeias et al. (1994); Folks et al. (1995)

Fig. 7. Oxidation of proteins by hypochlorous acid

HOCl (Harrison and Schultz, 1976; Michaelis et al., 1992). As is illustrated in Fig. 7, HOCl has been shown to oxidize methionine residues to Met(O), to chlorinate tyrosine residues, to form chloramine derivatives of amino groups of lysine residues, to oxidize sulfhydryl groups to sulfenic acid derivatives, and to oxidize lysine amino groups to carbonyl derivatives. In addition, HOCl has been shown to convert lysine amino groups of proteins to nitrogen-centered radicals (Hawkins and Davies, 1998). The further observation that reaction of HOCl with $O_2^{\bullet-}$ leads to formation of $^{\bullet}OH$ provides another mechanism for the initiation of protein oxidation as described in Fig. 1. Because $^{\bullet}OH$ is also formed during the oxidation of ferrocyanide to ferricyanide by HOCl, it was proposed that the oxidation of Fe(II) to Fe(III) may provide another mechanism for the generation of $^{\bullet}OH$ (Candeis et al., 1994).

Other markers of protein oxidation

In addition to carbonyl levels, methods are now available for the assay of other types of ROS-mediated protein damage. These include methods for the estimation of *di-tyrosine* (Giulivi and Davies, 1993; Henicke et al., 1993; Huggins et al., 1993; Jacob et al., 1996; Leeuwenburgh et al., 1997), *3-nitro-tyrosine* (Hensley et al., 1997; Uppu et al., 1998), *chlorine derivatives* (Kettle, 1996), and various *mono- or di-hydroxy phenylalanine*(DOPA) derivatives (Armstrong and Dean, 1995; Hensley et al., 1997; Huggins et al., 1993). In view of the multiplicity of factors involved and complexity of the interrelationships between ROS generation, antioxidant activities, biological targets, and especially differences in the susceptibility of individuals to ROS damage, no single marker of oxidative stress is all inclusive. Nevertheless, because carbonyl groups can be formed by so many different mechanisms and because protein carbonyl levels are orders of magnitude greater than any other type of amino acid oxidation (Dean et al., 1997), the carbonyl level is probably the best overall marker of oxidative stress-induced cellular damage. For review, see Levine (2002). Even so, because various forms of ROS differ in their abilities to oxidize a given amino acid residue, further studies to identify the amino acid modifications associated with a given physiological disorder may lead to a better understanding of the ROS involved.

Accumulation of oxidized proteins

The steady-state level of oxidatively modified proteins is dependent on a multitude of factors that influence the rates of ROS generation, the ability of cells to scavenge ROS, and also the levels and activities of the 20 S proteasome and other proteases that catalyze the degradation of oxidized proteins. For reviews see: Oliver et al. (1981, 1987); Rivett et al. (1985); Stadtman (1988, 1988); Rivett (1986); Levine (1989); Davies (1985, 1986, 1987); Wolf et al. (1986); Dean et al. (1984). It is noteworthy that oxidative modifications of some proteins, especially those leading to generation of cross-linkages, lead to derivatives that are not only resistant to degradation by the proteasome, but inhibit the ability of the proteasome to degrade oxidized forms of other proteins (Rivett, 1986; Friguet et al., 1994; Grant et al., 1992; Davies, 1987). Observations that an age-related decrease in proteasome activity is sometimes accompanied by an age-related increase in the levels of oxidized proteins (Starke-Reed and Oliver, 1991; Carney et al., 1991; Stadtman et al., 1991) suggests that the two events may be related.

Oxidation of free amino acids

There is substantial evidence that the oxidative modification of free amino acids by ionizing radiation involves mechanisms similar to those described above for oxidation of amino acids in proteins (Garrison, 1987). However, the oxidation of free amino acids by the Fenton system (e.g. by $Fe(II)/H_2O_2$) is almost completely dependent on the presence of bicarbonate ion and is stimulated by substoichiometric concentrations of various chelating agents, but is inhibited by higher concentrations of chelators (Stadtman and Berlett, 1991). These metal-catalyzed reactions involve oxidative deamination of the amino acid to form CO_2, NH_4^+, and a carboxylic acid containing one less carbon atom. This can occur by two independent pathways illustrated by reactions 1–8.

Pathway A

$$RCHNH_3^+COO2 + H_2O_2 \longrightarrow RCOCOO2 + NH_4^+ + H_2O \quad (1)$$

$$RCOCOO2 + H_2O_2 \longrightarrow RCOO2 + CO_2 + H_2O \quad (2)$$

$$RCHNH_3^+COO2 + 2H_2O_2 \longrightarrow RCOO2 + CO_2 + NH_4 + 2H_2O \quad (3)$$

Pathway B

$$RCHNH_3^+COO2 + H_2O_2 \longrightarrow RCHO + NH_4^+ + HCO_32 \quad (4)$$

$$RCHO + H_2O_2 \longrightarrow RCOO2 + H^+ + H_2O \quad (5)$$

$$RCHN_3COO2 + 2H_2O_2 \longrightarrow RCOO2 + CO_2 + NH_4^+ + 2H_2O \quad (6)$$

In addition, some of the amino acid is converted an oxime (reaction 7):

$$RCHNH_3^+COO^- + 2H_2O_2 \longrightarrow RCH{=}NOH + 3H_2O \quad (7)$$

In the course of these studies on the metal-catalyzed oxidation of free amino acids, it was discovered that complexes formed between an amino acid, Fe(II), or Mn(II) and physiological concentrations of bicarbonate are able to catalyze the disproportion of H_2O_2, reaction (8) (Stadtman et al., 1990; Berlett et al., 1990; Yim et al., 1990).

$$2H_2O_2 \longrightarrow O_2 + 2H_2O \quad (8)$$

Thus, $Mn(II)/HCO_3^-/$ amino acid complexes are able to exhibit catalase-like antioxidant activity.

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