

Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants

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Abstract

Plant phenolic compounds such as flavonoids and lignin precursors are important constituents of the human diet. These dietary phytochemicals have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species including $O_2^{\bullet-}$, H_2O_2 , $^{\bullet}OH$, and 1O_2 . Here we review our current understanding of the antioxidant and prooxidant actions of phenolics in plant cells. In plant systems, phytochemicals can act as antioxidants by donating electrons to guaiacol-type peroxidases (GuPXs) for the detoxification of H_2O_2 produced under stress conditions. As a result of such enzymatic as well as non-enzymatic antioxidant reactions, phenoxyl radicals are formed as the primary oxidized products. Until recently, phenoxyl radicals had been difficult to detect by static electron spin resonance (ESR) because they rapidly change to non-radical products. Application of Zn exerts spin-stabilizing effects on phenoxyl radicals that enables us to analyze the formation and decay kinetics of the radicals. The ESR signals of phenoxyl radicals are eliminated by monodehydroascorbate radical (MDA) reductase, suggesting that phenoxyl radicals, like the ascorbate radical, are enzymatically recycled to parent phenolics. Thus, phenolics in plant cells can form an antioxidant system equivalent to that of ascorbate. In contrast to their antioxidant activity, phytochemicals also have the potential to act as prooxidants under certain conditions. For example, flavonoids and dihydrocinnamic acids can nick DNA via the production of radicals in the presence of Cu and O_2 . Phenoxyl radicals can also initiate lipid peroxidation. Recently, Al, Zn, Ca, Mg and Cd have been found to stimulate phenoxyl radical-induced lipid peroxidation. We discuss the mechanism of phenoxyl radical prooxidant activity in terms of lifetime prolongation by spin-stabilizing agents. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Plant phenolic compounds such as flavonoids and lignin precursors are potent antioxidants.

Vitamin C (ascorbate) functions as a ubiquitous antioxidant in both animals and plants by scavenging reactive oxygen species via enzymatic and non-enzymatic reactions. The concept that flavonoids are efficient antioxidants is not a novel idea itself but has a long research history. In 1937, Szent-Györgyi, a Nobel prize winner who isolated ascorbate, demonstrated that the flavonoid hesperidine can behave similar to ascorbate in maintaining capillary permeability (Bentsáth et al., 1937). Based on this observation he proposed that flavonoids are essential human nutrients, i.e. the short-lived vitamin P (permeability) concept (Bentsáth et al., 1937). Although the vitamin concept of flavonoids did not gain broad acceptance, the idea that flavonoids can complement the functions of ascorbate has renewed interest in the antioxidant hypothesis. After the recent explosion of research in the pharmacology of food phytochemicals, a great number of reports have established that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutagenic and anticarcinogenic effects (Middleton and Kandaswami, 1994; Rice-Evans et al., 1997).

There have also been a number of reports suggesting that dietary phenolics exhibit prooxidant and cytotoxic properties under certain conditions (Summers and Felton, 1994; Yamanaka et al., 1997; Sugihara et al., 1999). The antioxidant/prooxidant activity of phytochemicals can depend on such factors as metal-reducing potential, chelating behavior, pH, and solubility characteristics (Decker, 1997). In this review, we describe how plant phenolics may act as antioxidants or prooxidants in plant cells from the point of view of phenoxyl radical lifetime prolongation.

2. Biosynthesis of phytochemicals

Phenolics are chemical compounds characterized by at least one aromatic ring (C₆) bearing one or more hydroxyl groups. Hydroxycinnamic acids (HCAs) and flavonoids have the basic carbon skeletons C₆–C₃ and C₆–C₃–C₆, respectively. HCAs and flavonoids are produced from phenylalanine, via the shikimate pathway, general

phenylpropanoid pathway and specific flavonoid pathway. Fig. 1 summarizes biosynthetic relationships and basic structures of HCAs and flavonoids.

Flavonoids commonly accumulate in epidermal cells of plant organs such as flowers, leaves, stems, roots, seeds and fruits, being found in glycosidic forms (glycosides) and non-glycosidic forms (aglycones). Subcellular localization of the glycosides is mainly confined to hydrophilic regions such as vacuoles and apoplasts (McClure, 1975; Wollenweber and Dietz, 1981). Glutathione *S*-transferases (GST) function in the recognition and transport of anthocyanins into the plant cell vacuoles (Edwards et al., 2000). Glutathione *S*-conjugation of medicarpin (an isoflavonoid) by a GST (Li et al., 1997) and of cinnamic acid by an ascorbate peroxidase (Walczak and Dean, 2000) have been shown to permit their entry into membrane vesicles via glutathione *S*-conjugate pumps. A few flavonoid glycosides have also been found in chloroplasts and etioplasts (Weissenböck, 1973; Ruzieva et al., 1980). The aglycones have been reported to be localized in lipophilic regions such as oil glands and waxy layers (Wollenweber and Dietz, 1981).

3. Antioxidant functions in plants

3.1. Stress protectant function of phytochemicals

Most plants constitutively synthesize phenylpropanoids including flavonoids and HCAs. However, accumulation of phenolics in plants can be induced by abiotic and biotic stresses, e.g. UV (ultra violet) radiation, high-light illumination, low temperatures, wounding, low nutrients, and pathogen attack (Dixon and Paiva, 1995; Yamasaki et al., 1995). Abiotic stress has been postulated to promote production of harmful active oxygen species within the cells (Alscher et al., 1997; Draper, 1997). In addition to the stress-induced production of active oxygens, it has been conclusively shown that active oxygen species are formed even during regular metabolism in plants (Rich and Bonner, 1978; Halliwell and Gutteridge, 1985). In plants, photosynthetic electron

transport is a major source of active oxygen production (Asada and Nakano, 1978). Superoxide ($O_2^{\bullet-}$), the primary produced active oxygen, can be disproportionated to hydrogen peroxide (H_2O_2), a reaction catalyzed by superoxide dismutase (SOD) (Asada and Takahashi, 1987; Asada, 1994). Unlike other active oxygen species, H_2O_2 is able to diffuse across membranes due to its high stability and membrane-permeability. H_2O_2 toxicity itself is weak compared with other reactive oxygen species, but in the presence of transition metals, H_2O_2 produces the hydroxyl radical ($\bullet OH$), the most reactive active oxygen. Thus, scavenging of H_2O_2 is essential to avoid oxidative damage of plant cells.

Normally, the H_2O_2 by-product of photosynthetic electron transport is detoxified to water by the ascorbate-glutathione cycle (Foyer, 1993). In this scavenging system ascorbate acts as the elec-

tron donor for ascorbate peroxidase (APX) to remove H_2O_2 , and ascorbate is regenerated by monodehydroascorbate (MDA) reductase (Asada, 1997). It has recently been shown that flavonoids and HCAs are capable of scavenging H_2O_2 by acting as electron donors for guaiacol peroxidases (GuPXs), such as horseradish peroxidase (HRP) (Yamasaki et al., 1997; Sakihama et al., 2000). Under conditions of severe stress, the scavenging capacity of the chloroplast may be exceeded when plastid ascorbate pools become oxidized (Yamasaki et al., 1995, 1999; Grace and Logan, 2000). It has been proposed that phytophenolics, particularly polyphenols, function as antioxidants to support the primary ascorbate-dependent detoxification system as a backup defense mechanism of vascular plants (Yamasaki et al., 1995, 1999).

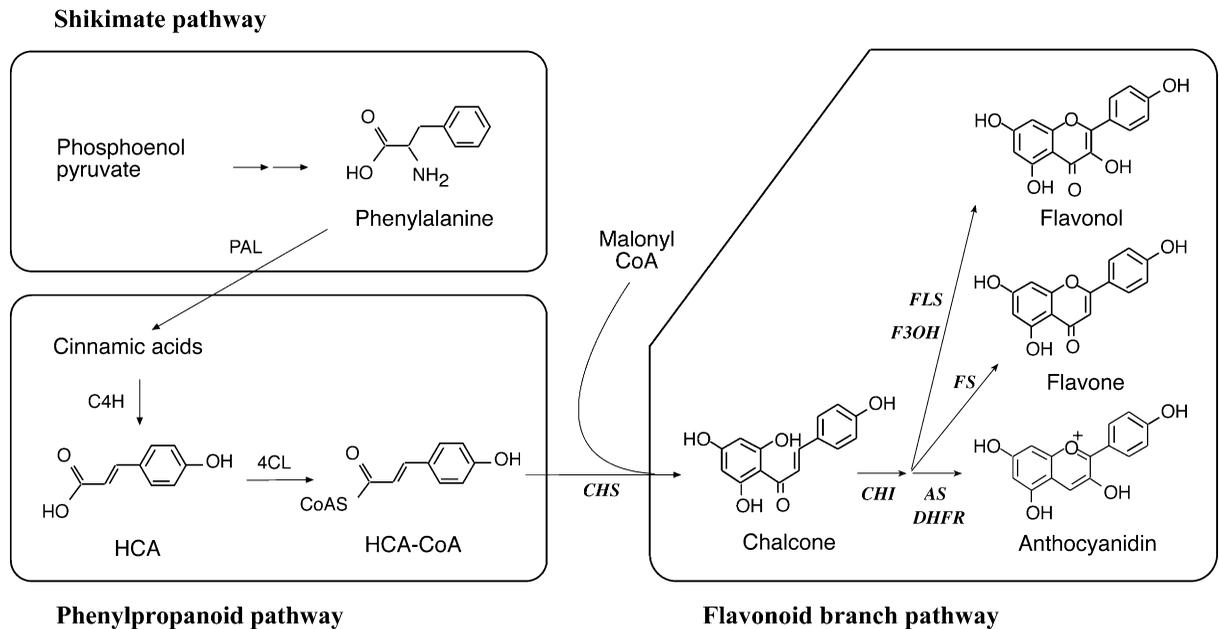


Fig. 1. Diagram of the phytophenolics biosynthetic pathway. HCAs and flavonoids are derived from cinnamic acid which is formed from phenylalanine by phenylalanine ammonia-lyase (PAL) (Harborne, 1988; Herrmann, 1995). Cinnamic acid is modified to HCA by cinnamate-4-hydroxylase (C4H) and *o*-methyltransferase. The enzyme 4-coumarate:CoA ligase (4CL) catalyzes the formation of hydroxycinnamoyl CoA (HCA-CoA) and these activated intermediates are used in the biosynthesis of flavonoid and lignin. Flavonoids synthesis starts with the condensation of HCA-CoA and three molecules of malonyl CoA catalyzed by chalcone synthase (CHS). Chalcone is further converted to other flavonoid subgroups, including flavone, flavonol and, anthocyanin by chalcone isomerase (CHI), flavanone 3-hydroxylase (F3OH), isoflavone synthase (ISF), flavone synthase (FS), flavonol synthase (FLS), dihydroflavonol reductase (DHFR) and, anthocyanin synthase (AS) (Douglas, 1996; Shirley, 1996).

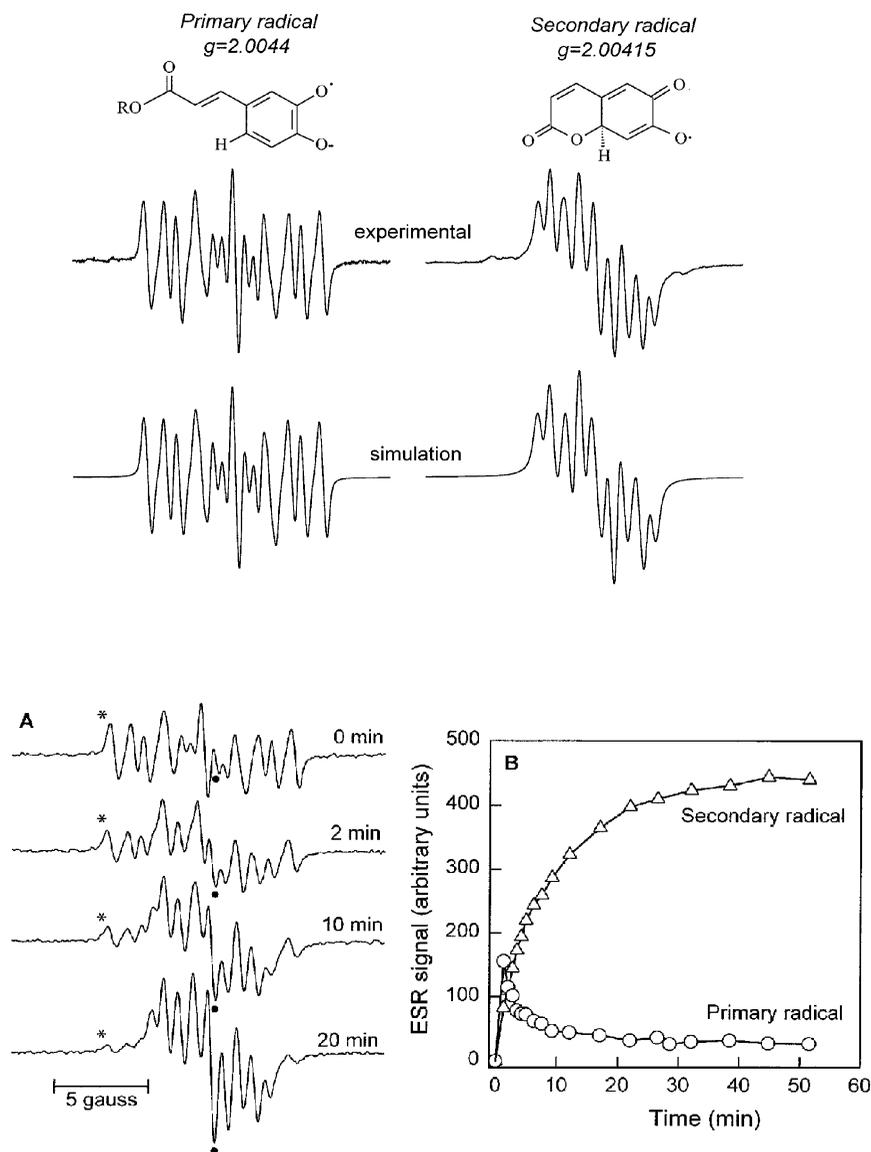


Fig. 2. *Top Panel.* ESR spectra of CGA radicals formed by oxidation in the horseradish peroxidase reaction and proposed structures of the radical products. The reaction contained 10 mM CGA, 14 mM H_2O_2 , and 0.1 nM HRP in 50 mM MES-NaOH buffer, pH 5.6, and 200 mM ZnSO_4 as a spin stabilizing agent. The ESR spectra were recorded 1 min (primary radical) and 30 min (secondary radical) after addition of H_2O_2 . Simulated ESR spectra were obtained using software provided from the National Institute of Environmental Health Science, USA (<http://epr.niehs.nih.gov>). The hfsc values for the primary radical are 0.87, 1.36, 2.52, and 3.00 gauss and for the secondary radical are 0.53, 0.68, 0.81, 1.55, and 2.22 gauss. R = quinate. *Bottom panel.* (A) Time-dependent changes in the ESR spectrum during the HRP-catalyzed oxidation of CGA. (B) Kinetics of radical transition; (○) primary radical, (△) secondary radical. The changes in the ESR signal intensities were determined at $g \approx 2.0073$ for the primary radical (marked *) and $g \approx 2.0039$ for the secondary radical (marked ●) where the spectral overlap is minimal. Redrawn from Grace et al. (1999).

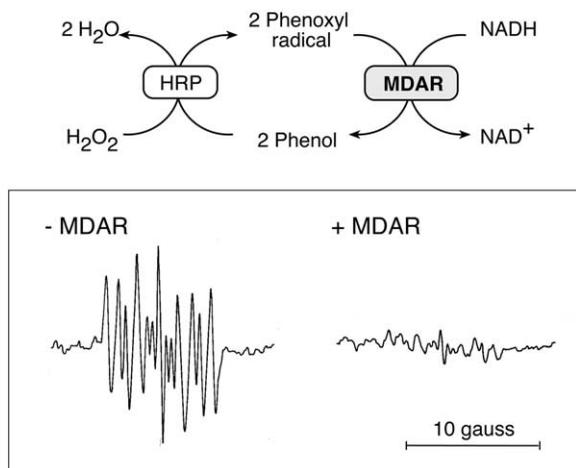


Fig. 3. Quenching of the CGA radicals by MDA reductase (MDAR) detected with ESR. Redrawn from Sakihama et al. (2000).

3.2. Phenoxyl radical produced via antioxidant function

When phenolics function as antioxidants either by enzymatic or direct radical scavenging mechanisms, they are univalently oxidized to their respective phenoxyl radicals (Kagan and Tyurina, 1997). However, until recently, these radicals had been difficult to detect by static electron spin resonance (ESR) because they rapidly change to non-radical products. Application of divalent metals such as Zn^{2+} exerts spin-stabilizing effects on phenoxyl radicals that enables analysis of their structural and kinetic properties (Kalyanaraman, 1990). We have applied the technique of spin stabilization to analyze radicals derived from chlorogenic acid (CGA) in several oxidative enzyme systems (Yamasaki and Grace, 1998; Grace et al., 1999). Information about the structure of these radicals was obtained from a computer-based simulation of their ESR spectra and analysis of the hyperfine splitting constants (hfsc). Two distinct radical species have been identified in the oxidation of CGA by HRP (Fig. 2). The primary CGA radical is a short-lived species ($t_{1/2} = 45$ s) that is also formed in the tyrosinase reaction (Grace et al., 1999). The ESR spectrum of this radical indicates hyperfine interaction between the

unpaired electron and the three aromatic protons plus one proton from the side chain. These data are consistent with the formation of an *o*-semiquinone anion (pK_a 3–4) that decays rapidly to non-radical products even in the presence of Zn.

With prolonged incubation a stable secondary radical species appears in the HRP reaction coinciding with the loss of the primary *o*-semiquinone. The intensity of this new, secondary radical species increases as the reaction proceeds and reaches a stable value after 20 min (Fig. 2, bottom panel). Unlike the primary radical, the ESR spectrum of the secondary radical indicates hyperfine interactions between the unpaired electron and *five* protons, consistent with a cyclized structure in which the unpaired electron is delocalized over a more extended π -conjugated system in comparison with the primary radical. Based on these data, we have proposed that the primary CGA radical is converted to a more stable secondary radical following cyclization, homolytic cleavage and a second peroxidase reaction (Grace et al., 1999). Such metal-stabilized phenoxyl radicals could exert prooxidant effects in plant cells.

3.3. Phytophenolics regeneration system

Phenoxyl radicals can be reduced to their parent compounds by non-enzymatic reactions with ascorbate (Takahama and Oniki, 1992; Yamasaki and Grace, 1998). It has recently been shown that phenoxyl radicals can also be reduced to parent phenolics by MDA reductase (Sakihama et al., 2000). MDA reductase, an enzyme of the ascorbate-glutathione cycle, catalyzes the reduction of MDA radical, the univalent oxidized product of ascorbate, to regenerate the parent compound ascorbate. The ESR signal of the CGA primary radical is suppressed by MDA reductase in conjunction with the substrate NADH, whereas NADH alone does not show such a suppressive effect (Fig. 3). Although MDA reductase reduces the primary CGA radical, the enzyme does not reduce further oxidized products (Sakihama et al., 2000). These results demonstrate that MDA reductase, which had been considered to be highly

specific to the MDA radical, is capable of reducing phenoxyl radicals to the parent phenols.

Fig. 4 presents a scheme showing cooperativity between ascorbate and phytophenolics in the mechanism for scavenging H_2O_2 in plant cells. Ascorbate functions as an essential antioxidant to scavenge active oxygen species such as $O_2^{\bullet-}$, H_2O_2 , $\bullet OH$, and 1O_2 through direct chemical reactions (Bodannes and Chan, 1979; Felton, 1995; Conklin et al., 1996). In addition to non-enzymatic scavenging, ascorbate also detoxifies H_2O_2 through the enzymatic reaction of APX. Although ascorbate is found in chloroplasts at high concentrations of more than 10 mM (Asada and Takahashi, 1987), from the known rates of $O_2^{\bullet-}$ and H_2O_2 photoproduction, it can be calculated that ascorbate is consumed within only 80 s under illumination (Asada, 1994). Thus, the ascorbate regeneration systems, including the non-enzymatic reaction by photoreduced ferredoxin (Miyake and Asada, 1994) and the enzymatic reaction cata-

lyzed by MDA reductase (Hossain and Asada, 1984), are indispensable for plant cells to maintain ascorbate antioxidant activity. Like ascorbate, phytophenolics can also scavenge active oxygen species through direct reaction (Bors et al., 1990; Yamasaki et al., 1996) and enzymatic reaction (Yamasaki et al., 1997; Sakihama et al., 2000). Furthermore, phytophenolics can be regenerated from phenoxyl radicals by non-enzymatic reaction with ascorbate (Takahama, 1989; Yamasaki and Grace, 1998) and enzymatic reaction of MDA reductase (Sakihama et al., 2000). Hence, it is now clear that phytophenolics can function cooperatively with ascorbate as antioxidants, particularly in vacuoles and apoplasts where they co-accumulate with GuPX (Yamasaki et al., 1997).

4. Toxic effects of phenolics

4.1. Involvement of phenolics in defense mechanisms

Polyphenols, particularly flavonoids and tannins, have long been associated with plant defense against herbivores. We have recently found that in fronds of the aquatic fern *Azolla* anthocyanin levels negatively correlate with palatability, and that accumulation of anthocyanin occurs in response to long-term feeding by tadpoles (Cohen et al., 2002). Similar increases in phenolics have also been observed in poplar and oak trees in response to feeding and wounding (Hammerschmidt and Schultz, 1996). Oxidation of phenolics usually occurs as a consequence of tissue damage, but in the outer layers of onions, oxidation of quercetin by peroxidases forms end products like 3,4-dihydroxybenzoic acid as part of a pre-formed defense against fungal ingress (Takahama and Hirota, 2000). Phenoxyl radicals formed via oxidative processes have multiple roles in plant defense owing to their ability to initiate free-radical chain reactions in the membrane and their propensity to cross-link with a variety of molecules (Appel, 1993; Cohen et al., 2001). The midgut of insect herbivores is a highly oxidizing environment. Accordingly, diet supplementation of *Helicoverpa zea* larvae with phenolic acids was found to in-

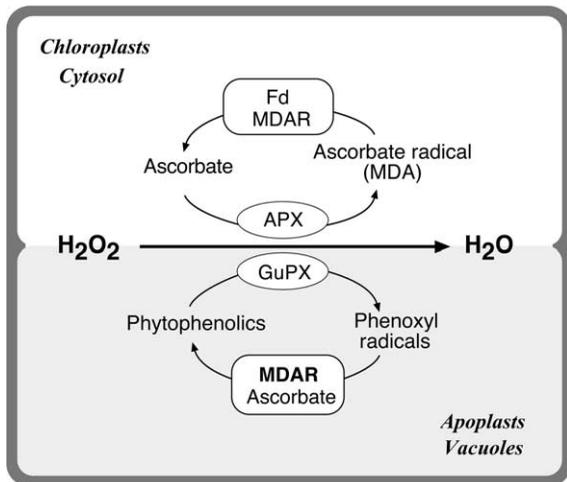


Fig. 4. A scheme showing a similarity between ascorbate and phytophenolics in scavenging H_2O_2 produced in plant cells. In the chloroplast and cytosol, ascorbate is oxidized to the MDA radical by APX to detoxify H_2O_2 . MDA radical can be reduced to ascorbate by non-enzymatic reaction of ferredoxin (Fd) or enzymatic reaction of MDAR. Similarly, phytophenolics can detoxify H_2O_2 as electron donors for GuPX localized in apoplasts or vacuoles, which results in the formation of phenoxyl radicals. Phenoxyl radicals can be reduced to parent phenolics by non-enzymatic reaction of ascorbate as well as by enzymatic reaction of MDAR.

crease various indicators of oxidative stress in midgut tissues (Summers and Felton, 1994).

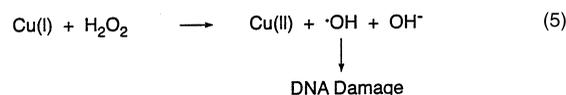
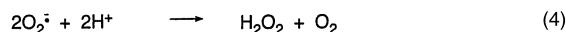
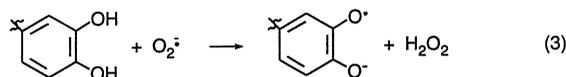
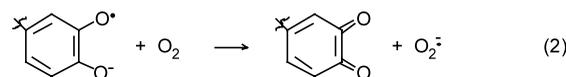
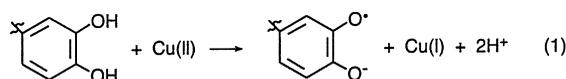
There is growing evidence of an association between exposure to metals and phenolic accumulation. Spraying leaves of the herb *Phyllanthus tenellus* with a copper sulfate solution resulted in antimicrobial phenolics being accumulated at high concentrations in vacuoles of spongy layer cells prior to apoptosis, a process very similar to the hypersensitive response induced by pathogen attack (Santiago et al., 2000). When grown in heavy metal-contaminated medium, the waterlily *Nymphaea* accumulates the metal together with polyphenols, some of which are antibacterial, in epidermal glands that have a high constitutive peroxidase activity (Lavid et al., 2001). Likewise, mustard (*Brassica* spp.) can sequester molybdenum by chelation to anthocyanins in epidermal tissue (Hale et al., 2001). Ayala-Silva and Al-Hamdani (1997) found that *Azolla caroliniana* plants incubated with $Al_2(SO_4)_3$ accumulated nine times more anthocyanins than control plants, however, no attempt was made to determine co-localization. It is likely that accumulated metals, at least in some plant species, complement the cytotoxic action of phenolics in defense against herbivores and pathogens (Boyd and Martens, 1998).

4.2. Prooxidant reaction of phenoxyl radicals

Phytophenolics have the potential to act as prooxidants in systems containing redox-active metals. In the presence of O_2 , transition metals such as Cu and Fe catalyze the redox cycling of phenolics, leading to the formation of reactive oxygen species and other organic radicals that can damage DNA, lipids, and other biological molecules (Rahman et al., 1989; Li and Trush, 1994; Yamanaka et al., 1997). It has been suggested that the interaction of phytophenolics with DNA-associated copper can result in a spectrum of DNA lesions, including oxidative base modifications, strand breaks, and formation of DNA adducts (Li and Trush 1994). Xenobiotic phenolics and their quinone derivatives are known to exert genotoxic and mutagenic effects as a result of their prooxidant properties (Flowers et al., 1997).

To assess the prooxidant activity of phytophenolics, we compared the ability of three plant catechols with similar structures, CGA, caffeic acid, and dihydrocaffeic acid (DHCA), to nick DNA in the presence of Cu ions. Although these compounds have similar redox potentials (Boyer et al., 1988; Jovanovic et al., 1994), they vary considerably in their ability to nick DNA. Exposure of DNA to DHCA in the presence of Cu results in the greatest extent of damage in this system, with a dose-dependent increase in both single and double strand breaks (Fig. 5). Caffeic acid causes less severe damage under these conditions (single strand breaks only), and CGA causes only minimal damage.

The reactive species responsible for DNA nicking activity of phenolics is the $\cdot OH$ radical or a species with similar oxidative potential (Li and Trush, 1994). In the presence of Cu the $\cdot OH$ radical is formed by the following series of reactions:



The initial oxidation of catechols by Cu(II) generates a semiquinone (reaction 1) that can react with O_2 to form $O_2^{\cdot -}$ (reaction 2). This reaction has an autocatalytic character since $O_2^{\cdot -}$ will oxidize the parent compound to regenerate the semiquinone and H_2O_2 (reaction 3). H_2O_2 can also form by the disproportionation of $O_2^{\cdot -}$ (reaction 4). In the presence of Cu(I), H_2O_2 is rapidly converted to the $\cdot OH$ radical in a Fenton-type reaction (reaction 5).

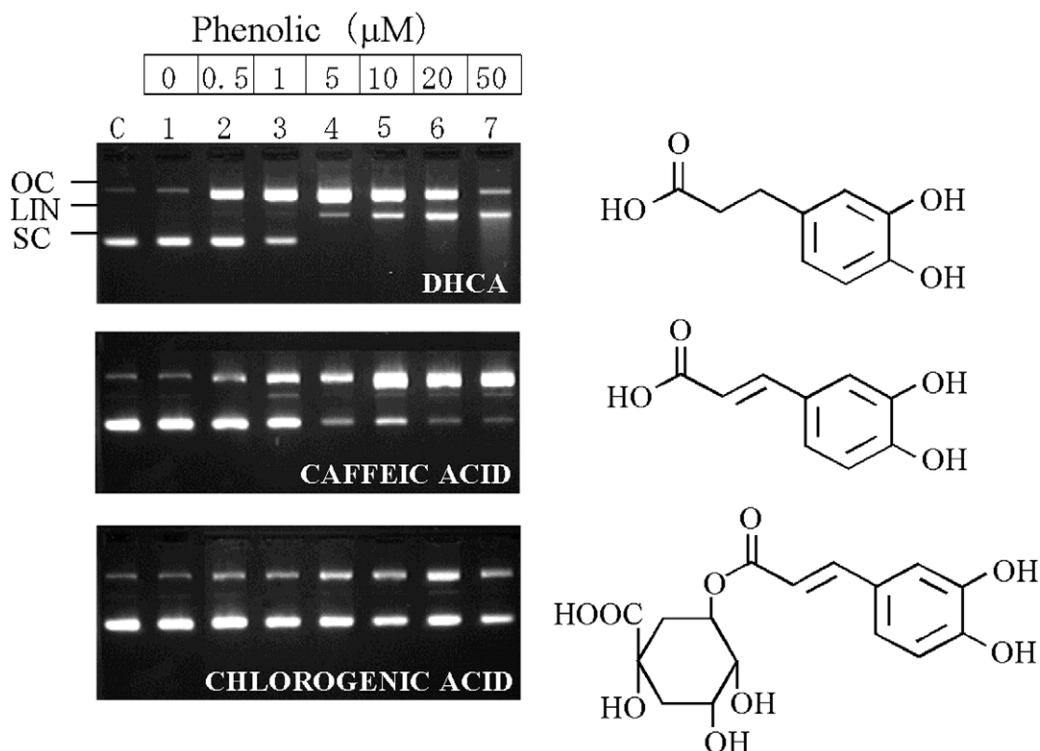


Fig. 5. DNA nicking activity of Cu-phenolic chelates. Supercoiled (SC) plasmid DNA (pBR322, 0.5 μg) was incubated with 10 μM CuCl_2 and the indicated concentrations of phenolics at 37 $^\circ\text{C}$ for 1 h in 10 mM Tris-HCl buffer, pH 7.4. The formation of open circular (OC) and linear (LIN) forms of DNA were detected by mobility shifts in 0.9% agarose gels containing 0.5 $\mu\text{g}/\text{ml}$ ethidium bromide. These oxidized forms of DNA are caused by single stranded and double stranded breaks, respectively.

We confirmed the formation of $\cdot\text{OH}$ radicals in the metal-phenolic system using an ESR spin trap method and dimethyl sulfoxide (DMSO) as a primary target molecule. Oxidation of DMSO by the $\cdot\text{OH}$ radical generates the methyl radical ($\cdot\text{CH}_3$), which reacts rapidly with α -(pyridyl-4-*N*-oxide)-*N*-*tert*-butylnitron (POBN) to form a stable spin adduct with a characteristic six-line ESR spectrum (Gunther et al., 1995). In the presence of Cu(II) the highest levels of spin adduct were formed by co-incubation with DHCA (Fig. 6). Lower levels of spin adduct were observed in the presence of caffeic acid and no spin adducts were observed in the presence of CGA. Both O_2 uptake and $\cdot\text{OH}$ radical formation were suppressed by the Cu(I) chelator bathocuproine and catalase (not shown), confirming the involvement of Cu(I) and H_2O_2 in a metal-catalyzed Fenton reaction.

The prooxidant activity of phenolics depends on their metal reducing properties, chelating behavior, and O_2 -reducing capacity. This latter property depends on the redox potential of the oxidized species and the lifetime of the phenoxyl radicals. We measured the stability of phenoxyl radicals by ESR in the absence of spin stabilizing metals. Whereas the caffeic acid and CGA radicals decay rapidly, DHCA forms a persistent semiquinone radical when generated enzymatically by HRP (Fig. 7). The ability of the DHCA radical to reduce O_2 was confirmed in O_2 uptake measurements (Grace and Logan, 2000). These data clearly show that phenoxyl radical lifetime is an important determinant of the O_2 reducing potential of phenolics. Two factors that apparently limit this prooxidant potential are resonance delocalization of the phenoxyl radical by the side

chain double bond (e.g. caffeic acid) and esterification of the terminal carboxyl group (e.g. CGA).

Because of the nature of the biosynthetic pathway for phytophenolics (Fig. 1), DHCA is rarely found in plants. Caffeic acid is a more common phenolic metabolite, although it is normally conjugated as a sugar or organic acid ester. In con-

trast, CGA is an extremely widespread plant metabolite that appears to provide protection against certain forms of stress (Grace and Logan, 2000). Although phytophenolics may normally function as antioxidants in plants, it is possible that they may be converted to prooxidants by ester hydrolysis and bioreductive metabolism during microbial and/or insect attack.

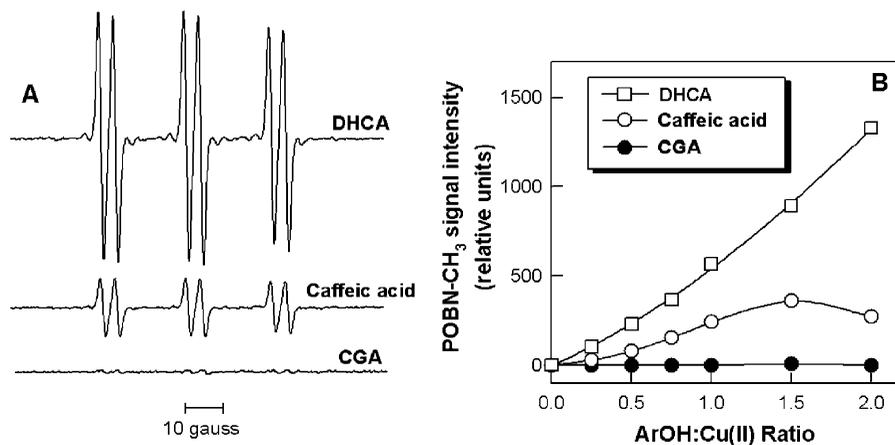


Fig. 6. Formation of $\cdot\text{OH}$ radicals by Cu-phenolic chelates as detected by ESR spin trapping. (A) Phenolics (2 mM) were incubated with CuSO_4 (0.4 mM) for 1 h in chelexed sodium phosphate buffer (0.1 M), pH 7.4 containing 2% DMSO and 25 mM POBN. Redrawn from Grace and Logan (2000). (B) Dose response of $\cdot\text{CH}_3$ radical adduct formation in the presence of increasing concentrations of phenolics.

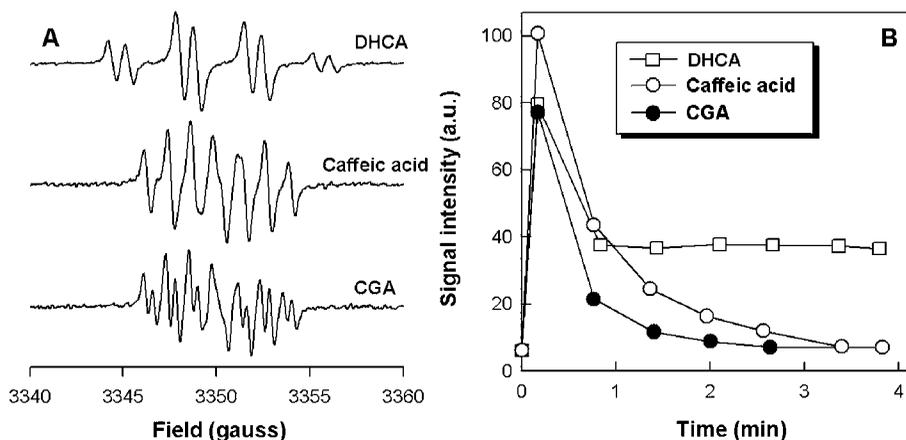


Fig. 7. Lifetime of phenoxyl radicals of DHCA, caffeic acid, and CGA in the absence of spin stabilizing metals. (A) ESR spectra of radicals. (B) Kinetics of radical decay. Solutions contained 10 mM H_2O_2 and 5 mM of the indicated compounds in 0.1 M sodium phosphate, pH 7.4. Radicals were generated by the addition of 3 nM HRP. Signal intensity was measured by double integration of the ESR spectra. ESR conditions were as follows: power 10 mW, gain 5×10^3 , modulation amplitude 0.4 gauss, sweep time 20 gauss/min, time constant 0.128 s.

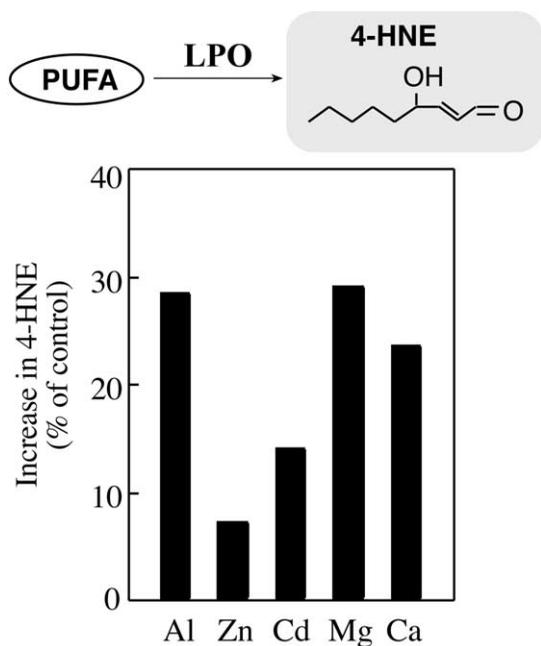


Fig. 8. Enhancement of phenolic-induced lipid peroxidation by metals. Lipid peroxidation is represented by increase in 4-HNE formation of soybean liposome. The reaction medium (1 ml) contained 0.14 M sodium acetate–0.06 N acetate buffer (pH 5.0), 2.5 mM H₂O₂, 100 μM CGA, 2% w/v liposomes and 100 μM metals. HRP (1 U/ml) was added to the reaction mixture and incubated at 25 °C for 5 min. LPO, lipid peroxidation; PUFA, polyunsaturated fatty acid.

4.3. Heavy metal-mediated phenoxyl radical toxicity

In vitro experiments have clearly shown that phenoxyl radicals are potential prooxidants for living systems (Grace and Logan, 2000). However, there is little information on the effects of endogenously produced phenoxyl radicals on plant physiology. It has been recently demonstrated that redox-inactive metals can enhance the prooxidative effect of phenolics. Although the reduced form of CGA exhibits antioxidant activity (Rice-Evans et al., 1997), its oxidized form has been shown to initiate lipid peroxidation of soybean liposomes that can be detected by the formation of 4-hydroxynonenal (4-HNE), a decomposition product of lipid peroxide (Sakihama and Yamasaki, 2002). 4-HNE is specifically produced from ω-6 polyunsaturated fatty acids (PUFAs)

including linoleic acid and arachidonic acid (Esterbauer and Cheeseman, 1990). Al enhances lipid peroxidation induced by the oxidized form of CGA (Sakihama and Yamasaki, 2002). Unlike transition metals such as Fe and Cu, Al is redox inactive and, thus, does not have potential to initiate lipid peroxidation (Ono et al., 1995). It has been shown that Al exhibits a spin-stabilizing effect on the phenoxyl radical (Kalyanaraman, 1990). Enhancement of the prooxidant nature of CGA by Al can be accounted for by stabilization of the radical. In fact, other spin-stabilizing metals produce effects similar to Al. Zn, Cd, Mg and Ca, all of which have been reported to have the spin-stabilizing effects, stimulate lipid peroxidation induced by the oxidized form of CGA (Fig. 8). Because these metals without CGA radicals do not induce significant in vitro lipid peroxidation (Sakihama and Yamasaki, 2002), direct interactions between metals and lipids can be considered negligible in the stimulation mechanism. These results suggest that even redox inactive metals mediate lipid peroxidation by enhancing the prooxidant free radical state of phenolics. Fig. 9 depicts a model for how the synergistic interactions of phenoxyl radicals and heavy metals may act to induce oxidative stress metabolism in plants, presenting the case of Al as an example.

5. Concluding remarks

Reduced forms of phytochemicals are powerful antioxidants equivalent to ascorbate. In contrast, the phenoxyl radical produced through antioxidative reactions and in lignin biosynthesis, is a potential prooxidant. Under normal growth conditions phenoxyl radicals usually do not show harmful prooxidant activity because they are rapidly changed to non-radical products by polymerization reactions or enzymatic (as well as non-enzymatic) reduction of the radicals. However, phenoxyl radicals would exhibit cytotoxic prooxidant activity when the lifetime of the radicals is prolonged by effectors of spin-stabilization (Fig. 10).

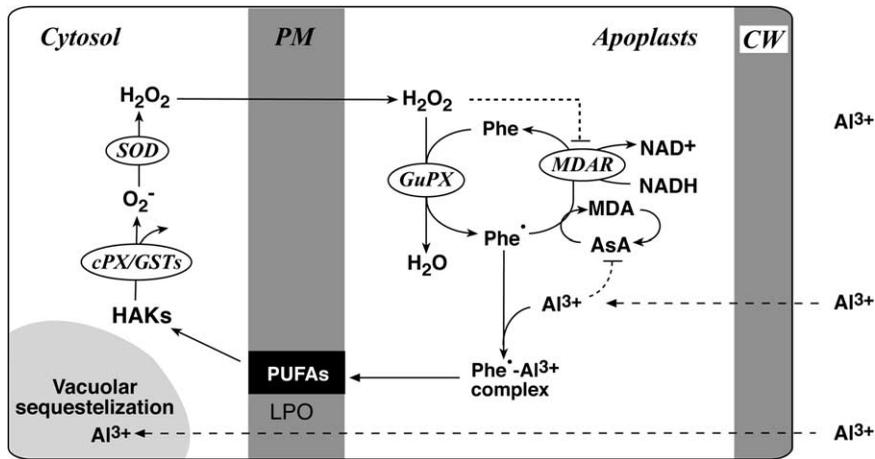


Fig. 9. A hypothetical scheme for plant Al toxicity mediated by phenolics. Under acidic conditions Al can infiltrate into plant tissues as Al^{3+} resulting in a dramatic decline in ascorbate (AsA) concentrations (Lukaszewski and Blevins, 1996). Loss of AsA should shift the equilibrium of the phenolic (Phe)/ H_2O_2 cycle towards increased phenoxyl radical (Phe^\bullet) levels. Complexation of Al^{3+} stabilizes the Phe^\bullet and greatly enhances their ability to initiate peroxidation of PUFAs of the cell membrane lipids (Sakihama and Yamasaki, 2002) forming hydroxyalkenals (HAKs). Exposure to Al is known to increase expression of cytosolic peroxidases (cPX) (Cakmak and Horst, 1991; Ezaki et al., 1996) which, in conjunction with induced GSTs (Richards et al., 1998), are thought to detoxify the lipid peroxidation products (Skipsey et al., 1997; Edwards et al., 2000). These enzymatic detoxification processes release $\text{O}_2^{\bullet-}$ which is reduced to H_2O_2 by superoxide dismutase (SOD), another enzyme that shows elevated activity in response to Al^{3+} (Cakmak and Horst, 1991). MDA reductase localized in the apoplast may contribute to reduce toxicity mediated by phenolics (Sakihama et al., 2000). PM, plasma membrane; CW, cell wall.

The spin stabilization of phenoxyl radicals by metal ions also has potential implications for the etiology of Alzheimer's and Parkinson diseases, both of which are characterized by abnormal accumulations of metals (Meglio and Oteiza, 1999; Smith et al., 2000) and 4-HNE formation (Yoritaka et al., 1996; Ando et al., 1998) in affected areas of the brain. Growing evidence indicates that the catecholamine neurotransmitter dopamine, when oxidized, can act as a direct causative agent of Parkinson disease neurodegeneration (Conway et al., 2001; Stokes et al., 1999). The resulting dopamine radicals, stabilized by metal ions, could then directly initiate oxidative damage on neurons without the requirement for reactive oxygen species formation. Not only to understand plant physiology but also to overcome these diseases, the spin-stabilizing effect may be an important clue for exploring the cytotoxicity potential of phenolic compounds.

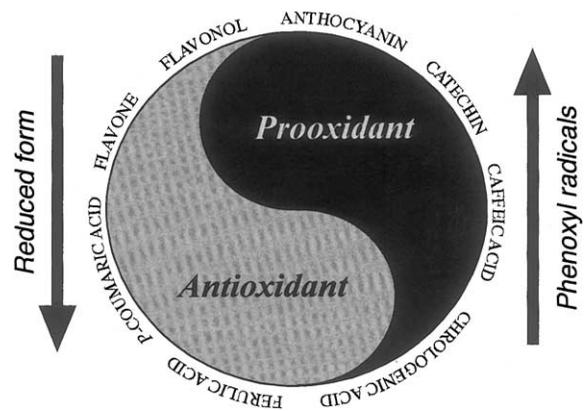


Fig. 10. The Yin-Yang symbol represents the balance between antioxidant and prooxidant characteristics of phytophenolics. Although the reduced forms of phytophenolics act as antioxidants, the oxidized form, phenoxyl radicals, can exhibit prooxidant activities under conditions that prolong the radical lifetime.

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