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Review

Non-phenolic radical-trapping antioxidants

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Abstract

Objectives The aim of this review article is to introduce the reader to the mechanisms, rates and thermodynamic aspects of the processes involving the most biologically relevant non-phenolic radical-trapping antioxidants.

Key findings Antioxidant defences in living organisms rely on a complex interplay between small molecules and enzymes, which cooperate in regulating the concentrations of potentially harmful oxidizing species within physiological limits. The noxious effects of an uncontrolled production of oxygen- and nitrogen-centered radicals are amplified by chain reactions (autoxidations), sustained mainly by peroxyl radicals ($ROO^{\bullet}$), that oxidize and alter essential biomolecules such as lipids, lipoproteins, proteins and nucleic acids.

Summary Non-phenolic antioxidants represent an important and abundant class of radical scavengers in living organisms. These compounds react with peroxyl radicals through various mechanisms: (i) formal H-atom donation from weak X-H bonds (X = O, N, S), as in the case of ascorbic acid (vitamin C), uric acid, bilirubin and thiols; (ii) addition reactions to polyunsaturated systems with formation of C-radicals poorly reactive towards O_2, for example β-carotene and all carotenoids in general; (iii) co-oxidation processes characterized by fast cross-termination reactions, for example γ-terpinene; and (iv) catalytic quenching of superoxide ($O_2^{\bullet-}$) with a superoxide dismutase-like mechanism, for example di-alkyl nitroxides and $FeCl_3$. Kinetic data necessary to evaluate and rationalize the effects of these processes are reported. The mechanisms underlying the pro-oxidant effects of ascorbate and other reducing agents are also discussed.

Keywords antioxidants; ascorbate; autooxidation; pro-oxidants; radicals

Introduction

'Living organisms are exposed to much more severe oxidative stress than is food in a refrigerator. Nevertheless, they do not become rancid until they, in their turn, become food.'
Burton and Ingold 1986

There are various lines of evidence supporting the conclusion that the process of autoxidation of biomolecules (lipids, lipoproteins, proteins, nucleic acids) is deleterious to living organisms, including humans.[1] The oxidant species responsible for such effects is dioxygen (O_2). Aerobic respiration and several enzymes produce a lot of 'activated' oxygen, that is oxygen (and nitrogen) radicals that react with various cellular components and produce toxic compounds.[1,2] The harm caused by such radicals can be judged by the fact that, for instance, the immune system makes use of oxygen radicals to kill pathogens.[2] Small quantities of oxygen and nitrogen radicals are also necessary for signalling transduction.[3] Indeed, in healthy subjects, there exists a fine balance between production and removal of oxygen radicals by means of endogenous and exogenous antioxidants and the inherent ability of cells to repair oxidative damage.[3] Oxidative stress arises from an imbalance in this equilibrium, when there is an augmented production of radicals that overwhelm existing antioxidant defences. This imbalance is caused by various pathological events and, if not corrected, can lead to cell death (apoptosis).[1]

Air oxidation also causes considerable economic damage because most (if not all) man-made products suffer extensive degradation of their chemical and physical properties after prolonged contact with O_2.[4] Incidentally, it is important to point out that pharmaceuticals are not immune from O_2-mediated degradation and many antioxidants are commonly used in pharmaceutical formulations.[5] Oxidation processes are however of fundamental importance

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for the degradation of the enormous quantities of anthropogenic and biogenic pollutants released into the environment.[6]

Air oxidation of a substrate can be drastically slowed down by the addition of antioxidants.[4,7] In principle, oxidative stress and its pathological effects could be prevented by supplying effective antioxidants, but several recent clinical trials have cast doubts on this.[8] Many compounds have antioxidant abilities and, among them, the phenolic compounds stand out for their efficacy.[4,7] The properties of the latter have been described in a previous review published in this Journal in 2007 in a special issue dedicated to the 'Chemistry and Biology of Antioxidants'.[7] We now want to expand the work to comprise other fundamental classes of compounds, including thiols, aromatic amines, hydrocarbons, ascorbic acid and metal ions, to give a wider, though still limited, view of the 'antioxidant world'.

Antioxidants are usually divided into two major groups, preventive and chain-breaking, according to whether they reduce the rate of chain-initiation (the former) or capture the alternating alkyl ($R^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals responsible for the oxidative propagation in organic matter (the latter).[9,10] This division, however, is often formal because many antioxidants can participate in different inhibitory stages. Generally, preventive antioxidants act by converting hydroperoxides to non-radical products (e.g. glutathione peroxidises, catalase, pyruvate, sulfur(II)-containing compounds, phosphites), by deactivating metal ions (e.g. transferrin, ferritin, EDTA) or by absorbing UV light (which can generate free radicals) or quenching singlet oxygen (e.g. carotenes, bilirubin, glutathione).[4,7,9] Chain-breaking antioxidants reduce the oxidation rate by reacting with $ROO^{\bullet}$ radicals (often compounds with relatively weak O-H, N-H and S-H bonds) or, more rarely, with carbon-centered radicals $R^{\bullet}$ (e.g. quinones, stable nitroxyl radicals). We will essentially focus here on chain-breaking non-phenolic antioxidants fundamental for preserving life in mammals, giving a general overview of their up-to-date mechanisms of action. The process of autoxidation of organic matter is also briefly recalled.

Autoxidation of hydrocarbons

The slow oxidation of organic materials by triplet ground-state molecular oxygen ($^3O_2$) at low to moderate temperatures is termed autoxidation or peroxidation.[4] At high temperatures, the process becomes extremely violent and destructive and is accompanied by the emission of light and large quantities of heat (combustion). The mechanism of autoxidation of hydrocarbons or lipid chains was first elucidated after the Second World War and is now known in great detail.[4] It can be used as a prototypical process to understand and describe the aerobic oxidation of other classes of organic compounds.

The major initial products of air oxidation of a hydrocarbon (RH) in solution and at low temperatures are (in most cases) hydroperoxides (ROOH).[4] The overall reaction, whose driving force is given by the $\Delta_r G_1^0$ value ($\Delta_r G_1^0 = \Delta_r H_1^0 - T\Delta_r S_1^0$), can be represented as follows:

$$RH + {}^3O_2 \longrightarrow ROOH \qquad (1)$$

The enthalpies of formation of ROOH and (corresponding) RH show that reaction 1 is exothermic by about 17–31 kcal/mol (in the gas phase at 298 K), according to the C-H bond being broken.[4,11,12] The exothermicity is greater for weaker C-H bonds, that is, the value of $|\Delta_r H_1^0|$ increases along the series of C-H bonds: 1° < 2° < 3° < benzyl. In contrast, the entropy change of reaction 1 is expected to be less sensitive to changes in the C-H bond. Given that the standard molar entropies are known only for few ROOH, we can for simplicity assume that $S_m^0(\mathrm{ROOH}) \approx S_m^0(\mathrm{ROH})$ (the entropies of alcohols are available and, although the latter are slightly smaller than those of ROOH, the error introduced is minor).[12] Thus, $\Delta_r S_1^0 \geq S_m^0(\mathrm{ROH}) - S_m^0(\mathrm{RH}) - S_m^0(\mathrm{O_2}) \approx 11 - 49 = -38$ cal/mol K (gas phase, 298K). Despite the largely negative entropy, the enthalpy of reaction 1 yields a favourable driving force to the reaction since at 298 K, $\Delta_r G_1^0 \leq -(5 \div 20)$ kcal/mol.

Fortunately, the large thermodynamic driving force does not greatly alter the intrinsic chemical inertia of reaction 1. Air oxidation at room temperature of a hydrocarbon or other organic material usually occurs slowly.[4] The apparent simplicity of reaction 1 is deceptive. We can 'exclude' *a priori* a molecular mechanism because it does not obey the rule of spin conservation. Indeed, the ground state of dioxygen is an 'unreactive' triplet state (diradical) while hydroperoxide and RH are in a singlet state. Reaction 1 can instead occur with the (excited) singlet oxygen molecules, $^1O_2$, since in this case the reaction is spin-allowed ($\Delta s = 0$).[4] The actual mechanism of reaction 1 involves a complex chain process carried by peroxyl radicals, $ROO^{\bullet}$,[4] (see Figure 1), in which $ROO^{\bullet}$ radicals are produced by initiating reactions (start) and disappear by recombination (end).

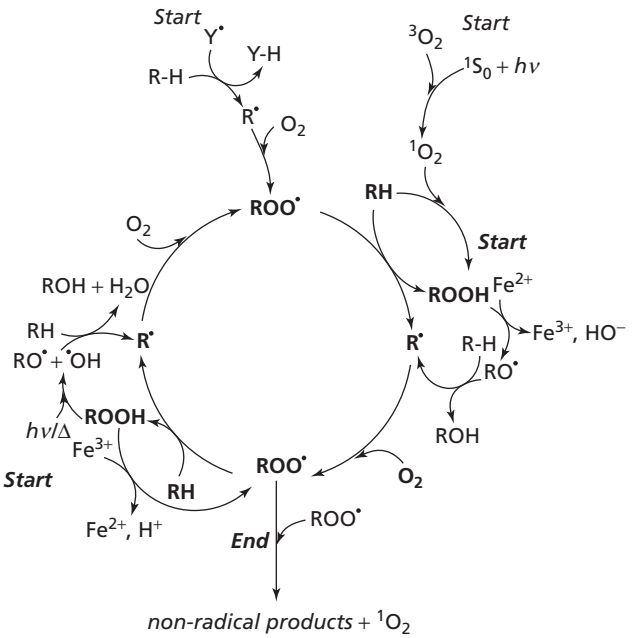

Figure 1 Simplified scheme of autoxidation of a hydrocarbon RH. The process is initiated by free radicals produced by various reactions, such as decomposition of azo-compounds or peroxides, homolysis of hydroperoxides due to heat, metal catalysis or UV light. Hydroperoxides can also be generated by singlet oxygen sensitizer, $^1S_0$ (photosensitized oxidation). Transition metal ions (for instance, Fe^{2+} and Fe^{3+}) have an active role in accelerating the process by decomposing ROOH to free radicals.

In the absence of initiating agents, formation of these radicals occurs by reactions with dioxygen,[4] mainly H-atom abstractions, reaction 2. These reactions are very slow because of their high endothermicity.

$$RH + O_2 \longrightarrow R^{\bullet} + HOO^{\bullet} \xrightarrow{O_2} ROO^{\bullet} + HOO^{\bullet} \qquad (2)$$

In reality, in most systems, radical chain processes are started by light, ionizing radiations (radon gas, cosmic radiation, diagnostic X-rays), heat, hydroperoxides and transition metals (usually present in traces in all organic materials). In living organisms, many enzymes are able to initiate autoxidation in cell membranes and lipoproteins by releasing $O_2^{\bullet-}$ radicals.[1] In kinetic studies, an efficient production of $ROO^{\bullet}$ is achieved by the use of radical initiators, ROOH, transition metals, light, ionizing radiations and singlet oxygen sensitizers.[7] All these agents ultimately yield a high flux of free radicals $Y^{\bullet}$ that efficiently initiate oxidation chains, reactions 3a and 3b.

$$\text{Source of radicals} \xrightarrow{R_i} Y^{\bullet} \qquad (3a)$$

$$Y^{\bullet} + RH \longrightarrow YH + R^{\bullet} \xrightarrow{O_2} YH + ROO^{\bullet} \qquad (3b)$$

However, when constant and reproducible rates of initiation R_i are required, it is best to use azo-compounds, for example azo-bis(isobutyronitrile) ($NC(CH_3)_2C\text{-}N{=}N\text{-}C(CH_3)_2CN$, AIBN), because they thermally form C-radicals at a constant rate, which are then transformed (at diffusion rates) into peroxyl radicals by O_2 addition.[7]

Peroxyl radicals formed in the initiation step propagate autoxidation through reactions 4 and 5.

$$RH + ROO^{\bullet} \xrightarrow{k_p} R^{\bullet} + ROOH \qquad (4)$$

$$R^{\bullet} + O_2 \underset{(\leftarrow)}{\overset{fast}{\longrightarrow}} ROO^{\bullet} \qquad (5)$$

When the hydrocarbon RH has H-atoms in allylic or benzylic position, reaction 4 is comparatively fast; peroxyl radicals may also add to double bonds (see below).[4] On the other hand, reaction 5 is, in most cases, diffusion-controlled, that is $k_5 \sim 10^9\ M^{-1}s^{-1}$, and essentially irreversible (there are exceptions, see below).[13] This causes the stationary concentration of alkyl radicals in solution at $P_{O_2} > 100$ mmHg to be very small. The combination of reactions 4 and 5 overcomes the difficulties highlighted above with a molecular mechanism for reaction 1. Indeed, both reactions are spin-allowed. The cyclic mechanism yields the 'final' product of autoxidation, that is ROOH (reaction 4), and regenerates the major chain-carrying radical, $ROO^{\bullet}$, (reaction 5) at the expense of RH and O_2. Thus, we can summarize that a peroxyl radical $ROO^{\bullet}$ functions as a 'catalyst' of reaction 1, oxidizing a large number (the chain length) of RH until it combines with another $ROO^{\bullet}$, reaction 6.

$$ROO^{\bullet} + ROO^{\bullet} \xrightarrow{2k_t} \text{non-radical products} \qquad (6)$$

Under steady rate of initiation, the rate law for uninhibited autoxidation in many cases conforms to equation 7 where, for AIBN-initiated autoxidations, $R_i = \alpha[AIBN]$ (α is a constant).[14]

$$R_p = -\frac{d[O_2]}{dt} = \frac{k_p}{(2k_t)^{1/2}}[RH] \times R_i^{1/2} \qquad (7)$$

Thus, the rate of autoxidation will depend, in this case, only on the propagation and termination rate constants, and the concentrations of RH and AIBN (the reaction displays zeroth-order kinetics in O_2 provided that $P_{O_2} > 100$ mmHg and reaction 5 is not reversible).

Despite the foregoing reactions giving an adequate description of the autoxidation process in hydrocarbons and in other classes of compounds, a comprehensive picture of the real process is far more complex (see Figure 1) and significant differences can be found among the various classes of organic compounds.[4] Oxidation of biomolecules such as proteins and DNA is a particularly complex subject matter.[4] Oxidation of proteins mediated by for example $HO^{\bullet}$, $NO^{\bullet}$, metal ions, $ONOOH/ONOO^{-}$ and HOCl, may determine proteolysis, alteration of the amino acid residues and inactivation of enzyme activity. The DNA strands can be cleaved by H-atom abstraction from the deoxyribose sugar moiety by $HO^{\bullet}$ radicals and the DNA bases are modified because of $HO^{\bullet}$ addition. A description (even superficial) of all these reactions exceeds the limits of the present review.

Inhibition of autoxidation

The rate of autoxidation (R_p) of a substrate RH (equation 8) is determined by the pseudo-first-order rate constant of propagation, $k_p[RH]$, (reaction 4), and by the stationary concentration of peroxyl radicals $[ROO^{\bullet}]_{ss}$.

$$R_p = k_p[RH] \times [ROO^{\bullet}]_{ss} \qquad (8)$$

Under steady-state conditions, the $[ROO^{\bullet}]_{ss}$ is calculated by equating the rate of initiation to the rate of termination, that is $R_i = R_t$. If the self-quenching reaction 6 is the major termination process, then $R_t = 2k_t\ [ROO^{\bullet}]^2_{ss}$ and thus, $[ROO^{\bullet}]_{ss} = (R_i/2k_t)^{1/2}$ and equation 8 gives equation 7.

Some compounds (antioxidants), AH, have the ability to reduce the rate R_p by interfering with the chain mechanism of oxidation even at low concentrations. In the final analysis, these compounds are able to reduce the $[ROO^{\bullet}]_{ss}$. This decrease in $[ROO^{\bullet}]_{ss}$ may be consequent to: (i) decomposition of the initiating agents without formation of radicals (preventive antioxidants);[7,9] or (ii) more efficient termination processes of $ROO^{\bullet}$. One major mechanism of $ROO^{\bullet}$ quenching relies on the ability of certain antioxidants (chain-breaking antioxidants)[10] to donate a H-atom to $ROO^{\bullet}$, reaction 9.

$$AH + ROO^{\bullet} \xrightarrow{k_9} A^{\bullet} + ROOH \qquad (9)$$

The radical $A^{\bullet}$ is usually resonance-stabilized and therefore unable to propagate the oxidative chains, reactions 10 and 11.

$$A^{\bullet} + RH \xrightarrow{slow} AH + R^{\bullet} \qquad (10)$$

$$A^{\bullet} + O_2 \xrightarrow{very\ slow} AOO^{\bullet} \qquad (11)$$

It is eventually eliminated from the system by reaction with a second peroxyl radical, reaction 12, or by self-quenching, reaction 13.

$$A^{\bullet} + ROO^{\bullet} \xrightarrow{fast} \text{non-radical products} \quad (12)$$

$$A^{\bullet} + A^{\bullet} \xrightarrow{fast} \text{dimer or other products with or without antioxidant activity} \quad (13)$$

In heterogeneous systems, diffusive processes combined with chemical transformations may also provide an escape mechanism for $A^{\bullet}$ or the 'putative' $A^{\bullet}$-derived radical outside the oxidation site.[15] Finally, in biological systems, the radical $A^{\bullet}$ may be reduced back to its precursor AH. In a few cases, the $A^{\bullet}$ radical is persistent enough to be detected by electron spin resonance spectroscopy, which allows the study of its structure and spin distribution (see Figure 2 for two representative phenolic antioxidants).

The rate constant k_9 is a quantitative measure of the antioxidant ability of AH (k_9 is often indicated in the literature as k_{inh}). The stoichiometric factor, n, that is the number of peroxyl radicals quenched per AH molecule, is another important parameter and a useful indicator of the relative importance of reactions 10–13. For many antioxidants, n is 2 or close to 2, indicating that the decay of $A^{\bullet}$ is dominated by reaction 12 (in the case of catechols and hydroquinones, for which $n = 2$, the decay of $A^{\bullet}$ may also occur by disproportionation with regeneration of the parent phenols).[7] On the other hand, a value of $n = 1$ may indicate a fast self-quenching of the radical $A^{\bullet}$ (reaction 13) with formation of inactive compounds. Cases of antioxidants with $n < 1$ are known[16] and are usually attributed to the occurrence of chain-transfer reactions such as reaction 11.

Given that reactions 9 and 12 are in most cases the major termination reactions, then $R_i = R_t \approx nk_9[AH] \times [ROO^{\bullet}]_{ss}$ and hence equation 8 becomes:

$$R_p \approx \frac{k_p[RH]R_i}{nk_9[AH]} \quad (14)$$

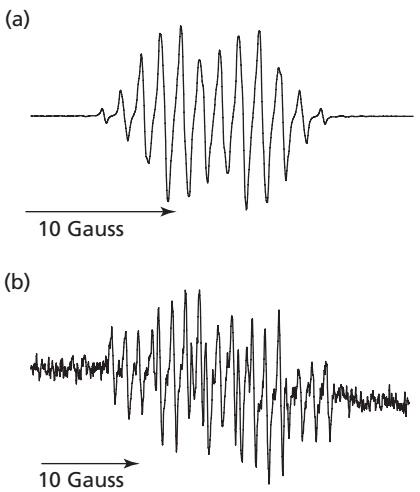

Figure 2 Electron spin resonance spectra of phenoxyl radicals. Spectra were obtained by irradiating a benzene solution of 2,4-dimethoxyphenol (a) or sesamol (3,4-methylenedioxyphenol) (b) in the presence of di-*tert*-butylperoxide. The smaller signal-to-noise ratio in the case of sesamol (3,4-methylenedioxyphenol) indicates a faster decay of the phenoxyl radicals from sesamol.

which represents the rate law for inhibited autoxidation via H-atom transfer to $ROO^{\bullet}$ radicals. Equation 14 shows that the ability of AH to inhibit autoxidation, that is to give $R_p \approx 0$, depends on k_p and on the oxidizable substrate concentration [RH]. Unsaturated lipids in cell membranes and plasmatic lipoproteins are characterized by relatively large k_p (e.g. the k_p of methyl linoleate is 60 $M^{-1}s^{-1}$ at 30°C),[17] which thus requires antioxidants with $k_9 > 10^4$–10^5 $M^{-1}s^{-1}$ and/or in sufficiently large amounts.

Another mechanism of $ROO^{\bullet}$ quenching by AH involves reaction 9 followed by the reaction sequence:

$$A^{\bullet} + O_2 \rightleftarrows AOO^{\bullet} \quad (15)$$

$$AOO^{\bullet} + RH \xrightarrow{k_{16}} AOOH + R^{\bullet} \quad (16)$$

$$AOO^{\bullet}/A^{\bullet} + ROO^{\bullet} \xrightarrow{k_{17}} \text{quenching} \quad (17)$$

The antioxidant effect is generated by fast cross-quenching reactions of $ROO^{\bullet}$ with $AOO^{\bullet}$ or $A^{\bullet}$ radicals, reaction 17, that is $k_{17} >> 2k_t$.[18] The radical $AOO^{\bullet}$ contributes to the oxidative chains, reaction 16, but its quenching effect in reaction 17 overwhelms the pro-oxidation ability and thus the steady-state concentrations of $ROO^{\bullet}$ decreases. Kinetic analysis of these reactions and several further simplifications lead to the following approximated, though acceptable, rate law,[18] which shows that this antioxidant mechanism necessitates large concentrations (≈1 mM) of AH to produce a detectable effect.

$$R_p \approx \frac{k_p[RH]R_i^{1/2}}{\sqrt{2k_t + \frac{2k_{17}k_9[AH]}{k_{16}[RH]}}} \quad (18)$$

H-atom donors

The mechanism of action of antioxidant compounds structurally related to phenols, such as aromatic amines, phenothiazines, pyridinols, pyrimidinols and thiols, which all contain 'weak' X-H bonds (X = O, N, S), can be rationalized on the basis of considerations similar to those adopted for phenols.[4,7] That is, their antioxidant action relies on their capability of donating a H-atom from the X-H bond to the $ROO^{\bullet}$ radicals, reaction 9. They can therefore be grouped under the term 'H-atom donors', even though this classification in some instances is only formal because an electron transfer (ET) followed or preceded by a proton transfer (PT) can underlie the actual mechanism (see below).

Phenols

Phenols (ArOH) are prominent examples of chain-breaking antioxidants characterized by large values of k_9 (range 10^4–10^7 $M^{-1}s^{-1}$).[7] Their aryloxyl radicals ($ArO^{\bullet}$) are able in most cases to trap a second $ROO^{\bullet}$ radical (reaction 12) or to self-quench (reaction 13) often with the formation of active compounds, that is $n = 2$ and sometimes $n > 2$,[19] but not to continue autoxidation, reactions 10 and 11 (one exception is represented by α-tocopheroxyl radicals isolated in human

low-density lipoprotein (LDL)).[15] For all these reasons, phenols have a privileged role among chain-breaking antioxidants and have in fact been the subject of extensive investigations over the past decades.[4] Structure–activity relationships are now available that allow us to predict the k_9 values for any phenol.[20–22]

Pyridinols and pyrimidinols

3-Pyridinols and 5-pyrimidinols (Figure 3) represent a recent advancement in the development of new antioxidants for technological and possibly medical purposes.[23–25]

These heterocyclic antioxidants are air-stable, while the most active phenolic antioxidants tend to be unstable and turn brownish on standing in air.[24] The aryloxyl radicals of tellurium-containing 3-pyridinols are reduced back to their parent compounds by *N*-acetylcysteine in a biphasic system.[26] Thus, these antioxidants function as catalysts of the otherwise slow reaction: *N*-acetylcysteine + $ROO^{\bullet}$.

As with phenols, the values of k_9 of these compounds are dependent on the bond dissociation enthalpy (BDE) of the O-H group (see Figure 4).[21,27,28] The rate constants k_9 can therefore be predicted on the basis of the O-H BDE which, in turn, can be estimated by applying additive rules[20] or quantum mechanics.[23,24] Electron-donating substituents (CH_3, OCH_3, NH_2) in *ortho* and *para* positions to the reactive O-H favour H-atom donation. This is because these substituents lower the O-H BDE by stabilizing the aryloxyl radical formed upon H-atom abstraction (see Figure 4).

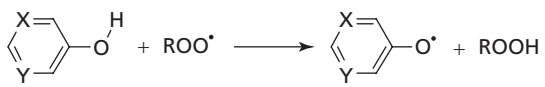

Figure 3 Reaction of peroxyl radicals with phenol (X = Y = CH), pyridinols (X = N; Y = CH) and pyrimidinols (X = Y = N)

Aromatic amines and compounds with weak N-H bonds

Aromatic amines containing amino hydrogens function as inhibitors of hydrocarbon autoxidation by donating a H atom from the N-H bond to $ROO^{\bullet}$.

$$Ar_2N\text{-}H + ROO^{\bullet} \longrightarrow Ar_2N^{\bullet} + ROOH \qquad (19)$$

The overall process of inhibition is frequently more complex than inhibition by phenols. The aminyl radical is able, in fact, to react with a second $ROO^{\bullet}$ radical to give non-radical products (reaction 12) but it may also give rise to the following reaction:

$$Ar_2N^{\bullet} + ROO^{\bullet} \longrightarrow Ar_2NOOR \longrightarrow Ar_2NO^{\bullet} + RO^{\bullet} \qquad (20)$$

which is a chain-transfer reaction. The resulting rate law may depend (as actually observed in a few cases) on $R_i^{1/2}$ and $[AH]^{-1/2}$ (see equation 18).[14,29]

Ethoxyquin (see Figure 5) is a common amine antioxidant used as a preservative in pet foods because it efficiently quenches peroxyl radicals with a rate constant k_9 of 2.0×10^6 $M^{-1}s^{-1}$ (in benzene at 30°C) and a stoichiometric factor

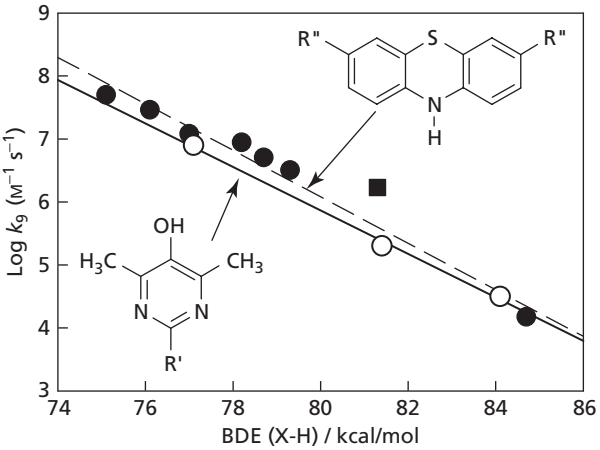

Figure 4 Plot of log k_9 versus the corresponding X-H bond dissociation enthalpy (BDE). Ethoxyquin (X = N; ■) at 30°C, 4,6-di-methylpyrimidinols (X = O; ○) at 50°C and phenothiazines (X = N; ●) at 50°C with different R′ and R″ substituents.

of 2. This large k_9 has been attributed to the low BDE of the N-H bond (81.3 kcal/mol) and to the lack of steric crowding around the reactive N atom.[27] The use of this amine has however been limited by toxicity concerns. In 1997, the US Food and Drug Administration requested to manufacturers of ethoxyquin and trade associations for the pet food industry that the maximum level contained be lowered from 150 ppm to 75 ppm.[30] Other aromatic amines with antioxidant abilities include phenothiazines, which are a class of drugs used to treat mental and emotional disorders.[31] Unsubstituted representatives on the N atom behave as good H-atom donors since the aminyl radical formed upon H-atom abstraction is stabilized by resonance with two aromatic rings and by the presence of the electron-releasing S atom (see Figures 4 and 5). In the case of the simple phenothiazine reported in Figure 5, $k_9 = 8.8 \times 10^6$ $M^{-1}s^{-1}$ (50°C in benzene) and the N-H BDE is 78.2 kcal/mol.[28]

A mechanism of formal H-atom donation to peroxyl radicals can also be invoked to explain the antioxidant properties of uric acid (Figure 6) and bilirubin (Figure 7). Uric acid is a biologically relevant antioxidant that contributes about 20–30% to the 'total antioxidant capacity'

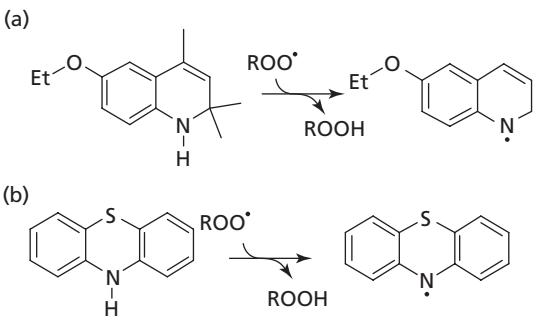

Figure 5 Antioxidant mechanism of ethoxyquin (a) and phenothiazine (b)

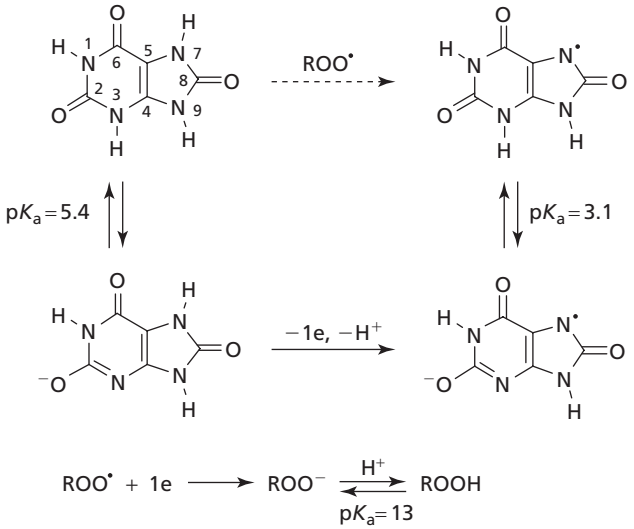

Figure 6 Ionization of uric acid and reaction scheme with peroxyl radicals. The reaction mechanism between urate and peroxyl radicals is believed to be an electron transfer followed by fast protonation of the hydroperoxide anion. The dotted arrow indicates the H-atom transfer reaction from neutral uric acid to peroxyl radicals which, at neutral pH, is irrelevant.

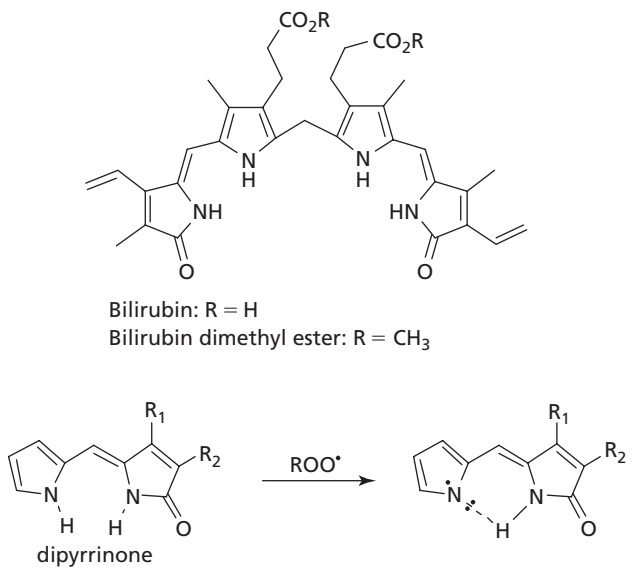

Figure 7 Structure of bilirubin and of its dimethyl ester. Formation of a hydrogen bond in the pyrrolyl radical of a dipyrrinone after H-atom abstraction.

of plasma.[32] In this regard, the cause of the increased serum total antioxidant capacity observed after the ingestion of red wine or apples is most likely due to changes in the plasmatic urate concentration and not to the polyphenols in red wine or apple.[33,34] At physiological pH, uric acid is almost entirely ionized to the monoanion (urate), $pK_a = 5.4$,[35] which accumulates in human plasma to concentrations normally over the range of 0.2–0.4 mM before being excreted in the urine.[1] This anion reacts with peroxyls by an electron transfer–proton transfer mechanism (see Figure 6) with a rate constant of 3×10^6 $M^{-1}s^{-1}$.[35,36] Unlike the ascorbate anion, the urate anion is not able to reduce the α-tocopheroxyl radical to its parent compound.[37,38]

The human body produces about 275 mg of bilirubin per day, which is derived from the catabolism of haemoglobin (~80%) and from other haeme sources (~20%).[1] Bilirubin is transported in the bloodstream by albumin (to which it is bound in a 1 : 1 stoichiometry) because it is insoluble in aqueous media at neutral pH.[1] Thus, the evaluation of its in-vivo antioxidant activity is difficult. However, in-vitro experiments seem to indicate that bilirubin bound to albumin contributes by about 5–10% to the 'total antioxidant capacity' of blood plasma.[39] Bilirubin presumably reacts in apolar media with peroxyl radicals via H-atom transfer from the N-H bonds (see Figure 7).[40]

The small increase in the antioxidant activity of bilirubin dimethyl ester (Figure 7) observed on passing from apolar ($k_9 = 22.5 \times 10^4$ $M^{-1}s^{-1}$, $n = 2$ in styrene/chlorobenzene at 30°C) to polar solvents ($k_9 = 37.9 \times 10^4$ $M^{-1}s^{-1}$, $n = 1.8$, in 1.74 M styrene/11.1 M methanol at 30°C) was tentatively explained by a switching of the reaction mechanism from H-atom transfer to an electron transfer–proton transfer mechanism.[40–42] Intramolecular hydrogen bonding in the intermediate pyrrolyl radical of dipyrrinones has been shown to have a major role for the antioxidant activity of these compounds because it stabilizes the radical (see Figure 7). The formation of a similar hydrogen bonding might also influence the antioxidant activity of biliverdin (bilirubin precursor) and possibly bilirubin.[40]

Ascorbic acid

Ascorbic acid has long been known to have antioxidant properties especially in support of oil-soluble antioxidants (tocopherols).[43] Its salts of Na and Ca and the palmitate and stearate esters are commonly used as food additives.[44] Ascorbic acid has four stereoisomeric forms of the chemical formula $C_6H_8O_6$. The L-ascorbic acid stereoisomer (see Figure 8), commonly known as vitamin C, is present in the human plasma over the range 30–100 μM.[1,2] Ascorbic acid is a relatively strong acid ($pK_a = 4.04$) and at pH 7.4 is entirely ionized; thus, the anion is the species involved in the antioxidant chemistry of vitamin C.[45] L-Ascorbate (hereafter simply ascorbate) reacts with free radicals via a concerted electron and proton transfer mechanism (see Figure 8).[46]

The ascorbyl radical anions $Asc^{\bullet-}$ so formed disproportionate rapidly with a rate constant in water of 3×10^6 $M^{-1}s^{-1}$ yielding dehydroascorbic acid (DHA) and regenerating ascorbate,[46] reaction 21.

$$2Asc^{\bullet-} + H^+ \longrightarrow DHA + AscH^- \qquad (21)$$

Surprisingly, in anhydrous organic solvents (perhaps because of the lack of H^+ ions)[46] the radical $Asc^{\bullet-}$ becomes persistent (for hours, room temperature).[46] *In vivo*, the radical $Asc^{\bullet-}$ and DHA can be converted back to ascorbate by tissue enzymes at the expense of glutathione (GSH) and NADH.[1]

Ascorbate is probably the most effective water-soluble antioxidant in the plasma. Frei *et al.*[47] showed that ascorbate

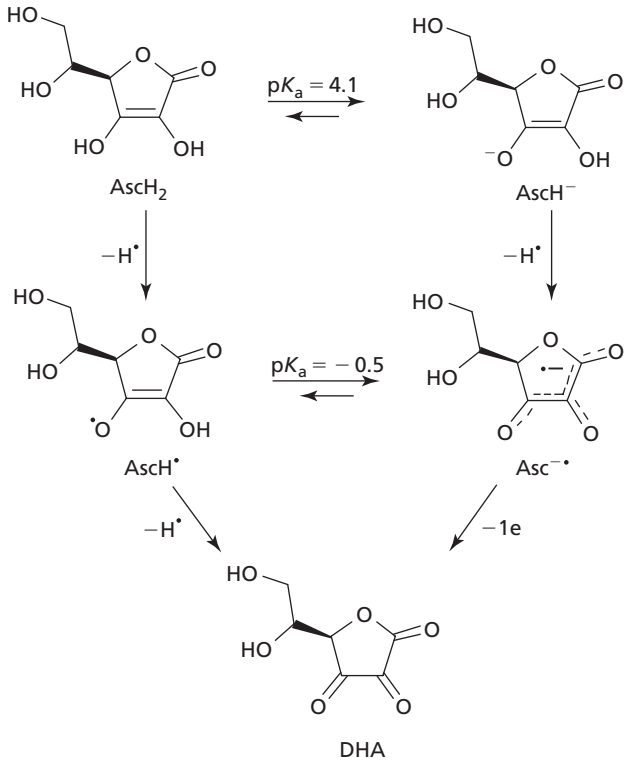

Figure 8 Acid–base equilibria of ascorbic acid ($AscH_2$) and its radicals. The electron released by $AscH^-$ is accepted by the $ROO^\bullet$ radical in the reaction $AscH^- + ROO^\bullet \rightarrow Asc^{\bullet-} + ROO^- + H^+ \rightarrow Asc^{\bullet-} + ROOH$.

protects plasma lipids against AAPH-initiated autoxidation better than other antioxidants such as urate or α-tocopherol. This is due to the fact that the AAPH initiator is water-soluble and produces, by thermal decomposition, positively charged peroxyl radicals[48] in the aqueous phase (reaction 22), which are promptly intercepted by the ascorbate anions (reaction 23). This delays the initiation of autoxidation in the lipid phase until ascorbate is entirely consumed (lag phase).

$$^+(NH_2)_2C(CH_3)_2CN{=}NC(CH_3)_2C(NH_2)_2^+ \xrightarrow[-N_2]{\Delta} \underset{(\mathbf{AAPH})}{}$$

$$2\ ^+(NH_2)_2C(CH_3)_2C^\bullet \xrightarrow{O_2} {}^+(NH_2)_2C(CH_3)_2COO^\bullet \qquad (22)$$

$$^+(NH_2)_2C(CH_3)_2COO^\bullet + AscH^- \longrightarrow {}^+(NH_2)_2C(CH_3)_2COOH + Asc^{\bullet-} \qquad (23)$$

Indeed, ascorbate reacts rapidly with peroxyls in water: with $CH_3OO^\bullet$, the rate constant at pH 7, obtained by pulse radiolysis, is 1.7×10^6 $M^{-1}s^{-1}$.[49] In SDS micelles containing linoleic acid, the rate constant k_9 for the reaction of ascorbate with $ROO^\bullet$ at the water/lipid boundary reduces to 3.2×10^3 $M^{-1}s^{-1}$ while for the lipophylic analogue ascorbyl palmitate, $k_9 = 2.1 \times 10^5$ $M^{-1}s^{-1}$.[50] Ascorbate is also able to scavenge other water-soluble radicals among which are $O_2^{\bullet-}/HOO^\bullet$, $HO^\bullet$, the urate radical and nitroxide radicals, and non-radical species such as HOONO/$ONOO^-$ and HOCl.[1] Most importantly, ascorbate can regenerate α-tocopherol from the α-tocopheroxyl radical at the lipid/water interface of LDL particles.[51]

The high reactivity of ascorbate towards free radicals is due to the low dissociation enthalpy of its O-H bond. Recent thermochemical measurements have set this O-H BDE at about 70 kcal/mol in acetonitrile and ≥ 74 kcal/mol in water.[46] For comparison, the O-H BDE of α-tocopherol (the major lipid-soluble chain-breaking antioxidant in the human plasma) is 77 kcal/mol.[20,22]

Pro-oxidant effects of ascorbate

Pro-oxidant activity of ascorbate has occasionally been observed in in-vitro and in-vivo experiments.[1] Recent studies have shown that ascorbate, at pharmacologic concentrations of 10 mM, is capable of generating a hydrogen peroxide-dependent cytotoxicity towards a variety of cancer cells *in vitro*.[52] These pro-oxidant effects are most likely linked with the presence of trace redox-active metal ions,[53,54] such as Fe^{3+} or Cu^{2+}, although other mechanisms not involving metal ions have also been invoked (see below). Cupric ion increases greatly the cytotoxicity[55] of ascorbate and its rate of autoxidation.[54] In fact, ascorbic acid undergoes metal-catalysed autoxidation according to the following reactions:[56,57]

$$AscH^- + O_2 \xrightarrow{Metal\ ions} Asc^{\bullet-} + H^+ + O_2^{\bullet-} \qquad (24)$$

$$AscH^- + O_2^{\bullet-} + H^+ \longrightarrow Asc^{\bullet-} + H_2O_2 \qquad (25)$$

$$Asc^{\bullet-} + O_2 + 2H^+ \longrightarrow DHA + H_2O_2 \qquad (26)$$

The presence of metal ions is essential since the treatment of the solutions with the Chelex-100 resin for the removal of contaminating metals results in the suppression of ascorbate autoxidation.[54] Formation of superoxide anion $O_2^{\bullet-}$ and H_2O_2 can explain the pro-oxidant activity of $AscH^-$, especially in conjunction with the Fenton reactions 27 and 28.[58–60]

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^\bullet + HO^- \qquad (27)$$

$$Cu^+ + H_2O_2 \longrightarrow Cu^{2+} + HO^\bullet + HO^- \qquad (28)$$

Hydrogen peroxide is also released in the singlet oxygen quenching[61] by $AscH^-$ and in the catalytic oxidation of the latter by quinones[62,63] (reactions 29 and 30). Quinones (Q) are in fact known to be highly cytotoxic because of enzymatic redox-cycling mechanisms.[62,63] Non-enzymatic reactions may also involve ascorbate at physiological concentrations, reactions 29 and 30.

$$AscH^- + Q \longrightarrow Asc^{\bullet-} + H^+ + Q^{\bullet-} \qquad (29)$$

$$Q^{\bullet-} + O_2 \longrightarrow Q + O_2^{\bullet-} \qquad (30)$$

Finally, nitrous acid is reduced by ascorbate to NO radical,[64] which in the presence of $O_2^{\bullet-}$ produces peroxynitrite anion $ONOO^-$. Its conjugated acid HOONO decomposes rapidly yielding $HO^\bullet$ and $NO_2^\bullet$ radicals.[65]

Thiols and sulfenic acids

Alkyl and aryl thiols react with most radicals by H-atom transfer.[66] Typical values of the rate constants with C-centred ($R^{\bullet}$),[66] $HO^{\bullet}$[66] and $ROO^{\bullet}$[67,68] radicals are 10^8, $>10^9$ and 10^3–10^5 $M^{-1}s^{-1}$, respectively. The resulting thiyl radicals can give rise to the reactions reported in Figure 9,[1,4,66] among which the reaction with dioxygen is remarkable. This reaction is reversible,[66] with the forward rate constant of the order of 10^7 $M^{-1}s^{-1}$ for alkyl thiyl radicals and $<10^4$ $M^{-1}s^{-1}$ for aryl thiyl radicals. The $RS^{\bullet}$ + $RS^{\bullet}$ self-reaction is essentially diffusion-controlled, $k \sim 10^9$ $M^{-1}s^{-1}$; the $RS^{\bullet}$ radical can also react rapidly with the anion RS^- but the pH influences the observed rate constant since the concentration of RS^- is pH-dependent. Thus, the prevailing process of decay of the radical $RS^{\bullet}$ depends on the ratio $[RS^{\bullet}]/[O_2]$ and on the pH. The thiyl radical can also add reversibly to olefins, dienes and β-carotene and thus it may be responsible for a catalysed *cis* → *trans* isomerization of *cis*-unsaturated phospholipids.[69] In this regard, retinol was found to be effective in preventing $RS^{\bullet}$-mediated *cis–trans* isomerization of lipids.[70] Sulfur can expand its valence shell to form sulfonyl ($RS^{\bullet}O_2$) and sulfonyl peroxyl ($RSO_2OO^{\bullet}$) radicals; sulfinyl ($RSO^{\bullet}$) radicals are formed by the reaction of thiyl peroxyls ($RSOO^{\bullet}$) with thiols, see Figure 9.[66]

Thiols, disulfides and more in general sulfur(II)-containing compounds reduce ROOH to ROH, therefore acting as preventive antioxidants.[4] The processes are rather complex and necessitate the presence of protic solvents (acid catalysts).[4] Dialkylsufides are oxidized by ROOH to sulfoxides $R'S(O)R'$ and then to sulfones $R'SO_2R'$, whereas ROOH is reduced to ROH. The reaction is acid-catalysed; in aprotic solvents the ROOH molecules can also act as a catalyst.[4]

The most biologically relevant example of a thiol with antioxidant properties is glutathione (Figure 10), which in its reduced form (GSH) is able to remove hydroperoxides formed by cell metabolism under aerobic conditions with the catalysis of glutathione peroxidase (GPx), reaction 31.

$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O \qquad (31)$$

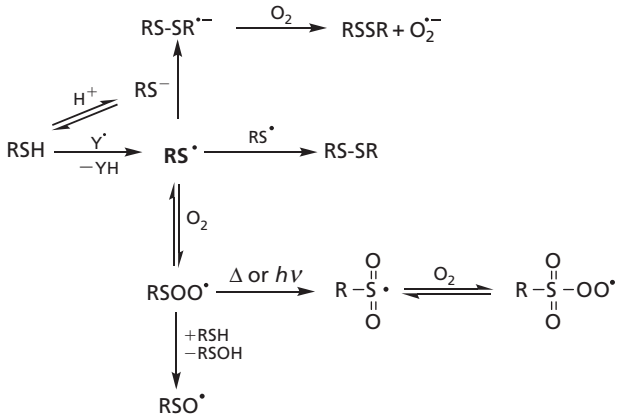

Figure 9 Decay reactions of the thiyl radical in the presence of dioxygen

GSH reacts with any oxygen[71,72] radical (see above) but also with non-radical species such as $^1O_2$ (singlet oxygen),[1] HOCl (hypochlorous acid)[1] and $ONOO^-$ (peroxynitrite).[73] The $GS^{\bullet}$ radical at physiological pH can generate the superoxide anion through the reaction sequence:

$$GSH \xrightarrow{-H} GS^{\bullet} \xrightarrow{GS^-} GSSG^{\bullet-} \xrightarrow{O_2} GSSG + O_2^{\bullet-} \qquad (32)$$

Hence, to minimize the pro-oxidant potential of GSH *in vivo*,[1] there might be the need for co-assistance by superoxide dismutase (SOD) (to remove $O_2^{\bullet-}$) or ascorbate (to quench the $GS^{\bullet}$ radical), reaction 33.

$$AscH^- + GS^{\bullet} \longrightarrow Asc^{\bullet-} + GSH \qquad (33)$$

The *R*-stereoisomer of lipoic acid, in its amide form, is an essential component of various enzymes such as the pyruvate dehydrogenase complex, and thus is essential for the aerobic metabolism.[1] In the reduced form (dihydrolipoic acid, Figure 10), lipoic acid is a potent reductant that reacts with a wide range of free radicals and other oxidants (e.g. $HO^{\bullet}$, $O_2^{\bullet-}$, $ROO^{\bullet}$, HOCl). It is also able to reduce GSSG to GSH and ubiquinone-10 (coenzyme Q-10) to ubiquinol-10.[74] Similar to other strong reductants, in the presence of transition metals it behaves as a pro-oxidant, following a reaction pathway similar to that previously reported for ascorbate.[74] The low concentration of lipoic acid in human tissues, however, makes unlikely its relevance as an endogenous antioxidant.[1]

Sulfenic acids (RSOH) are produced by sulfides contained in garlic and onion and are emerging as a novel class of powerful peroxyl radical scavengers.[75] On chopping fresh garlic cloves, the secondary metabolite alliin is converted into allicin by the enzyme alliinase *via* 2-propene sulfenic acid (see Figure 10).[76] The latter is also produced by allicin decomposition during storage (Figure 10).[75] Given the very low RSO-H BDE of about 69 kcal/mol,[75] sulfenic acids react with the $ROO^{\bullet}$ radical at diffusion rates, while allicin or garlic-derived disulfides lack any antioxidant activity.[77]

Antioxidants with mechanisms other than H-atom donation

Several compounds exhibit antioxidant properties that cannot be traced back to reaction 9, that is, to $ROO^{\bullet}$ quenching via H-atom donation from weak A-H bonds. These compounds include hydrocarbons, lactones, trisubstituted aromatic amines and even transition metal ions. We briefly describe several of these compounds in the following sections; for further information on those not included please refer to the original works.[78–80]

Hydrocarbons

There are two general mechanisms by which certain readily peroxidizable hydrocarbons (AH) may display antioxidant ability.[18] Hydrocarbons in class 1 form carbon-centred radicals, reaction 9, strongly stabilized by resonance, $A^{\bullet}$. The reaction of $A^{\bullet}$ with dioxygen (reaction 34) is thus reversible and the equilibrium favours $A^{\bullet}$ + O_2 rather than $AOO^{\bullet}$. The radical $A^{\bullet}$ is also able to quench rapidly the chain-carrying

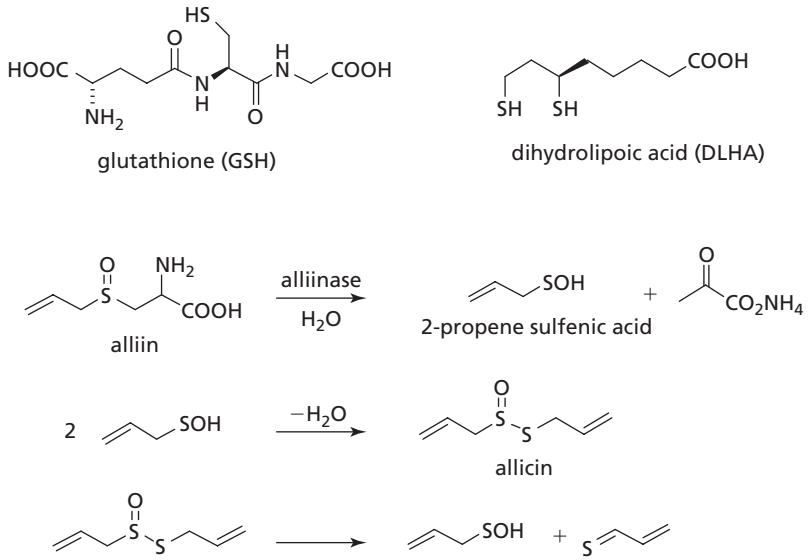

Figure 10 Sulfur-containing antioxidants: glutathione, dihydrolipoic acid and sulfenic acids. The reactions leading to the production of 2-propene sulfenic acid from garlic-derived alliin and allicin are shown.

peroxyls $ROO^{\bullet}$. The entire antioxidant mechanism can be illustrated via the following reactions:

$$A^{\bullet} + O_2 \overset{\rightarrow}{\leftarrow} AOO^{\bullet} \qquad (34)$$

$$ROO^{\bullet} + A^{\bullet} \longrightarrow \text{non-radical products} \qquad (35)$$

$$ROO^{\bullet} + AOOH \longrightarrow ROOH + AOO^{\bullet} \qquad (36)$$

The reversibility of oxygen addition to $A^{\bullet}$ explains why the antioxidant effectiveness of AH is higher at low oxygen partial pressure.[81] Reaction 36 followed by reactions 34 and 35 lets us foresee the ability of the hydroperoxide AOOH to work as an antioxidant (especially at low oxygen partial pressure) in conjunction with AH. Typical class 1 hydrocarbon antioxidants are triphenylmethane,[81] β-carotene[81] and some lactones recently introduced by Scaiano and coworkers.[78,79]

Class 2 hydrocarbon antioxidants (see section: Inhibition of autoxidation) decrease the steady-state concentration of $ROO^{\bullet}$ because the rate of the cross-reaction between $AOO^{\bullet}$ and $ROO^{\bullet}$ is much greater than the rate of the self-reaction $ROO^{\bullet} + ROO^{\bullet}$. Faster terminations reduce the rate of autoxidation of the substrate RH. Many examples are reported in the literature on the reduction of the autoxidation rate of hydrocarbons (cumene) by other oxidizable substrates such as tetralin[82] or diallyl disulfide.[77] A remarkable example regarding the inhibition of the autoxidation of linoleic acid by a class 2 hydrocarbon is given by γ-terpinene (and 1,4-cyclohexadiene), a monoterpene hydrocarbon present in essential oils.[18] It retards the AIBN-initiated autoxidation of linoleic acid with an activity comparable with that of a phenol with a rate constant k_9 of $(1–3) \times 10^3$ $M^{-1}s^{-1}$, for example C_6H_5OH. γ-Terpinene oxidizes to *p*-cymene in a chain-reaction in which the chain-carrier is $HOO^{\bullet}$. In the autoxidation of linoleic acid the chain-carrier is $LOO^{\bullet}$ and the

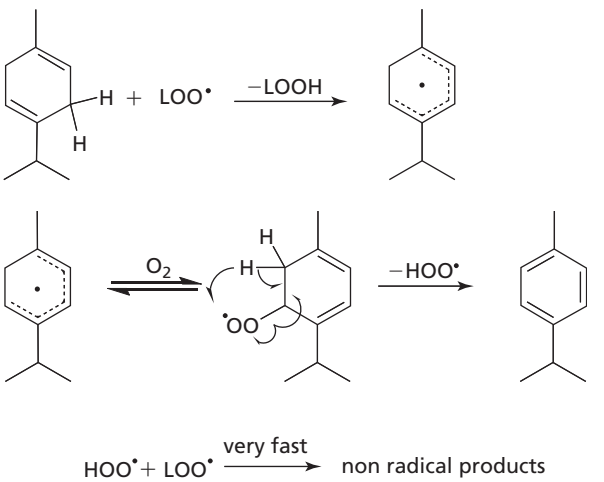

Figure 11 Antioxidant mechanism of γ-terpinene. LOO are peroxyl radicals from linoleic acid.

self-reaction $LOO^{\bullet} + LOO^{\bullet}$ occurs with a rate constant of about 3×10^7 $M^{-1}s^{-1}$ (in cyclohexane at 50°C). On the other hand, the cross-reaction $HOO^{\bullet} + LOO^{\bullet}$ was suggested to occur, on mechanistic grounds, at close to the diffusion-controlled limit. The overall mechanism of action is represented by the reactions reported in Figure 11. It is therefore clear that addition of small quantities of γ-terpinene to linoleic acid or other edible lipids may provide an alternative or supplementary strategy for obtaining large increases in their oxidative stability and shelf life.

β-*Carotene*

β-Carotene is a polyunsaturated hydrocarbon present in the hydrophobic domains of LDL, proteins, cells and tissues.[1] In

human plasma its concentration is over the range 0.3–0.6 μM.[1] Although the structure of β-carotene (and of all carotenoids) could suggest quite an important role for protecting polyunsaturated fatty acids against autoxidation, there is a general consensus on the fact that the major role of β-carotene is to serve as precursor of vitamin A.[1] However, β-carotene is able to quench various radicals including the $NO_2^{\bullet}$ radical,[4] which is quenched by electron transfer with a rate constant of 1.1×10^9 $M^{-1}s^{-1}$, the $GS^{\bullet}$ radical[4] quenched by double-bond addition, with a rate constant of about 2×10^8 $M^{-1}s^{-1}$, the peroxyl radicals (see below) but not the α-tocopheroxyl radical.[83] β-Carotene is also, as with most carotenoids, a powerful quencher of singlet oxygen, $^1O_2$.[84] Peroxyl radicals add rapidly to polyenes when the resulting C-radicals are strongly stabilized by resonance (see Figure 12), the rate constants being of the order of 10^4–10^5 $M^{-1}s^{-1}$.[85,86]

At low oxygen partial pressure, the β-carotene$^{\bullet}$-OOR radical may quickly quench a second peroxyl radical. In fact, addition of dioxygen to β-carotene$^{\bullet}$-OOR occurs reversibly[81] and is characterized by relatively low rate constants. El-Agamey and McGarvey[87] have recently reported that the rate constants of oxygen addition to carotenoid radicals, k_{O_2}, display a moderate dependence on the number of conjugated double bonds present in the radical. For carotenoid radicals with 7–11 double bonds, the measured values of k_{O_2} are over the range 10^3–10^4 $M^{-1}s^{-1}$.

The above mechanism explains why the antioxidant effectiveness of β-carotene strongly depends on the oxygen partial pressure, P_{O_2}. At low oxygen partial pressure typical of human tissues ($P_{O_2} \leq 50$ mmHg), in-vitro experiments suggest that β-carotene can function as an antioxidant, while with increasing P_{O_2} the pro-oxidant effects may overcome its antioxidant potential.[88] Since P_{O_2} is greatest in the outermost cells of the lung, these cells might be particularly subject to the toxic effects of β-carotene. Recently developed human chemoprevention trials have shown that administration of β-carotene actually increases lung cancer incidence and mortality in human smokers.[8,89,90] The compounds formed by fragmentation of the β-carotene oxidation products may contribute to its in-vivo toxic effects.[4]

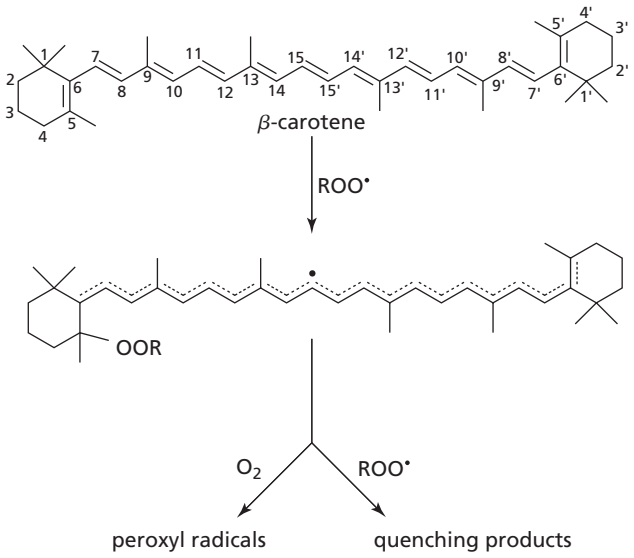

Figure 12 Reaction of β-carotene with peroxyl radicals

SOD-like antioxidants

Nitroxides

Some small molecules exert an antioxidant action by accelerating the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2, thus mimicking the endogenous enzyme SOD.[1] This mechanism can be exemplified by cyclic nitroxides with tertiary alkyl groups linked to the nitrogen atom ($R_2NO^{\bullet}$), a well known class of persistent radicals (Figure 13).[91] These nitroxides can in principle be either reduced (path A)[92] or oxidized (path B)[93] by superoxide to hydroxylamines (R_2NOH) or oxoammonium cations (R_2NO^+), respectively, which are in turn converted to the parent nitroxides by another molecule of superoxide (see Figure 13). For instance, the nitroxide OXANO (2-ethyl-2,5,5-trimethyl-3-oxazolidin-1-oxyl) catalyses superoxide dismutation via path A,[92] while TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) follows[93,94] path B. The catalytic efficiency of nitroxides following the reaction path B is reported to be 100-fold greater.[93] The occurrence of either mechanism seems to be dependent at physiologic pHs on the reduction potentials of the species involved in the catalytic cycle.[93] Salen–manganese complexes (see Figure 13) capable of promoting both $O_2^{\bullet-}$ and H_2O_2 dismutation, by mimicking the SOD and catalase activities,[95] were found to be effective against ageing processes[96] and pathologies related to oxidative stress.[97,98]

Recently, it has been recognized that TEMPO also possesses chain-breaking activity, due to electron transfer to peroxyl radicals, with the subsequent protonation of the peroxide anions by water. Measured k_9 values range from 1.4×10^4 $M^{-1}s^{-1}$ in acetonitrile/water [13] to 2.8×10^7 $M^{-1}s^{-1}$ in pure water.[99] Nitroxides can be reduced *in vivo* to the corresponding hydroxylamines (R_2NOH),[91] which react with peroxyl radicals with a rate constant of about 5×10^5 $M^{-1}s^{-1}$ in apolar solvents.[100]

Redox active transition metals

The ability of transition metal ions to promote autoxidation in organic materials has been recognized for a long time. Transition metals decompose ROOH and H_2O_2 with the formation of highly reactive radicals.[57,101] Rather surprisingly, however, a few metal ions have been claimed to also possess antioxidant properties,[102] though controversial opinions have been expressed on this.[103,104] However, it is worth noting the fact that all antioxidant enzymes (e.g. catalase, superoxide dismutase, glutathione peroxidase) contain metal ions essential to their activity. Examples of documented antioxidant activity of free metal ions *in vitro* do exist. Foti and Ingold[105] have recently reported that low concentrations of $FeCl_3$ can retard the autoxidation of γ-terpinene to *p*-cymene in acetonitrile with a SOD-like mechanism (see Figure 14). $CuCl_2$ also displays remarkable antioxidant properties but its effects are poorer (see Figure 14).[105] Conversion of

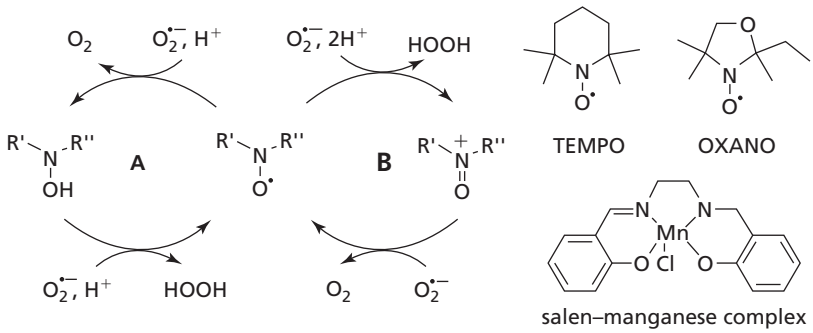

Figure 13 Superoxide dismutase-like antioxidant activity of stable dialkyl nitroxides and structure of a prototypal salen–manganese complex

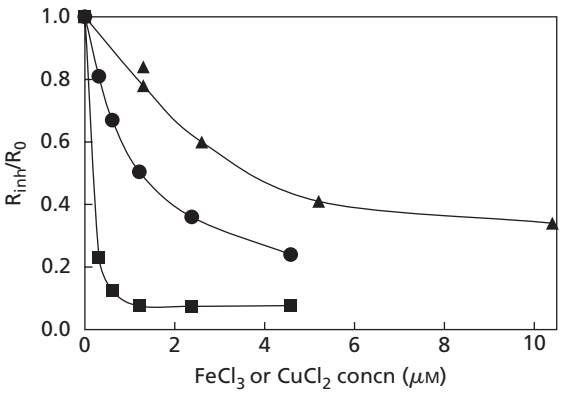

Figure 14 Metal ion inhibited rates, R_{inh}, plotted relative to the rate in the absence of metals, R_0. AIBN (8 mM) initiated autoxidation of γ-terpinene (50 mM) in CH_3CN at 50°C in the presence of different concentrations of $FeCl_3$ during the induction period (lag phase) (■), after the induction period (●), and in the presence of different concentrations of $CuCl_2$ 5 min after the start of the reaction (▲).

γ-terpinene (TH_2) to *p*-cymene (Cy) occurs via a chain-reaction having $HOO^{\bullet}$ as a chain-carrier.

$$TH_2 + O_2 \xrightarrow{HOO^{\bullet}} Cy + H_2O_2 \qquad (37)$$

Addition of micromolar concentrations of $FeCl_3$ generates a lag-phase (during which autoxidation of γ-terpinene is very slow) followed by oxidation at an almost steady rate (slower than the rate observed in the absence of $FeCl_3$). These effects were interpreted in terms of faster chain-terminations due to direct reactions of the metal ions with the $HOO^{\bullet}$ radical.

There is, in fact, experimental evidence indicating that peroxyl radicals can complex (reversibly) with transition metal ions. In the case of the hydroperoxyl radical, chain-termination may occur through the following fast processes:

$$Fe^{3+} + HOO^{\bullet} \rightleftarrows [Fe^{IV}\text{-}OOH]^{3+} \xrightarrow{HOO^{\bullet}} Fe^{3+} + H_2O_2 + O_2 \qquad (38)$$

$$Cu^{2+} + HOO^{\bullet} \longrightarrow Cu^{+} + O_2 + H^{+} \qquad (39)$$

$$Cu^{+} + HOO^{\bullet} + H^{+} \longrightarrow Cu^{2+} + H_2O_2 \qquad (40)$$

in which the metal ions behave catalytically. Unlike $FeCl_3$, $CuCl_2$ does not give rise to a SOD-like mechanism because as the $[H_2O_2]$ builds up, reactions 37 and 40, the Cu^{+} ion formed in reaction 40 will induce a chain-transfer via the rather fast (in water) reaction 41.[105]

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + HO^{-} + HO^{\bullet} \qquad (41)$$

Conclusions

Much of the chemistry involved in the mechanisms of action of antioxidants in biological systems is now fairly well understood due to the exciting work started in the fields of polymer and food stabilization. We now recognize that dietary and endogenous antioxidants are essential to healthy living. In 1986, Burton and Ingold[106] emphasized the extreme importance of antioxidants in preserving all forms of life by the acute though dramatic observation that: 'living organisms are exposed to much more severe oxidative stress than is food in a refrigerator. Nevertheless, they do not become rancid until they, in their turn, become food. What this means is that living organisms have some mechanism or mechanisms by which they protect themselves against autoxidation.' Thus, the equations: oxidative stress = illness and antioxidants = health were considered to be absolute truths until recently. Nowadays, the limitations of this over-simplified view are clear, and the complexity of this research field is being fully disclosed. Some chemical observations valid *in vitro* may lose their strength when applied to living organisms. For instance, the concept of 'total antioxidant capacity', which is undoubtedly useful in oil and polymer stabilization, may lose meaning in biological systems since regeneration of reducing species by enzymes plays a fundamental role.[107] Moreover, it is now clear that the reactive oxygen species are not only involved in harmful oxidative reactions, but are also responsible for the regulation of important biological processes, such as the fine balance between proliferation and apoptosis.[3] It is therefore possible that an excessive intake of antioxidants can lead to unexpected and even undesired outcomes.[8,89,90]

In this review, the interactions between various antioxidants and possible pro-oxidant effects have been outlined and the chemical grounds for a deeper comprehension of the processes have been given. For instance, ascorbate is one of the most potent water-soluble reductant/antioxidants in

nature, but at high concentrations and in the presence of redox-active metal ions it may become a source of hydrogen peroxide and superoxide. Unexpected antioxidant effects are displayed by oxidizable hydrocarbons such as γ-terpinene, which favours the decay of alkylperoxyl radicals by releasing hydroperoxyl radicals during autoxidation. Besides these 'sacrificial' antioxidants, which irreversibly react with free radicals, several antioxidant compounds that act catalytically with SOD-like mechanisms are gaining increasing interest. Alkylnitroxides, salen–Mn complexes and $FeCl_3$ are interesting examples of these catalytic antioxidants. Indeed, a deeper knowledge of the chemistry of radicals and antioxidants can give a valuable contribution to the development of novel antioxidant compounds.

Declarations

Conflict of interest

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