Lipid peroxidation induced by phenolics in conjunction with aluminum ions

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Abstract

Using the whole plant and model systems, we demonstrate that the aluminum ions (Al³⁺) stimulate phenolic-dependent lipid peroxidation. Lipid peroxidation in barley (Hordeum vulgare L. cv. Donor) roots was 30% higher under AlCl₃ treatment than without Al. Major decomposition product of lipid peroxidation was 4-hydroxynonenal (4-HNE) but not thiobarbituric acid reactive substances (TBARS), a widely used markers for lipid peroxidation. Similarly, AlCl₃ stimulated lipid peroxidation of soybean liposomes in the presence of chlorogenic acid (CGA) and H₂O₂/horseradish peroxidase system which can oxidize phenolics. Al³⁺ was found to enhance lipid peroxidation induced by oxidized CGA. Intermediates of lignin biosynthesis in plants, including p-coumaric acid, ferulic acid, sinapic acid and coniferyl alcohol, also showed similar effects. These results suggest that Al³⁺ has a potential to induce oxidative stress in plants by stimulating the prooxidant nature of endogenous phenolic compounds.

Additional key words: barley, chlorogenic acid, coniferyl alcohol, ferulic acid, 4-hydroxynonenal, liposomes, p-coumaric acid, sinapic acid, soybean.

Introduction

Phenolic compounds, such as flavonoids, are abundant in plants. They have been widely appreciated as powerful antioxidants (Bors et al. 1990, Yamasaki et al. 1996). The flavonoid quercetin is capable of scavenging superoxide (O₂⁻), singlet oxygen (¹O₂), hydroxyl radical (OH⁻), alkoxyl radical (RO·) and peroxyl radical (LOO·). Similarly, anthocyanins have a capability of scavenging those radical species without any aid of enzymes (Yamasaki et al. 1996). A structural basis for the antioxidant action of flavonoids is largely ascribed to dihydroxy group(s) (catechol moiety) of the B-ring (Bors et al. 1990, Yamasaki et al. 1997). Thus, structurally simpler phenolic compounds including a catechol moiety, such as derivatives of hydroxymacronic acid, also exhibit radical scavenging activities (Castelluccio et al. 1995, Rice-Evans et al. 1996).

Since lifetime of radical species are normally short and these cannot diffuse across the membranes (Takahashi and Asada 1983), the radical scavenging activities of phenolics should occur only at the site where radicals are generated (Yamasaki et al. 1997). Therefore, the radical scavenging function could be a localized event, perhaps only in the vacuole and apoplastic space. Unlike such radical species, however, the non-radical active oxygen hydrogen peroxide (H₂O₂) that can diffuse over the cells, would be destroyed in the vacuole and apoplast through the reaction of guaiacol type peroxidase (GuPX) using phenolics as the electron donors (Takahama 1988, Yamasaki 1997, Yamasaki et al. 1997). This delocalized H₂O₂ detoxification hypothesis has been proposed as a secondary plant antioxidant system to support the ascorbate/ascorbate peroxidase (APX) system under severe abiotic and biotic stress (Yamasaki et al. 1997, Sakihama et al. 2000).

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Abbreviations: APX - ascorbate peroxidase; CA - coniferyl alcohol; p-CA - p-coumaric acid; CGA - chlorogenic acid; FA - ferulic acid; GuPX - guaiacol type peroxidase; 4-HNE - 4-hydroxynonenal; HRP - horseradish peroxidase; MDA - malondialdehyde; SA - sinapic acid; TBARS - thiobarbituric acid reactive substances.

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Either through non-enzymatic or enzymatic oxidation, phenoxy radical (Ph+) is primarily produced as the result of antioxidative reactions (Grace et al. 1999, Jørgensen et al. 1999). Ph+ rapidly decouples to non-radical species as in lignin formation (Takahama and Oniki 1992), thereby usually being harmless for living organisms. However, there is increasing number of reports suggesting that these phytophenolics may exhibit both prooxidant and cytotoxic properties in living systems (Decker 1997, Ohshima et al. 1998). Yamanaka et al. (1997) have demonstrated that flavonoids stimulate in vivo Cu-induced low density lipoprotein oxidation. It has also been reported that prooxidant activity of phenolics can be elicited by metal ions (Summers and Felton 1994) or alkalinization (Moran et al. 1997). In contrast to our improved knowledge of antioxidant reactions, the elicitation mechanisms for the prooxidant activities of phenolics remains unclear.

Here we demonstrate that aluminum ions (Al3+) elicits prooxidant nature of phenolics. Physiological significance of the Al-induced oxidative stress mediated by phenolics is discussed in terms of Al cytotoxicity in plants.

Materials and methods

Sample preparation: Seeds of barley (Hordeum vulgare L. cv. Donor) were germinated in distilled water at 25 °C for 2 d in darkness. The germinated seedlings were subsequently incubated in a growth chamber in a hydroponic culture with Ruakura medium [0.1 mM MgSO4, 0.3 mM KNO3, 0.2 mM NaCl, 0.15 mM NH4NO3, 0.5 mM CaSO4, 5 μM FeCl3, 5 μM (NH4)2HPO4, 5 μM H3BO3, 1 μM ZnSO4, 1 μM MnSO4, 0.2 μM CuSO4, 0.2 μM CoCl3] adjusted to pH 4.3 with HCl (Snowden et al. 1995), without FeCl3 at a 16-h photoperiod (irradiance of 100 μmol m−2 s−1) day/night temperature of 20/15 °C. After 2 d the growth medium was replaced with one containing 100 μM AlCl3. Following a 3-h incubation the roots were homogenized for 30 s at 0 °C with a known volume of a 20 mM potassium phosphate (pH 7.0), 5 mM butylated hydroxytoluene solution. The homogenate was filtered through two layers of gauze and the filtrate centrifuged at 20 000 g for 2 min. The supernatant obtained was used for the in vitro assay of lipid peroxidation.

Liposomes were prepared by vigorous shaking of soybean lecithins (10 % w/v) in 20 mM potassium phosphate (pH 7.0) under the nitrogen gas. The reaction medium (1 cm3) contained 0.14 M sodium acetate -0.06 N acetate buffer (pH 5.0), 2 % w/v liposomes, 100 μM AlCl3, 100 μM chlorogenic acid (CGA), 2.5 mM H2O2 and 1 U cm−3 HRP. After a 20-min (Figs. 1 and 2) or a 5-min (Fig. 3) incubation at 25 °C the reaction mixture was combined with 1 cm3 chloroform:methanol (2:1, v/v). The solution was vortexed and centrifuged at 2 000 g for 10 min. The separated chloroform phase (0.2 cm3) was dried with a stream of nitrogen gas and then 20 mM potassium phosphate (pH 7.0) was added. This suspension was used for the in vitro assay of lipid peroxidation.

Measurement of lipid peroxidation: The amounts of 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) were determined with Bioxytech LPO 586 (Oxis International, Portland, USA) using the methanesulfonic acid and HCl procedures (Oxis International 1997). The amount of thiobarbituric acid reactive substances (TBARS) was determined using the thiobarbituric acid method as described previously (Esterbauer and Cheeseman 1990).

Chemical reagents: Chlorogenic acid (CGA), coniferyl alcohol (CA), ferulic acid (FA) and sinapic acid (SA) were purchased from Sigma-Aldrich (St. Louis, USA), p-coumaric acid (p-CA) and phosphatidylcholines from Nacalai tesque (Kyoto, Japan), horseradish peroxidase (HRP) from Toyobo (Tokyo, Japan), N-methyl-2-phenylindole from Wako (Osaka, Japan) and methanesulfonic acid from Kanto (Tokyo, Japan).

Results and discussion

As previously reported (Ono et al. 1995), thiobarbituric acid reactive substance (TBARS) including malondialdehyde (MDA), which are widely used for the assays of lipid peroxidation, showed no significant increase in barley roots during the Al treatment (Fig. 1). There is accumulating evidence that 4-HNE, another decomposition product of lipid peroxide, is produced in animal cells by oxidative stress (Esterbauer et al. 1991). We examined whether 4-HNE is produced by the Al treatment similar to lipid peroxidation in animal cells, and found that Al treatment did indeed increase 4-HNE to a level 30 % higher than without Al (Fig. 1A). These results indicate that 4-HNE, but not TBARS or MDA, is the major decomposition product of lipid peroxide in barley roots.

Lipid peroxidation can be initiated by radical species (e.g., alkoxyl and hydroxyl radicals) as well as the redox active metals (e.g. Fe2+ and Cu2+) (Hall 1996, Nakano