Reactive species are also associated with important mediators in a wide range of biological processes such as signaling, for proper synaptic plasticity, and normal memory [3, 6–9]. Additionally, nitroxidative species contribute to pain and central sensitization [10, 11]. The amounts of reactive species during neurodegenerative diseases and aging increase to higher levels than the antioxidants present in a cell can handle. The reactive species that participate in a large number of reactions in diseases [4, 12–17] include both free radicals and non-radical species (Table 1.1) [18–20]. Reactive oxygen species (ROS) include superoxide anion ($O_2^-$), hydroperoxyl ($HO_2^-$), alkoxyl (RO•), peroxyl (ROO•), hydroxyl radical (•OH), hydrogen peroxide ($H_2O_2$), ozone (O₃), singlet oxygen ($^1O_2$), and hypochlorous acid (HOCI). The ROS initiate many reactions, for example, the primary mitochondrial ROS, $O_2^*$, reacts with superoxide dismutase (SOD) to form $H_2O_2$, which then reacts further with metal ions or their complexes (Fenton and Fenton-like reactions) to produce •OH. Other intermediates are reactive nitrogen species (RNS), which include nitric oxide (NO•), nitrogen dioxide radical (NO2•), peroxynitrite (OONO•), peroxynitrous acid (OONOH), alkylperoxynitrite (ROONO), and nitrosyl (NO•). ROS and RNS are interconnected and cause protein damage in biological processes. $O_2^*$, NO•, and ONOO• are associated with neuroimmune activation,
<table>
<thead>
<tr>
<th>Free Radicals</th>
<th>Nonradicals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reactive oxygen species (ROS)</strong></td>
<td></td>
</tr>
<tr>
<td>Superoxide, ( \cdot \mathrm{O}_2 )</td>
<td>Hydrogen peroxide, ( \cdot \mathrm{H}_2 \mathrm{O}_2 )</td>
</tr>
<tr>
<td>Hydroxyl, ( \cdot \mathrm{OH} )</td>
<td>Hypobromous acid, ( \cdot \mathrm{HOBr} )</td>
</tr>
<tr>
<td>Hydroperoxyl, ( \cdot \mathrm{HO}_2 ) (protonated superoxide)</td>
<td>Hypochlorous acid, ( \cdot \mathrm{HOC}_\text{I} )</td>
</tr>
<tr>
<td>Carbonate, ( \cdot \mathrm{CO}_3 )</td>
<td>Ozone, ( \cdot \mathrm{O}_3 )</td>
</tr>
<tr>
<td>Peroxyl, ( \cdot \mathrm{RO}_2 )</td>
<td>Singlet ( \cdot \mathrm{O}_2 \uparrow\uparrow )</td>
</tr>
<tr>
<td>Alkoxyl, ( \cdot \mathrm{RO} )</td>
<td>Organic peroxides, ( \cdot \mathrm{ROOH} )</td>
</tr>
<tr>
<td>Carbon dioxide radical, ( \cdot \mathrm{CO}_2 )</td>
<td>Peroxynitrite, ( \cdot \mathrm{ONOO} )</td>
</tr>
<tr>
<td>Singlet ( \cdot \mathrm{O}_2 \uparrow\uparrow )</td>
<td>Peroxynitrinate, ( \cdot \mathrm{O}_3 \mathrm{NOO} )</td>
</tr>
<tr>
<td></td>
<td>Peroxynitrous acid, ( \cdot \mathrm{ONOOH} )</td>
</tr>
<tr>
<td></td>
<td>Peroxysoperoxycarbonate, ( \cdot \mathrm{HOOC}_2 )</td>
</tr>
<tr>
<td></td>
<td>Nitrosoperoxycarbonate, ( \cdot \mathrm{ONOOCO}_2 )</td>
</tr>
</tbody>
</table>

| Reactive nitrogen species (RNS) | | |
| Nitric oxide, \( \cdot \mathrm{NO} \) | Nitrous acid, \( \cdot \mathrm{HNO}_2 \) |
| Nitrogen dioxide, \( \cdot \mathrm{NO}_2 \) | Nitrosyl cation, \( \cdot \mathrm{NO}^+ \) |
| Nitrate, \( \cdot \mathrm{NO}_3 \) | Nitroxy anion, \( \cdot \mathrm{NO}^- \) |
| | Dinitrogen tetroxide, \( \cdot \mathrm{N}_2 \mathrm{O}_4 \) |
| | Dinitrogen trioxide, \( \cdot \mathrm{N}_2 \mathrm{O}_3 \) |
| | Peroxynitrite, \( \cdot \mathrm{ONOO} \) |
| | Peroxynitrinate, \( \cdot \mathrm{O}_3 \mathrm{NOO} \) |
| | Peroxynitrous acid, \( \cdot \mathrm{ONOOH} \) |
| | Nitriunm cation, \( \cdot \mathrm{NO}_2^+ \) |
| | Alkyl peroxynitrites, \( \cdot \mathrm{ROONO} \) |
| | Alkyl peroxynitrates, \( \cdot \mathrm{RO}_2 \mathrm{ONO} \) |
| | Nitryl chloride, \( \cdot \mathrm{NO}_2 \mathrm{Cl} \) |
| | Peroxyacetyl nitrate, \( \cdot \mathrm{CH}_3 \mathrm{C}(\mathrm{O})\mathrm{ONO} \) |

| Reactive chlorine species (RCS) | | |
| Atomic chlorine, \( \cdot \mathrm{Cl} \) | Hypochlorous acid, \( \cdot \mathrm{HOC}_\text{I} \) |
| | Nitryl chloride, \( \cdot \mathrm{NO}_2 \mathrm{Cl} \) |
| | Chloramines |
| | Chlorine gas (\( \cdot \mathrm{Cl}_2 \)) |
| | Bromine chloride (\( \cdot \mathrm{BrCl} \)) |
| | Chlorine dioxide (\( \cdot \mathrm{ClO}_2 \)) |

| Reactive bromine species (RBS) | | |
| Atomic bromine, \( \cdot \mathrm{Br} \) | Hypobromous acid (\( \cdot \mathrm{HOBr} \)) |
| | Bromine gas (\( \cdot \mathrm{Br}_2 \)) |
| | Bromine chloride (\( \cdot \mathrm{BrCl} \)) |

“ROS” is a collective term that includes both oxygen radicals and certain nonradicals that are oxidizing agents and/or are easily converted into radicals (\( \cdot \mathrm{HOC}_\text{I}, \cdot \mathrm{HOBr}, \cdot \mathrm{O}_3, \cdot \mathrm{ONO} \)). All oxygen radicals are ROS, but not all ROS are oxygen radicals. Peroxynitrite and \( \cdot \mathrm{H}_2 \mathrm{O}_2 \) are frequently erroneously described in the literature as free radicals, for example. “RNS” is a similar collective term that includes \( \cdot \mathrm{NO} \) and \( \cdot \mathrm{NO}_2 \) as well as nonradicals such as \( \cdot \mathrm{HNO}_2 \) and \( \cdot \mathrm{N}_2 \mathrm{O}_4 \). “Reactive” is not always an appropriate term: \( \cdot \mathrm{H}_2 \mathrm{O}_2 \), \( \cdot \mathrm{NO} \), and \( \cdot \mathrm{O}_2 \) react fast with few molecules, whereas \( \cdot \mathrm{OH} \) reacts fast with almost everything. Species such as \( \cdot \mathrm{RO}_2 \), \( \cdot \mathrm{NO}_2 \), \( \cdot \mathrm{RO} \), \( \cdot \mathrm{HOC}_\text{I}, \cdot \mathrm{HOB}_\text{r}, \cdot \mathrm{CO}_2 \), \( \cdot \mathrm{CO}_2 \), \( \cdot \mathrm{NO}_2 \), \( \cdot \mathrm{ONO}_2 \), \( \cdot \mathrm{NO}_3 \), and \( \cdot \mathrm{O}_3 \) have intermediate reactivities.

\( \cdot \mathrm{HOBr} \) and \( \cdot \mathrm{BrCl} \) could also be regarded as RCS. \( \cdot \mathrm{HOC}_\text{I} \) and \( \cdot \mathrm{HOBr} \) are often included as ROS; although \( \cdot \mathrm{HOC}_\text{I} \) is also an RCS. \( \cdot \mathrm{NO}_2 \) is also produced in vivo by myeloperoxidase and from \( \cdot \mathrm{ONO} \) \([19]\). Ozone might also be produced in vivo, although the chemistry involved is unclear \([20]\). \( \cdot \mathrm{ONO}_2 \), \( \cdot \mathrm{O}_3 \mathrm{NOO} \), and \( \cdot \mathrm{ONOOH} \) are often included as ROS but are also classifiable as RNS. \( \cdot \mathrm{NO}_2 \) can also be regarded as a RNS.

Adapted from Halliwell \([18]\) with the permission of the International Society of Neurochemistry.
supraspinal descending facilitation, and nitrooxidative stress [21]. The $\text{O}_2^\cdot$ species is produced from mitochondria and NADPH oxidase, while NOS enzymes synthesize NO$^\cdot$ through enhanced nociception and the activation of the $N$-methyl-D-aspartate receptor. Both $\text{O}_2^\cdot$ and NO$^\cdot$ form ONOO$^-$, which inactivates the glutamate transporter, manganese superoxide dismutase (MnSOD), and glutamate synthase, which increases the production of additional nitrooxidative species [10]. In addition to ROS and RNS, other reactive species also involved in various biological activities include the carbonate radical ($\text{CO}_3^\cdot$) and the organic radical, $\text{R}^\cdot$ (thiyl and protein radicals). Metals such as Cr, Mn, and Fe in their high-valent states are also involved in reactions with molecules of biological importance. Reactive intermediates may also be produced by UV radiation in the presence of oxygen [22].

1.1 DISEASES

1.1.1 Neurodegenerative Diseases

Generally, there are four common features in neurodegenerative diseases, which are interrelated with one another [23–25]. These include (1) both ROS and RNS working together to cause damage in the degenerative disease and also to create a vicious cycle by stimulating proinflammatory gene transcription in glia; (2) participation of redox-active (e.g., Cu and Fe) and redox-inactive (e.g., Zn) metal ions; (3) abnormal functioning of mitochondria; and (4) accumulation of misfolded or unfolded proteins in brain cells, which leads to Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), frontotemporal labor degeneration (FTLD), multiple sclerosis, and amyotrophic lateral sclerosis (ALS) (Table 1.2) [26]. A recent study demonstrated the role of RNS in protein misfolding, mitochondrial dysfunction, and synaptic injury [27]. Most of the folded proteins display toxicity toward cultured neuronal cells in vitro and, hence, may be related to the degeneration and loss of nerve cells in vivo. The molecular mechanism of toxic effects is not fully understood, but changes in membrane permeability, influx of Ca$^{2+}$, and oxidative damage induction, followed by apoptosis have been suggested [26].

The chemistry of neurotoxicity is presented in Figure 1.1 [23]. The $\text{O}_2^\cdot$ species is produced from mitochondrial proteins and mutationally altered or damaged proteins, which subsequently generate $\text{H}_2\text{O}_2$. The $^\cdot\text{OH}$ species is generated through reactions of $\text{H}_2\text{O}_2$ with $\text{O}_2^\cdot$ (Haber–Weiss reaction) and transition metal ion (generally Cu(I) and Fe(II)) (Fenton reaction). The resulting oxidized metal ions (Cu(II) and Fe(III)) can be reduced by cellular reductants such as thiols, vitamin C, and vitamin E. SOD also transforms $\text{O}_2^\cdot$ to O$_2$ and $\text{H}_2\text{O}_2$, while catalase (CAT) removes $\text{H}_2\text{O}_2$. Oxidation of protein side chains generate hydroxylated and carbonyl products (Chapters 4 and 5). Oxidation also occurs through halogenated species (e.g., HOCl) (Chapter 3). The
TABLE 1.2. Misfolded Proteins and Their Associated Neurodegenerative Diseases

<table>
<thead>
<tr>
<th>Protein(s)</th>
<th>Disease(s)</th>
<th>Lesion(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ and tau protein</td>
<td>AD, Down's syndrome</td>
<td>Senile plaques, NFTs</td>
</tr>
<tr>
<td>o-Synuclein</td>
<td>Parkinson's disease, dementia with Lewy bodies</td>
<td>Lewy bodies and Lewy neurites</td>
</tr>
<tr>
<td>Tau protein</td>
<td>FTLD</td>
<td>Tau inclusions</td>
</tr>
<tr>
<td>PrP</td>
<td>Transmissible prion disease</td>
<td>Amyloid plaques and prion rods</td>
</tr>
<tr>
<td>Huntington</td>
<td>HD</td>
<td>Intranuclear inclusions</td>
</tr>
<tr>
<td>ABri/ADan and tau protein</td>
<td>British and Danish familial dementias</td>
<td>Amyloid plaques and NFTs</td>
</tr>
<tr>
<td>SOD-1</td>
<td>Motor neuron disease</td>
<td>SOD-1 inclusions</td>
</tr>
<tr>
<td>TDP-43</td>
<td>FTLD, motor neuron disease</td>
<td>Intracellular inclusions</td>
</tr>
</tbody>
</table>

Mutations in the genes encoding these proteins invariably give rise to familial forms of neurodegenerative disease.

Aβ, amyloid β-peptide; ADan, Danish dementia peptide; SOD-1, superoxide dismutase 1; TDP-43, TAR DNA-binding protein 43; NFT, neurofibrillary tangle.

Adapted from Allsop et al. [26] with the permission of the Biochemical Society.

**Figure 1.1.** Scheme of oxidative stress biochemistry and neurotoxicity. HNE, 4-hydroxy-2-nonenal; ONE, 4-oxo-2-nonenal (adapted from Sayre et al. [23] with the permission of the American Chemical Society).
roles of reactive species in common neurodegenerative diseases, Alzheimers disease (AD) and Parkinson’s disease (PD), as well as in aging, are summarized below.

1.1.1.1 Alzheimers Disease. AD was first discovered in 1907 and is an irreversible disease, which causes unusual behavior, memory loss, personality changes, and a decline in the ability to concentrate [28]. AD directly affects ~10% of humans by age 65 and ~50% by age 85 [28, 29]. Two major hypotheses have been proposed to explain AD. The first suggests that an abnormal processing of the amyloid precursor protein (APP) takes place during the neurodegeneration, which results in generation, aggregation, and deposition of the Aβ peptide [30]. This process may facilitate neurofibrillary tangle (NFT) peptide formation and, consequently, cell death (see Table 1.2), and is classified as an amyloid cascade hypothesis. This hypothesis is reasonably supported by genetic studies [31–33]. APP encodes the Aβ peptide, while the protein genes, PS1 and PS2, encode transmembrane proteins. The second hypothesis suggests cytoskeletal changes take place during neurodegeneration, in which hyper-phosphorylation and aggregation of tau processes contribute to the activation of cell death [34]. The amyloid cascade hypothesis has been investigated extensively [28, 35–38]. Recently, mass spectrometry (MS) has been applied in vitro and in vivo to learn the role of the Aβ peptide in AD [39]. Studies include elucidating the structure of the Aβ peptide and its interaction with metals (e.g., Cu(II)) [28]. Copper, iron, and zinc have been determined in amyloid plaques from the brains of those with AD [40].

The size of the Aβ peptide varies from 39 to 43 amino acids, produced from the sequential β- and γ-secretase processing of APP [39]. Cu(II) binds to the Aβ peptide through tyrosine (Tyr10) and histidine (His13, His14, and His6) [41]. The Aβ complex of Cu(II) has a high positive reduction potential. The neurotoxicity of the Aβ peptide has been suggested to relate to the production of •OH in the copper-mediated oxidation of ascorbate (AScH−) in the presence of oxygen and H2O2 (Eqs. 1.1–1.4) [42]:

\[
\begin{align*}
\text{Aβ-Cu(II)} + \text{AScH}^- & \rightarrow \text{Aβ-Cu(I)} + \text{ASc}^+ + \text{H}^+ \\
\text{Aβ-Cu(II)} + \cdot + \text{ASc}^+ & \rightarrow \text{Aβ-Cu(I)} + \text{ASc} \\
\text{Aβ-Cu(I)} + \text{H}_2\text{O}_2 & \rightarrow \text{Aβ-Cu(II)} + \cdot\text{OH} + \text{OH}^- \\
\text{Aβ-Cu(I)} + \text{O}_2 & \rightarrow \text{Aβ-Cu(II)} + \text{O}_2^-.
\end{align*}
\]

1.1.1.2 Parkinson’s Disease. PD is a neurodegenerative movement disorder in which pathophysiological features include the accumulation of intracellular inclusions and degeneration and death of dopaminergic neurons of substantia nigra (SN), a part of the midbrain [43]. Mitochondrial dysfunction, oxidative stress, abnormal protein accumulation, and protein phosphorylation are important molecular mechanisms that compromise dopamine neuron function.
REACTIVE SPECIES

and survival in the pathogenesis of both sporadic and familial PD [44]. The role of ROS in sporadic PD is shown in Figure 1.2 [43]. Derangements in complex I (NADH CoQ10 reductase) and oxidative stress lead to aggregation and accumulation of \( \alpha \)-synuclein [43]. Dysfunction of complex I results in the formation of free radicals and a decrease in the level of the formation of ATP, which ultimately result in neuronal depolarization and excitotoxic neuronal injury. The involvement of RNS also increases oxidative stress and injury (see Fig. 1.2) [45].

The role and significance of glutathione (GSH) in PD has been studied in detail [36, 37, 46]. A cell has a redox equilibrium, which involves GSH and GSH disulfide [47]. Under oxidative stress, the equilibrium moves toward disulfide and, hence, depletes GSH. A significant decrease in levels of GSH in SN has been observed in PD, which lowers the activity of the mitochondrial complex I [48–50]. This process accelerates the buildup of defective proteins, which impairs the ubiquitin–proteasome pathway during protein degradation, resulting in cell death of the SN dopaminergic neurons. The presence of abnormal protein aggregates is a characteristic of neurodegeneration during PD. The role of iron in PD has been reported [49, 51]. Iron transporters, divalent metal transporter 1 (DMT1) and ferroportin, may be involved in dopaminergic brain areas where the production of \( H_2O_2 \) favors the Fenton reaction. The chelation of iron may be effective in preventing or delaying the progression of PD [52].

1.1.2 Metals in Human Diseases

In biological systems, redox metals such as Cu, Fe, Cr, and Co are involved in redox cycle reactions and generate reactive intermediates like \( O_2^- \) and \( NO^+ \), which cause the disruption of homeostasis. This creates an excess amount of
ROS and RNS over available antioxidant biomolecules that subsequently induce modification of proteins, peroxidation of lipid, and damage of DNA resulting in diseases such as cancer, cardiovascular disease, diabetes, and atherosclerosis [35]. The significance of Cu and Fe in AD and PD is described in the previous subsection. Elevated levels of copper have been detected in tissues of serum and tumors of cancer patients in comparison to healthy persons. The ratios of Cu:(Fe, Zn, and Se) have also been detected at higher levels in cancer patients than in normal subjects [53]. Copper can catalyze the formation of ROS and also decrease the levels of GSH [54, 55]. Increased levels of copper could play an important role in the development of various cancers. The interaction between Cu and homocysteine may be involved in atherosclerosis [56]. This interaction generates free radicals, which oxidize low-density lipoprotein (LDL), and oxidized products have been found in atherosclerosis plaques.

Excess levels of iron can be toxic due to its role in producing hydroxyl radicals (Fenton reaction, Fig. 1.3), which are involved in oxidizing DNA. More than 100 oxidized products, both carcinogenic and mutagenic, have been identified. The most common DNA oxidized product is 8-hydroxyguanine (Fig. 1.3) [35]. The lipid peroxidation process, involved in coronary artery disease, is catalyzed by iron [57] (Fig. 1.3). The initial formation of the peroxy radical (ROO•) rearranges into endoperoxides through a cyclization reaction, which leads to the formation of malondialdehyde (MDA). The other major product of the lipid peroxidation process is 4-hydroxy-2-nonenal. Reactions between MDA and DNA bases produce adducts (Fig. 1.3) such as M1C, M1A, and M1G formed from DNA bases cytosine, adenosine, and guanine, respectively [58]. A significant increase in the levels of 8-OH-G, 2-hydroxy-adenine, and 8-hydroxy-adenine adducts have been detected in rectum and colon biopsies [59].

Chromium(VI) at high levels is considered to be a risk to human health. Some compounds of Cr(VI) may cause skin cancer when they come in contact with skin. Further details on the toxic effects of Cr and related mechanisms are discussed in Chapter 6. Vitamin B12 is 4% cobalt by mass, which contributes to toxic and carcinogenic effects. Cobalt in the presence of O2 generates Co(I)-OO•, which, through catalysis by SOD, forms Co(I) and H2O2 [60, 61]. The reaction between Co(I) and H2O2 results in •OH, which induces damage to DNA and inhibits DNA repair [62].

The redox inactive metals (Zn, Cd, and Pb) generate toxic effects when undergoing bond formation with sulfhydryl groups of proteins and depletion of GSH. The exposure of Pb to adolescents represents a serious health threat worldwide [63]. The toxic effects of Pb include hypertension, cognitive impairments, and neurological disorders [64, 65]. Both ROS and RNS have been implicated in hypertension due to their exposure to humans [66]. A number of studies have shown arsenic is carcinogenic [67]. Adverse health effects of arsenic from contaminated water include anemia, skin lesions, peripheral
Evidence of both ROS and RNS involvement has been detected in the metabolism of arsenic [60, 69, 70]. In recent years, the use of metal nanoparticles in various applications has increased greatly and further studies are in progress to evaluate their toxicity [71, 72]. For example, silver nanoparticles and Ag\(^+\) ions have been shown to promote oxidative stress and DNA damage.

Figure 1.3. ROS formation and the lipid peroxidation process (adapted from Jomova and Valko [35] with the permission of the Elsevier Inc.). See color insert.

neuropathy, and tumors [68]. Evidence of both ROS and RNS involvement has been detected in the metabolism of arsenic [60, 69, 70].

In recent years, the use of metal nanoparticles in various applications has increased greatly and further studies are in progress to evaluate their toxicity [71, 72]. For example, silver nanoparticles and Ag\(^+\) ions have been shown to...
generate ROS [73, 74, 74–77]. It has been suggested that ROS, produced by silver nanoparticles, may disrupt the production of ATP and damage cell membranes. The specific type of DNA damage or chromosomal aberrations needs to be investigated. DNA damage response in silver nanoparticle-treated cells by the upregulation of damage response proteins may be identified by conducting protein expression analysis [78]. Recently, there has been more emphasis on protein–nanoparticle interactions for the development of functional and safe nanoparticles [79]. Significantly, a role of modified fullerenes and cerium oxide nanoparticles has been demonstrated in protecting mammalian cells against damage, which can possibly be caused by ROS and RNS [80].

The biological consequences of various reactive species, mentioned above, are determined by rates of their formation and decay in different intra- and extracellular environments. Steady-state concentrations of reactive species can be estimated using their reaction rates with various constituents of environments. Proteins are made of ~70% of the mass of organic constituents related to living matter and are an important target of reactive species. The modifications of proteins by reactive species are governed by factors such as structure, redox properties, and acid–base chemistry of proteins [81]. At a molecular level, assessing the protein structure is critical to understand the modifications induced by oxidation processes and thus the function of proteins [82, 83]. The next section describes the progress that has been made in revealing structures of proteins. The role of redox properties of proteins in oxidative reactions is explained for thiols as an example in Section 1.3.5. The influence of $pK_a$ and the speciation of reactive amino acids and their moieties in proteins are presented in Chapter 2. Redox potentials, reaction rates, and oxidative mechanisms of reactive species are given in Chapters 3–6. The involvement of reactive species in environmental processes (e.g., disinfection and remediation) is also presented in Section 1.4 and in Chapters 3–6.

### 1.2 PROTEIN STRUCTURE

The details of protein structure are very important in understanding biochemical functions such as energy conversion, transport, enzyme catalysis, and host defense [84, 85]. Studies on protein structure are also imperative to understanding various *in vivo* processes, such as cell–cell communication, ligand binding, folding, and transport of proteins across membranes [86–88]. A number of techniques have been applied to determine the structure of proteins at all structural levels [87, 89–93]. Conformational changes in proteins in response to alternations in the environment of a solvent can be observed by using calorimetric and optical methods [94]. Optical methods include UV–vis, infrared, and fluorescence spectroscopy, and circular dichroism (CD), which have been applied in measuring protein thermodynamic stabilities based on the denaturant-induced unfolding transition [95]. X-ray crystallography has been used extensively to obtain high-resolution structural
information on proteins [96]. This technique has limitations because partially unfolded proteins do not crystallize, and hence, it is not always clear whether conformations in the solid state are identical to the bulk solution phase structures [97]. Time-resolved X-ray techniques may shed more light on protein dynamics [96]. Nuclear magnetic resonance (NMR) spectroscopy can provide insight on the structure and dynamics of proteins in solution [98–100]. Modern instruments have eliminated the limitations of high concentrations of proteins using traditional NMR spectroscopy when collecting data. However, determining structural information for proteins larger than 40–50 kDa remains a challenge for NMR spectroscopy. Progress in the mapping of proteins is summarized below, which includes the application of oxidative labeling of proteins.

1.2.1 Oxidative Labeling

1.2.1.1 Carbonyl Labeling. Proteins containing carbonyls are produced by reacting them with 2,4-dinitrophenylhydrazine (DNPH), followed by separation using gel electrophoresis [90]. The identification and quantification of derivatized protein carbonyls can also be carried out by ratiometric Raman spectroscopy [101]. Another approach of protein carbonyl labeling is by the reaction with biotin hydrazide under mild pH conditions [102]. One drawback of labeling is that both approaches cannot distinguish primary and secondary carbonyls as well as carbonyls from glycation [103, 104]. It is also possible that both reactants may not react with all types of oxidized amino acids [105].

1.2.1.2 Cysteine Residue Labeling. Labeling of the free SH group by a reagent based on iodoacetamide, maleimide, and 5,5′-dithiobis-(2-nitrobenzoic acid) allows indirect assessment of the oxidation of Cys [106–108]. Increased Cys oxidation causes a decrease in the amount of labeling. One other approach uses the blocking of reduced thiols present in solution before the reduction of oxidized thiols with dithiothreitol (DTT), followed by labeling with iodoacetamidofluorescein for separation using two-dimensional gel electrophoresis [109]. Thiol-specific biotin-HPDP (N-[6-(Biotinamido)hexyl]-3′-(2′-pyridyldithio)propionamide) labeling has also been demonstrated for proteins containing reversibly oxidized cysteines [110].

1.2.1.3 Reactive Species Labeling. Oxidized forms of chlorine, bromine, and iodine readily react with Cys, Met, and aromatic amino acids to modify the side chains of proteins (Fig. 1.4) [111, 112]. Aside from the side chains shown in Figure 1.4, cystine also reacts to yield N-dischlorocystine. The stoichiometric amounts of oxidizing agents used in the reaction mixtures determine the extent of oxidation of Cys, Met, and cystine. The reaction between halide anions and oxidizers such as H₂O₂ and •OH may also form oxidized halide intermediates. Significantly, intermediates may react with water to form
hydroxylated products as well as products of adjacent peptide bond cleavage. Examples include simultaneous oxidative halogenation and cleavage of C-terminal peptide bonds of Trp and Tyr. The size of reactants, pH, and redox potential of either Trp or Tyr determine the selectivity of the cleavage reaction [113]. HOCl and hypobromous acid (HOBr) form mostly halogenated Trp, Tyr, and 2-oxo Trp [114–116]. RNS preferentially oxidize Met, Cys, His, Tyr, Phe, and Trp. The reaction of NO in oxygenated Cys resulted in nitrosation of the thiol group [117]. RNS are selective for site-directed labeling of peptides and proteins. For example, the reduction of nitrated Trp or Tyr to their corresponding aromatic amines is a suitable approach for site-directed labeling [118, 119].
Most of the labeling of peptides and proteins has been performed using ROS, particularly hydroxyl radicals [87]. The detailed chemistry of reactive halogen, nitrogen, and oxygen species-mediated oxidation of side chains is described in Chapters 3–5.

1.2.1.4 Hydroxyl Radical Labeling. The probing of the solvent-accessible surface area (SASA) of proteins using reactions with \( \cdot \)OH radical and subsequent identification of oxidized sites by MS has been widely used for the oxidative labeling of proteins [86, 87, 120–129]. Because of the low specificity of the \( \cdot \)OH radical, a number of target sites in the protein are modified, resulting in an incorporation of oxygen atoms into amino acid side chains. This yields characteristic +16 Da adducts in the mass spectrum. The oxidative labeling of the target sites in proteins is influenced by intrinsic reactivity and solvent accessibility [87, 130]. Hydroxyl radicals preferentially react with sulfur-containing heterocyclic and aromatic amino acids with rate constants varying from \( 5 \times 10^{-9} \) to \( 1 \times 10^{10}/\text{M/s} \) [1, 12, 131–134]. However, \( \alpha \)-carbon hydrogen atoms, such as those found in Gly and Ala, have been found to react slower with the \( \cdot \)OH radical (\( k \sim 10^9/\text{M/s} \)) [131]. More is discussed in Chapter 4. The reactivity of amino acid residues with \( \cdot \)OH radicals and the ability of MS to detect oxidized products under aerobic conditions have the potential to probe the structure of proteins. The primary oxidation products for the amino acid side chains are presented in Table 1.3 [87, 135, 136]. Various methods of \( \cdot \)OH generation have been described, including Fenton chemistry, electrochemistry, coronal discharge methods, photochemistry, radiolysis, and pulse electron beams (Chapter 4) [87, 87, 137, 138].

A scheme of \( \cdot \)OH radical probing of the protein is presented in Figure 1.5 [87]. In this approach, the side chains of the protein and its complexes are modified using \( \cdot \)OH radicals, followed by X-ray exposure and digestion with proteases [82]. Quantification by MS determines the extent of modification. A tandem MS technique is applied to assess particular modified sites. This quantitation provides information about the solvent accessibility of each peptide in both the isolated and complexed states of the protein. The slower rate of binding proteins compared to the free protein indicates the influence of the binding process on the peptide containing the reactive side chains. Furthermore, allosteric changes in the conformation during binding may also produce an increase in reactivity [87]. This phenomenon can be observed in dose–response experiments. The use of a freeze-drying technique for the removal of \( \text{H}_2\text{O}_2 \) to determine the conformation of a protein was demonstrated to be unsuitable due to the oxidation of proteins under cold conditions [139].

There has been recent focus on the duration of either electron or laser pulses to the protein in order to prevent structural equilibrium during the timescale of exposure [140–143]. The use of timescales in submicroseconds has been determined for oxidative protein surface mapping [140, 142, 143]. A continuous-flow capillary setup has also been employed with an objective of exposing individual molecules of a protein to only a single \( \cdot \)OH pulse [123,
### TABLE 1.3. Primary Products and Corresponding Mass Changes for Various Amino Acid Side Chains Subjected to Radiolytic Modification and Detectible by Mass Spectrometry

<table>
<thead>
<tr>
<th>Side Chain</th>
<th>Side-Chain Modification and Mass Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys</td>
<td>Sulfonic acid (+48), sulfinic acid (+32), hydroxy (−16)</td>
</tr>
<tr>
<td>Cystine</td>
<td>Sulfonic acid, sulfinic acid</td>
</tr>
<tr>
<td>Met</td>
<td>Sulfoxide (+16), sulfone (+32), aldehyde (−32)</td>
</tr>
<tr>
<td>Trp</td>
<td>Hydroxy- (+16, +32, +48, etc.), pyrrol ring-open (+32, etc.)</td>
</tr>
<tr>
<td>Tyr</td>
<td>Hydroxy- (+16, +32, etc.)</td>
</tr>
<tr>
<td>Phe</td>
<td>Hydroxy- (+16, +32, etc.)</td>
</tr>
<tr>
<td>His</td>
<td>Oxo- (+16), ring-open (−22, −10, +5)</td>
</tr>
<tr>
<td>Leu&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Ile&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Val&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Pro</td>
<td>Hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Arg</td>
<td>Deguanidination (−43), hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Lys</td>
<td>Hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Glu</td>
<td>Decarboxylation (−30), hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Gln</td>
<td>Hydroxy- (+16), carbonyl (+14)</td>
</tr>
<tr>
<td>Asp</td>
<td>Decarboxylation (−30), hydroxy- (+16)</td>
</tr>
<tr>
<td>Asn</td>
<td>Hydroxy- (+16)</td>
</tr>
<tr>
<td>Scr&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hydroxy- (+16), carbonyl (−2-or +16-H₂O)</td>
</tr>
<tr>
<td>Thr&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hydroxy- (+16), carbonyl (−2-or +16-H₂O)</td>
</tr>
<tr>
<td>Ala</td>
<td>Hydroxy- (+16)</td>
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</tbody>
</table>

<sup>a</sup> For aliphatic side chains, +14 Da products are normally much less than +16 Da products.

<sup>b</sup> For Ser and Thr, only trivial amounts of +16 and −2 Da products were found.

Adapted from Xu and Chance [87] with the permission of the American Chemical Society.

Different oxidation products detected in model proteins, ubiquitin and apomyoglobin, using a nanosecond laser-induced photochemical oxidation method are presented in Table 1.4 and Table 1.5 [141]. A single shot of the laser resulted in 70% and 90% oxidation of ubiquitin and apomyoglobin, respectively, and different surface amino acids residues were oxidized. The liquid chromatography (LC)/MS/MS analysis of trypsin-digested apomyoglobin showed 99.3% sequence coverage. Extensive protein surface modifications of ubiquitin and β-lactoglobulin were seen in one submicrosecond electron beam pulse [142]. Fast photochemical oxidation of protein (FPOP) utilizing ‘OH radicals has also been used to characterize the epitope of the serine protease thrombin [145].

#### 1.2.2 Other Techniques

In the last two decades, the approach of side-chain modification coupled with proteolysis and MS has been applied extensively to analyze protein
Protein cleavage can be performed either enzymatically or chemically for MS analysis. Common proteases used are trypsin, chymotrypsin, pepsin, savinase, proteinase K, endoproteinase Asp-N, endoproteinase Lys-C, and metalloendopeptidase Lys-N [90, 92, 146]. A mixture of several proteases has been used to achieve cleavage at a wide range of sites in proteins [147]. However, protease is large and may not have access to the structural backbone of protein for cleavage in order to probe the structure precisely. A microwave method has also been applied to protein digestion [148]. Cyanogen bromide

Figure 1.5. Schematic representation of hydroxyl radical footprinting (adapted from Xu and Chance [87] with the permission of the American Chemical Society).
TABLE 1.4. Number of Oxidations Detected by LC/FT-MS and Reactive Amino Acid Residues Identified by LC/MS/MS for Ubiquitin

<table>
<thead>
<tr>
<th>Peptide Residues</th>
<th>Measure Mass (Da) by LC/FT-MS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Oxidation Detected by LC/FT-MS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sites of Oxidation by LC/MS/MS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Solvent-Accessible Surface Area by NMR (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–6</td>
<td>780.4201</td>
<td>M + O</td>
<td>M1</td>
<td>23.57</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Q2</td>
<td>69.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T12</td>
<td>39.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P19</td>
<td>65.70</td>
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<td></td>
<td></td>
<td></td>
<td>S20</td>
<td>70.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N25</td>
<td>61.76</td>
</tr>
<tr>
<td>12–27</td>
<td>1802.9145</td>
<td>M + O</td>
<td>I36</td>
<td>32.28</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>P37</td>
<td>67.15</td>
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<td>P38</td>
<td>36.52</td>
</tr>
<tr>
<td>34–42</td>
<td>1070.5102</td>
<td>M + 2O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43–47</td>
<td>699.3591</td>
<td>M + O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48–63</td>
<td>1794.8742</td>
<td>M + O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64–72</td>
<td>1082.6081</td>
<td>M + O</td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup> Six oxidized peptides were found in LC/FT-MS analysis, whereas three oxidized peptides were detected in LC/MS/MS analysis. The solvent-accessible surface areas were calculated using GETAREA 1.1 [337] using NMR structure of bovine ubiquitin [338].

Adapted from Aye et al. [141] with the permission of the American Chemical Society.

(CNBr) probes the C-terminal side of Met if it is not oxidized (MetO); however, CNBr is highly toxic [90].

There have been several reviews on the role of MS in the field of proteomics [89–91, 149–154]. Analysis of MS is based on the mass-to-charge ratio (m/z) of gaseous ions or their fragments and corresponding signal intensities. The m/z of the resulting gas phase ions can be correlated to structural properties of proteins in solution. Electrospray ionization (ESI)-MS and matrix-assisted laser desorption/ionization (MALDI)-MS techniques have been applied to determine the structure of peptides and proteins [155–167]. ESI produces multiple charged peptides, while MALDI generates singly charged peptides, and their fragmentation has been carried out by postsource decay (PSD) [168, 169]. High-quality MS/MS spectra of peptides are produced by high- and low-energy collision-induced dissociation (CID) on time-of-flight (TOF)/TOF instruments [170]. MALDI-MS analysis has also been enhanced by combining it with the ion trap, Fourier transform mass spectrometer, and triple quadrupole (Q<sub>3</sub>Q<sub>LIT</sub>).

Figure 1.6 shows the fragmented ions that may result from high- and low-energy CID. Generally, high-energy CID results in all types of fragmented ions, while only b, y, a, and z fragments are observed in low-energy CID spectra.
Loss of $\text{H}_2\text{O}$, CO, and ammonia from the sequence fragmented ions can usually be observed by comparing the two high- and low-energy CID spectra [154]. The fragmentation efficiency of the chemical modification of a peptide can significantly improve in low-energy CID. A protonated basic amino acid residue or positively charged derivative located at the N-terminus of a peptide promotes the fragmentation of b-ion fragments (see Fig. 1.6). Quaternary ammonium and phosphonium groups have been shown to enhance fragmentation [171, 172]. The sulfonation of peptides by reagents such as chlorosulfonylacetyl chloride, sulfobenzoic acid cyclic anhydride, 4-sulfophenyl isothiocyanate, and 3-sulfopropionic acid $N$-hydroxysuccinimide ester display

Table 1.5. Number of Oxidations Detected by LC/FT-MS and Reactive Amino Acid Residues Identified by LC/MS/MS for Apomyoglobin

<table>
<thead>
<tr>
<th>Peptide Residues</th>
<th>Measure Mass (Da) by LC/FT-MS</th>
<th>Oxidation Detected by LC/FT-MS</th>
<th>Site of Oxidation Detected by LC/MS/MS</th>
<th>Solvent-Accessible Surface Area by NMR ($\text{A}^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–16</td>
<td>1830.8891</td>
<td>M + O</td>
<td>S3</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E6</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>W7</td>
<td>19.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Q8</td>
<td>83.7</td>
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<td></td>
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<td></td>
<td>L11</td>
<td>42.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>K16</td>
<td>47.8</td>
</tr>
<tr>
<td>17–31</td>
<td>1637.8371</td>
<td>M + 1O</td>
<td>Q26</td>
<td>19.3</td>
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<tr>
<td></td>
<td>1653.8321</td>
<td>M + 2O</td>
<td>E27</td>
<td>54.1</td>
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<td>32–42</td>
<td>1286.6506</td>
<td>M + 1O</td>
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<tr>
<td></td>
<td>1302.6455</td>
<td>M + 2O</td>
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<td></td>
</tr>
<tr>
<td>43–47</td>
<td>699.3591</td>
<td>M + O</td>
<td></td>
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</tr>
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<td>51–62</td>
<td>1366.6286</td>
<td>M + O</td>
<td>M55</td>
<td>7.3</td>
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<td>64–77</td>
<td>1521.9242</td>
<td>M + 1O</td>
<td>H64</td>
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<td>1537.9191</td>
<td>M + 2O</td>
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<td>P88</td>
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<td>L89</td>
<td>48.6</td>
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<td>S92</td>
<td>23.9</td>
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<td>H96</td>
<td>55.9</td>
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<tr>
<td>119–133</td>
<td>1517.6568</td>
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<td>M131</td>
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<td>1533.6517</td>
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<td>134–139</td>
<td>763.4228</td>
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<td>140–145</td>
<td>646.3286</td>
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<td>148–153</td>
<td>665.3020</td>
<td>M + O</td>
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</tbody>
</table>

*Eleven peptides are detected as oxidized peptides in LC/FT-MS analysis, although six oxidized peptides are observed in LC/MS/MS. The solvent-accessible surface areas were calculated using GETAREA 1.1 [337] using NMR structure of horse heart myoglobin [339]. Adapted from Aye et al. [141] with the permission of the American Chemical Society.
an enhancement of fragmentation along the peptide chain [161, 173–176]. The interpretation of spectra was simplified because only y-ions were detected.

The amino group of the lysine residue in peptides was selectively modified by reactions with peroxycarbonate [167]. The lysine peroxycarbamates formed undergo hemolytic fragmentation under low-energy CID in ESI and MALDI-MS. The fragmentation of deuterated analogues of modified lysine resulted in the formation of a-, c-, or z-types of ions with MS, which suggested the involvement of a free radical mechanism in the fragmentation. The results in MALDI-MS are shown in Table 1.6 [157]. Mostly, the fragments of a- and z-ions were observed at the Lys site with one Lys or multiple Lys in the sequence of peptides. Entry 4 showed the disulfide bond remains intact; however, the peptide bond near the Lys was cleaved. Entry 1 in Table 1.6 showed the loss of a side chain of Val at the N-terminus in the fragment with m/z 961.45. Side-chain losses of Lys, Tyr, and Ser were also observed (entries 2, 3, and 5 in Table 1.6).

Other dissociation and ionization techniques besides CID have also been applied in the MS-based structural determination of peptides and proteins. These include ultraviolet photodissociation, nanoelectrospray ionization (nanoESI), easy ambient sonic spray ionization (EASI), easy ambient sonic spray ionization-membrane interface mass spectrometry (EASI-MIMS), laser ablation electrospray ionization (LAESI), desorption electrospray ionization (DESI), electrospray-assisted laser desorption/ionization (ELDI), electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD), atmospheric pressure chemical ionization (APCI), and ambient liquid mass spectrometry (ALMS) [162, 163, 165–167, 177–189]. CID and IRMPD normally produce heterolytic cleavage of the amide bonds in the polypeptide chain and resulting b and y fragments having N- and C-termini, respectively [190]. ECD cleaves the N–Cα bonds giving mainly fragmented ions of the N-terminus c’ and C-terminus z’. A recent study compared top-down CID, CID, and IRMPD of nitrated proteins: hen egg-white lysozyme (HEWL), myoglobin, and cytochrome c [191]. The total number of fragments in ECD was greater than those in IRMPD or CID, but
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<td>NH=CH-CO-</td>
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<td>831.39</td>
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<tr>
<td>KKALRRQETVDAL</td>
<td>z&lt;sub&gt;12&lt;/sub&gt;&lt;sup&gt;z&lt;/sup&gt;</td>
<td>NH=CH-CO-</td>
<td>1382.78</td>
<td>1382.91</td>
<td>1455.82</td>
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<td>1382.91</td>
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<td></td>
<td></td>
<td>KALRRQETVDAL&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>YEVHHQKLVFF</td>
<td>[a&lt;sub&gt;7&lt;/sub&gt; + Na]&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>916.52</td>
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<td>EVHHQKLVFP&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>CGNLSTCMLGTEDFNKFH</td>
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<td>SYSMEHFRWGKPVGKKR</td>
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<td>NH=CH-CO-</td>
<td>1381.65</td>
<td>1762.85</td>
<td>1890.98</td>
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<tr>
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<td>a&lt;sub&gt;15&lt;/sub&gt;</td>
<td>a&lt;sub&gt;16&lt;/sub&gt;</td>
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<td>1431.66</td>
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<tr>
<td>SYSMEHFRWGKPVGKRRPVKVYP</td>
<td>a&lt;sub&gt;13&lt;/sub&gt;</td>
<td>a&lt;sub&gt;15&lt;/sub&gt;</td>
<td>a&lt;sub&gt;16&lt;/sub&gt;</td>
<td></td>
<td>1381.65</td>
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</table>

<sup>a</sup> Loss of side chain of N-terminus.

Adapted from Yin et al. [157] with the permission of the American Society of Mass Spectrometry.
more cleavages were generated in the vicinity of the sites of nitration in CID than IRMPD and ECD. ELDI is suitable for MS and top-down MS/MS of large proteins up to 29 kDa [164, 165]. The use of LC ESI tandem mass spectrometry (MS/MS) to sequence peptides generated from a digest of proteome sample has also been demonstrated [158].

MS-based approaches are generalized in Figure 1.7 [90]. In the bottom-up approach (A), tryptic proteolysis is followed by MS and CID-MS/MS analysis of relatively small +1 and +2 charged species, while further species proteolysis is followed by MS and ETD-MS/MS of larger +2, +3, and +4 charged species in the middle-down approach. A top-down approach involves ESI-MS on the intact protein to determine the total protein mass followed by ECD-MS/MS of a selected protein species with the modification of interest. The bottom-up approach is the most commonly used method for peptide mass analysis. Several labeling and separation methods are applied in the analysis of complex protein mixtures.

Figure 1.7. MS-based approaches A, B, and C. The gray boxes symbolize what takes place inside the MS equipment; the stars denote a certain peptide or protein (from an oxidized sample), chosen for CID, ETD, or ECD (adapted from Törnvall [90] with the permission of the Royal Society of Chemistry). See color insert.
1.3 REACTIVE SPECIES

Amino acids play many roles in the metabolic activities of humans [192]. For example, amino acids are involved in the efficiency of utilizing dietary proteins, the cell-specific metabolism of nutrients, cell signaling, and oxidative stress [193, 194]. Reactive intermediates produced in the body influence metabolic activities by altering side chains of amino acids and modifying protein backbones (polypeptide chains) [195]. This section summarizes the involvement of reactive intermediates in biological systems. The generation and reactivity of the intermediates are described in Chapters 3–6.

1.3.1 Halogen Species

The heme enzyme myeloperoxidase (MPO) is linked to atherosclerosis [196]. Reactions between halide/pseudohalide (Cl\(^{-}\), Br\(^{-}\), and SCN\(^{-}\)) ions and hydrogen peroxide (H\(_2\)O\(_2\)) are catalyzed by MPO to produce HOCl, HOBr, and hypothiocyanous acid (HOSCN) [197]. The concentrations of Cl\(^{-}\), Br\(^{-}\), and SCN\(^{-}\) in plasma are in the ranges of 100–140 mM; 20–100 μM, and 20–250 μM, respectively. HOCl and HOBr are powerful oxidants and react at multiple targets (Chapter 3) [198]. These oxidants can cause tissue damage at excessive levels. HOCl and HOBr have been implicated in kidney disease, inflammatory bowel disease, cardiovascular disease, asthma, cystic fibrosis, rheumatoid arthritis, and neurodegenerative conditions [199, 200]. SCN\(^{-}\) reacts much faster with H\(_2\)O\(_2\) (730-fold) than with Cl\(^{-}\); hence, most of H\(_2\)O\(_2\) is transferred into HOSCN. A high level of HOSCN has been observed in smokers due to their elevated levels of SCN\(^{-}\) [197]. HOSCN is less reactive compared to HOCl and HOBr, but is highly selective. Reactions of HOSCN are predominately with thiols (e.g., GSH) (Chapter 3). The ratio of [GSH]/[oxidized glutathione (GSSG)] may be useful to detect early atherosclerosis [201]. Overall, the concentration of SCN\(^{-}\) determines the damage induced by MPO. The chemistry of HOCl, HOBr, and HOSCN and their oxidation of amino acids including thiols are discussed in Chapter 3. Halamines (e.g., RNHCl and RHBr) can be produced as intermediates in reactions of HOCl and HOBr and may participate in reactions of biological systems. Hydrolysis, halogen transfer, and reduction of halamines are presented in Chapter 3.

Chlorine dioxide (ClO\(_2\)) is an important biocide and bleach [202]. Generation of ClO\(_2\) using different methods including that from chlorite ion at neutral pH under mild conditions in the presence of water-soluble, manganese porphyrins and porphyrazines [203] is discussed in Chapter 3. Mechanisms of decomposition of ClO\(_2\) under neutral and alkaline conditions are also given in Chapter 3. Finally, oxidation of inorganic and organic compounds (e.g., amino acids, peptides, and proteins) by ClO\(_2\) is summarized in Chapter 3.
1.3.2 Oxygen Species

ROS play fundamental roles in maintaining health and in developing diseases, and estimation of their generation in isolated mitochondria has been evaluated as 0.1–2.0% of all consumed oxygen [204]. Mitochondria can produce $\text{O}_2^{\cdot\cdot}$ at complex I (NADH-coenzyme Q reductase) and complex III (ubiquinone–cytochrome $c$ oxireductase) [13, 205]. The generation of ROS in mitochondria is monitored by NO*, which is transferred to other RNS (see Fig. 1.1). Thus, a low concentration of NO* can increase the production of $\text{O}_2^{\cdot\cdot}$ and $\text{H}_2\text{O}_2$, while the formation of $\text{OONO}^-$ results from the reaction of $\text{O}_2^{\cdot\cdot}$ and NO* [206]. Other major cellular sources of ROS are lipoxygenases, Nox family of enzymes, peroxisomers, uncoupled nitric oxide synthase (NOS), cyclooxygenases, xanthine oxireductase, and cytochrome P450 family proteins [207]. Levels of ROS were shown to increase in mouse hepatic cells in culture and in mouse liver by cytochrome P450 enzyme-mediated processes [208].

Significantly, the basic biology of cells and tissues is affected by SOD, $\text{O}_2^{\cdot\cdot}$, and $\text{H}_2\text{O}_2$ [209]. Due to the importance of SOD, several studies have been performed on their structures and their involvement in the dismutation of $\text{O}_2^{\cdot\cdot}$ [210–216]. MnSOD is an essential enzyme affecting levels of ROS in mitochondria and the protection of cells from damage by ROS [210, 216]. Nickel superoxide dismutase (NiSOD) has also been studied to elucidate its role in the disproportionation of $\text{O}_2^{\cdot\cdot}$ [217–219]. Part of the $\text{O}_2^{\cdot\cdot}$ produced in the mitochondria may be converted to $\text{H}_2\text{O}_2$ by Cu,Zn-SOD. The mechanistic aspects of the reactions of SOD with $\text{O}_2^{\cdot\cdot}$ are described in Chapter 4. Under unregulated conditions, ROS accumulate and result in oxidative damage to cellular proteins, lipids, and nucleic acids [5, 7, 220, 221]. However, SOD, CAT, and thiol-based redox couples in molecular systems neutralize threats from ROS to cells. Chapter 4 discusses the oxidation of functional groups on proteins by ROS ($\text{O}_2^{\cdot\cdot}$, $^1\text{O}_2$, $\text{O}_3$, and $^\cdot\cdot\text{OH}$). Mechanisms of reactions are also given in Chapter 4.

Carbonate radical ($\text{CO}_3^{\cdot\cdot}$) may also be considered as ROS due to its selectivity. The reactivity of $\text{CO}_3^{\cdot\cdot}$ with amino acids is less than that of $^\cdot\cdot\text{OH}$, but it is more selective and may also be a mediator of protein modification in cellular environments under conditions of oxidative stress. Carbonate radicals may also play a role in the decomposition of peroxynitrite in the presence of bicarbonate. Reactions of $\text{CO}_3^{\cdot\cdot}$ are discussed in Chapter 5. Modifications of proteins by peroxymonocarbonate ($\text{HCO}_4^{\cdot\cdot}$) and carboxy radical ($\text{CO}_2^{\cdot\cdot}$) are briefly presented in Chapter 4. Other species that may also be considered ROS are peroxyl ($\text{ROO}^\cdot$) and alkoxyl ($\text{RO}^\cdot$) radicals, which are formed from the reaction of carbon-centered radicals with oxygen. Peroxidation has been demonstrated to go through chain reactions and to generate these radicals. The breakdown of these radicals into carbonyl hydperoxides, alcohols, and carbonyl groups suggests their importance in biological systems.

Sulfite and sulfate radicals ($^\cdot\cdot\text{SO}_3$ and $\text{SO}_4^{\cdot\cdot}$) may be other ROS because of their possible reactions with amino acids and peptides (Chapter 5). $\text{SO}_4^{\cdot\cdot}$ is a
strong oxidant and its redox potential is similar to that of \( \cdot \text{OH} \) [222]. Both \( \text{SO}_4^{\cdot -} \) and \( \cdot \text{OH} \) can possibly react with phosphate ions to produce phosphate radicals (Chapter 5). Reactions of phosphate radicals with aromatic amino acids and their peptides are discussed in Chapter 5.

1.3.3 Nitrogen Species

Nitric oxide (\( \cdot \text{NO} \)) has been studied extensively due to its importance in biological chemistry. A low level of \( \cdot \text{NO} \) production is involved in several processes such as immune system control, blood pressure modulation, signal transduction, smooth muscle relaxation, memory, and inhibition of platelet aggregation [21, 223]. However, high levels of \( \cdot \text{NO} \) can result in injury to tissues [224]. Recent studies hypothesized that dietary nitrite may form \( \cdot \text{NO} \) in the human stomach [225, 226]. One of the reasons of \( \cdot \text{NO} \) toxicity may be its reaction with \( \text{O}_2^{\cdot -} \) to form peroxynitrite. Peroxynitrite can oxidize as well as nitrate a number of biological molecules including proteins. Peroxynitrite can either directly promote one- and two-electron oxidations in molecules or decompose to secondary radicals such as \( \cdot \text{OH} \) and \( \cdot \text{NO}_2 \) (Chapter 5). \( \cdot \text{NO}_2 \) is a mild and selective oxidant (Chapter 5). The secondary radicals thus result in nitration and lipid peroxidation [227]. In the occurrence of high levels of bicarbonate in interstitial (30 mM) and intracellular (12 mM) fluids, the formation of a short-lived adduct between peroxynitrite and \( \text{CO}_2 \) occurs. This adduct decomposes into oxidizing intermediates, \( \text{CO}_3^{\cdot -} \) and \( \cdot \text{NO}_2 \). The kinetics of nitrogen species and nitration products of free amino acids and proteins are presented in Chapter 5.

1.3.4 Sulfur Species

Thiol proteins play key roles in diverse physiological processes [228, 229]. Thiol proteins are also important molecules by which reactive species involve themselves in cellular transduction pathways [230]. During oxidative stress, oxidation of protein thiols takes place. The reactions of protein thiols (Pr-SH) can occur through one-electron and two-electron pathways (Fig. 1.8) [230]. In two-electron pathways, sulfenic acid (Pr-SOH) is initially formed, which can undergo several secondary reactions. Sulfenic acid can also form mixed disulfides (Pr-SS-G) by reacting with GSH. The formation of sulfenic acid is a prominent feature in several acute and chronic diseases. Significantly, more than 200 different cellular proteins have been shown to undergo cysteine oxidation [231].

One-electron oxidants such as radicals and transition metal ions produce the corresponding thyl radical (Pr-S\(^*\)). This radical may react with either other biomolecules or may participate in further reactions with thiol molecules and oxygen. The reaction with oxygen ultimately forms superoxide and hydrogen peroxide. A recent study on the oxidation of methionine-lysine peptide by \( \cdot \text{OH} \) in the presence of CAT resulted in the formation of methionine
Figure 1.8. Oxidation of protein thiols by one-electron and two-electron pathways. PUFA, polyunsaturated fatty acids (adapted from Winterbourn and Hampton [230] with the permission of Elsevier Inc.) See color insert.

sulfoxide through a one-electron transfer [232]. Recently, the reactivity of simple thiols and protein thiols with $\text{H}_2\text{O}_2$ has been explained in detail [228]. Generally, the nucleophilic attack of thiolate on an electrophile occurs and rate constants vary by seven orders of magnitude ($k(\text{cysteine}) = 2.6 \times 10^9/\text{M/s}; k(\text{peroxiredoxin 2}) = 1.0 \times 10^8/\text{M/s})$ [233, 234]. Thiyl radicals are involved in enzymatic and detoxifying pathways, and in recent years, the oxidizing power of the thiyl radicals produced by one-electron oxidations has been studied. In a recent work, cysteine thiyl radicals were shown to be involved in reversible intramolecular hydrogen transfer reactions with amino acid residues in peptides and proteins [235].

The reduction potential is the key parameter to express oxidation power and is pH dependent. The reduction potential of a one-electron couple, expressed as a midpoint electrode potential, $E_m(\text{GS}^*, \text{H}^+$/GSH) for GSH ($\gamma$-glutamylcysteineglycine) in water as a function of pH has been determined (Fig. 1.9) [236]. $E_m$ is an experimentally useful midpoint potential where the sum of concentrations of oxidants is equal to the sum of reductants [237]. In Figure 1.9, the solid line is for a simple thiol in which only thiol/thiolate ionization was considered with $pK_a = 9.2$ and assuming $E^0(\text{RS}^*/\text{RS}^-) = 0.8V$. However, the dashed line represents the considered four macroscopic $pK_a$ values of the reductant (GSH, $pK_{1-4}$), consisting of 2.05, 3.40, 8.72, and 9.49, and three $pK_a$ values of the oxidant (GS*, $pK_{5-3}$), consisting of 1.8, 3.2, and 9.08, and also assuming $E^0(\text{RS}^*/\text{RS}^-) = 0.8V$ for the fully deprotonated GSH [236]. The value of $E_m(\text{GS}^*, \text{H}^+$/GSH) = 0.92 at pH 7.4 was determined and compared to other biologically relevant free radicals (Table 1.7). The value of
Figure 1.9. Expected form of the dependence upon pH of the midpoint electrode potential of the thiyl radical/thiol couple $E_m(\text{RS}^\bullet, \text{H}^+/\text{RSH})$ (adapted from Madej and Wardman [236] with the permission of Elsevier Inc.).

$E_m$ at pH 7 for the thiyl radical of hydrogen sulfide is similar to the value of GS* at pH 7.4. This is an indication of the moderate oxidizing power of GS*. However, GS* has a higher oxidizing power than do radicals of 8-oxo-7,8-dihydro-2'-deoxyguanosine, urate, and ascorbate [238–240]. The order of $E_m$ in Table 1.7 suggests GS* would oxidize 8-oxo-7,8-dihydro-2'-deoxyguanosine but not guanosine [240]. The order also suggests NO2 can oxidize GSH [238].

The role of thiyl radicals can be studied through analysis in biological systems [241]. However, thiyl radicals are highly reactive with half-lives on the order of microseconds; therefore, their direct detection is challenging in biological systems. Rate constants for hydrogen transfer reactions of the thiyl radical of cysteamine with amino acids and peptides are up to $10^7$/M/s [242–245]. The reactions of thiyl radicals from GSH, cysteine, and pencillamine with NO* are in the range of $(2–3) \times 10^9$/M/s [246]. A recent analysis of thiyl radicals suggests a spin trapping electron paramagnetic resonance technique using 5,5-dimethyl-1-pyrrolone N-oxide as a trapping agent is a promising approach for the analysis of thiyl radicals in biosystems [241].

In addition to thiyl radicals, the reactions of protein radicals with ascorbate have also been studied to determine if the loss of ascorbate in a living organism can be related to these reactions [247]. Protein radicals were generated on tryptophan and tyrosine residues of insulin, β-lactoglobulin, pepsin, chymotrypsin, and bovine serum albumin ($k = 2.9–19$) $\times 10^9$/M/s. The tryptophanyl
TABLE 1.7. Oxidizing Power of Some Biological Free Radicals (Midpoint Electrode [Reduction] Potentials, $E_m$ of Radicals, pH 7.0–7.4 vs. Norman Hydrogen Electrode [NHE]) (Values of the $E_m$ of the Couple Were Taken from Madej and Wardman [236] with the Permission of Elsevier Inc.)

<table>
<thead>
<tr>
<th>Radical Obtained from Oxidation of</th>
<th>Couple$^a$</th>
<th>$E_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bi)carbonate</td>
<td>CO$_3^-$, H$^+/HCO$_3^-$</td>
<td>1.74</td>
</tr>
<tr>
<td>Adenosine</td>
<td>AdO$^+$/Ado</td>
<td>1.42</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met$^+$/Met$^b$</td>
<td>$-1.2$–$-1.5$</td>
</tr>
<tr>
<td>Guanosine</td>
<td>G(–H)$^+$, H$^+/G$</td>
<td>1.29</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp$^+$, H$^+/TrpH$</td>
<td>$-1.05$</td>
</tr>
<tr>
<td>Nitrite</td>
<td>NO$_2^-$/NO$_3^-$</td>
<td>$-1.04$</td>
</tr>
<tr>
<td>Glutathione</td>
<td>G$^+$, H$^+/GSH$</td>
<td>0.92</td>
</tr>
<tr>
<td>Hydrogen sulfide</td>
<td>S$^-$, H$^+/HS$</td>
<td>0.92</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>TyrO$^+$, H$^+/TyrOH$</td>
<td>$-0.90$</td>
</tr>
<tr>
<td>8-Oxo-7,8-dihydro-2$^-$-deoxyguanosine</td>
<td>8-OHdG(–H)$^+$, H$^+/8-OHdG$</td>
<td>0.74</td>
</tr>
<tr>
<td>5-Hydroxyindole</td>
<td>5-Indoxyl$^+$/5-hydroxyindole</td>
<td>0.64</td>
</tr>
<tr>
<td>Urate</td>
<td>UH$^-$, H$^+/UH_2^-$</td>
<td>0.59</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>Asc$^-$, H$^+/AscH^-$</td>
<td>0.30</td>
</tr>
</tbody>
</table>

$^a$ Predominant forms of radical/reductant couples at pH 7–7.4 are shown.

$^b$ Irreversible process with rapid deprotonation from the radical cation.

Radicals in chymotrypsin, pepsin, and β-lactoglobulin reacted with monohydrogen ascorbate with rate constants of $1.6 \times 10^8$/M/s, $1.8 \times 10^9$/M/s, and $2.2 \times 10^7$/M/s, respectively. Comparatively, the corresponding reaction of protein tyrosyl radicals had rate constants an order of magnitude slower. The rate constants of the reactions were not significantly affected by the location of radicals in the protein chains [247]. A separate study estimated a rate constant of $2.2 \times 10^9$/M/s for the tyrosyl radical with GSH, which is about two orders of magnitude slower than the reaction of the tyrosyl radical with ascorbate [248]. This indicates GSH plays a minor role in radical “repair,” while the loss of ascorbate may be due to its reactions with protein radicals under physiological conditions.

Overall, the chemistry of reactive sulfur species is complex. In addition to cysteine and GSH, a biological role for H$_2$S is also being studied (Chapter 5) [249–251]. Physiological levels of H$_2$S are 50–160μM and 10–100μM in mammalian brain tissues and human plasma, respectively [252]. The comparison of sulfahydryl radicals derived from H$_2$S is compared with other thiol radicals in Chapter 5.

1.3.5 High-Valent Cr, Mn, and Fe Species

Transition metal ions play important roles in a number of biological processes [35, 36, 253, 254]. Metal oxo and metal hydroxo moieties have been recognized
in different biological oxidation and electron transfer reactions such as hydroxylation, epoxidations, sulfoxidation, dehalogenation, and deamination [255, 256]. The interactions of high-valent metal ions with proteins and DNA may lead to oxidative deterioration of biological molecules. For example, high-valent compounds of Cr pose serious danger to biological systems and are considered both toxic and carcinogenic (Chapter 6). The properties and reactivity of Cr species are discussed in Chapter 6. Chapter 6 also highlights the role of high-valent Cr species in carcinogenicity, genotoxicity, and cytotoxicity.

High-valent species of Mn and Fe have been reported as active intermediates in oxidation events of metalloenzymes [35, 257, 258]. Various complexes of Mn and Fe have been synthesized to mimic biologically important processes (Chapter 6). High-valent oxo complexes of Mn have been prepared to elucidate the role of Mn V-oxo species in water oxidation at the oxygen-evolving center in photosystem II [259]. The aqueous chemistry of oxo compounds of Mn is presented in Chapter 6. The rates of reactions between Mn(VII) and amino acids are influenced by colloidal MnO2 and phosphate species in solution (Chapter 6). Pathways of oxidation reactions carried out by Mn(VII) with amino acids and aminopolycarboxylates are described in Chapter 6.

High-valent iron complexes as intermediates have been invoked in reactions of heme and nonheme enzymes [260–264]. In recent years, several complexes of iron(IV) and iron(V) have been synthesized as models to mimic catalytic centers of enzymes [253, 265–270]. Properties of these complexes are presented in Chapter 6. Rate studies have revealed that metal ions influence the electron transfer from reductants to iron(IV) complexes (Chapter 6). A summary of the recent work on the kinetics and products of reactions of iron(IV) complexes with amino acids and peptides is presented in Chapter 6.

1.4 REACTIVE SPECIES IN ENVIRONMENTAL PROCESSES

1.4.1 Atmospheric Environment

Free radical species play a critical role in combustion, plasma environments, interstellar clouds, and atmospheric chemistry [271]. Reactions of radical species have been shown to have an adverse effect on human health and vegetation [272]. Species such as chlorine atoms, O3, "OH, and NO3 can react with inorganic and volatile organic compounds of the atmosphere [273–276]. For example, the reaction of CO with "OH is important in combustion reactions [271]. ROS are also formed in the reaction of O3 with aerosol particles [277]. In recent years, reactions of NO3 have also been studied. NO3 radicals are formed through the reaction of NO2 with O3 (Eq. 1.5):

\[
O_3 + NO_2 \rightarrow NO_3 + O_2.
\]  

(1.5)

Kinetic studies on the reactions of "OH and NO3 radicals with a number of 1-alkenes [CH2=CHR] and 2-methyl-1-alkenes [CH2=C(CH3)R], where
R = (CH\textsubscript{2})\textsuperscript{n}CH\textsubscript{3} have demonstrated the carbon number of substituent group, \( n \), increases the rate constants up to \( n < 7 \), followed by no further increase at \( n \geq 7 \). Significantly, substituent group carbon number \( n \) had no affect on the reaction of O\textsubscript{3} with the same alkenes. Steric and ring strain effects appear to control the rate constants of the reactions of these alkenes with \(^{•}\text{OH}, \text{NO}_3^{•}\), and O\textsubscript{3} [278].

\text{NO}_3^{•}\) is a nighttime atmospheric oxidant and its reactivity with most organic molecules is many orders of magnitude higher than of O\textsubscript{3} or \text{NO}_2 [279]. Reaction mechanisms of reactions of \text{NO}_3^{•} with molecules include electron transfer, hydrogen abstraction, and addition to the \( \pi \) system [279]. The reaction between \text{NO}_3^{•} and \text{NO}_2 can take place to yield N\textsubscript{2}O\textsubscript{5} in the absence of organic reactants, and N\textsubscript{2}O\textsubscript{5} acts as a reservoir for \text{NO}_3^{•} [279]. Recently, the reactions of \text{NO}_3^{•} with \( N^{-} \) and \( C^{-} \)-protected aromatic amino acids in the presence of O\textsubscript{2}, O\textsubscript{3}, \text{NO}_2, and N\textsubscript{2}O\textsubscript{5} have been studied [280, 281]. Consistent with the chemistry of \text{NO}_3^{•}, the initial electron transfer step at the aromatic ring occurred, followed by multi-steps, which finally yield nitroaromatic compounds [280]. The formation of products was not influenced by the presence of O\textsubscript{2} when reactions of \text{NO}_3^{•} with tyrosine and phenylalanine were studied. In the case of tryptophan, an intramolecular oxidizing cyclization involving the amide moiety leads to the formation of tricyclic products. Results suggest \text{NO}_3^{•} may cause damage to the peptides lining the respiratory tract, ultimately causing pollution-driven diseases [281].

1.4.2 Disinfection By-Products (DBP)

Dissolved organic nitrogen (DON) in the aquatic environment is of interest due to the bioavailability and connections between the carbon and nitrogen biogeochemical cycles [282]. The concentrations of nitrogen-containing substances in aquatic environments have increased due to inputs from agricultural runoff, deposition of \text{NO}_3, and wastewater effluents [283–285]. Dissolved free amino acids are one of the constituents of DON [286]. Amino acids have been found in a number of water resources including drinking water, lakes, rivers, marshes, and groundwaters [287–289]. Amino acids may also be found in industrial effluents because of their many uses and applications. Proteins have been detected in wastewater treatment effluents [290].

A number of disinfectants/oxidants are used in the treatment of water. Redox potentials of oxidants are provided in Table 1.8 [291–294]. Under acidic conditions, the redox potential of \(^{•}\text{OH}\) is one of the highest of any oxidant (Table 1.8). However, the potential is low under basic conditions. Under acidic conditions, the order of redox potentials of oxidants without chlorine and transition metal species are \(^{•}\text{OH} > \text{SO}_4^{2•} > \text{NO}_3^{•} > \text{O}_3 > \text{H}_2\text{O}_2 > \text{CO}_3^{•} > \text{HSO}_5 > \text{O}_2\). Redox potentials of some oxidants (\text{SO}_4^{2•}, \text{NO}_3^{•}, \text{CO}_3^{•}, \text{HSO}_5, \text{ClO}_2, \text{and Cl}_2) have no pH dependence. Ozone has a high redox potential in alkaline medium. Among the high-valent iron and manganese species, redox potentials of Fe(VI) (Fe\textsuperscript{6+}/Fe\textsuperscript{3+}) and Mn(VI) (Mn\textsuperscript{6+}/MnO\textsubscript{2}) are similar and higher than Mn(VII).
This suggests both Fe(VI) and Mn(VI) have strong oxidizing power, particularly under acidic conditions.

Chlorine is a commonly used disinfectant in drinking water because it is readily available and effective. However, chlorine can react with amino acids and peptides to form DBPs, which have negative health effects \([295–299]\). The kinetics and mechanisms of the formation of DBPs are presented in Chapter 3. The chlorination products of amino acids include cyanogen chloride, chloroform, dichloroacetonitrile, monochloroamines, aldehydes, and nitriles \([300–303]\). Chlorinated products of peptides are more stable than chlorinated derivatives of amino acids; therefore, peptides in water are also of concern \([300]\). The chlorination of waters containing bromide and iodide ions may produce hypobromous (HOBr) and hypoiodous (HOI) acids \([304, 305]\). Thus, both HOBr and HOI may also result in DPBs \([304, 305]\).

Monochloroamine (NH₂Cl) as a chlorine alternative is applied to reduce the formation of DPBs. During chloramination, lower concentrations of DBPs,

### TABLE 1.8. Redox Potentials for the Oxidants/Disinfectants Used in Water Treatment [291–294]

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Reaction</th>
<th>(E^o) (V/NHE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl radical</td>
<td>(\cdot\text{OH} + \text{H}^+ + e^- \rightleftharpoons \text{H}_2\text{O})</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>(\cdot\text{OH} + e^- \rightleftharpoons \text{OH}^-)</td>
<td>1.89</td>
</tr>
<tr>
<td>Sulfate radical</td>
<td>(\text{SO}_4^{2-} + e^- \rightleftharpoons \text{SO}_3^{2-})</td>
<td>2.43</td>
</tr>
<tr>
<td>Nitrate radical</td>
<td>(\text{NO}_3^+ + e^- \rightleftharpoons \text{NO}_3)</td>
<td>2.30</td>
</tr>
<tr>
<td>Manganese</td>
<td>(\text{MnO}_4^{2-} + 4\text{H}^+ + 2e^- \rightleftharpoons \text{MnO}_2 + 2\text{H}_2\text{O})</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>(\text{MnO}_4^{2-} + 8\text{H}^+ + 4e^- \rightleftharpoons \text{Mn}^{2+} + 4\text{H}_2\text{O})</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>(\text{MnO}_4^{2-} + 2\text{H}_2\text{O} + 2e^- \rightleftharpoons \text{MnO}_2 + 4\text{OH}^-)</td>
<td>0.60</td>
</tr>
<tr>
<td>Ferrate(VI)</td>
<td>(\text{FeO}_4^{2-} + 8\text{H}^+ + 3e^- \rightleftharpoons \text{Fe}^{3+} + 4\text{H}_2\text{O})</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>(\text{FeO}_4^{2-} + 4\text{H}_2\text{O} + 3e^- \rightleftharpoons \text{Fe(OH)}_3 + 5\text{OH}^-)</td>
<td>0.70</td>
</tr>
<tr>
<td>Ozone</td>
<td>(\text{O}_3 + 2\text{H}^+ + 2e^- \rightleftharpoons \text{O}_2 + \text{H}_2\text{O})</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td>(\text{O}_3 + \text{H}_2\text{O} + 2e^- \rightleftharpoons \text{O}_2 + 2\text{OH}^-)</td>
<td>1.24</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>(\text{H}_2\text{O}_2 + 2\text{H}^+ + 2e^- \rightleftharpoons 2\text{H}_2\text{O})</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td>(\text{H}_2\text{O}_2 + 2e^- \rightleftharpoons 2\text{OH}^-)</td>
<td>0.88</td>
</tr>
<tr>
<td>Carbonate radical</td>
<td>(\text{CO}_3^{2-} + e^- \rightleftharpoons \text{CO}_3^{2-})</td>
<td>1.59</td>
</tr>
<tr>
<td>Permanganate</td>
<td>(\text{MnO}_4^{2-} + 8\text{H}^+ + 5e^- \rightleftharpoons \text{Mn}^{2+} + 4\text{H}_2\text{O})</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>(\text{MnO}_4^{2-} + 2\text{H}_2\text{O} + 3e^- \rightleftharpoons \text{MnO}_2 + 4\text{OH}^-)</td>
<td>0.59</td>
</tr>
<tr>
<td>Hypochlorite</td>
<td>(\text{ClO}^- + \text{H}^+ + 2e^- \rightleftharpoons \text{Cl}^- + \text{H}_2\text{O})</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>(\text{ClO}^- + \text{H}_2\text{O} + 2e^- \rightleftharpoons \text{Cl}^- + 2\text{OH}^-)</td>
<td>0.84</td>
</tr>
<tr>
<td>Peroxysulfate</td>
<td>(\text{HSO}_3^- + e^- \rightleftharpoons \text{HSO}_3^-)</td>
<td>1.40</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>(\text{ClO}_4^- + 8\text{H}^+ + 8e^- \rightleftharpoons \text{Cl}^- + 4\text{H}_2\text{O})</td>
<td>1.39</td>
</tr>
<tr>
<td>Chlorine</td>
<td>(\text{Cl}_2 + 2e^- \rightleftharpoons 2\text{Cl}^-)</td>
<td>1.36</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>(\text{O}_2 + 4\text{H}^+ + 4e^- \rightleftharpoons 2\text{H}_2\text{O})</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>(\text{O}_2 + 2\text{H}_2\text{O} + 4e^- \rightleftharpoons 4\text{OH}^-)</td>
<td>0.40</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>(\text{ClO}_2(\text{aq}) + e^- \rightleftharpoons \text{ClO}_2^-)</td>
<td>0.95</td>
</tr>
</tbody>
</table>
such as trihalomethane and haloacetic acids, are produced than in chlorine-treated water [300]. Similar to chlorination, chloramination also produces haloacetonitriles and haloketones, which include dichloroacetonitrile, trichloroacetonitrile, and 1,1,1-trichloro-2-propanone [300]. Other disinfectants, ClO$_2$ and O$_3$, are also applied as water treatments. Both disinfectants have high reactivities with amino acids and proteins (Chapters 3 and 4). The use of ClO$_2$ is beneficial in minimizing the formation of trihalomethanes, but ClO$_2$ itself reduces to ClO$_2^-$ and ClO$_3^-$, which may cause hemolytic anemia and other health effects. Ozone is an efficient disinfectant, but it may react with Br$^-$ in water to produce the carcinogenic bromate ion. Mn(VII) and Fe(VI) are other alternate disinfectants [306] and their reactions with amino acids, peptides, and proteins are presented in Chapter 6.

$N$-Nitrosodimethylamine (NDMA) is also of considerable concern as an environmental contaminant and has been detected in air, beverages, food products, and water [307–309]. NDMA is classified as a “probable human carcinogen” by the International Agency for Research on Cancer (IARC). A number of studies have shown that both chlorination and chloramination produce NDMA [310–312]. Ozonation has also been shown to result in the production of NDMA [313, 314]. The formation of NDMA in water usually occurs from the reaction of disinfectants (ClO$_2$, O$_3$, *OH, Mn(VII), and Fe(VI)) with nitrogen-containing precursors such as dimethylamine, tertiary amines, amine-containing polymers, and dimethylsulfamide [315]. The destruction of NDMA can be accomplished by oxidation, which include electrochemical, photolytic, photocatalytic, and chemical methods [315].

### 1.4.3 Oxidation Processes for Purifying Water

Oxidation processes using H$_2$O$_2$, ozone, the Fenton reaction, electron beam radiation, and ultrasound have been applied to degrade recalcitrant and emerging contaminants in water [316–318]. Generally, oxidation processes involve the formation of *OH, which reacts nonselectively with organics (see Chapter 4) [319, 320]. The reactions of O$_3$ with organics are selective (see Chapter 4). Generation of *OH to oxidize compounds can also be achieved by applying UV/TiO$_2$, UV/H$_2$O$_2$, TiO$_2$-photocatalyzed, photoassisted Fenton, and electro-Fenton systems [291, 321, 322]. In recent years, studies have focused on photocatalysts under visible light to produce *OH [322–324]. Sulfate radicals have also received attention in oxidation processes to destroy refractory organic contaminants, pharmaceutical and personal care products [222, 325–327]. More details on *OH and SO$_4^{2-}$ are presented in Chapter 4.

Among the high-valent metals, ferrate(VI) (Fe(VI), Fe$^{VI}$O$_4^{2-}$) has been shown to oxidize a number of inorganic and organic compounds in water [328–330]. Oxidations carried out by Fe(VI) are completed in shorter time periods than oxidations performed by Mn(VII) and Cr(VI) [331]. More details of the chemistry of high-valent compounds of iron, manganese, and chromium and their role in oxidizing organic compounds including amino acids and
peptides are described in Chapter 6. Unlike chromium and manganese, processes using Fe(VI) are considered environmentally benign. Other oxidation states of iron, ferrate(V) (Fe(V)) and ferrate(IV) (Fe(IV)), have shown much higher reactivity than that of Fe(VI) and also have potential uses in oxidation processes [300, 332–334]. Synthetic methods of ferrates are summarized in Chapter 6. The reactivity of ferrates with molecules is highlighted for amino acids and peptides (Chapter 6). The generation of Fe(V) species in the Fe(VI)-TiO₂-UV system has also been suggested as a method to degrade pollutants and organisms [335]. The iron-tetraamidomacrocyclic ligand (Fe-TAML), a catalyst in trace amounts, activates H₂O₂ to produce reactive species, Fe⁴⁺=O and Fe⁵⁺=O, which oxidize numerous compounds of environmental interest [336].

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