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Review

The oxidative environment and protein damage

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Abstract

Proteins are a major target for oxidants as a result of their abundance in biological systems, and their high rate constants for reaction. Kinetic data for a number of radicals and non-radical oxidants (e.g. singlet oxygen and hypochlorous acid) are consistent with proteins consuming the majority of these species generated within cells. Oxidation can occur at both the protein backbone and on the amino acid side-chains, with the ratio of attack dependent on a number of factors. With some oxidants, damage is limited and specific to certain residues, whereas other species, such as the hydroxyl radical, give rise to widespread, relatively non-specific damage. Some of the major oxidation pathways, and products formed, are reviewed. The latter include reactive species, such as peroxides, which can induce further oxidation and chain reactions (within proteins, and via damage transfer to other molecules) and stable products. Particular emphasis is given to the oxidation of methionine residues, as this species is readily oxidised by a wide range of oxidants. Some side-chain oxidation products, including methionine sulfoxide, can be employed as sensitive, specific, markers of oxidative damage. The product profile can, in some cases, provide valuable information on the species involved; selected examples of this approach are discussed. Most protein damage is non-repairable, and has deleterious consequences on protein structure and function; methionine sulfoxide formation can however be reversed in some circumstances. The major fate of oxidised proteins is catabolism by proteosomal and lysosomal pathways, but some materials appear to be poorly degraded and accumulate within cells. The accumulation of such damaged material may contribute to a range of human pathologies.

Keywords: Protein oxidation; Free radical; Oxidant; Fragmentation; Cross-linking; Methionine; Methionine sulfoxide

1. Generation of oxidants

Radicals and non-radical oxidants can be generated by a wide variety of different processes in biological systems. These range from the deliberate, and highly controlled, generation of radicals within the active site of enzymes to the unintended, essentially random, formation of oxidants in cells and tissues exposed to high-energy radiation. A number of endogenous systems are known to generate radicals and other non-radical oxidants (e.g. peroxides, hypochlorous acid, peroxynitrite, singlet oxygen), with these including a range of enzymes (e.g. nitric oxide synthases, NADPH oxidases, ribonucleotide reductase, prostaglandin synthase, myeloperoxidase, other heme enzymes, lipoxygenases), and electron transport chains (e.g. those of mitochondria and the cytochrome P_{450} system). Oxidant generation can also occur as a response to a wide range of exogenous agents including radiation (X-ray, gamma, UV, or visible light in the presence of a sensitizer), metal ions, solvents, particulate matter (asbestos, airborne material), nitrogen oxides, ozone, smoke of various forms, etc. The formation of such oxidants has been reviewed (see, e.g. Ref. [1]).

2. Reactions of oxidants with biological targets

Most highly reactive oxidants, including many radicals, react with virtually all biological molecules, including DNA, RNA, cholesterol, lipids, carbohydrates, proteins and antioxidants. Some less reactive species, such as peroxides (e.g. H_2O_2, lipid- or protein-peroxides), are much

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more selective in the targets that they damage, with modifications occurring to specific molecules at particular sites. The extent of damage to particular targets depends on a number of factors including: the concentration of target, the rate constant for reaction of oxidant with the target, the location of the target when compared to the site of oxidant formation, the occurrence of secondary damaging events (chain reactions and damage transfer processes), the occurrence of oxidant-scavenging reactions, and repair reactions. The first two of these determine the rate at which any particular reaction is likely to occur; the others modulate the extent of reaction that occurs.

This review concentrates on the reactions of oxidants with proteins, as these are the major component of most biological systems whether this is at the tissue, cellular or biological fluid level (cf. data in Fig. 1). In each of these examples, proteins comprise the major, non-water, component. Even in materials, such as low-density lipoproteins (LDL) and cell membrane fractions, which are often considered as lipid-rich environments, proteins are still major components, with, for example, the single protein in LDL—apolipoprotein B-100—contributing approximately 25% of the mass of the particle [2] and the plasma membrane of many cells comprising 50–60% protein by dry mass.

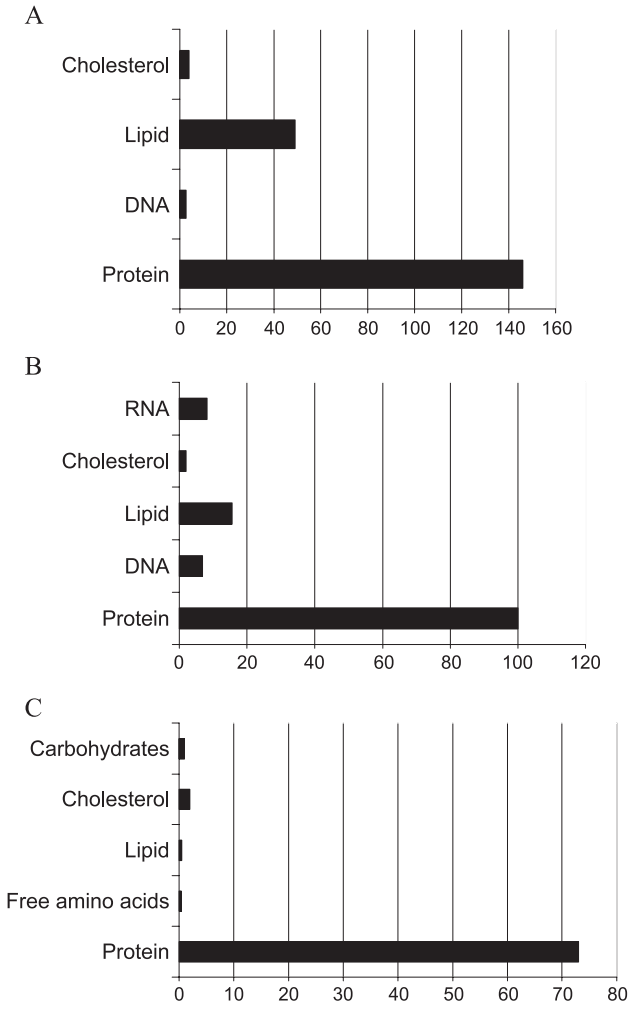


Fig. 1. Approximate constitution of various biological materials. (A) Liver (g per kg wet weight); (B) leukocytes (g per 10^{12} cells); (C) plasma (g per dm^3).

Table 1
Rate constants for reaction of $HO^{\bullet}$ with macromolecules at pH ca. 7

Substrate	Rate constant ($dm^3\ mol^{-1}\ s^{-1}$)
DNA	8×10^8
RNA	1×10^9
Hyaluronan	7×10^8
Linoleic acid	9×10^9
Collagen	4×10^{11}
Albumin	8×10^{10}
Ascorbate	1×10^{10}
GSH	1.4×10^{10}
Trolox C (water-soluble vitamin E analogue)	6.9×10^9

Selected data from Ref. [3].

The rate constants for reaction of a range of reactive radicals with biological macromolecules offer a further indication of the potential importance of proteins as targets for oxidants. The data given in Table 1 shows that the rate constants for reaction of species, such as the hydroxyl radical ($HO^{\bullet}$), with macromolecules varies to a relatively small extent, and therefore the overall rate of reaction (which is the product of the rate constant and the concentration of target), will be driven, in the main, by the concentration of the target. Furthermore, when these rate constants are compared with those for a range of biologically relevant antioxidants (or models thereof, Table 1), it can be seen that there is also little difference in these numbers. This data underscores the difficulty in designing antioxidant strategies that can compete successfully with damage to biological targets, given that most antioxidants are present at micromolar concentrations, when compared to the millimolar–molar concentrations of these targets. A number of compilations of kinetic data are available for a wide range of species including: $HO^{\bullet}$, hydrogen atoms ($H^{\bullet}$) and hydrated electrons [3], superoxide radicals and its protonated form ($O_2^{-\bullet}/HOO^{\bullet}$; [4]), carbon-centred radicals [5], a wide range of inorganic radicals (e.g. $NO_2^{\bullet}$, $CO_2^{-\bullet}$, $CO_3^{-\bullet}$, $Br_2^{-\bullet}$, $N_3^{\bullet}$; [6]), peroxyl radicals ($ROO^{\bullet}$; [7]), singlet oxygen ($^1O_2$; [8]), and hypochlorous and hypobromous acids (HOCl and HOBr; [9–11]). A regularly updated list of rate constant data for the above species, and many others, can be found at http://kinetics.nist.gov/solution/index.php.

Knowledge of such kinetic and abundance data can be used in computational models to *predict* sites of damage. Data arising from such analyses for a typical cell (leukocytes) and two reactive species—$HO^{\bullet}$ and $^1O_2$—are shown in Fig. 2. This modelling data needs, obviously, to be treated with great caution, as it does not take into account a range of other factors that are known to affect oxidant reactivity with particular targets—these include the role of location (of both

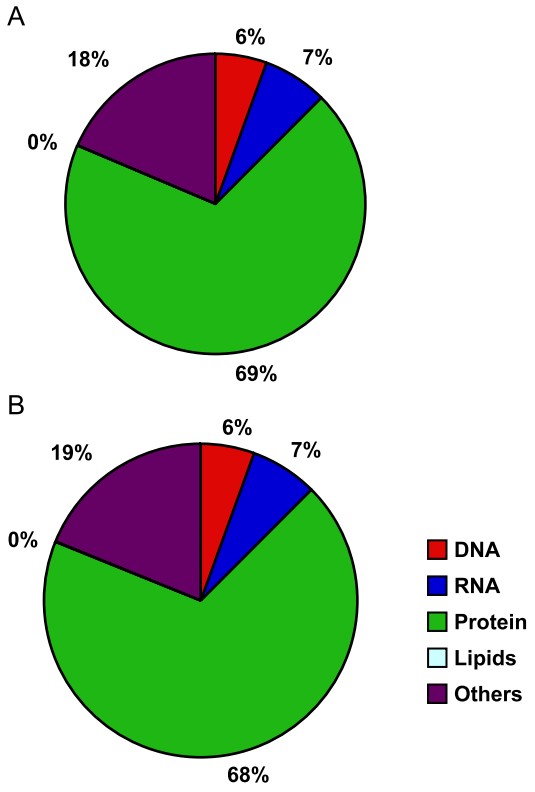


Fig. 2. Calculated consumption of: (A) hydroxyl radicals, and (B), singlet oxygen by various cellular components in leukocytes from kinetic and abundance data. See text for further details.

oxidant and target), the occurrence of chain reactions and the effects of repair processes. This said, such data could provide important clues as to the potential importance of damage to particular species.

As might be expected, there is a large variation in the selectivity of damage between different oxidants. In general the most reactive radicals tend to be the least selective. Thus the variation in the rate constants for reaction of $HO^{\bullet}$ with different side-chains is relatively small (k 10^7–10^{10} dm^3 mol^{-1} s^{-1}; Table 2), with the result that all side-chain sites are oxidised to a greater or lesser extent, though the aromatic and sulfur-containing side-chains would be expected to be depleted the most rapidly. Hydrogen abstraction from backbone sites by $HO^{\bullet}$ occurs with k ca. 10^9 dm^3 mol^{-1} s^{-1} [3] consistent with this radical causing both side-chain and backbone damage (and hence protein fragmentation).

Less reactive oxidants are much more selective in the site that they oxidise so, for example, the rate constants for reaction of the substituted peroxyl radical $CCl_3OO^{\bullet}$ with amino acid side-chains vary from ca. 9×10^7 dm^3 mol^{-1} s^{-1} for Trp residues to below the measurable detection limit, consistent with much more selective damage by this oxidant. Thus it is generally true that the less reactive an oxidant, the more selective the damage induced by such a species will be. For this reason a number of relatively weak oxidants can give rise to very selective damage to proteins, and a number of reagents are known which selectively target single residues. Comparable kinetic data is also available for non-radical oxidants; for example $^1O_2$ reacts with a considerable degree of selectivity, with Trp, Tyr, His, Met and Cys residues being the major targets [8]. As many of the reactive species generated in biological systems are electron-deficient (oxidising, electrophilic) there is a general pattern for most rapid reaction with electron-rich side-chains (i.e. Trp, Tyr, His, Met, Cys, Phe residues). There are however a few important nucleophilic or reducing species (hydrated e^-, $Ph^{\bullet}$, $CO_2^{-\bullet}$) with these having a markedly different selectivity; reaction with O_2 is a particularly rapid and important reaction in most cases.

Though kinetic data is of great use in determining the *overall* rate of reaction of a particular oxidant with a target, the techniques that are usually employed to obtain such data (typically pulse radiolysis with UV detection), do not usually yield information on the selectivity of damage at different sites *within* a molecule, unless it is possible to monitor specific absorptions from particular transients or products. For technical reasons such an approach is usually only possible for aromatic and sulfur-containing residues, as most aliphatic side-chains, and the carbon-centred radicals derived from them by hydrogen abstraction, do not have readily detectable absorptions. Data on the selectivity of attack at different sites within a particular side-chain can however be obtained by two other methods—electron paramagnetic resonance (EPR or ESR) spectroscopic studies of the radicals formed, and product analyses. Neither

Table 2
Rate constants for reaction of $HO^{\bullet}$ with free (zwitterionic) amino acids, and small peptides, at pH ca. 7

Substrate	Rate constant (dm^3 mol^{-1} s^{-1})
Alanine	7.7×10^7
Arginine	3.5×10^9
Asparagine	4.9×10^7
Aspartic acid	7.5×10^7
Cysteine	3.4×10^{10}
Cystine	2.1×10^9
Glutamine	5.4×10^8
Glutamic acid	2.3×10^8
Glycine	1.7×10^7
Histidine	1.3×10^{10}
Isoleucine	1.8×10^9
Leucine	1.7×10^9
Lysine	3.4×10^8
Methionine	8.3×10^9
Phenylalanine	6.5×10^9
Proline	4.8×10^8
Serine	3.2×10^8
Threonine	5.1×10^8
Tryptophan	1.3×10^{10}
Tyrosine	1.3×10^{10}
Valine	7.6×10^8
Cyclo(Gly)2 (backbone attack)	7.8×10^8
N-Ac-Gly–Gly (backbone attack)	7.8×10^8

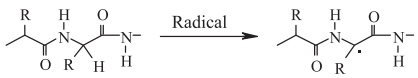
Selected data from Ref. [3].

approach is perfect, and both have major drawbacks particularly when anything larger than small peptides are examined, as the analysis of the data obtained becomes immensely complex and the occurrence of transfer reactions (see below) can cause problems with interpretation. A number of factors are known to influence which sites are most favoured. These include: the stability of the incipient radical in the case of radical-mediated oxidation (with the order of stability tertiary>secondary>primary, and species with potential for electron delocalisation on to hetero-atoms favoured over those without such possibilities); statistical factors (i.e. the number of each particular type of C–H bond/sites of addition); and possible steric and charge interactions, which might favour or disfavour reaction at a particular position.

Despite the accumulating evidence that protein oxidation may be a key process in many biological systems, the study of this area had lagged behind that of lipid and DNA oxidation. This is not due to a late start, with the first detailed studies on the oxidation of amino acids appearing in 1906 from H.D. Dakin [12]. Follow-up papers in 1908 from this same group occupied approximately half of the total page-count of J. Biol. Chem. for the entire year, which is probably the only occasion when protein oxidation has been so dominant. Various reasons can be readily discerned for the dominance of lipid and DNA oxidation over studies on proteins, including the complexity of proteins as targets (20 different side-chains plus the backbone, as potential targets compared to the more limited number of reactive sites in DNA and lipids), the complexity of the products that are formed, and the complexity of the mechanisms that can occur. Furthermore, until relatively recently, there has been a paucity of sensitive, stable, readily detectable markers of damage to proteins which has hindered in vivo studies of protein oxidation. In the following sections, some of these areas are explored in more detail. As the multiple sites on proteins that can be damaged by oxidants can be broadly categorised into backbone and side-chain sites, they will be discussed in this manner.

3. Sites and selectivity of damage on proteins—overview of backbone damage

The rates of reaction of most non-radical oxidants with protein backbone sites are slow and hence little damage is usually observed at this position. In contrast, radicals react rapidly with the backbone of proteins, with this occurring primarily via hydrogen atom abstraction at the α-carbon site (the site of attachment of the side-chains; reaction (1)), to give a stabilised (via electron delocalisation by the neighbouring carbonyl and nitrogen functions) carbon-centred radical. These species appear to have two major fates—reaction with another radical (which would be expected to be a limited process in most situations due to the low radical flux encountered in most biological systems and steric/electronic interactions) and reaction with O_2 to give a peroxyl radical (reaction (2)). The fate of such peroxyl radicals is not fully elucidated. One major pathway appears to be via an elimination reaction that releases $HO_2^{\bullet}$ and generates an imine, which subsequently undergoes hydrolysis and hence gives rise to backbone fragmentation (reaction (3) [13]). An alternative fate appears to be hydrogen atom abstraction from another species to give rise to a hydroperoxide (reaction (4) [14]). Subsequent decomposition of these hydroperoxides to radicals can also result in backbone fragmentation via an alkoxyl-radical mediated process (reaction (4) [14]); this gives rise to alternative products to the imine pathway. The key role of peroxyl radicals in this chemistry is underscored by the observation that little backbone fragmentation is detected in the absence of O_2 [13].



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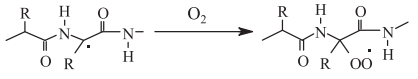

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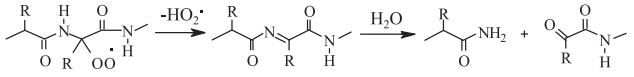

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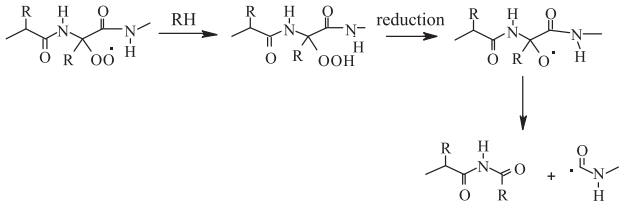


(4)

Reducing species (such as hydrated electrons and other powerful reductants) can give rise to electron attachment at the carbonyl group of the peptide bond (reviewed in Ref. [13])—this is, however, a relatively specialised situation as many of these reducing species also react at diffusion-controlled rates with O_2 (see data in Ref. [3]) and this reaction would be expected to compete effectively except under anoxic conditions.

4. Sites and selectivity of damage on proteins—overview of side-chain damage

The 20 different types of amino acids side-chains (excluding unusual amino acids and any post-translational modifications) result in a huge variety of potential reaction sites and products on reaction with oxidants. For ease of discussion, these have been split into four different catego-

ries—aliphatic, aromatic, Cys and cystine residues, and Met. The first three of these are discussed briefly, whereas the oxidation of Met, which is the subject of this special edition, is discussed in more depth in a separate section.

4.1. Aliphatic residues

Reaction with most of the aliphatic residues in proteins occurs to a major extent with only the most reactive of radicals, though there are some exceptions. One of the most notable is the two-electron halogen-transfer reactions of hypohalous acids (HOCl, HOBr) with Lys and Arg residues (and to a much lesser extent Asn and Gln) to give the corresponding unstable chloramines/chloramides and bromamines/bromamides (Table 3; Refs. [9,11,15]). Subsequent decomposition of these species can give rise to both nitrogen-centred radicals and carbonyl groups; this chemistry has been reviewed [16].

The majority of the radical-mediated reactions are hydrogen atom abstraction processes that give rise to carbon-centred radicals. With highly reactive radicals such as $HO^{\bullet}$, reaction can occur at all available sites, resulting in the formation of a range of radicals. The ratio of intermediates formed at different C–H positions varies with the attacking radical, with limited information available on the ratio of attack at different sites from (direct, rapid-flow) EPR studies ([17], see also data reviewed in Ref. [18]).

There appear to be three major fates for these carbon-centred radicals. Firstly, dimerisation with another radical—this is likely to be an uncommon reaction in the presence of O_2 (see below) and in situations where the radical flux is low. Such processes may however be of significance in anoxic systems where cross-links are detected within and between proteins and peptides; these reactions may play a major role in this aggregation. Secondly, it has been demonstrated that carbon-centred radicals can be repaired, albeit at a relatively slow rate, by thiols with resulting formation of a thiyl radical [19–21]. It should be noted that these reactions are equilibria and the extent of repair, versus oxidation of the side-chain by thiyl radicals, is dependent on the exact nature of the species involved. The third major pathway, and probably the most important in oxygenated conditions, is reaction with O_2 to give a peroxyl radical. This type of reaction usually occurs at, or near, the diffusion-controlled limit (i.e. k ca. 10^9 dm^3 mol^{-1} s^{-1} [5,7]) and hence competes effectively against most other reactions even at the low oxygen tensions in metabolically active tissues.

The fate of peroxyl radicals formed on proteins is incompletely defined. It is known from solution studies with model compounds that such species can undergo a range of radical–radical termination reactions that can generate alcohols and carbonyl compounds (see Table 3), or alternatively alkoxyl radicals and O_2. Alkoxyl radicals once formed can undergo rapid hydrogen atom abstraction reactions (to give alcohols) or rearrangement/fragmentation reactions which give carbonyl compounds. These processes have been reviewed [18,22]. The quantitative significance of such radical–radical reactions in biological systems may be limited due to low radical fluxes and the high concentration of other targets with which these peroxyl radicals can undergo hydrogen atom abstraction reactions; the latter yield hydroperoxides and a further radical species on the oxidised target. These reactions constitute propagation processes in protein chain oxidation reactions [18,22,23]. Evidence has been presented for the oxidation of a wide range of other biological targets by such peroxides including DNA [24–27], lipids (Davies et al, unpublished data) and other proteins [28,29]. These reactions can occur via both radical and non-radical (two-electron) reactions.

A number of studies have quantified the yield of hydroperoxide groups formed on oxidised amino acids, peptides and proteins, and it is clear that these materials are major products with many side-chains and a wide

Table 3
Stable and unstable products formed on oxidation of aliphatic and sulfur-containing amino acids and protein side-chains

Amino acid	Product
Glutamic acid	hydroperoxides (unstable)
Leucine	hydroperoxides (unstable)
	alcohols
	α-ketoisocaproic acid
	isovaleric acid
	isovaleraldehyde
	isovaleraldehyde oxime
	carbonyls
Glycine	aminomalonic acid
Valine	hydroperoxides (unstable)
	alcohols
	carbonyl compounds
Lysine	hydroperoxides (unstable)
	alcohols
	chloramines (unstable—from HOCl)
	bromamines (unstable—from HOBr)
	carbonyl compounds
Proline	hydroperoxides (unstable)
	alcohols
	5-hydroxy-2-aminovaleric acid
	carbonyl compounds
Arginine	hydroperoxides (unstable)
	5-hydroxy-2-aminovaleric acid
	carbonyl compounds
	chloramines (unstable—from HOCl)
	bromamines (unstable—from HOBr)
Isoleucine	hydroperoxides (unstable)
	alcohols
	carbonyl compounds
Methionine	methionine sulfoxide
	methionine sulfone
Cysteine	cystine (disulfide)
	oxyacids (RSO_2H, RSO_3H)
	sulfonamides
	sulfenyl chloride (unstable—from HOCl)

Selected data from Refs. [18,39].

range of oxidising systems (e.g. γ-radiation, X-rays, UV light, visible light in the presence of a sensitizer, metal ion-peroxide or metal ion-ascorbate systems, peroxyl radical sources, peroxynitrite, activated white cells, and hemoprotein–peroxide systems) (Table 3) [30–32]. Quantification of the peroxides based on the initial oxidant concentrations have given yields of up to 70% [25,26,31,32]. Decomposition of these hydroperoxides has been shown to give rise to further radicals (alkoxyl, carbon-centred and superoxide radicals) as well as further alcohols and carbonyl compounds [14,23,33,34]. Further information on the formation and subsequent reactions of these peroxides can be found in [18,22]. The formation of carbonyl compounds has been employed as a generic marker of protein oxidation both in vitro and in vivo, with a number of assays developed for the quantitation of these species (e.g. Refs. [35–37]). Care has to be taken in using such methods with complex biological samples, such as those from diabetic patients, where glycation and glycoxidation reactions can give rise to erroneous data (see discussion in Refs. [38,39]). Specific alcohols have also been used as stable markers of oxidative damage (e.g. Refs. [33,34,40]). This area has been reviewed [18,22,39,41,42].

4.2. Aromatic residues

The major reaction of most oxidants with aromatic side-chains is addition, though substitution reactions are known in the case of reaction at heteroatom substituents (e.g. reaction of HOCl with His residues gives short-lived *N*-chlorinated materials [16]). Although electron transfer reactions to give transient radical-cations are known with powerful oxidants (e.g. with $SO_4^{-\bullet}$ [43]), these are likely to occur via very rapid addition–elimination sequences. These radical-cations usually react very rapidly with water, and hence give rise to hydroxylated products, though some fragmentation and other reactions are also known [43].

Reaction of a wide range of radicals and other oxidants (e.g. peroxidase–H_2O_2 systems) with Tyr residues gives rise to Tyr phenoxyl radicals. This can occur via either direct oxidation of the ring and subsequent deprotonation at the hydroxyl group, or via addition–elimination reactions (e.g. with $HO^{\bullet}$). One of the major fates of this radical is self-dimerisation, with the formation of the cross-linked product di-tyrosine [18,22,38]. This can result in the formation of intra- or inter-molecular cross-links in proteins [18,22]. An alternative fate for such phenoxyl radicals is reaction with a hydrogen atom donor with resultant repair of the amino acid lesion; such reactions can occur with a range of compounds including thiols, other phenols and ascorbate [18,22,38].

As addition to the major reaction with these residues, the range of products that can be formed is very diverse as most of the products incorporate elements of the original oxidant. Thus reaction of chlorinating species (HOCl or Cl_2) gives rise to chlorinated aromatics such as 3-chlorotyrosine and chlorinated tryptophan, and reaction with nitrating species (e.g. ONOOH, NO_2^+) gives nitrated products [18,22,38,39]. The majority of these reactions are unaffected (or only marginally affected) by the presence of O_2, so a similar spectrum of materials is often observed under both oxic and anoxic conditions. The nature and mechanisms of formation of a number of these species have been determined and this information is reviewed in Refs. [18,22]. A number of the products formed (e.g. Table 4) have been employed as stable markers of protein oxidation in vitro and in vivo (reviewed in Refs. [39,44,45]).

Table 4
Stable and unstable products formed on oxidation of aromatic and related amino acids and protein side-chains

Amino acid	Product
Phenylalanine	ortho-tyrosine (2-hydroxyphenylalanine)
	meta-tyrosine (3-hydroxyphenylalanine)
	tyrosine
Tyrosine	3,4-dihydroxyphenylalanine (DOPA) (unstable)
	di-tyrosine (carbon-carbon dimer and carbon-oxygen dimer, and higher species)
	3-chlorotyrosine
	3,5-dichlorotyrosine
	3-bromotyrosine
	3,5-dibromotyrosine
	3-nitrotyrosine
	hydroperoxides (unstable—from $^1O_2$)
	alcohols and cyclized products (from $^1O_2$)
Tryptophan	*N*-formylkynurenine
	kynurenine
	5-hydroxytryptophan
	7-hydroxytryptophan
	hydroperoxides (unstable—from $^1O_2$)
	alcohol and cyclized products (from $^1O_2$)
Histidine	2-oxohistidine
	chlorinated materials (unstable—from HOCl)
	hydroperoxides (unstable—from $^1O_2$)
	alcohol and carbonyl products (from $^1O_2$)

Selected data from Refs. [18,39,183–185].

4.3. Cysteine and cystine residues

The ease of oxidation of sulfur centres makes Cys, cystine and Met residues major sites of oxidation within proteins. Such oxidation can arise either via direct interaction with the oxidant, or via radical transfer processes within a protein after oxidation at remote sites; these transfer reactions, which can also be thought of as repair processes, are discussed further below.

Oxidation of Cys residues is facile with a very wide range of oxidants. Reaction can occur via both one- (radical-mediated) and two-electron processes; the end products of both types of reaction are often similar (Table 3). The rate of oxidation varies enormously with the oxidant involved, and rate constants are known for a wide

range of these processes (see references above for specific values). With most radical oxidants, thiyl radicals ($RS^{\bullet}$) are formed. The fate of these species is complex with dimerisation to give the disulfide (cystine) and reaction with O_2 to give a peroxyl radical being competitive processes [46]. The latter reaction is usually in equilibrium with the reverse reaction, so the quantitative significance of these two processes is dependent on the conditions involved. The ultimate fate of the peroxyl radical is usually formation of oxyacids (RSO_2H and RSO_3H), though this chemistry is complex and incompletely understood for proteins [46]. Reaction of an initial thiyl radical with another radical can occur with a range of other species so mixed dimers are a common product. As these reactions involve the interaction of two radicals, the rate of these processes is very situation-dependent, and is dramatically affected by, for example, steric factors when a thiyl radical is formed on a protein.

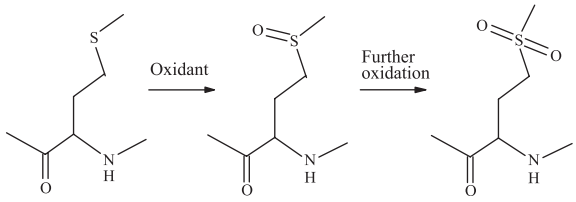
Reaction of Cys residues with two-electron oxidants often results in the initial formation of an adduct species (e.g. RSCl species from reaction of Cys with HOCl or Cl_2, RSNO species from Cys with NO^+, RSOH from Cys plus peroxides). Many of these species are short-lived and undergo hydrolysis reactions to give oxyacids (as above) or reaction with another thiol group to give a dimer species (e.g. cystine or mixed disulfide). Such disulfides are one of the few lesions generated on proteins by oxidation that can be readily repaired, as most biological systems have efficient reductase and disulfide isomerase systems to maintain Cys residues in their reduced form [22,38]. There is now considerable evidence that the controlled oxidation of Cys residues, and reduction of cystine, constitutes a redox switching mechanism that controls the structure and function of a number of key proteins (reviewed in Ref. [47]).

Oxidation of cystine residues is less well studied than Cys, but this can also occur readily, and may involve either one- or two-electron chemistry. Oxidation by many reactions involves initial formation of an adduct species and rapid subsequent reaction to give (ultimately) oxygenated species such as the mono-sulfoxide (RSS(=O)R) [18]. Radical oxidation can also give rise to formation of carbon-centred radicals at the neighbouring alpha carbon site, and some evidence has been obtained for cleavage of the disulfide bond [18,22,38].

5. Sites and selectivity of damage on proteins—oxidation of methionine residues

The thioether group (R-S-R′) function of Met residues is readily oxidised by a large number of species due to its low oxidation potential. Rate constants for oxidation of Met by a number of these species are given in Table 5. Comparison of the data in this table with the data for other amino acid side-chains and the backbone (cf. data in Table 2), shows that this side-chain is a major target for many oxidants. Of particular note is the high rate constant for molecular reaction of free Met with HOCl and HOBr, which underlies the common usage of this material as a scavenger of these oxidants (e.g. Ref. [16]).

The major product that arises from oxidation of this residue in most peptides, or proteins, is the corresponding sulfoxide (reaction (5)); further oxidation of this species gives the sulfone, though this occurs to a much lesser extent (e.g. Ref. [48]). The sulfoxide is the major product with a wide range of oxidants regardless of whether the initiating species is a radical or a two-electron oxidant, though the mechanisms by which this species is formed are



(5)

obviously different. It is important to note that two stereoisomers are generated of the sulfoxide group- the S- (D-) and R- (L-) forms [49–51], with this having important repercussions for the repair of this lesion as reduction is stereospecific (see Section 8 below). The ratio of the two isomers has been shown to vary between different oxidants, and also between different Met residues on proteins; current evidence suggests that the protein structure, at least in vitro, is the most important controlling factor in determining

Table 5
Rate constants for reaction of oxidants with methionine at pH ca. 7 in water (except where indicated)

Oxidant	Rate constant $dm^3\ mol^{-1}\ s^{-1}$
$HO^{\bullet}$	$7.4–8.5\times10^9$
$H^{\bullet}$ (at acidic pH)	3.5×10^8
$HOO^{\bullet}$	$<4.9\times10^1$
$O_2^{-\bullet}$	<0.33
$Cl_2^{-\bullet}$ (at acidic pH)	3.9×10^9
$Br_2^{-\bullet}$	2.5×10^9
$I_2^{-\bullet}$	$<1\times10^6$
$N_3^{\bullet}$	$<1\times10^6$
$SO_4^{-\bullet}$	1.1×10^9
$CO_3^{-\bullet}$	$2\times10^7–1.2\times10^8$
e_{aq}^-	$3.5–4.5\times10^7$
$(SCN)_2^{-\bullet}$	$<1\times10^6$
$CCl_3OO^{\bullet}$ (in H_2O/isopropanol)	2.9×10^7
$CF_3CHClOO^{\bullet}$ (in H_2O/isopropanol)	1.4×10^6
Singlet oxygen	$8.6\times10^6–4.2\times10^7$
Ozone	$6.3\times10^4–5\times10^6$
Hydrogen peroxide	6×10^{-3}
HOCl	3.8×10^7
HOBr	3.6×10^6
Various chloramines	$3.9\times10^1–1.97\times10^2$
ONOOH	2×10^3
$ONOO^-$	0.2

Selected data from NDRL/NIST database (http://kinetics.nist.gov/solution/index.php) and Refs. [9,11,67,186–188].

which stereoisomer predominates at any particular Met residue within a protein [50]. Whether this is also true in vivo is more difficult to ascertain as repair and protein turnover can affect the population detected.

With powerfully oxidising radicals, such as $HO^{\bullet}$, the initial reaction is addition at the sulfur atom to give a transient R-$^{\bullet}$S(OH)-R (hydroxy sulfuranyl) adduct species. This adduct undergoes further rapid reactions which ultimately give rise to the sulfoxide, though this can occur via multiple pathways. It is well established that the initial adduct species can lose HO^- to give a monomeric radical cation; this can dimerise with an additional parent molecule to give a dimeric radical cation which contains a two-centre, three-electron bond [52,53]. The monomeric radical-cation species can undergo reaction with superoxide radicals to give the sulfoxide [54], or can be stabilised by other nucleophiles including amine, hydroxyl, halide and carboxyl groups; such reactions result in the formation of further short-lived species containing S-N, S-O, and S-halide two-centre, three-electron bonds [55,56]. As might be expected, the formation of all of these species is critically dependent on the neighbouring residues and the sequence of a peptide [56–58]. The chemistry of these species is reviewed extensively elsewhere in this volume by C. Schoneich, and hence will not be covered further here. A limited extent of oxidation is also seen at the C–H positions flanking the sulfur atom with formation of carbon-centred radicals [53]. These species undergo typical carbon-centred radical chemistry (see above).

With two electron oxidants short-lived adduct species are also likely to be generated, though direct evidence for some of these species is lacking. Thus reaction of Met residues with H_2O_2, or other peroxides, is believed to occur via the formation of R-S^+(OH)-R species which rapidly deprotonate to give the sulfoxide, and reaction with hypohalous acids (HOCl, HOBr) are likely to occur via analogous R-S^+(Cl/Br)-R species with subsequent rapid hydrolysis. The mechanisms of oxidation of Met by a range of other oxidants are less well characterised. With $^1O_2$, the intermediacy of a zwitterionic peroxidic adduct species with structure R-S^+(OO^-)-R has been proposed. Subsequent reaction of this species with a second molecule of Met has been shown to result in the formation of two molecules of the sulfoxide [59,60]. Whether similar reactions occur on proteins where the initial zwitterion may be sterically isolated, and poorly accessible to a second Met molecule, is unclear, though there is good evidence for the formation of the sulfoxide as the final product [50].

Exposure of peptides and proteins containing Met residues to ozone (O_3) has been shown to give rise to consumption of the parent amino acid and generation of the sulfoxide [61–64]. In some of these systems, but not others [61,62], oxidation appears to occur selectively at this residue, even in the presence of lipids [63]. The mechanism by which the sulfoxide is formed in these reactions is not completely clear, though evidence has also been presented for the formation of singlet oxygen in these reactions, in near quantitative yield, consistent with cleavage of an initial adduct species into the sulfoxide and singlet oxygen [65].

Peroxynitrite reacts rapidly with Met residues and gives rise to both the sulfoxide and ethylene (ethene) [66]. These oxidations could potentially occur via three possible processes. The first of these involves the formation of $HO^{\bullet}$ and $NO_2^{\bullet}$ from decomposition of ONOOH, and subsequent reaction of these with the target; such reactions may be the source of ethylene (though see below) [66]. The second mechanism is via direct reaction of the protonated form of peroxynitrite with Met, probably via the formation of an initial adduct R-S^+(OH)-R species with release of NO_2^-, and subsequent deprotonation of the former to give the sulfoxide as described above, and the third process is via addition of the peroxynitrite anion to the sulfur function and subsequent decomposition of this species to the sulfoxide. Of the latter two pathways, the reaction involving ONOOH occurs at a more rapid rate than with $ONOO^-$ (see Table 5) [66,67]. Studies using a range of compounds that are known to react rapidly with hydroxyl radicals, have shown that the presence of these materials has only marginal effects on the formation of both the sulfoxide and ethylene, consistent with "free" radicals (as opposed to caged radicals) being only minor contributors [66].

In the presence of physiological levels of CO_2 an adduct is formed with $ONOO^-$. The resulting $ONOOCO_2^-$ has been shown to oxidise Met at a much slower rate than ONOOH or $ONOO^-$, suggesting that the extent of Met oxidation observed in vivo will be modulated to a major extent by local CO_2 levels [68,69]. An analogue of Met where the sulfur centre is replaced by a selenium atom (MetSe), reacts with $ONOO^-$/ONOOH much more rapidly than the parent amino acid, with the rate constants reported to be 10- to 1000-fold higher [67]. These reactions occur by two pathways—one yields the selenoxide (i.e. the analogue of the sulfoxide), whereas the other gives rise to ethylene (ethene); the former has been reported to be a two-electron process, whereas the latter product has been postulated to arise via radical reactions [67]. The rapid kinetics of reaction of this seleno analogue rationalise the protective effect of this species [67,70].

Direct two-electron oxidation of Met residues (both free and within proteins) by peroxides has been the subject of considerable interest, as this process offers a mechanism by which peroxides (e.g. H_2O_2, lipid- and protein-peroxides) can initiate oxidative damage in the absence of metal ions that catalyse radical formation. The presence of redox-active metal ions in normal and pathological samples has been the subject of considerable controversy; there is now a reasonable consensus that the levels of "free", redox-active, iron and copper in most tissues is minimal under normal conditions, but that low levels of these materials can be present in pathological samples (see, e.g. Ref. [71]). It is well established that H_2O_2 can oxidise Met residues in the

active sites of a number of enzymes and that such oxidation can result in loss of enzyme activity (reviewed in Refs. [72,73]). Alkyl peroxides, such as ^{t}BuOOH, can also induce such Met oxidation in some proteins [74], but not others [75]. Free Met residues can be oxidised by some peptide and protein peroxides [76], but not others [77]; this probably relates to the identity of the species involved and their position within the protein structure. Lipid peroxides can also oxidise protein-bound Met residues to the sulfoxide; this has been demonstrated with (poorly characterised) peroxidised lipids [78], oxidised lipid–protein mixtures [48], and lipid hydroperoxides in low- and high-density lipoproteins [79–81].

6. Transfer of damage within proteins, and from proteins to other targets

There is now good evidence from a number of studies for the transfer of damage within proteins, and from proteins to other biological targets. Such reactions can occur with a wide range of species including Met residues. The following discussion will provide a brief overview of transfer reactions involving residues other than Met, before discussing the involvement of the latter in more detail.

Evidence has been presented for the transfer of damage from initial side-chain sites to the backbone α-carbon position in peptides and proteins as a result of the stability of the delocalised α-carbon radical [82–86]. Thus alkoxyl radicals formed at the β-carbon (C-3 position) have been shown to undergo rapid β-scission to give an α-carbon radical with the release of the former side-chain as an aldehyde or ketone [87–89]. These reactions can occur in high yield and have been shown to occur with a number of different initial side-chains and on intact proteins exposed to a wide range of oxidants [87–89]. The resulting α-carbon radical would be expected to result in backbone cleavage (see above) thereby providing a mechanism by which initial side-chain oxidation can be converted into backbone fragmentation. Abstraction of the hydrogen atom at the α-carbon position would also be expected to occur with alkoxyl radicals at more remote positions, particularly when favourable five- or six-membered transition states can be formed. These processes may provide alternative methods of transferring damage from the side-chain to the backbone.

Thiyl radicals have been shown to be capable of abstracting α-carbon hydrogen atoms though these reactions are finely balanced thermodynamically and are usually equilibrium processes [90–92]. Thus the extent of "repair" of α-carbon radicals by thiols is balanced by the potential induction of backbone cleavage by thiyl species. The extent of these reactions is markedly affected by the occurrence of alternative reactions of the thiyl radical, as well as polar and conformational effects. This area has been reviewed elsewhere [46,93].

Side-chain to side-chain transfer reactions have been reported, with the majority of these involving one-electron reactions. A number of processes have been identified which involve transfer from the initial site of oxidation to readily oxidised residues such as Trp, Tyr, His, Cys, cystine and Met residues. These processes have been shown to occur over very large distances in suitable protein structures and are known to be important in a number of enzyme activities (reviewed in Refs. [94,95]). A number of these processes are equilibria, and the thermodynamics and rate constants for these cascade reactions have been determined (see, e.g. Refs. [96–103]). Electron transfer reactions have also been shown to occur to oxidised heme groups from surrounding proteins structures with this resulting in the formation of Tyr, Trp, His, and Cys derived radicals [104–111].

A number of studies have shown that radical transfer reactions can occur from other residues, such as Tyr and Trp, to oxidised Met residues in peptides and proteins. These include Met-derived species such as the initial adduct species formed with the hydroxyl radical, the corresponding monomer or dimeric radical cations formed by one-electron oxidation, and sulfur–oxygen/sulfur–nitrogen (two-centre/three electron) species [100,112]. These studies have been reviewed [97] and are discussed in more detail elsewhere in this volume by C. Schoneich.

It has been shown that initial oxidation of Met residues by hydroxyl radicals can result in subsequent fragmentation of N-terminal Ser and Thr residues as a result of intramolecular proton transfer from these residues to the initial hydroxy sulfuranyl radical formed from the Met residue [113]. This type of reaction is however limited in scope to free N-terminal residues of this type and hence these processes are relatively rare [113]. These studies have however been extended to examine potential transfer reactions of Met radical cations formed by one-electron oxidation of the sulfur function. These species can be stabilised by oxygen and nitrogen atoms on neighbouring residues with the formation of two-centre, three-electron bonds. Sulfur–oxygen bonds are kinetically preferred, but on longer time scales, these can be converted into sulfur–nitrogen species, which can eventually be transformed into α-carbon radicals formed on the backbone [58]. This type of process is therefore another example of how initial side-chain oxidation can result in subsequent damage to the backbone of proteins and peptides.

Transfer of reducing species can also occur through proteins after initial electron attachment. These processes often involve Cys and cystine groups. These reactions have been reviewed [114,115].

The reactions discussed above are true transfer reactions in that a single oxidising (or reducing) species is transferred through the protein, with the original site of oxidation (or reduction) being repaired by the transfer reaction; in other cases (see below) radical transfer can occur which results in residual alteration at the initial site of oxidation, and hence damage at multiple sites.

7. Consequences of oxidation of proteins

It is well established that oxidation of proteins can have a wide range of downstream consequences. The general principles are reviewed briefly here, before a more extended discussion of specific effects arising from Met oxidation in peptides and proteins.

7.1. Fragmentation and dimerisation

As will be obvious from the above sections, oxidation can result in major physical changes in protein structure ranging from fragmentation of the backbone to oxidation of the side-chains. The latter can result in either direct, or subsequent, dimerisation or aggregation. Direct cross-linking can occur via intermolecular radical–radical dimerisation reactions of Tyr phenoxyl radicals to give inter-protein di-tyrosine cross-links. Secondary dimerisation has been proposed to occur via a number of reactions, including reactions of amine-containing (e.g. Lys and Arg) residues with carbonyl groups arising from oxidation reactions, via (uncharacterised) His oxidation products, and from the reaction of quinones (e.g. arising from oxidation of DOPA generated from Tyr residues) with nucleophiles such as Cys on other proteins [116–121]. The exact nature of some of these cross-linking reactions, and the species formed, remains to be established.

7.2. Formation of further reactive species and the occurrence of chain reactions

Oxidation of both the backbone and the side-chains can result in the formation of further reactive species. These include the formation of hydroperoxides or peroxides, chloramines/chloramides and bromamines/bromamides, DOPA (and quinone species derived from this), and other short-lived intermediates such as R-S-X and R-S^{+}(X)-R species (where X=OH, Cl, Br, etc.). The peroxides can arise from hydrogen abstraction reactions of peroxyl radicals formed at either the α-carbon site or at C–H bonds on the side-chain [14,23]. They can also be formed from reaction of Trp, Tyr and His side-chains with $^{1}O_2$ (reviewed in Ref. [122]). It has been shown that reaction of peroxides, chloramines/chloramides, and bromamines/bromamides with reducing metal ions, or UV light can give rise to further radicals [14–16,23]. Some of these decomposition reactions, particularly those of the chloramines/chloramides, and possibly the related bromo species as well, can be catalysed by superoxide radicals, which may be of particular significance at sites of inflammation where both HOCl and superoxide radicals are generated simultaneously [123]. Reaction of DOPA formed from Tyr (either as the free amino acid, or on proteins), with oxidising metal ions (e.g. Fe^{3+} or Cu^{2+}) can result in semi-quinone radical-anion formation [30,124]. Such radicals react rapidly with molecular O_2 to give superoxide radicals (and hence H_2O_2 on dismutation). The chemistry of these quinone/semi-quinone/diol systems has been reviewed [125,126].

It should be noted that each of these processes results in the formation of further reactive species and hence that protein oxidation can occur via chain reactions (i.e. oxidation of multiple sites per single initiating event). The length of the chains that are formed are likely to be much shorter than in lipid peroxidation, as chain propagation is more complex and less efficient, but it is clear from the limited data already available that the yield of protein oxidation products can be larger than the number of initiating species (e.g. Refs. [87,127]). These reactive species can also induce damage to other biomolecules including DNA and other proteins via both radical and molecular reactions [24–29,128].

7.3. Unfolding and conformational changes, and effects on structure and function

It is well established that oxidation of protein side-chains can give rise to unfolding and conformational changes in proteins, and that this can have consequential effects on biological function. A number of studies have suggested that oxidation of surface-exposed residues has less influence on protein conformation than oxidation of buried residues, though the latter are often much less rapidly oxidised (and often require harsher conditions) than surface-exposed residues when the initiating oxidant is present in the bulk aqueous phase (e.g. Refs. [129–131]). The majority of oxidation processes that occur on side-chains, result in an increase in hydrophilicity of the target residue. There are some notable exceptions including the decarboxylation of Asp/Glu residues [23] and deamination of Lys residues [15] induced by hydroperoxide decomposition and chloramine decomposition, respectively. Thus many C–H bonds on aliphatic (hydrophobic) side-chains are converted into peroxide, alcohol or carbonyl groups that have larger dipole moments and can participate in hydrogen bonding to solvent molecules. Oxidation of many aromatic residues can also result in the incorporation of polar or charged groups (e.g. hydroxyl-, peroxyl-, nitro-, chloro-, and bromo-substituents, amongst others). Thus there is a driving force for such oxidised residues to be present on an external, water-accessible, surface of the protein and this can result in changes in protein conformation [38,132].

The above general arguments are also true for Met residues. Thus many solvent accessible Met residues are much more readily oxidised than buried species (e.g. Refs. [129–131]), but oxidation of exposed residues does not appear to have marked effects on the conformation of most proteins [131], unless this residue is within an enzyme active site or in a binding pocket (e.g. in α1-proteinase inhibitor [133], reviewed in Ref. [72]). The Met side-chain is however relatively non-polar when compared to many other amino acid side-chains, and is therefore often found buried within hydrophobic regions of protein structures, and

its conversion to the more polar sulfoxide can have marked effects on conformation [131,132]. A commonly used polarity scale, which employs calculated free energies for the transfer of a residue from an α-helix from inside a membrane interior to the aqueous phase, has Met side-chains having the second highest transfer free energy, with only Phe having a higher value [134]. Conversion of Met to the sulfoxide would be expected to decrease this value dramatically and consequentially there is driving force for alteration to the protein structure that places this oxidised residue in a more polar environment. Thus it is not surprising that oxidation of initially buried Met residues can have consequential effects on protein structure; the flexibility of this side-chain may also allow local changes in structure to occur more readily than with larger rigid groups, where greater changes in overall structure might be required to allow access of the aqueous phase to the oxidised residue.

It has been shown for example that the oxidation of surface-exposed Met residues on a number of proteins, including Ca^{2+}-dependent modulator protein [129], has no effect on the structure of these proteins. In contrast, oxidation of other Met residues can result in detectable changes in structure and/or interaction with other materials. Oxidation of one of the eight Met residues (Met385) in α1-proteinase inhibitor results in the loss of the inhibition of elastase by this species. Similarly, oxidation of one of the vicinal (Met146 or Met147) residues in the C-terminal domain of calmodulin has been shown to give rise to a 30-fold decrease in calcium affinity, and the extent of oxidative modification correlates with the loss of calmodulin-dependent activation of plasma membrane Ca^{2+}-ATPase [135]. Oxidation of Met residues present at positions 412, 432 and 446 in plasma fibronectin decreases the interaction of this material with gelatin; the presence of the latter compound prevents the oxidation of Met residues in fibronectin implying their presence within the binding site [136]. Oxidation of Met residues in actin have also been shown to prevent actin polymerisation, and to induce changes in the stability of actin filaments [137]. Oxidation of Met residues in surface-exposed helices of small heat shock proteins has also been reported to result in the loss of chaperone activity [138].

Oxidation of the Met ligand (Met-80) at the heme centre in cytochrome *c* does not affect its role as a substrate for cytochrome *c* oxidase, and the oxidised species does not autoxidise at neutral pH. The reduction potential of the protein is however altered with this changing from 260 mV in the native form to 184 mV in the oxidised material. This oxidised form is almost completely inactive with succinate-cytochrome *c* reductase however, and it therefore appears that one of the major roles of the Met residue is to maintain a closed hydrophobic pocket which is essential for the maintenance of the necessary reduction potential of the protein [139]. In some cases the key role of Met oxidation in the loss of activity has been addressed by use of site-directed mutagenesis; thus with subtilisin, mutagenesis of a key Met residue (Met222) to Ser, Ala, or Leu has been shown to make the mutant protein highly resistant to inactivation induced by high concentrations of H_2O_2 [140].

Not all oxidative events are however deleterious and a number of examples are known where oxidation of Met residues to the sulfoxide can result in an increased biological activity. Thus oxidation of the Met-1 residue of ubiquitin to the sulfoxide has been reported to give rise to a 50% increase in reactivity, whereas further oxidation to the sulfone resulted in a 50% decrease in activity at low concentrations [49]. In a similar manner, oxidation of Met residues to the sulfoxide in the native fifth component of human complement results in the conversion of this protein into its active form which binds component C6 [141].

These examples represent only a small fraction of the work that has been carried out on the effect of Met oxidation on enzyme activity; this area has been reviewed extensively, and the reader is referred to these other works for further details and examples [72,73].

7.4. Alterations in cellular handling and turnover

Oxidation of Met residues, like other side-chains, has been proposed as a marker for protein degradation by cellular machinery. It has been proposed that oxidative modification results in enhanced turnover, although there is also evidence for a decreased rate of turnover for some heavily modified proteins [142–149]. With calmodulin oxidised at specific Met residues, there is evidence for an enhanced rate of degradation by the 20S proteosome [150], with the rate of degradation dependent on the extent of modification of the protein (as assessed by the extent of Met oxidation) and decrease in secondary structure (as detected by circular dichroism and fluorescence spectroscopy) [150]. In contrast, when the surface hydrophobicity of the protein was altered, the rate of degradation was not enhanced, suggesting that specific changes need to occur before the modified protein is recognised and subject to degradation [150].

8. Repair of protein damage

Most of the protein damage outlined above (see, e.g. Tables 3 and 4) does not appear to be repaired by cells, and the fate of proteins which contain such oxidative damage is catabolism (reviewed in Refs. [22,38,143,146]). The only exceptions appear to be the formation of cystine, and mixed disulfides, which can be readily reduced back to the corresponding thiols by a battery of reductases and isomerases, and Met sulfoxide. A number of proteins have been characterised, originally in *E. coli* and later in many other cell types including mammalian, which are able to reduce Met sulfoxide back to the parent amino acid [151–161]. Some of these methionine sulfoxide reductase (Msr) proteins are specific for free Met sulfoxide, whereas others

reduce only the peptide-/protein-bound species [151–161]. The latter form is known to be present intra-cellularly in many cell types, and some isoforms have been shown to be membrane-associated (e.g. Ref. [156]), but Met sulfoxide reducing activity does not appear to be present extracellularly [152–154,161]; if this is correct, quantification of Met sulfoxide levels in plasma may be a potentially useful marker of in vivo oxidative damage (see below). There is good evidence that the isoforms of this enzyme are stereospecific with the MsrA isoform only reducing the S-(D-) isomer, and the more recently discovered MsrB form reducing the R-(L-) form [50,51,155,156,158–160]. This topic area is reviewed elsewhere in this volume by Moskovitz, and also previously [152–154,157,159]. The (at least partial) reversibility of Met oxidation to the sulfoxide has resulted in the suggestion that Met residues on proteins can act as sacrificial antioxidants [131,159,162], and that the interconversion of Met to Met sulfoxide may act as a reversible redox switch to control the activity and function of proteins [73]. The recent development of a Met sulfoxide reductase knock-out mouse should allow these facets to be explored in greater detail in vivo [163].

Evidence for the key role that Met sulfoxide reductase can play in protecting cells and tissues against oxidative damage has been provided by studies where this enzyme has been knocked out or over-expressed. Thus over-expression of peptide Met sulfoxide reductase in yeast (and also in human T lymphocytes) has been shown to make these organisms resistant to a range of oxidant stresses (e.g. H_2O_2, paraquat and peroxyl radicals), but not to UVA radiation exposure [164]. The latter insult also results in Met oxidation [165–167], suggesting that some damage to Met residues is not functionally deleterious [164]. Conversely, a null mutant strain with non-functional Met sulfoxide reductase has been shown to be more sensitive to oxidative stress and to contain higher levels of free and protein-bound Met sulfoxide [153].

9. Methionine oxidation in disease

As might be expected from the above discussion, elevated levels of Met sulfoxide have been detected in a number of human diseases and other in vitro and in vivo systems. A number of these are reviewed elsewhere in this volume, so only a few selected examples are listed here. Elevated levels of Met sulfoxide have been detected in tissues (lungs, heart, lived, kidney and jejunum) from rats subjected to high doses of ionising radiation [168], in ageing human erythrocytes [169], in bronchoalveolar lavage fluid from smokers [170], in crystallin and other lens proteins from human and animal eyes with increasing age [171] and increasing oxidative stress [172–174], in cataractous human lenses [175] with this increasing as the disease progresses [176], in human skin collagen with increasing age (but not diabetes) [177], in calmodulin obtained from young versus old animals [178,179], in human plasma samples from patients with inflammatory disorders (P.E. Morgan, A. Sturgess, and M.J. Davies, unpublished data), on proteins from activated neutrophils [180,181] and in murine tissues in an animal model of iron-overload disease [182].

10. Concluding remarks

There is now good evidence that proteins are major targets for oxidants, be they radicals or molecular oxidants, and there is now a large body of evidence to suggest that protein oxidation plays a major role in a number of human diseases. Kinetic studies are consistent with Met residues in proteins being important targets for oxidation, and it is known that such oxidation can play a key role in regulating the structure, function and activity of proteins. Data is also available for the presence of elevated levels of Met sulfoxide, the major oxidation product of Met, in a number of human and animal pathologies, as well as in in vitro systems subject to increased oxidative stress. The discovery of a specific enzymatic activity which repairs this lesion—one of only two oxidative lesions on proteins which are known to be repaired—further emphasises the potential biological importance of regulation of the oxidation state of this key amino acid. The (at least partial) reversible nature of this oxidative process has also been postulated as a redox switch to control the activity of a number of key enzymatic reactions and hence cell regulation.

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