Chapter 8
Radiolytic and Photolytic Production of Free Radicals and Reactive Oxygen Species: Interactions with Antioxidants and Biomolecules

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Abstract This chapter discusses a variety of free radicals and other reactive oxygen species that are biologically and medically relevant. Radiolytic and/or photochemical methods of production for each reactive oxygen species are shown and for each type of reactive oxygen species some antioxidant and/or biomolecule interactions are discussed. Additionally, the techniques of laser flash photolysis and pulse radiolysis are described in detail and a comparison of the two techniques is made

8.1 Introduction

Other than the obvious exceptions, such as pigmented skin cells, the rods and cones in the eye and photosynthetic cells in plants and bacteria, most cells are not very sensitive to light. However, the presence of a photosensitiser, which can be exogenous or a cellular component, may induce damage possibly leading to cell death.

Photosensitisation in the presence of molecular oxygen can be classified into two pathways (shown in Fig. 8.1). A type I mechanism occurs *via* electron or hydrogen atom transfer from a substrate (RH) to the triplet state of a sensitiser (^{3}sens*); this produces a carbon-centred radical ($R^\bullet$). This carbon-centred radical can then go on to react with oxygen producing a peroxyl radical ($RO_2^\bullet$). A chain reaction follows, with $RO_2^\bullet$ attacking other substrates, thus, generating more carbon-centred radicals, propagating the oxidation. A type II mechanism generates singlet oxygen *via* energy transfer from ^{3}sens* to ground state molecular oxygen.

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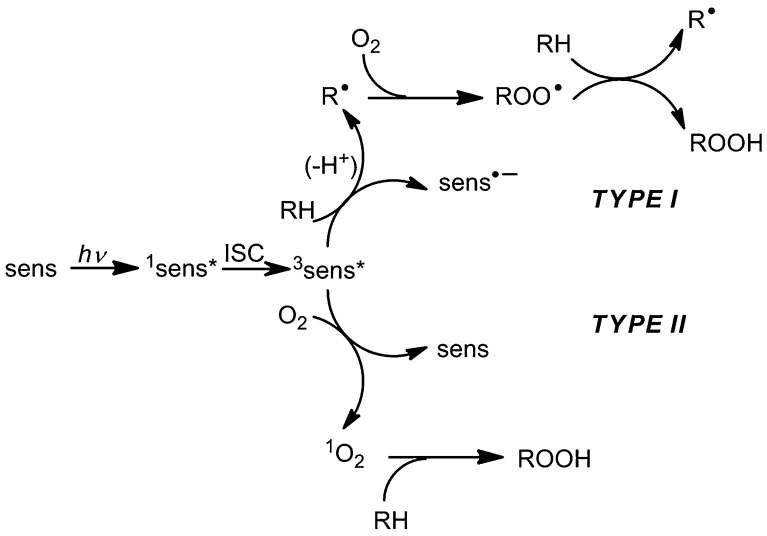

Fig. 8.1 Reaction scheme for photosensitised oxidation, showing type I and type II initiation

Additionally, many free radicals are produced in the body during normal metabolic processes (see, for example, [1, 2]) and they are usually very reactive species, having one unpaired electron and a spin multiplicity of two. Radicals can be neutral, positively or negatively charged, and are, essentially, either oxidising or reducing species, depending on their reduction potentials and those of the substrates they react with.

Lipids, proteins and DNA are all susceptible to attack by both singlet oxygen and free radicals [3, 4]. Cellular damage is both dependent on the location of the sensitiser and permeation of the sensitiser to the target site. It has been suggested that many diseases, including atherosclerosis [5], cancer [4], age-related macular degeneration [6] and neurological disorders [4], such as Alzheimer's disease, as well as the ageing process in general [7], are all associated with singlet oxygen and/or free radical production.

A range of defense systems have evolved to scavenge these species and to repair slight cellular damage. Substances which afford these defense mechanisms are known as antioxidants and can be classified into two categories, preventative antioxidants and scavenging antioxidants.

The preventative antioxidants include superoxide dismutase (SOD), catalase and glutathione peroxidase, as well as metal chelators. There are three types of SOD enzyme, containing different metals, which all catalyse the dismutation of superoxide [8]. Due to SOD enzymes generating H_2O_2, catalase and glutathione peroxidase work together with SOD to remove H_2O_2 by converting it to harmless compounds. Catalyse converts H_2O_2 to oxygen and water, while glutathione peroxidase oxidises reduced glutathione and also generates water [9]. Metal chelators, such as protoporphyrin IX and albumin, are used to prevent iron or copper from catalysing the Haber–Weiss reaction [10], which produces the hydroxyl radical.

The radical scavenging antioxidants are non-enzymatic and are found in plasma, lipoproteins and membranes. It is these types of antioxidant reactions that this Chapter will concentrate on, since many of these antioxidants can be increased by dietary supplementation and, therefore, could be used medicinally to prevent oxidation by radicals and other reactive oxygen species.

This category of antioxidants can be subdivided into water soluble and lipid soluble antioxidants. Nevertheless, they all act in a similar manner when scavenging reactive oxygen species. Radicals usually react with these antioxidants as a redox system, hence, the difference in reduction potentials of the species involved is a factor in determining how efficiently the reaction proceeds. An antioxidant can either react by donating an electron or a hydrogen atom to the radical, thus producing a stable compound and an antioxidant-derived radical (non-redox reactions can also occur, such as addition).

In the electron transfer case, the antioxidant radical cation can subsequently deprotonate producing the neutral radical, and there is much evidence to suggest that the interaction of antioxidants with peroxyl radicals proceeds *via* this mechanism [11]. However, for the antioxidant to be effective its radical must either be stable and thus, not initiate other chain reactions by attacking other substrates, or it must be recycled by an enzyme or another antioxidant compound whose radical has a lower reduction potential. Scavenging antioxidants also react with singlet oxygen in two ways, either physical or chemical quenching [12]. Physical quenching occurs *via* electron exchange energy transfer producing the triplet state of the antioxidant and molecular oxygen. This reaction must predominate over chemical quenching, which will destroy the antioxidant, to give prolonged protection.

The major water-soluble antioxidant present in plasma, is ascorbic acid (vitamin C) [13]. At physiological pH it is present as ascorbate ($AscH^-$) and, due to its strong reducing potential, it is capable of scavenging many reactive oxygen species. Other water soluble antioxidants capable of radical scavenging include uric acid, albumin-bound billirubin and glutathione [13, 14]. The lipid-soluble antioxidants include ubiquinol, carotenoids, flavonoids and other polyphenols, as well as the tocopherols (vitamin E) [14, 15]. Indeed, α-tocopherol is generally considered to be the most important lipophilic antioxidant [16].

This chapter will describe both pulse radiolytic and flash photolytic methods for the generation of a variety of biologically relevant free radicals and reactive oxygen species in solution, giving examples of antioxidant/biomolecule interactions with each species.

8.2 Experimental Techniques: Laser Flash Photolysis and Pulse Radiolysis

Flash photolysis is a useful technique for studying transient species, such as excited states and radicals, which are too short lived to be detected by conventional absorption spectroscopy [17].

An intense short pulse of UV or visible radiation is used to electronically excite the sample, and the subsequent absorption changes are probed spectrophotometrically. The technique was first introduced by Norrish and Porter in 1949 [18] and at this time gas-filled discharge lamps were used, limiting the time resolution, which is principally governed by the duration of the excitation pulse, to microseconds. This is now usually termed conventional flash photolysis. However, with the development of laser pulsed techniques in place of flash excitation, the time resolution has been progressively reduced to subpicosecond, particularly with the use of mode-locked solid state lasers. Much current work utilises nanosecond time resolution with pulsed lasers such as ruby, neodymium and excimer lasers.

One advantage of laser excitation is that monochromatic light allows excitation selectivity. Hence, laser flash photolysis has become an extremely useful method which is widely used in the investigation of many transient species, including biradicals, photoisomers, and photo tautamers, as well as excited states and radicals. Laser flash photolysis is also discussed in more detail in Chaps. 14 and 15.

Pulse radiolysis is another technique for generating free radicals and excited states *in vitro* [17, 19, 20]. This technique is complementary to laser flash photolysis and has the ability to generate radicals in high yields, which is often impossible by laser flash photolysis. Thus, pulse radiolysis is often the preferred method for generation of radicals while flash photolysis is preferred for generating electronic excited states.

Pulse radiolysis emerged about ten years after laser flash photolysis and uses similar principles to study the effects of high energy radiation upon molecules. The laser excitation source used in flash photolysis is often replaced by a beam of electrons in pulse radiolysis, although X-rays, γ-rays, and other energetic particles, such as protons, neutrons, and α- or β-particles may also be used. The beam and sample are housed in a shielded room due to the potentially lethal effects of the ionising radiation and the detection equipment is outside this shielding. The energy of the pulsed beam can be varied as can the pulse length. Hence, the dose of radiation which the sample receives can be altered *via* variation of these parameters.

The transient species produced by either technique are usually monitored *via* the transient absorption changes induced, though other methods of detection can also be used, such as Raman spectroscopy and electron paramagnetic resonance spectroscopy (EPR). Briefly, a monitoring light, e.g. a xenon arc lamp (possibly pulsed) is focused through the sample cell, which, for pulse radiolysis, is normally a flow cell which can be operated from outside the shielding, since the sample is destroyed by the radiation and, therefore, a fresh sample is needed after each pulse, unlike with laser flash photolysis (unless the sample photodegrades rapidly). The monitoring light passes through the sample cell perpendicular to the laser or ion beam and, after passing through the sample, it is collimated into a monochromator and a photodetector, for pulse radiolysis it is first reflected by planar front mirrors out of the shielded radiation area. Changes in the photodetector current are recorded as changes in voltage on an oscilloscope, which can be PC interfaced for analysis and storage of the data.

Even though the experimental apparatus for pulse radiolysis and laser flash photolysis are very similar their initial effects on the samples are very different. In pulse radiolysis, unlike laser flash photolysis where it is the solute which is excited, the energy from the ionising radiation is absorbed by the most abundant species, which in dilute solutions is the solvent. Upon absorption of the radiation the solvent-derived intermediates can interact with the solute thus forming solute transient intermediates. Hence, in pulse radiolysis the choice of solvent is extremely important in determining the type of species formed.

However, despite the initial differences in the two techniques they can both ultimately produce the same species, although the efficiency of their generation is expressed differently. In laser flash photolysis the efficiency is expressed by the quantum yield (ϕ) which is equivalent to the number of excited intermediates formed per absorbed photon. In contrast, in pulse radiolysis the effects of the ionising radiation are measured in G values, which are the number of excited intermediates produced per 100 eV of absorbed energy.

Solute excited states and radicals produced using pulse radiolysis can be formed *via* recombination, direct excitation, or energy transfer from excited solvent and sub-excitation electrons. Mechanisms for some common solvents are discussed below, since they are solvent specific. Thus, by appropriately choosing the experimental conditions, specific radicals or excited states can be generated.

Non-polar solvents, such as hexane and benzene, produce high yields of excited states *via* ion recombination, and relatively low yields of radical ions. In contrast, polar solvents like methanol, acetonitrile and water support high yields of radical ions with low excited state yields, due to solvation and stabilisation of the initial ions, particularly the electrons, leading to a slow rate of ion recombination. In intermediate polarity solvents, such as acetone, approximately equal amounts of radicals and excited states are generated. Hence, generally it is better to study solute excited states with pulse radiolysis in non-polar solvents and solute radicals or radical ions in polar solvents. This is often not possible due to insolubility in the preferred solvent, but if the transients are being monitored *via* transient absorption spectroscopy and they have high molar absorption coefficients then low yields need not be problematic.

8.2.1 Radiolytic generation of radicals and excited states in various solvents

8.2.1.1 Water

The radiolysis of water occurs in two stages, firstly excited states (H_2O^*), cations and electrons are produced (reaction 8.1), then a variety of reactions occur, also generating hydrogen atoms and hydroxyl radicals (reactions 8.2–8.4) and the electron loses energy *via* excitation and ionisation of other molecules and becomes solvated (reaction 8.5).

$$H_2O \rightsquigarrow H_2O^{\bullet+} + e^- + H_2O^* \tag{8.1}$$

$$H_2O^{\bullet+} + H_2O \rightarrow {}^{\bullet}OH + H_3O^+ \tag{8.2}$$

$$H_2O^{\bullet+} + e^- \rightarrow H_2O \tag{8.3}$$

$$H_2O^* \rightarrow {}^{\bullet}OH + H^{\bullet} \tag{8.4}$$

$$e^- \rightarrow e^-_{(therm)} \rightarrow e^-_{(aq)} \tag{8.5}$$

Reaction 8.2 occurs in 1.6×10^{-14} s [17] which is faster than the recombination of $H_2O^{\bullet+}$ and $e^-_{(aq)}$ (reaction 8.3). These species can then rapidly react with each other so that further hydrogen atoms are generated *via* reaction 8.6 and reactions 8.7–8.9 produce hydrogen and hydrogen peroxide.

$$e^-_{(aq)} + H^+ \rightarrow H^{\bullet} \quad k = 2.2 \times 10^{10} \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.6}$$

$$H^{\bullet} + H^{\bullet} \rightarrow H_2 \quad k = 1 \times 10^{10}\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.7}$$

$$e^-_{(aq)} + e^-_{(aq)} \rightarrow H_2 + 2OH^- \quad k = 5 \times 10^9\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.8}$$

$${}^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad k = 6 \times 10^9\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.9}$$

Many of the radicals formed will recombine to form water and the protons and hydroxide ions eventually neutralise one another. Thus, the ultimate products of water radiolysis (within a ns) in an argon or nitrogen saturated solution are given in reaction 8.10 with the G values shown in parentheses [17, 21].

$$H_2O \rightsquigarrow \begin{array}{c} {}^{\bullet}OH(2.7) + H^{\bullet}(0.55) + e^-(2.65) \\ + H_2O_2(0.7) + H_2(0.45) + H_3O^+(2.7) \end{array} \tag{8.10}$$

Of these products it is the three radical species which are the most reactive. The solvated electron and hydrogen atom have reduction potentials (E_0) of −2.87 and −2.30 V versus the standard hydrogen electrode (SHE), respectively [22], and hence they are extremely reactive reductants. The hydroxyl radical is a highly oxidising species with a reduction potential (E_0) of 2.65 V *vs* SHE [22].

Since a restricted radical source is needed for many studies, specific scavengers can be utilised to produce exclusively reducing or oxidising conditions. In order to selectively produce reduced products of the solute, sodium formate can be added to the solution in a high concentration. The formate anion reacts with the oxidising hydroxyl radical and with the hydrogen atom (reaction 8.11) forming $CO_2^{\bullet-}$ which is reducing and has a reduction potential of −1.9 V *vs* SHE [23], so it is not as reactive as the solvated electron.

$${}^{\bullet}OH(H^{\bullet}) + HCO_2^- \rightarrow H_2O(H_2) + CO_2^{\bullet-} \tag{8.11}$$

Alternatively, alcohols such as isopropanol or *tert*-butanol can be used to remove hydroxyl radicals (reactions 8.12 and 8.13). Isopropanol also effectively

scavenges hydrogen atoms ($k = 5 - 7 \times 10^7$ mol dm^{-3} s^{-1} [24, 25]) whereas *tert*-butanol does not ($k = 2 \times 10^5$ mol dm^{-3} s^{-1} [25]).

$$^{\bullet}OH(H^{\bullet}) + (CH_3)_2CHOH \rightarrow H_2O(H_2) + (CH_3)_2C^{\bullet}OH \quad (8.12)$$

$$^{\bullet}OH(H^{\bullet}) + (CH_3)_3COH \rightarrow H_2O(H_2) + {}^{\bullet}CH_2(CH_3)_2COH \quad (8.13)$$

Predominantly oxidising conditions can be produced by saturating the solution with nitrous oxide gas (N_2O), which reacts with the solvated electron to generate further oxidising hydroxyl radicals (reaction 8.14). The reducing hydrogen atoms also react with nitrous oxide, producing more $OH^{\bullet}$ and nitrogen, though with a much slower rate constant ($k = 2.1 \times 10^6$ mol dm^{-3} s^{-1} [26, 27]).

$$e^-_{(aq)} + N_2O \rightarrow {}^{\bullet}OH + N_2 + OH^- \quad k = 9.1 \times 10^9 \text{ mol dm}^{-3} \text{ s}^{-1} \quad (8.14)$$

Nitrous oxide saturation can also be used with other solvents to remove the electron although the $O^{\bullet-}$ produced may not generate $OH^{\bullet}$ as it does in water but little is known about the reactions of N_2O in other solvents. However, in N_2O saturated cyclohexane, nitrogen and hydrogen are produced together with the oxygenated product, cyclohexanol [28].

In some cases when oxidising conditions are required, milder oxidants may be needed, because the hydroxyl radical can react with the solute forming adducts as well as *via* electron transfer. Hydroxyl radicals can be converted into milder (one-electron) oxidants by the addition of halides, thiocyanate or azide ions (reactions 8.15–8.17). In fact, halide radical reactions occur in atmospheric chemistry, particularly in urban cloud droplets, as well as in marine water radical reactions [29].

$$^{\bullet}OH + Br^-(SCN^-) \rightarrow Br^{\bullet}(SCN^{\bullet}) + OH^- \quad (8.15)$$

$$Br^{\bullet}(SCN^{\bullet}) + Br^-(SCN^-) \rightarrow Br_2^{\bullet-}\left((SCN)_2^{\bullet-}\right) \quad (8.16)$$

$$^{\bullet}OH + N_3^- \rightarrow N_3^{\bullet} + OH^- \quad (8.17)$$

8.2.1.2 Methanol

Methanol is a useful polar solvent for solutes which are insoluble in water. The radiolysis of methanol yields a number of intermediates including $CH_3O^{\bullet}$, $H^{\bullet}$, $^{\bullet}OH$, and $CH_3^{\bullet}$ as well as $e^-_{(MeOH)}$ and $^{\bullet}CH_2OH$. The first four radicals above all react with methanol itself, yielding more $^{\bullet}CH_2OH$. Hence, the initial reaction reduces to reaction 8.18.

$$CH_3OH \rightsquigarrow {}^{\bullet}CH_2OH + e^-_{(MeOH)} \quad (8.18)$$

Both of the species, $e^-_{(MeOH)}$ and $^{\bullet}CH_2OH$ are reducing and will react with the solute to generate its radical anion.

Also useful for samples insoluble in water are detergents, since it is possible to study oxidation and reduction reactions *via* electron transfer through the water-detergent interface.

8.2.1.3 Hexane

This non-polar aliphatic hydrocarbon solvent has relatively short-lived excited states ($\tau_{s1} < 1$ ns), and as such, upon absorption of radiation the major process is solvent ionisation (reaction 8.19) producing the hexane radical cation ($C_6H_{14}^{\bullet+}$) and the electron (e^-). As the electron is not readily solvated in hexane, it will either recombine with the hexane radical cation or react with the solute (S) (reactions 8.20 and 8.21). The parent radical cation can also react with the solute, as in reaction 8.22.

$$C_6H_{14} \rightsquigarrow C_6H_{14}^{\bullet+} + e^- \tag{8.19}$$

$$C_6H_{14}^{\bullet+} + e^- \rightarrow C_6H_{14} \tag{8.20}$$

$$e^- + S \rightarrow S^{\bullet-} \tag{8.21}$$

$$C_6H_{14}^{\bullet+} + S \rightarrow C_6H_{14} + S^{\bullet+} \tag{8.22}$$

Fast recombination of solute radical anions and cations (reaction 8.23) or of solute radical anions with hexane radical cations (reaction 8.24) yields first excited singlet and triplet states of the solute (S*). Further solute triplet states may be produced *via* intersystem crossing.

$$S^{\bullet-} + S^{\bullet+} \rightarrow 2S^* \tag{8.23}$$

$$S^{\bullet-} + C_6H_{14}^{\bullet+} \rightarrow S^* + C_6H_{14} \tag{8.24}$$

Two types of ion are involved in ion recombination. Geminate ions, which constitute 90 % of the total, recombine within a few nanoseconds since the positive and negative ions which are formed do not escape each others influence. The other 10 % of the ions do escape each others influence and are termed 'free' or non-geminate. They recombine over microsecond time scales. The high percentage of geminate ions in hexane explains why non-polar solvents support high yields of excited states and low yields of radical ions.

8.2.1.4 Benzene

The aromatic hydrocarbon benzene differs from hexane since it has relatively long-lived excited singlet and triplet states. ($\tau = 20$ ns and 3 μs, respectively). Thus, solute excited states may be generated *via* energy transfer from the benzene

excited states. Again, intersystem crossing may occur, yielding more triplet states. The following reactions (8.25–8.29) illustrate the radiation chemistry of benzene with a solute (S). (Reactions 8.20–8.24 can also occur, with benzene replacing hexane).

$$C_6H_6 \rightsquigarrow C_6H_6^{\bullet +} + e^- \tag{8.25}$$

$$C_6H_6 + e^- \rightarrow C_6H_6^{\bullet -} \tag{8.26}$$

$$C_6H_6^{\bullet +} + C_6H_6^{\bullet -} \rightarrow 2\,^1C_6H_6{}^* \left(\text{or } 2\,^3C_6H_6{}^*,\ \text{or } {}^1C_6H_6{}^* + {}^3C_6H_6{}^*\right) \tag{8.27}$$

$$^1C_6H_6{}^* + S \rightarrow C_6H_6 + {}^1S^* \tag{8.28}$$

$$^3C_6H_6{}^* + S \rightarrow C_6H_6 + {}^3S^* \tag{8.29}$$

8.3 Production of Radicals and Reactive Oxygen Species and their Reactions

8.3.1 Hydroxyl radical

As discussed above (in Sect. 8.2.1.1) the hydroxyl radical is one of the primary products in the radiolysis of water and can almost be exclusively produced by saturating the solution with N_2O. Other methods of $^{\bullet}OH$ production include, the photolysis of dilute solutions of hydrogen peroxide [30] and the metal-ion catalyzed Haber–Weiss reaction which can also occur *in vivo* [10].

The hydroxyl radical is a highly oxidising species, having a reduction potential of 2.31 V *vs* SHE at pH 7 [31], higher in acidic solutions [22]. Thus, it is capable of oxidising many organic compounds, such as the flavour compound methional [32] and the anti-inflammatory drug metiazinic acid [33]. It can also abstract hydrogen atoms from C to H groups e.g. in aliphatic amino acids [34] and add across C=C double bonds e.g. in the purine bases [35] and in the spin traps often used to detect it, such as DMPO [36]. It has a pK_a of 11.9 and so forms $O^{\bullet -}$ in highly basic solutions, which can sometimes react *via* a different mechanism. For example, a study by Neta *et al.* has shown that for aromatic compounds with aliphatic chains $^{\bullet}OH$ will preferentially add to the aromatic ring whilst $O^{\bullet -}$ will abstract a hydrogen atom from the aliphatic chain [37].

A wide range of flavonoid antioxidants have been studied for their ability to scavenge $^{\bullet}OH$ radicals produced by photolysis of hydrogen peroxide and analysed using spin-trapping and HPLC [36]. It was found that those flavonoids containing the most hydroxyl groups in the aromatic B-ring were the best scavengers. They found that the C-3 hydroxyl group was the most important, as did a more recent study using a salicylate probe for detection in a modified CUPRAC (cupric ion

reducing antioxidant capacity) assay [38]. Additionally, the presence of a carbonyl group in the C-4 position increased the reactivity significantly, with catechin shown to quench only 2/3 the amount of $^{\bullet}OH$ as quercetin. In the spin-trapping study [36] the flavones were shown to exhibit similar quenching capacities to the flavanone, naringenin, suggesting that the presence of a double bond between the C-2 and C-3 groups has no effect on the scavenging capacity. However, the newer study showed that the presence of this double bond increased scavenging [38].

The reactions of $^{\bullet}OH$ radicals with the polyphenolic antioxidant bergenin have been monitored using pulse radiolysis [39]. Multiple reaction pathways have been shown to occur, with radical addition being the major process and one electron oxidation only a minor process. Both radical addition and hydrogen abstraction were shown to produce reducing radicals that react readily with oxygen to yield peroxyl radicals, suggesting that bergenin may act as a prooxidant.

8.3.2 Superoxide Radical Anion and its Protonated Form

Superoxide can be produced in a number of ways, radiolitically, photochemically, electrochemically [40], enzymatically (*via* xanthine oxidase) [41] or prepared from potassium superoxide [42]. Biologically it is generated mainly in phagocytic cells helping them to inactivate foreign bodies, such as viruses and bacteria [43]. When these cell types are activated for phagocytosis an increase in oxygen consumption (of at least 10 fold) is triggered and there is rapid reduction of the oxygen to superoxide. This reaction is catalysed by plasma membrane-bound NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase, reaction 8.30.

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^{\bullet -} \tag{8.30}$$

Several subcellular organelles, including mitochondria, microsomes and chloroplasts, generate superoxide during electron transport, usually via the autooxidation of various biomolecules, such as reduced cytochrome C and reduced flavins, as well as haemoglobin and myoglobin (see, for example, [1]).

Photochemical production of $O_2^{\bullet -}$ can be achieved in two ways. Firstly, by the photolysis of concentrated hydrogen peroxide solutions, where the initially produced hydroxyl radicals go on to react with the hydrogen peroxide to produce superoxide [44]:

$$H_2O_2 \xrightarrow{h\nu} 2^{\bullet}OH \tag{8.31}$$

$$^{\bullet}OH + H_2O_2 \rightarrow H^+ + O_2^{\bullet -} + H_2O \tag{8.32}$$

The other method is to generate it *via* reduction of a donor triplet, such as a flavin, to its radical anion, which will reoxidise simultaneously reducing molecular oxygen [45].

Radiolytic production of $O_2^{\bullet-}$ is achieved in oxygen saturated aqueous solutions containing formate. Of the primary radicals produced upon water radiolysis, both the hydrated electron and the hydrogen atom react rapidly with oxygen to produce $O_2^{\bullet-}$. The hydroxyl radicals (and the hydrogen atom) react with the formate to produce the carbon dioxide radical anion and this radical anion reacts with oxygen generating further $O_2^{\bullet-}$ [46].

$$e^-_{(aq)} + O_2 \rightarrow O_2^{\bullet-} \quad k = 2 \times 10^{10} \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.33}$$

$$H^{\bullet} + O_2 \rightarrow O_2^{\bullet-} + H^+ \quad k = 2 \times 10^{10}\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.34}$$

$$HCO_2^- + {}^{\bullet}OH \rightarrow CO_2^{\bullet-} + H_2O \quad k = 3.5 \times 10^9\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.35}$$

$$HCO_2^- + H^{\bullet} \rightarrow CO_2^{\bullet-} + H_2 \quad k = 1.3 \times 10^8\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.36}$$

$$CO_2^{\bullet-} + O_2 \rightarrow CO_2 + O_2^{\bullet-} \quad k = 2.4 \times 10^9\ \text{mol dm}^{-3}\ \text{s}^{-1} \tag{8.37}$$

In aqueous, and other protic media, superoxide is not very reactive, due to its negative charge, high activation energy and high energy of solvation (usually it acts as a mild reductant, although it can also act as an oxidant). However, it is the dissociated form of the hydroperoxyl radical ($HO_2^{\bullet}$), a weak acid, and this is more reactive. For example, $HO_2^{\bullet}$ is capable of initiating peroxidation of polyunsaturated fatty acids (PUFA), whereas $O_2^{\bullet-}$ cannot. The hydroperoxyl radical has a pK_a of 4.8 [47], thus at physiological pH only a small amount of superoxide will be present in the protonated form. However, in aqueous solutions both of these species ($HO_2^{\bullet}$ and $O_2^{\bullet-}$) can react with themselves or each other producing hydrogen peroxide which can then, in turn, react with superoxide generating the hydroxyl radical.

Many antioxidants have been shown to react with superoxide, such as ubiquinone [48], curcumin [49] and ascorbic acid/ascorbate [50]. A variety of flavonoids and other plant antioxidants have been tested for their superoxide scavenging ability [51], with those compounds containing ortho-trihydroxy groups showing the highest rate constants for scavenging. While those containing the ortho-dihydroxy (catechol) group have rate constants for superoxide scavenging of about one order of magnitude lower and the rate constants for those with only a monohydroxy group were shown to be 2–3 orders of magnitude lower. More recently, Silva *et al.* [52] have synthesised and studied some flavonoid derivatives, 3-alkylpolyhydroxyflavones, in which the C-3 hydroxyl group on the chromone ring has been replaced by an alkyl chain. *Via* pulse radiolysis studies of the reaction of superoxide with these compounds they have shown that different alkyl chain lengths allow the compounds to penetrate into the micelles to different depths, therefore, suggesting that cellular distribution can be selectively modified to improve the inhibitory effect on damage due to reactive oxygen species.

8.3.3 Singlet Oxygen

Ground state molecular oxygen has a spin multiplicity of 3 (*i.e.* it is in a triplet state, $^3\Sigma_g^-$) with the two unpaired electrons being in the degenerate pair of π^* orbitals. The two lowest electronic excited states of oxygen in the gas phase are singlet states ($^1\Delta_g$ and $^1\Sigma_g^+$) with the $^1\Delta_g$ state being the lower lying and as such being commonly referred to as singlet oxygen ($^1O_2$) [53].

$^1O_2$ can be produced in a number of ways, e.g. peroxide decomposition, high frequency discharge and energy transfer [53]. The most common mechanism for its production is *via* energy transfer from the excited state of a photosensitiser to ground state molecular oxygen. The low energy level of $^1O_2$ ($E = 0.98$ eV or 94.5 kJ mol^{-1}) means that many sensitisers have a high enough energy in their singlet and triplet states to convert molecular oxygen to its excited state. This means that the quantum yield of $^1O_2$ production can reach two. For both singlets and triplets to be quenched by molecular oxygen in this way, the singlet state lifetime must be long and the energy difference between the singlet and triplet state ($\Delta E(S_1 - T_1)$) and the triplet state energy must both be higher than $E(^1O_2)$. Hence, $^1O_2$ production most often occurs from triplet states only, since usually $\Delta E(S_1 - T_1)$ is too low and the lifetime of the singlet state is too short. Typical triplet sensitisers are dyes like methylene blue, rose bengal and eosin, although many other compounds are capable of sensitising singlet oxygen due to the relatively small energy difference between the ground state ($^3\Sigma_g^-$) and excited state ($^1\Delta_g$). Usually, the triplet state of the sensitiser is generated *via* laser flash photolysis (see Chap. 15) but pulse radiolysis can also be used [54, 55] and, in fact, can produce more accurate triplet-induced $^1O_2$ yields. This is because photolysis initially generates only excited singlet states, whereas radiolysis generates both triplet and singlet excited states (usually in about a 3:1 ratio), thus less singlet state quenching by oxygen can occur and therefore less additional sensitiser triplet states are produced (via oxygen-enhanced intersystem crossing or by energy transfer).

In biological systems, sensitisers such as porphyrins, chlorophylls and riboflavin can sensitise $^1O_2$ production and this can lead to deleterious effects including DNA damage and lipid peroxidation [56, 57]. Once produced $^1O_2$ can react with and oxidise many cellular substrates but it has a limited lifetime and, if no reaction occurs, it decays to the ground state either radiatively or by solvent-induced non-radiative deactivation. The non-radiative process dominates in solution, and is governed by the vibrational frequencies of the solvent molecule. Thus, the lifetime of singlet oxygen is greatly influenced by the solvent, varying from a few milliseconds to a few microseconds compared with a half-life of 45 min in the gas phase [58]. The radiative component of the deactivation of $^1O_2$ has a maximum around 1270 nm for the (0', 0) transition (varying only a few nm with the solvent) and this decay can be used for monitoring $^1O_2$ (see Chap. 15).

$^1O_2$ may be quenched either chemically or physically by antioxidants, with chemical quenching ultimately destroying the quencher. Physical quenching can occur either *via* collisional energy transfer, which is the reverse of the reaction by

which $^1O_2$ is formed and is the process by which carotenoids quench $^1O_2$ or via charge transfer with electron donors, such as amines [53]. Various antioxidants have been shown to quench singlet oxygen, for example the tocopherols [59]. One class of antioxidants which quench $^1O_2$ very efficiently is the carotenoids and many studies have been carried out on their quenching and on their protection against $^1O_2$ mediated photo-oxidation reactions. Foote and Denny [60] were the first to show that β-carotene inhibits photosensitised oxidation and was, therefore, able to efficiently quench $^1O_2$. Farmilo and Wilkinson [61] showed that electron exchange energy transfer quenching is the principal mechanism of carotenoid photoprotection against $^1O_2$, leading to the carotenoid triplet state (reaction 8.38), although, chemical quenching also occurs in a minor process destroying the carotenoid [62].

$$^1O_2 + CAR \xrightarrow{k_q} O_2 + {}^3CAR^* \tag{8.38}$$

Once produced ^{3}CAR* returns to the ground state dissipating the energy as heat or it can be quenched physically *via* enhanced intersystem crossing by oxygen.

Many carotenoids have been studied to investigate the influence of different structural characteristics on the ability to quench $^1O_2$ and it has been observed that the quenching ability increases with increasing number of conjugated double bonds, n, and the increasing wavelength of the $\pi\pi^*$ absorption maximum, reflecting increased exothermicity in the energy transfer as the energy of ^{3}CAR* decreases, see Fig. 8.2 [63, 64].

Studies have also been undertaken in more biologically relevant environments, such as micelles and dipalmitoylphosphatidylcholine (DPPC) liposomes [64, 65] where the quenching rate constants are still found to be high and in the liposome study [65] little difference was observed in the quenching when the $^1O_2$ was generated by either water or lipid soluble photosensitisers. Cellular studies have also shown carotenoids to be efficient quenchers of singlet oxygen, for example in isolated photosystem II reaction centres [66] and in protecting *ex vivo* lymphocytes from $^1O_2$ damage [67].

8.3.4 Peroxyl Radicals

Peroxyl radicals are formed in the oxidation of many organic and biological molecules and they can propagate chain reactions. They are usually formed *via* the reaction of oxygen with carbon-centered radicals. Lipid peroxyl radicals are produced during lipid peroxidation, which is a complex process but can be divided into stages [3]:

1. Initiation; production, and subsequent attack of a polyunsaturated fatty acid (PUFA) side chain by $R^\bullet$, $RO_2^\bullet$ or $^1O_2$, producing a lipid radical (capable of reacting with oxygen).

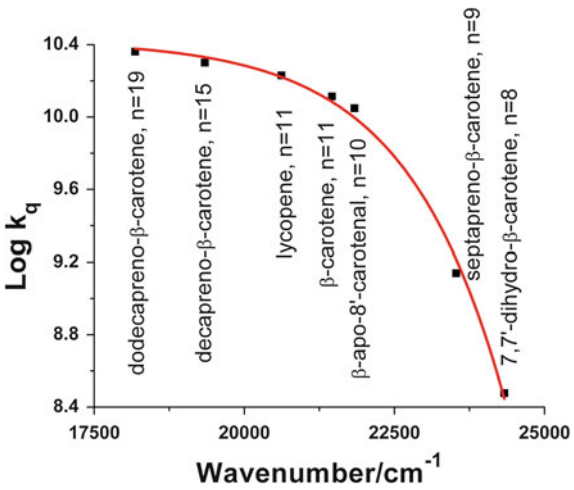

Fig. 8.2 Graph showing the relationship between the rate constant for $^1O_2$ quenching (k_q) and the wavenumber of the lowest energy ground state absorption maximum for a range of carotenoids in benzene, adapted from [64]

2. Propagation; the fatty acid peroxyl radical ($PUFAO_2^{\bullet}$) abstracts a hydrogen atom from another PUFA molecule.

$$PUFAO_2^{\bullet} + PUFA \rightarrow PUFAOOH + PUFA^{\bullet} \tag{8.39}$$

The resulting $PUFA^{\bullet}$ can react with oxygen and a chain reaction is initiated so that lipid hydroperoxides accumulate until:-

3. Termination; leads to non-radical products.

The accumulated lipid hydroperoxides can, however, react with metal complexes, generating even more alkoxyl and peroxyl radicals.

A wide range of peroxyl radicals can be produced both photochemically and radiolitically, *via* reaction of oxygen with the corresponding alkyl radical, and their methods of production and reaction rates with a variety of compounds have been detailed by Neta *et al.* [68].

The peroxyl radical that has been most extensively studied for its interactions with antioxidants is the trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$), which is produced during the metabolism of CCl_4 *via* reaction of the trichloromethyl radical ($CCl_3^{\bullet}$) with oxygen [69] and is known to cause hepatoxicity and other types of tissue injury. Pulse radiolysis is normally used to generate this radical and in primarily aqueous solutions it is prepared in air saturated solutions by adding carbon tetrachloride, 2-propanol and acetone and is produced *via* the following reactions [70].

$$^{\bullet}OH + (CH_3)_2CHOH \rightarrow H_2O + (CH_3)_2C^{\bullet}OH \tag{8.40}$$

$$^{\bullet}H + (CH_3)_2CHOH \rightarrow H_2 + (CH_3)_2C^{\bullet}OH \quad (8.41)$$

$$e^-_{(aq)} + (CH_3)_2CO + H^+ \rightarrow (CH_3)_2C^{\bullet}OH \quad (8.42)$$

$$(CH_3)_2C^{\bullet}OH + CCl_4 \rightarrow (CH_3)_2CO + CCl_3^{\bullet} + HCl \quad (8.43)$$

$$CCl_3^{\bullet} + O_2 \rightarrow CCl_3O_2^{\bullet} \quad (8.44)$$

$CCl_3O_2^{\bullet}$ reacts with ascorbic and uric acid [71], as well as bilirubin [72] and glutathione [73] *via* electron transfer. However, with tryptophan and carotenoids another reaction also occurs, suggested to be radical addition [74, 75]. For the carotenoids the proposed adduct decays to yield more radical cation and for the carotenoid, astaxanthin, the radical cation is not formed initially but is formed solely through the proposed addition radical [75]. The one electron reduction potential of astaxanthin radical cation has been shown to be higher than several other carotenoids [76], so it may be that it is very close to that of $CCl_3O_2^{\bullet}$ so that electron transfer is very slow.

8.3.5 NO_x

Nitrogen monoxide, or nitric oxide ($NO^{\bullet}$) as it is more usually called, is involved in many biological functions. It is formed in activated macrophages and neutrophils where it is produced from the amino acid L-arginine [77] and is involved in killing bacteria. It is also generated by a range of cells as an intercellular messenger and acts as a vasodilator [78]. When $NO^{\bullet}$ is present in excess it is thought to be cytotoxic [79] and humans are exposed daily to this substance from cigarette smoke as well as exhaust fumes (the cytotoxicity may well be mediated by other species derived from $NO^{\bullet}$). $NO^{\bullet}$ reacts readily with oxygen forming nitrogen dioxide $\left(NO_2^{\bullet}\right)$, which is also a major air pollutant and has been shown to trigger lipid peroxidation [80]. $NO^{\bullet}$ can also rapidly react with superoxide producing peroxynitrite ($OONO^-$) [81] and, since both radicals are generated in many cell types, there is a high likelihood of them being able to react. Peroxynitrite is stable at basic pH values, but is the salt of peroxynitrous acid, a weak acid with a pK_a of 6.8 [81], hence if produced *in vivo* nearly half will protonate to peroxynitrous acid. Rapid rearrangement of the peroxynitrous acid to H^+ and nitrate (NO_3^-) then occurs, with competing decomposition generating $NO_2^{\bullet}$ and $^{\bullet}OH$ [82]. The nitrate radical ($NO_3^{\bullet}$) can also be formed, *via* reaction of ozone with $NO_2^{\bullet}$ and, as with all NO_x, it is an air pollutant (see Chap. 5) and is found in cigarette smoke [83].

$NO^{\bullet}$ is stable as a gas in oxygen free environments and it can be selectively generated using pulse radiolysis in argon-saturated aqueous solutions *via* reaction of nitrite with $e^-_{(aq)}$, using formate to scavenge $OH^{\bullet}$ forming $CO_2^{\bullet-}$ *via* reaction 9.11 and the following reactions [84]:

$$NO_2^- + e^-_{(aq)} \rightarrow NO_2^{\bullet 2-} \rightarrow NO^{\bullet} + O^{2-} \quad (8.45)$$

$$NO_2^- + CO_2^{\bullet -} \rightarrow NO_2^{\bullet 2-}(+CO_2) \rightarrow NO^{\bullet} + O^{2-} \quad (8.46)$$

It can also be produced photolytically, for example from S-nitroso complexes [85] or nitrite [86].

$NO_2^{\bullet}$ is also a stable gas and can be produced radiolytically using a mixture of nitrate and nitrite in argon saturated water in a ~10:1 ratio [87]. The nitrite reacts with the hydroxyl radical and the nitrate reacts preferentially with the aqueous electron:

$$NO_3^- + e^-_{(aq)} \rightarrow NO_3^{\bullet 2-} \rightarrow NO_2^{\bullet} + O^{2-} \quad (8.47)$$

$$NO_2^- + OH^{\bullet} \rightarrow NO_2^{\bullet} + OH^- \quad (8.48)$$

Photochemically, $NO_2^{\bullet}$ has been produced directly from nitrite or *via* the nitrite reaction with the triplet state of nitronaphthalene [86, 88].

$ONOO^-$/ONOOH can be generated radiolytically either from reactions 8.47 and 8.48, above (using less nitrate so that the remaining $^{\bullet}OH$ can react with $NO_2^{\bullet}$ [87]) or in air saturated aqueous nitrite solutions containing formate. In this case reaction 8.45 will proceed as above but oxygen can compete for $CO_2^{\bullet -}$ and superoxide will be produced (reaction 8.49). This can then react with $NO^{\bullet}$ generating peroxynitrite (reaction 8.50) [89].

$$O_2 + CO_2^{\bullet -} \rightarrow O_2^{\bullet -} + CO_2 \quad (8.49)$$

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^- \quad (8.50)$$

$ONOO^-$/ONOOH can also be generated by photolysis of nitrite/formate solutions. $NO2^-$ is converted to $NO^{\bullet}$ and $^{\bullet}OH$, then $^{\bullet}OH$ reacts with formate (reaction 8.11) and reactions 8.49 and 8.50 proceed as above [90].

$NO_3^{\bullet}$ can be produced by pulse radiolysis of concentrated nitrate or nitrous acid solutions [91], so that $NO_3^{\bullet}$ is formed directly from the electron pulse, or *via* flash photolysis of ceric nitrate solutions [92]. Both of these methods present problems, as $NO_2^{\bullet}$ will also be produced when using the pulse radiolysis method (*via* reaction 8.47) and the ceric ion from the laser method has a high reduction potential (1.28 V *vs* SHE [93]) and so is also a powerful oxidising agent itself.

$NO^{\bullet}$ is not a highly reactive species and is relatively unreactive towards the antioxidants glutathione and ascorbate [85, 94]. Flavonoids were found to quench $NO^{\bullet}$ but the rate constants determined were also quite low (up to 4×10^2 mol dm^{-3} s^{-1}) [95]. In fact, $NO^{\bullet}$ has been shown to act as an antioxidant itself and can terminate the propagation process of lipid peroxidation [96]. Flavonoids have also been shown not to react efficiently with $ONOO^-$/ONOOH [97], though ascorbate is oxidised (by one electron) by it [98]. It does react with carotenoids and tocopherols [99, 100], though not *via* one electron transfer, and β-carotene has been shown to protect lymphocytes from $ONOO^-$/ONOOH induced damage [90].

$NO_2^{\bullet}$ and $NO_3^{\bullet}$ are both more powerful oxidising species, each reacting with a range of antioxidants. $NO_2^{\bullet}$ usually reacts by one-electron oxidation, as observed for β-carotene [101], though addition across double bonds is possible [102] and it has been shown that β-carotene in hexane is completely destroyed by 2 equimolar amounts of $NO_2^{\bullet}$, with the absorption spectra gradually decreasing and blue-shifting, possibly indicating a gradual decrease in conjugation [103]. Carotenoids are also able to protect lymphocytes from $NO_2^{\bullet}$ induced damage [67, 88, 90]. $NO_3^{\bullet}$ reactions are more complex and can occur *via* electron transfer, addition and hydrogen abstraction [91, 104, 105].

8.3.6 Carbonate Radical

The carbonate radical ($CO_3^{\bullet-}$) can be produced through the reaction of peroxynitrite with CO_2 (reaction 8.51) and this could occur *in vivo* [106]:

$$ONOO^- + CO_2 \rightarrow NO_2^{\bullet} + CO_3^{\bullet-} \tag{8.51}$$

$CO_3^{\bullet-}$ is a highly oxidising radical with a reduction potential of 1.59 V *vs* SHE [107] and it can be produced radiolytically very easily, *via* the quenching of $^{\bullet}OH$ by carbonate in nitrous oxide saturated solutions [107]:

$$^{\bullet}OH + HCO_3^-/\ CO_3^{2-} \rightarrow H_2O/OH^- + CO_3^{\bullet-} \tag{8.52}$$

It can also be produced by the photoionisation of carbonate or of carbonato metal complexes [108]. It has an optical absorption maximum at 600 nm with an absorption coefficient of 1830 $mol^{-1}\ dm^3\ cm^{-1}$ [108]. For a long time, it was assumed that this radical exists at neutral pH as the protonated form ($HCO_3^{\bullet}$), but it is now firmly established that above pH 0 the radical exists in the deprotonated state [109].

$CO_3^{\bullet-}$ reactions with antioxidants and biomolecules occur mainly *via* one electron transfer (for example the interaction with tea polyphenols [110]), and it has recently been shown to directly oxidise guanine bases [111] however, hydrogen abstraction and addition can also occur [112]. The rate constants for $CO_3^{\bullet-}$ reaction with amino acids were found to be lower for the aliphatic amino acids than for those containing sulfur, and aromatic amino acids and derivatives showed a range of reactivities, with the indole derivatives, such as tryptophan, reacting most efficiently [113]. Enzyme interactions with $CO_3^{\bullet-}$ were also monitored in this study and their reactivity reflected the reactivity of their constituent aromatic amino acids, with enzymes, such as lysozyme and trypsin (which contain tryptophan) having rate constants comparable to that of tryptophan itself and in ribnuclease A (which contains no tryptophan) the quenching was similar to that of tyrosine.

8.3.7 Sulfur-Containing Radicals

A wide range of sulfur radicals have been reported. Sulfur dioxide ($SO_2^{\bullet-}$), sulfite radical ($SO_3^{\bullet-}$), sulfate radical ($SO_4^{\bullet-}$) and peroxomonosulfate radical ($SO_5^{\bullet-}$) can all be formed from sulfur dioxide, which is an environmental air pollutant, as well as from sulfites and bisulfites used as preservatives [114–118] and methods of their production and their reactivity has been previously reviewed [119]. Briefly, $SO_2^{\bullet-}$ acts as a one electron reductant whereas the others are all oxidising species with $SO_4^{\bullet-}$ being the strongest one electron oxidant [117–119].

Many sulfur radical cations have also been reported and it has been suggested that they might be intermediates in biological redox processes. Their production and reaction has been the subject of an extensive review by Glass [120]. The most important biologically are sulfide ($RS^{\bullet+}$) and disulfide radical cations ($RSSR^{\bullet+}$) which are produced upon $^{\bullet}OH$ radical reaction with biological sulfides, such as the amino acid methionine, and so can be easily generated *via* laser flash photolysis and pulse radiolysis [121, 122]. These radicals have been shown to be oxidising but the reaction mechanisms can be complex. For example, a disulfide radical cation can be converted to the corresponding radical anion ($RSSR^{\bullet-}$) by reaction with thiolate as has been observed for cysteamine oxidation by lipoate radical cations [123]. This is because the reaction proceeds with $RSSR^{\bullet+}$ oxidising thiolate by one electron transfer forming a neutral thiyl radical ($RS^{\bullet}$) and this then equilibrates with excess thiolate to yield $RSSR^{\bullet-}$ (reactions 8.53 and 8.54), thus turning an oxidising species into a reducing one. $RSSR^{\bullet-}$ can also be generated *via* one electron reduction of disulfides [124].

$$RSSR^{\bullet+} + R'S^- \rightarrow RSSR + R'S^{\bullet} \tag{8.53}$$

$$R'S^{\bullet} + R'S^- \rightarrow R'SSR'^{\bullet-} \tag{8.54}$$

Neutral organosulfur (or thiyl) radicals ($RS^{\bullet}$) can also be produced *in vivo* by hydrogen abstraction from, or oxidation of, biological thiols (either *via* antioxidant repair mechanisms or *via* peroxidase catalysed oxidation), such as glutathione, the drug penicillamine and proteins containing the amino acid cysteine. They can also be easily generated by radiolysis and photochemically, for example from $^{\bullet}OH$ reaction with thiol, and the production, both *in vitro* and *in vivo*, and reactions of these radicals have been discussed in several reviews [124–126]. These neutral radicals react with oxygen to give thiol peroxyl radicals ($RSOO^{\bullet}$), these can then react with another thiol to give a sulfinyl radical ($RSO^{\bullet}$) or photoisomerise to sulfonyl radicals (with both oxygens bonded to sulfur, $RSO_2^{\bullet}$), which can also add oxygen to give sulfonyl peroxyl radicals ($RSO_2OO^{\bullet}$) [127].

Ascorbate and α-tocopherol can repair $RSSR^{\bullet+}$ and $RS^{\bullet}$ by electron transfer, and $RS^{\bullet}$ have been shown to abstract hydrogen from polyunsaturated fatty acids. [124, 126] $RSO^{\bullet}$ have been found to be relatively stable while $RSO_2^{\bullet}$ abstract hydrogen atoms, though at very slow rates, and $RSO_2OO^{\bullet}$ have been suggested to be much more reactive [127, 128], with sulfonyl peroxyl radicals from cysteine

reacting rapidly with DNA and free bases by both hydrogen abstraction and addition. [129] Destruction of β-carotene has been observed by xanthine-oxidase initiated $RS^{\bullet}$, with both ascorbic acid and the water soluble tocopherol analogue, Trolox, preventing its destruction [125]. The β-carotene reaction probably occurs *via* addition as seen by Everett *et al.* for the reaction of glutathione thiyl radicals. They also observed quenching of $RSO_2^{\bullet}$ by β-carotene with both an electron transfer and an addition reaction occurring [101].

8.3.8 Alkoxyl and Phenoxyl Radicals

Alkoxyl and phenoxyl ($RO^{\bullet}$) radicals are generated *in vivo* from complexed transition metals (M) and organic hydroperoxides (e.g. lipid hydroperoxides) *via* catalysed electron transfer reactions:

$$M^{(n-1)+} + ROOH \rightarrow M^{n+} + RO^{\bullet} + OH^{-} \tag{8.55}$$

The oxidised metal complex, M^{n+}, is then capable of breaking down peroxides, producing peroxyl radicals ($RO_2^{\bullet}$):

$$M^{n+} + ROOH \rightarrow M^{(n-1)+} + RO_2^{\bullet} + H^{+} \tag{8.56}$$

Aliphatic alkoxyl radicals have reduction potentials of about 1600 mV *vs* SHE at pH 7 making them better oxidising agents than alkyl peroxyl radicals ($E^7 \sim 1000$ mV *vs* SHE) [130]. Phenoxyl radicals usually have even lower reduction potentials, e.g. phenoxyl radical ($C_6H_5O^{\bullet}$) with E7 $\sim$ 900 mV *vs* SHE and tocopheroxyl radical with $E^7 \sim 500$ mV *vs* SHE [130], and these can also be produced *in vivo via* the oxidation of phenols, such as the amino acid tyrosine, flavenoids and other phenolic antioxidants (e.g. tocopherols), or *via* the reduction of quinones.

Radiolytically, $RO^{\bullet}$ can be produced *via* the electron reaction with hydroperoxides or quinones [131, 132] or *via* the one electron oxidation of phenolic compounds [133]. $RO^{\bullet}$ radicals can also be generated photolytically *via* photochemical reduction of quinones, photodecomposition of peroxides or *via* direct photolysis of phenols [134–136].

Alkoxyl radicals react with a variety of antioxidants and biological compounds, such as *t*-butoxyl radical reaction with a range of fatty acids, generating *t*-butanol and a fatty acid radical *via* hydrogen abstraction [135]. Introducing unsaturated bonds into the fatty acids was seen to increase the abstraction rate constant. These radicals have also been seen to react with the antioxidants quercetin, crocin and crocetin, ascorbate and Trolox, as well as with the DNA bases, thymidine and adenosine [131]. In the case of quercetin its phenoxyl radical was observed, and this would be expected to occur for Trolox as well, though the authors used competition kinetics to monitor this reaction rather than direct monitoring of the product.

Phenoxyl radicals of tyrosine in the enzyme lysozyme have been observed to react with α-tocopherol, ascorbate and urate, repairing the tyrosine amino acid

[137]. Again, this reaction with tocopherol produces another phenoxyl radical, the α-tocopheroxyl radical. However, antioxidant reactions with the α-tocopheroxyl radical have also been studied and it has been observed to be quenched by ubiquinol-10 [138], as well as by glutathione and ascorbate [139, 140] regenerating α-tocopherol. α-Tocopherol is an important antioxidant due to its position in the membrane, with the phytyl tail being anchored in the hydrophobic section and the chromane ring positioned near the membrane interface, thus, allowing reactions with free radicals in both the aqueous and lipid phases [14]. These repair reactions may be especially important *in vivo* as they can prevent α-tocopherol depletion. The repair by ascorbate is thought to occur *via* concerted electron and proton transfer and not by simple hydrogen atom transfer ($k = 3 \times 10^5$ mol dm^{-3} s^{-1} in lipid bilayers) [140]. The resulting ascorbate radicals are fairly unreactive and can be reconverted to ascorbate ($AscH^-$) and dehydroascorbate (Asc) [50]. The dehydroascorbate can then regenerate ascorbate *via* a glutathione peroxidase catalysed reaction with glutathione (GSH) yielding non-reactive, non-radical products [14]:

$$\mathrm{Asc} \; + \; 2\mathrm{GSH} \rightarrow \mathrm{AscH}^- + \; \mathrm{GSSG} \; + \; \mathrm{H}^+ \tag{8.57}$$

8.4 Conclusions

A large range of free radicals and other reactive oxygen species (ROS) can be produced biologically and *in vivo* and a variety of antioxidant species quench these ROS. Pulse radiolysis and laser flash photolysis are useful techniques for producing these radicals and ROS and for studying their reaction mechanisms.

The quenching reactions often generate another radical species, usually an antioxidant radical or radical ion, (though addition radicals are also possible) and these can then go on to react with other biomolecules or radicals. For example, carotenoid radical cations have been shown to oxidise the amino acids tyrosine and cysteine, so have pro-oxidant ability [141].

Each step in the reaction cascade that occurs upon the quenching of a free radical should generate a more stable and less reactive species. However, the presence of certain gases and metals can affect this and produce products which are more reactive, such as oxygen addition to carbon-centered radicals or to $RS^\bullet$ producing the more reactive peroxyl radicals (or sulfonyl radicals) [68, 127]; nitric oxides reaction with superoxide to give peroxynitrite [81], and the reaction of peroxynitrite with carbon dioxide to yield nitrogen dioxide and carbonate radicals [106]. Thus, an antioxidant can become pro-oxidant under certain conditions unless another antioxidant is present in sufficient amounts to quench the initial species produced in a competing reaction before any pro-oxidant reaction can occur. Therefore, knowing the reaction rates and mechanisms of antioxidant/biomolecule interactions with radicals and reactive oxygen species can help to predict biological anti/pro-oxidant capacity.

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