Free Radicals, Oxidants and Protein Damage

Michael Davies*

The Heart Research Institute, Newtown, Sydney, NSW 2042 and
Faculty of Medicine, University of Sydney, Sydney, NSW 2006
** Corresponding author: daviesm@hri.org.au*

Introduction

Free radicals (reactive species with an unpaired electron) and other reactive oxidants are generated in biological systems by both endogenous processes (e.g., metabolic pathways and enzymes) and exposure to external stimuli (**Table 1**). The damage induced by these oxidants has been linked not only to human disease, but also to changes in food quality and shelf life, agricultural yields, and the quality and efficacy of medicines, vaccines and antibodies (1).

Oxidants can be generated deliberately by enzymes to carry out particular functions, such as killing of invading pathogens by activated neutrophils, via the generation of superoxide radical, $O_2^{-\bullet}$, or the formation of the signalling molecule nitric oxide, $NO^{\bullet}$, by nitric oxide synthase enzymes. Other radicals arise unintentionally from metabolic reactions. Thus, $O_2^{-\bullet}$ is generated via electron leakage to molecular oxygen, O_2, from the electron transport chains of mitochondria and the endoplasmic reticulum. Exposure of cells and tissues to multiple external/exogenous stimuli can generate both radical and non-radical oxidants (**Table 1**). In many cases, formation of one oxidant or radical results in secondary reactions that generate further species (e.g., reaction of a radical with a molecule typically generates a second radical, thereby propagating damage), resulting in complex mixtures; this makes deciphering the roles of specific species complex.

Under normal circumstances, the formation and reactions of oxidants are kept in check by a battery of defensive and repair systems (1). These defences include:

1. low-molecular-mass scavengers (e.g., ascorbic acid, tocopherols, urate, thiols and various exogenous materials) that react with radicals to generate less reactive species that either undergo innocuous reactions or are repaired
2. enzymes that remove oxidant precursors (e.g., catalases, peroxiredoxins, and glutathione peroxidases that remove peroxides such as H_2O_2 and ROOH, which might otherwise act as sources of hydroxyl [$HO^{\bullet}$] or alkoxyl [$RO^{\bullet}$] radicals)
3. enzymes that directly scavenge oxidants (e.g., intra- and extra-cellular superoxide dismutases that convert $O_2^{-\bullet}$ to O_2 and H_2O_2)

Table 1. Sources of biological oxidants and examples of species formed.

Examples of processes that result in oxidant formation		Selected reactive oxidants postulated to be formed *in vivo*	
Endogenous	**Exogenous**	**Radicals**	**Two-electron oxidants**
Electron transport chains (mitochondria, endoplasmic reticulum, plasma membrane)	Radiation (high energy, UV, visible light + sensitiser, thermal)	Hydroxyl ($HO^{\bullet}$)	Hydrogen peroxide (H_2O_2)
Heme protein/enzyme reactions	Sulphur oxides	Superoxide radical anion ($O_2^{-\bullet}$)	Alkyl/aryl hydroperoxides (ROOH)
Peroxidases	Nitrogen oxides	Hydroperoxyl ($HOO^{\bullet}$)	Dialkyl/aryl/ endoperoxides (ROOR)
Nitric oxide synthases	Particulates (e.g., diesel particles)	Peroxyl ($ROO^{\bullet}$)	Peroxynitrite (ONOOH)
NADPH oxidases	Mineral fibres and dusts (e.g., asbestos)	Alkoxyl ($RO^{\bullet}$)	Peroxynitrosocarbonate ($ONOOCO_2^-$)
Xanthine oxidase	Ozone	Nitric oxide ($NO^{\bullet}$)	Singlet oxygen ($^1O_2$)
Lipoxygenases	Metabolism of chlorinated hydrocarbons	Nitrogen oxides ($NO_2^{\bullet}/NO_3^{\bullet}$)	Ozone (O_3)
Prostaglandin synthases	Metabolism of drugs (e.g., paracetamol, artemesin)	Nitrogen-centred radicals ($RNH^{\bullet}$)	Hypochlorous acid (HOCl)
Auto-oxidation of glucose	Metabolism of nitro compounds (e.g., metronidazole)	Sulphur trioxide radical anion ($SO_3^{-\bullet}$)	Hypobromous acid (HOBr)
Auto-oxidation of catecholamines	Metabolism of ethanol	Alkyl/aryl radicals ($R^{\bullet}$)	Hypothiocyanous acid (HOSCN)
'Misplaced' or excess metal ions	Oxidised foodstuffs	Carbonate radical anion ($CO_3^{-\bullet}$)	Excited state species
		Phenoxyl radicals (aromatic-$O^{\bullet}$)	α-Dicarbonyls
		Thiyl radicals ($RS^{\bullet}$)	α-Hydroxycarbonyls
		Perthiyl radicals ($RSS^{\bullet}$)	α-Aminocarbonyls
		Disulphide radical anions ($RSSR^{-\bullet}$)	α,β-Unsaturated aldehydes
		Semiquinone radicals	Quinones/quinimines

4. enzymes that repair damage (e.g., methionine sulfoxide reductases that convert oxidised methionine residues back to the parent, disulphide reductases that convert oxidised thiols back to the reduced form, and sulfiredoxins that reduce cysteine sulphinic acids back to cysteine)
5. enzymes and organelles that remove non-repairable materials (proteasomes, lysosomes, DNA repair enzymes, phospholipases)

Despite the range and diversity of these protective systems, there is considerable evidence to support the hypothesis that oxidative damage is widespread in mammals, plants and microorganisms. This damage may arise from an increased rate or extent of oxidant formation, a failure or decrease in activity of defence systems, or both simultaneously (1). This altered balance between formation of oxidants and their removal/repair in favour of higher oxidant levels is often termed 'oxidative stress'. In some cases, the cause of oxidative stress is clear (e.g., exposure to high energy radiation, genetic faults in repair enzyme synthesis), but in many cases, both factors appear to play a role. This is unsurprising, as many defensive systems are themselves subject to oxidation, or have a requirement for cofactors that can be readily depleted/oxidised, and cells have many layers of responses to oxidation typically resulting in changes in the activity or abundance of antioxidant/repair/catabolic enzymes.

The oxidant species outlined in **Table 1** vary markedly in their reactivity, and the damage that they induce is highly variable and complex. Some of these species, such as $HO^•$, are extremely reactive, with rate constants near the diffusion limit (**Table 2**). As a result of this reactivity and the abundance of biological targets, $HO^•$ primarily reacts with targets near where it is generated – i.e., damage can be site-specific.

Many of the oxidants in **Table 1** are less reactive than $HO^•$, and their corresponding rates of reaction with targets are slower and target/environment dependent. Thus, less reactive oxidants are more specific in their damage. Some species can therefore diffuse considerable distances from their sites of formation and cause remote damage. Furthermore, an initial oxidant can give rise to secondary species with markedly different reactivity and lifetimes. Thus, weakly reactive species such as $O_2^{-•}$ can be converted into highly damaging $HO^•$ via the formation and subsequent decomposition of H_2O_2 mediated by low-valency transition metal ions (e.g., the Fenton reaction involving Fe^{2+}, or Cu^+). The reactivity of some species is environment dependent; thus the protonated form of the superoxide radical $HO_2^•$ is a much more powerful oxidant (E°′ +1060 mV) than $O_2^{-•}$ (E°′ -160 mV), and reacts at much faster rates (2). The pK_a for this ionisation is 4.8, so at neutral pH, $O_2^{-•}$ predominates, but in acidic environments (e.g., within lysosomes or phagolysosomes), significant concentrations of $HO_2^•$ may be present, and enhanced oxidation may ensue.

The reduction potentials of oxidants, which are a measure of their ability to accept/donate an electron, provide information as to the likely occurrence of particular reactions (2). However, thermodynamically feasible reactions (i.e., reactions that have a favourable energy change) do not necessarily occur at rapid rates. This is exemplified by the reactions of H_2O_2 and hypochlorous acid (HOCl), the major component of household bleach. The former is the more powerful oxidant (E°′1.32 vs 1.28 V), and hence might be expected to induce greater damage, but their respective rates of reaction are dramatically different, with HOCl reacting with the amino acid methionine approximately 9 orders of magnitude (i.e., a billion times) more rapidly than H_2O_2 (rate constants, k, ~ 4×10^7 and ~ 2×10^{-2} $M^{-1} s^{-1}$, respectively). **Table 2** lists rate constants for reactions of a number of different oxidants with the same amino acid (methionine) to illustrate this range of reactivity. Such data must be treated with care, as the rate of a reaction can be altered markedly by the environment. This is clearly illustrated in the case of the reaction of the amino acid cysteine (Cys) with H_2O_2, with the rate constant for the reaction of the Cys34 residue of bovine serum albumin with H_2O_2 (k value ~3 $M^{-1} s^{-1}$) being ~7 orders of magnitude slower than for the same reaction in the active site of peroxiredoxin enzymes (k value ~10^7 $M^{-1} s^{-1}$). The peroxiredoxin active site appears to be exquisitely set up to enhance the rate of reaction via ionisation of the Cys residue and stabilisation of the transition state, consistent with this enzyme family playing a major role in rapid removal of H_2O_2 from cells (3). As the reactivity of particular oxidants can vary by more than 10 orders of magnitude, the *lifetimes* of biological oxidants also vary enormously (from microseconds through to many days).

Table 2. Rate constants for reaction of oxidants with the amino acid methionine.

Reactant	Rate constant ($M^{-1} s^{-1}$)
$HO^•$	7×10^9
$CO_3^{-•}$	1.2×10^8
HOCl	3.8×10^7
Singlet oxygen	2×10^7
Ozone	5×10^6
$CF_3CHClOO^•$	1.4×10^6
$N_3^•$	$< 10^6$
$ONOO^-$/ONOOH	3.6×10^2
$O_2^{-•}$	< 0.3
H_2O_2	2×10^{-2}
$NO^•$	Very slow

Despite the above caveats, rate constant data can provide valuable information as to the targets and fates of oxidants within biological systems. If biological systems are assumed to be homogenous (clearly an over-simplification), rate constant and abundance data can be used to predict oxidant targets and the extent of damage to different molecular classes. Calculations on cells indicate that for both $HO^•$ and the non-radical, excited state species, singlet oxygen ($^1O_2$), *proteins* are the major targets, consuming 65-70% of the available oxidants (4,5), with much lower extents of damage to DNA, RNA, lipids and antioxidants. Similar predictions have been made for plasma, tissues and other specialised systems (6). The key driving force in these calculations is the *abundance* of proteins (7). Whilst such data need to be treated with care, they do provide information as to likely targets. It should, however, be noted that *major* may not

necessarily equate to *important* with regards to a particular mechanism or pathology; the damage may merely be an unimportant consequence of another event.

Related calculations can provide data as to the distance over which a species may diffuse before it reacts (8). Values of < 0.2 mm have been calculated for $HO^{\bullet}$ and up to 1.5 mm for H_2O_2 (cf. a typical cell diameter of 20 mm). These calculations necessitate various assumptions, including free passage through cell membranes, and only unreactive oxidants (e.g., H_2O_2, $NO^{\bullet}$, and possibly $O_2^{-\bullet}$) are likely to do this without reacting. Overall, it is clear that the extent of reaction of an oxidant with a target is determined by multiple factors, including (but not limited to): the concentration of the target and the oxidant; the rate constants for the process under consideration versus competing events; the location of target versus oxidant; the occurrence of damage transfer reactions; and the extent and rate of oxidant repair/damage removal reactions.

Proteins as Targets for Oxidation

Radicals and two-electron oxidants can undergo a variety of reactions with amino acids, peptides and proteins, including hydrogen abstraction (removal of hydrogen by a radical), electron transfer (oxidation or reduction of the substrate), addition, fragmentation and rearrangement, dimerisation, disproportionation, and substitution (concerted addition and elimination) reactions. This area has been reviewed (7,9,10) and only a brief outline is presented here.

Oxidative damage can occur to both the protein backbone as well as the 20 common amino acid side-chains, most of which have multiple possible sites of attack, and the nature of the damage generated on a protein depends critically on the nature of the attacking species (**Table 3**). Most radicals and two-electron oxidants generated in biological systems (**Table 1**) are electrophilic (electron deficient) and therefore oxidise electron-rich targets. A few notable exceptions include disulphide radical anions ($RSSR^{-\bullet}$), semiquinone ($SQ^{\bullet}$) species and hydrated electrons (e_{aq}^-); each is a powerful reductant and undergoes rapid one-electron transfer with O_2 to give $O_2^{-\bullet}$. Given the prevalence of O_2 in most systems, this reaction is a major fate of these species. Some carbon-centred radicals, such as phenyl radicals, preferentially attack electron-deficient sites, but these species are relatively rare *in vivo* due to the rapid reaction of these species with O_2 to give peroxyl radicals, $ROO^{\bullet}$.

Reactions with Aliphatic Amino Acids

Hydrogen abstraction, the major process for most free aliphatic amino acids, occurs at sites *remote* from the deactivating α-amino group (and the terminal amine of the Lys side-chain), resulting in predominant formation of side-chain carbon-centred radicals (9). The ratio of attack at different side-chain sites is dictated by both the number of C-H bonds available and the stability of the resulting radical, with the order of stability being tertiary > secondary > primary carbon-centred radicals. Side-chain damage

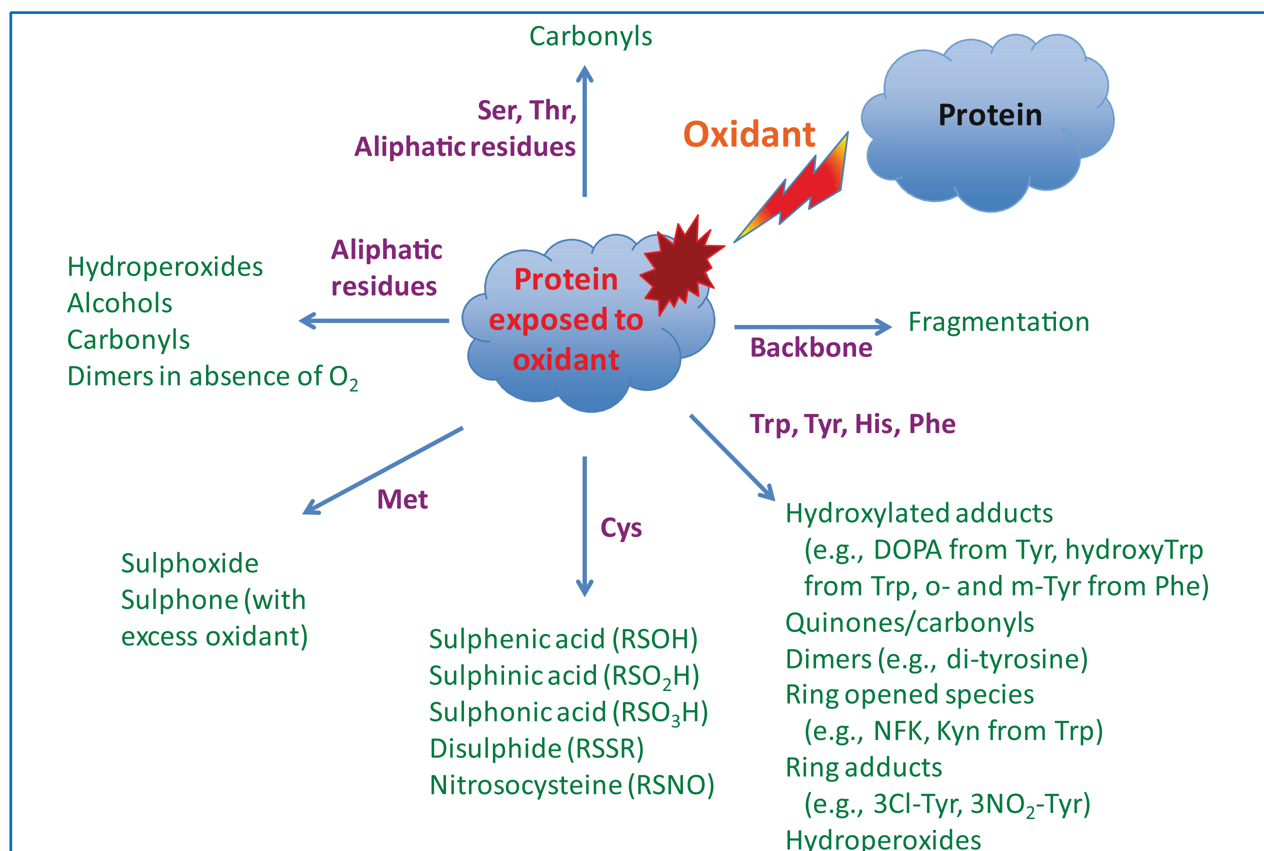

Fig. 1. Overview of products formed on proteins on exposure to oxidants.
Typical species generated from oxidation of particular side-chains are indicated; this list is not exhaustive and the species formed will depend on multiple parameters including the nature of the oxidising species. For further details, see text and (7,9,10,14). Abbreviations: DOPA, 3,4-dihydroxyphenylalanine; NFK, N-formylkynurenine; Kyn, kynurenine; 3Cl-Tyr, 3-chlorotyrosine; $3NO_2$-Tyr, 3-nitrotyrosine.

is also affected by groups that can stabilise or destabilise the resulting radical, with hydrogen abstraction occurring preferentially adjacent to hydroxyl groups in Ser and Thr, carboxyl and amide functional groups in Asp, Glu, Asn and Gln, and the guanidine group of Arg (7,9). These carbon-centred radicals typically undergo rapid reaction ($k \sim 10^9$ $M^{-1} s^{-1}$) with O_2 to give $ROO^\bullet$, or dimerisation. The latter is usually a minor process at most biological O_2 concentrations, but under anoxia, it is a major route to protein aggregation. The $ROO^\bullet$ species subsequently undergo a number of reactions, with a major pathway being hydrogen abstraction from another species to yield a hydroperoxide (ROOH) and a further radical; these processes are therefore *chain reactions*, albeit with relatively short chain lengths (4,11). Thus a *single* hit on a protein can result in damage to *multiple* residues. These hydroperoxides have significant lifetimes (minutes–hours), and can undergo further reactions to give additional radicals (via one-electron reduction by metal ions or cleavage by UV light to give alkoxyl radicals [$RO^\bullet$]), or two-electron reactions that result in reduction to alcohols. The latter process occurs rapidly with thiols (and can result in activation of thiol-dependent enzymes and GSH consumption) and yields a sulfenic acid (RSOH), disulphide (RSSR) or oxy acid (RS_xOH, x = 1-3). Other fates of $ROO^\bullet$ include termination reactions that give alcohols and carbonyls (12). Thus, initial attack at aliphatic side-chains results predominantly in formation of hydroperoxides, alcohols and carbonyls, with the first of these generating other secondary products. Most two-electron oxidants do not react to a major extent with aliphatic amino acids; notable exceptions are HOCl and HOBr, which react rapidly with the amine group of Lys residues to give chloramine (RNHCl) and bromamine (RNHBr) species, which are also oxidants (13). Major products formed from aliphatic side-chains are indicated in **Fig. 1**.

Reaction with Aromatic Amino Acids

Addition reactions of radicals are typically faster than hydrogen atom abstraction reactions, due to the more favourable transition state energies, and hence radical addition is a favourable process with Phe, Tyr, Trp and His (7,9,14). This results in the formation of a range of adduct radicals; with $HO^\bullet$, this yields various hydroxylated and quinone/carbonyl species. Ring cleavage can occur from an initial adduct, with this being significant with His and Trp. The latter results in a wide range of products including N-formylkynurenine and kynurenine from Trp. Two-electron oxidants give rise to diverse products as a result of addition reactions including peroxides (from $^1O_2$), nitrated species (from ONOOH) and halogenated materials (from HOCl and HOBr). Some of these materials are distinctive and useful biomarkers of damage and the formation of these specific oxidants (**Fig. 1**).

Reaction with Sulphur-containing Amino Acids

The sulphur atoms of Met, Cys and cystine are major oxidation sites with both radical and two-electron oxidants, and these processes are typically very fast (13,15). Two-electron oxidants (e.g., HOCl, HOBr, ONOOH, $^1O_2$) oxidise Met residues to the sulphoxide ($R_2S{=}O$) via the formation of initial short-lived adducts; this species can be repaired within cells by a family of methionine sulphoxide reductase enzymes (15). Radical-mediated oxidation of Met occurs via a complex series of reactions, but again results in significant yields of the sulphoxide (15). With Cys, radicals undergo rapid hydrogen abstraction from the thiol (-SH) group to give thiyl radicals ($RS^\bullet$). The chemistry of these species is complex (16); they can undergo direct dimerisation to give RSSR species with either the same thiol or another (these can subsequently be re-reduced by a wide range of enzymes), reversible reaction with O_2 to give a thiyl peroxyl radical, and reaction with another thiolate anion (RS^-) to give a radical anion ($RSSR^{-\bullet}$) that can subsequently reduce O_2 to $O_2^{-\bullet}$. One specific, but important, reaction of $RS^\bullet$ is with $NO^\bullet$ to give nitrosothiols (RS-NO); these species modulate thiol-dependent signalling processes. Two-electron oxidants also react very rapidly with Cys residues, with these processes usually resulting in the formation of the disulphide bond (homo- or mixed dimers), via short-lived adduct species. Oxidation of cystine residues is also highly complex, with this resulting in the formation of S-oxide species, and in some cases, cleavage products.

Reaction at Protein Backbone Sites

Unlike free amino acids, where reaction predominates at the side-chains, radicals (but not two-electron oxidants to any major extent) also react at the α-carbon of peptide (amide) bonds. This is a favourable process as a result of the captodative stabilisation of the resulting radical. The carbon-centred radicals resulting from hydrogen atom abstraction react rapidly with O_2 to give $ROO^\bullet$, and these in turn undergo additional reactions that give rise to either hydroperoxides or backbone fragmentation. Subsequent decomposition of backbone hydroperoxides to alkoxyl radicals ($RO^\bullet$) also results in backbone fragmentation.

Consequences of Protein Oxidation

As should be obvious from the above discussion, radical or two-electron oxidants can generate multiple species and have a wide range of effects on proteins (17). The extent of each of these pathways is dependent not only on the oxidant, but also reaction conditions (e.g., O_2 levels, pH, presence of metal ions, thiols, antioxidants), protein structure, accessibility of side-chain sites, and the occurrence of chain reactions. The chain reactions are

Table 3. Selectivity of damage to protein side-chains by reactive oxidants.

Oxidant	Major sites of damage
$HO^\bullet$	Most residues
$^1O_2$	Cys, Met, Trp, Tyr and His
HOCl/HOBr	Cys, Met, cystine, His, α-amino group Lys, Trp
Peroxynitrite	Cys, Tyr, Trp
UV light	Trp, Tyr, cystine
Reactive aldehydes	Arg, Lys, Cys, His, α-amino group
HOSCN	Cys

usually short (up to 15 amino acids modified per $HO^•$ generated (11)), compared to lipid peroxidation, where up to 100 amino acids can be modified, but still large enough to influence function. A range of other radical transfer processes have also been identified in proteins (7,9).

Selective damage can arise from oxidant formation at particular sites. This may arise from metal ion binding to particular side-chain sites (18) or, for example, from generation of excited states (e.g., $^1O_2$) by bound- or protein-associated chromophores. Reaction of peroxides, particularly H_2O_2, at the protoporphyrin centre of heme proteins (e.g., myoglobin, haemoglobin, heme peroxidases) is a well-established route to the generation of specific intermediates and oxidation products within proteins (19). In some cases, radical formation is intended and part of an enzymatic catalytic cycle; in other cases, the reactions are unintentional and damaging (e.g., with myoglobin and haemoglobin).

In addition to gross changes in protein structure arising from cross-linking and backbone fragmentation, which appear to occur predominantly at random sites, the formation of specific oxidation products can have marked effects on protein structure and function. Oxidation of key side-chains (e.g., active site Cys residues) can result in a loss or, less commonly, a gain of function. Oxidation of particular side-chains, with resulting changes in structure or charge/hydrophilicity, can result in changes in protein conformation and unfolding (17). This in turn can result in modulated recognition by binding partners and altered function and turnover (17). Exposure of previously buried hydrophobic residues appears to be a major recognition signal for protein degradation. The extent of damage required for such recognition is unclear and is likely to be protein and situation dependent.

Modulation of Protein Damage

Multiple exogenous compounds and processes have been identified that can modify the extent of protein damage. These include: enzymes and low molecular weight antioxidants that decrease the attacking oxidant flux; the presence or removal of catalytic metal ions; the O_2 concentration; the presence of agents (antioxidants) that can repair protein radicals once formed; and the actions of repair enzymes. With regard to the last of these, most protein oxidation appears to be irreversible, with the only well-characterised repair systems being limited to reduction of oxidised Cys residues (conversion of disulphides and RSNO species back to the thiol, and limited reversal of oxyacid, RSOH and RSO_2H, formation) and methionine sulphoxide reduction to the parent amino acid. Other damaged proteins are subject to catabolism (20), or in cases where this is not recognised or cannot be achieved, cellular accumulation; the latter has been associated with cellular dysfunction (e.g., in Alzheimer's disease).

Protein Oxidation in Disease

As proteins are major targets for oxidation, it is not surprising that an increased number of pathologies have been linked to changes in protein structure and function arising from oxidation (10,14). Evidence for a role for oxidation in these pathologies has arisen, at least in part, from studies of either gross changes in protein structure and function (e.g., changes in enzyme activity, formation of aggregates or fragments) or qualitative/quantitative assessment of various markers of protein damage. A number of the species listed in **Fig. 1** can be quantified in fluids, cells, tissues and whole organisms, and elevated levels of these materials are used to support the case for the occurrence of oxidative damage. It should, however, always be remembered that the formation of these oxidised materials may be a *consequence* rather than a *cause* of the disease/damage, and that some protein modifications are structurally and functionally unimportant and inconsequential. Thus, there are many protein modifications – particularly amino acid substitutions – that have no known structural or functional effect. It is, however, clear that in some cases, protein oxidation is *causative* in the disease process, and that understanding the mechanisms and processes involved may lead to advances in the mitigation, reversal or prevention of a number of important human diseases. Protein oxidation is not limited to human disease; oxidative damage to proteins is also of major significance in the food industry (quality of meat, cheese and milk products), the brewing industry, agricultural crop yields as a result of environmental stresses such as UV damage, the quality of agricultural materials (e.g., yellowing of wool fibre), and the quality and efficacy (shelf life) of medicines, particularly peptide and protein drugs, vaccines and antibodies.

References

1. Halliwell, B., and Gutteridge, J. M. C. (2007) *Free Radicals in Biology and Medicine*, 4th ed., Oxford University Press, UK
2. Wardman, P. (1989) *J. Phys. Chem. Ref. Data* **18**, 1637-1755
3. Rhee, S. G., and Woo, H. A. (2011) *Antioxid. Redox Signal.* **15**, 781-794.
4. Gebicki, J. M. (1997) *Redox Rep.* **3**, 99-110
5. Davies, M. J. (2004) *Photochem. Photobiol. Sci.* **3**, 17-25
6. Pattison, D. I., Hawkins, C. L., and Davies, M. J. (2009) *Chem. Res. Toxicol.* **22**, 807-817
7. Davies, M. J. (2005) *Biochim. Biophys. Acta* **1703**, 93-109
8. Winterbourn, C. C. (2008) *Nat. Chem. Biol.* **4**, 278-286
9. Hawkins, C. L., and Davies, M. J. (2001) *Biochim. Biophys. Acta* **1504**, 196-219
10. Dalle-Donne, I., Scaloni, A., Giustarini, D., Cavarra, E., Tell, G., Lungarella, G., Colombo, R., Rossi, R., and Milzani, A. (2005) *Mass Spectrom. Rev.* **24**, 55-99
11. Neuzil, J., Gebicki, J. M., and Stocker, R. (1993) *Biochem. J.* **293**, 601-606
12. Levine, R. L. (2002) *Free Radic. Biol. Med.* **32**, 790-796
13. Davies, M. J., Hawkins, C. L., Pattison, D. I., and Rees, M. D. (2008) *Antioxid. Redox Signal.* **10**, 1199-1234
14. Davies, M. J., Fu, S., Wang, H., and Dean, R. T. (1999) *Free Radic. Biol. Med.* **27**, 1151-1163
15. Schoneich, C. (2005) *Biochim. Biophys. Acta* **1703**, 111-119
16. Schoneich, C. (2008) *Chem. Res. Toxicol.* **21**, 1175-1179
17. Davies, K. J. (1993) *Biochem. Soc. Trans.* **21**, 346-353
18. Stadtman, E. R. (1993) *Annu. Rev. Biochem.* **62**, 797-821
19. Ortiz de Montellano, P. R. (1987) *Acc. Chem. Res.* **20**, 289-294
20. Davies, K. J., Lin, S. W., and Pacifici, R. E. (1987) *J. Biol. Chem.* **262**, 9914-9920