JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Slow-Binding Inhibition of Soybean Lipoxygenase-1 by Dodecyl Gallate

TAE JOUNG HA AND ISAO KUBO*

Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3114

Dodecyl gallate inhibited the soybean lipoxygenase-1 (EC 1.13.11.12, type-1) catalyzed peroxidation of linoleic acid with an IC_{50} of 0.007 μM without being oxidized. The progress curves for enzyme reactions were recorded by both spectrophotometric and polarographic methods, and the inhibition kinetics revealed competitive and slow-binding inhibition. Both the initial velocity and steady-state rate in the progress curve decreased with increasing dodecyl gallate. The kinetic parameters that described the inhibition by dodecyl gallate were evaluated by nonlinear regression fits.

KEYWORDS: Soybean lipoxygenase; dodecyl gallate; slow-binding inhibitor; competitive inhibitor

INTRODUCTION

Lipoxygenases (EC 1.13.11.12) are suggested to be involved in the lipid peroxidation that is one of the major factors in deterioration during the storage and processing of foods because it can lead to the development of unpleasant rancid or off-flavors as well as potentially toxic end products (*1*, *2*). For example, the oxidation of membrane lipids is implicated in the development of off-flavors (*3*), loss of fresh meat color (*4*), and the formation of harmful lipid peroxidation products (*5*) in muscle foods. The addition of antioxidants has become popular as a means of increasing shelf life of food products and improving the stability of lipid-containing foods by preventing loss of sensory and nutritional quality (*6*). Lipoxygenases are non-heme iron containing enzymes that catalyze the site-specific oxygenation of polyunsaturated fatty acids to produce hydroperoxides. Linoleic acid is the major/main target of lipid peroxidation.

For food protection, alkyl gallates are useful as antioxidative antimicrobial agents (*7*, *8*). Dodecyl (lauryl) gallate is one of the three alkyl gallates which are currently permitted for use as antioxidant additives in food (*9*). Its antioxidant activity has been extensively studied, but the emphasis has been mainly on the scavenging activity. Hence, the effects of dodecyl gallate on lipoxygenase-catalyzed lipid peroxidation were examined in detail using commercially available soybean lipoxygenase-1 (EC 1.13.11.12, Type 1).

MATERIALS AND METHODS

Chemicals. Dodecyl gallate (**1**) and octyl gallate were available from our previous work (*10*). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, dodecanol, and nordihydroguaiaretic acid (NDGA) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Soybean lipoxygenase-1 (EC 1.13.11.12, Type 1), dimethyl sulfoxide (DMSO), Tween-20, and linoleic acid (purity >99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Tris buffer was obtained from Fisher Scientific Co. (Fairlawn, NJ). Ethanol was purchased from Quantum Chemical Co. (Tuscola, IL). 13(*S*)-Hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13-HPOD: $\lambda_{max} = 234$ nm, $\epsilon = 25$ mM^{-1} cm^{-1}) was prepared enzymatically by a described procedure (*11*) and stored in ethanol at −18 °C.

Enzyme Assay. The inhibitory concentration leading to 50% activity loss (IC_{50}) was obtained by fitting experimental data to the logistic curve by the equation as follows (*12*):

$$\text{activity (\%)} = 100[1/(1 + ([I]/IC_{50}))] \quad (1)$$

where [I] is the inhibitor concentration.

In a spectrophotomeric experiment, the oxygenase activity of the soybean lipoxygenase was monitored at 25 °C by a Spectra MAX plus spectrophotometer (Molecular Devices, Sunnyvale, CA). The enzyme assay was performed as previously reported (*13*) with slight modification. In general, 5 μL of an ethanolic inhibitor solution was mixed with 54 μL of 1 mM stock solution of linoleic acid and 2.936 mL of 0.1 M Tris-HCl buffer (pH 8.0) in a quartz cuvette. Then, 5 μL of a 0.1 M Tris-HCl buffer solution (pH 8.0) of lipoxygenase (1.02 μM) was added. The resultant solution was mixed, and the linear increase of absorbance at 234 nm, which expresses the formation of conjugated diene hydroperoxide (13-HPOD, $\epsilon = 25000$ M^{-1} cm^{-1}), was measured continuously. A lag period shown in the lipoxygenase reaction (*14*) was excluded for the determination of initial rates. The stock solution of linoleic acid was prepared with methanol and Tris-HCl buffer at pH 8.0, and then, total methanol content in the final assay was adjusted below 1.5%.

Lipoxygenase-dependent O_2 uptake was performed using a Clark-type oxygen electrode (YSI 53, Yellow Springs Instrument Co., Yellow Springs, OH) at 25 °C as essentially the same procedures in the spectrophotomeric experiment. For obtaining IC_{50}, the final assay concentrations of the enzyme and the substrate were adjusted to 4.25 nM and 46 μM, respectively. All assays were conducted in separate triplicate experiments.

Progress Curve Determinations. All reactions were carried out using linoleic acid as a substrate in 0.1 M Tris-HCl buffer (pH8.0) at

* To whom correspondence should be addressed. Phone: (510) 643-6303. Fax: (510) 643-0215. E-mail: ikubo@uclink.berkeley.edu.

10.1021/jf0616457 CCC: $37.00
Published on Web 12/23/2006

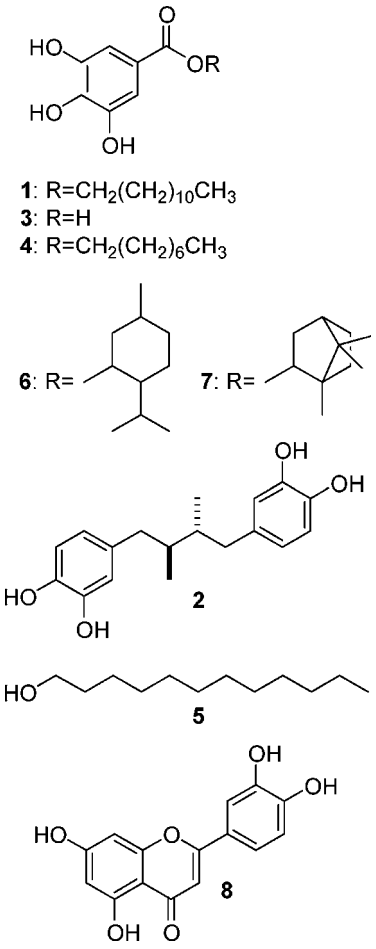


Figure 1. Chemical structures of dodecyl gallate and related compounds. Dodecyl gallate, **1**; nordihydroguaiaretic acid, **2**; gallic acid, **3**; octyl gallate, **4**; dodecanol, **5**; menthyl gallate, **6**; bornyl gallate, **7**; luteolin, **8**.

25 °C. Enzyme activities were measured 20 and 10 min continuously using a UV spectrophotometer and Clark-type oxygen electrode, respectively. To determine the kinetic parameters associated with slow-binding inhibition of soybean lipoxygenase-1, progress curves with 25 or more data points, typically at 2 s intervals, were obtained at several inhibitor concentrations and fixed concentration of substrate. The data were analyzed using the nonlinear regression program of Sigma Plot (SPSS Inc., Chicago, IL) to give the individual parameters for each progress curve; v_i (initial velocity), v_s (steady-state velocity), k_{obs} (apparent first-order rate constant for the transition from v_i to v_s), A (absorbance at 234 nm and/or O_2 uptake), A_0 (included to correct any possible deviation of the baseline), and K_i^{app} (apparent K_i) according to eqs 2 and 3 (*15*):

$$A = v_s t + (v_i - v_s)[1 - \exp(-k_{obs} t)]/k_{obs} + A_0 \quad (2)$$

$$k_{obs} = k_6 + [(k_5 \times [I])/(K_i^{app} + [I])] \quad (3)$$

To classify the type of inhibition of a time-dependent inhibitor, analysis of various linoleic acid concentrations on k_{obs} at a fixed dodecyl gallate concentration were performed. Kinetic parameters were also calculated by nonlinear regression, fitting the data to eq 2.

RESULTS

Lipoxygenases are a family of non-heme iron containing dioxygenases. These enzymes catalyze the reaction of oxygen with polyunsaturated fatty acids containing (1*Z*,4*Z*)-diene moieties to yield (2*Z*,4*E*)-conjugated hydroperoxides. Due to their free radical nature, fatty acid hydroperoxides can be quite active by themselves and are capable of producing membrane damage and promoting cell death (*16*). Thus, the quantitative methods used to assay the dioxygenase activity of soybean lipoxygenase-1 include an estimation of spectrophotometric recording of conjugated diene formation absorbing at 234 nm and oxygen uptake by means of a Clark-type oxygen electrode. One molecule of dodecyl gallate (**1**) (see **Figure 1** for structures) scavenged six molecules of DPPH radical. Dodecyl gallate inhibited soybean lipoxygenase-1 but is unlikely due to its capability of scavenging linoleic acid derived free radicals. Although intermediate free radicals are formed during the catalytic cycle of lipoxygenases (*14*), they remain tightly bound at the active site, thus not being accessible for free radical scavengers (*17*). In a preliminary assay, dodecyl gallate is a more potent lipoxygenase inhibitor than octyl gallate. The head and tail structure of these alkyl gallates suggests that optimization is possible through the synthetic approach. Hence, the inhibition mechanism of dodecyl gallate was investigated in detail.

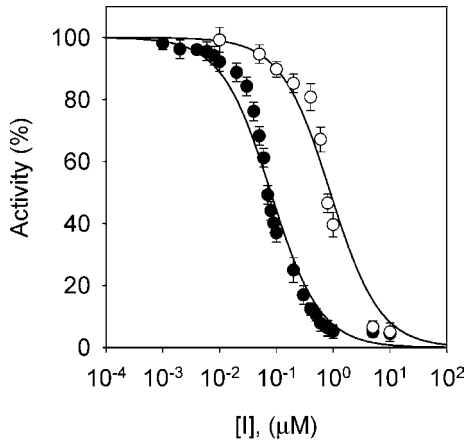


Figure 2. Inhibition of soybean lipoxygenase-1 activity by dodecyl gallate. Closed circles (●): 18 μM linoleic acid in 0.1 M Tris-HCl buffer (pH 8.0) at 25 °C, 1.3 nM soybean lipoxygenase-1 and recorded spectrophotometrically (UV 234 nm). Open circles (○): 46 μM linoleic acid in 0.1 M Tris-HCl buffer (pH 8.0) at 25 °C, 4.25 nM soybean lipoxygenase-1 and recorded oxygen consumption.

In the current study, linoleic acid was used as a substrate since linoleic acid is the main/major target of lipid peroxidation. Soybean lipoxygenase-1 (EC 1.13.11.12, Type 1) is known to catalyze the dioxygenation of (1*Z*,4*Z*)-diene moiety of linoleic acid. In plants, the primary dioxygenation product is 13(*S*)-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13-HPOD) (*2*). The enzyme assay was first performed using a UV spectrophotometer to detect the increase at 234 nm associated with the (2*Z*,4*E*)-conjugated double bonds newly formed in the product but not the substrate. As a result, dodecyl gallate showed a dose-dependent inhibitory effect on this oxidation as shown in **Figure 2**. As dodecyl gallate increased, the enzyme activity rapidly decreased with eventual complete suppression. The inhibitory concentration leading to 50% activity loss (IC_{50}) was estimated to be 0.07 μM. The data obtained was also compared with that of NDGA (**2**) used as a reference compound. Its IC_{50} was obtained as 0.2 μM. Gallic acid (**3**) did not show this inhibitory activity up to 200 μM (*17*). As the need arose, the assay was also monitored by using polarography (oxygen consumption) for comparison. The IC_{50} obtained was 0.89 μM, which is almost comparable with that measured by the spectrophotometric method. The difference of IC_{50} value of these methods is due

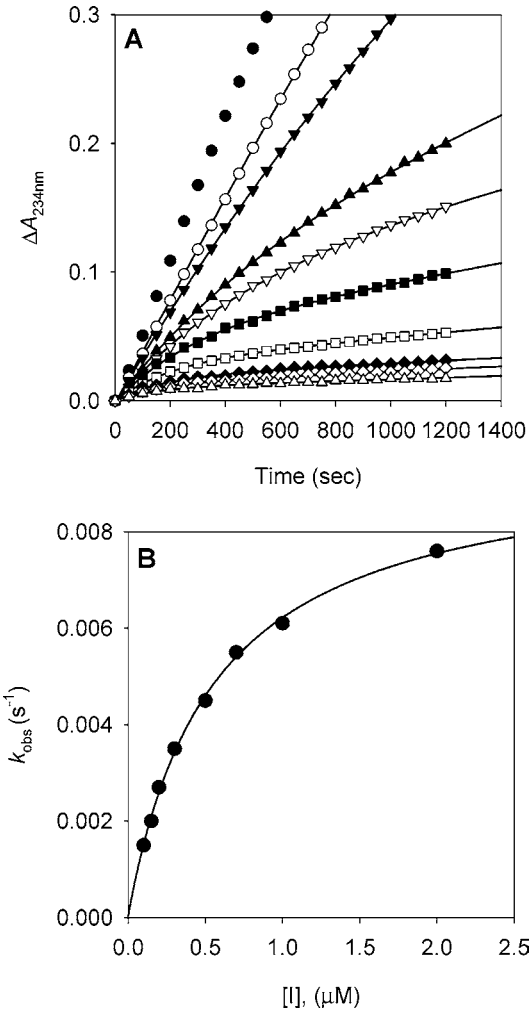


Figure 3. Time-dependent inhibition of soybean lipoxygenase-1 in the presence of dodecyl gallate. (A) Conditions were as follows: 40 μM linoleic acid, 1.3 nM lipoxygenase-1, and concentrations of dodecyl gallate for curves from top to bottom were 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, 0.7, 1.0, and 2.0 μM. The k_{obs} values at each inhibitor concentration were determined by fitting the data to eq 2. (B) Dependence of the values for k_{obs} on the concentration of dodecyl gallate. The k_{obs} values, determined in panel A, were fitted to eq 3.

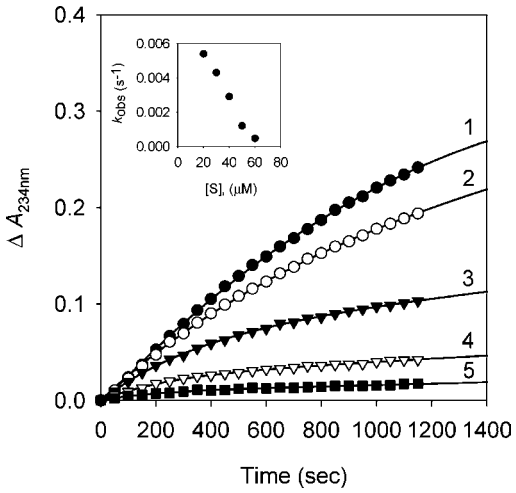
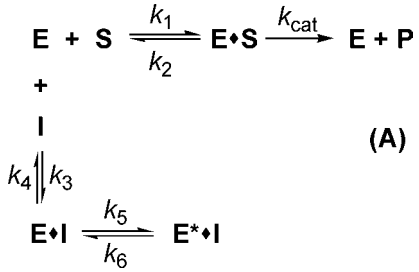


Figure 4. Progress curves for the competitive behavior of dodecyl gallate. Conditions were as follows: 1.0 μM dodecyl gallate, 1.3 nM lipoxygenase-1, and concentrations of linoleic acid for curves 1–5 were 60, 50, 40, 30, and 20 μM. (Inset) Dependence of the values for k_{obs} on the concentration of linoleic acid.

to a K_m for linoleic acid almost 3-fold larger using the oxygen monitor. This is in good agreement with previously reported observations (*18*, *19*).

To investigate the inhibitory effect of dodecyl gallate on dioxygenase enzyme, we assayed soybean lipoxygenase-1 activity with the inhibitor. Soybean lipoxygenase-1 showed time-dependent inhibition in the presence of dodecyl gallate (**Figure 3**, panel A). Increasing dodecyl gallate concentrations led to the decrease in both the initial velocity (v_i) and the steady-state rate (v_s) in the progress curve. The progress curves obtained using various concentrations of the inhibitors were fitted to eq 2 to determine v_i, v_s, and k_{obs}. The plot for k_{obs} versus [I] are shown in panel B in **Figure 3**. That plot showed a hyperbolic dependence on the concentration of the dodecyl gallate, so the inhibition of lipoxygenase-1 by dodecyl gallate followed mechanism A. The kinetic parameters, k_5, k_6, and K_i^{app}, were derived from the plots by fitting the results to eq 3. Thus, analysis of data according to eq 3 yielded the following values: $k_5 = 9.6 \times 10^{-3}$ s^{-1}, $k_6 = 3.7 \times 10^{-5}$ s^{-1}, and $K_i^{app} = 0.55$ μM.

The kinetic model can be written as



where E, S, I, and P denote enzyme, substrate, inhibitor (alkyl gallate), and product (13-HPOD), respectively. ES and EI are respective complexes. Because k_5 is greater than k_6, the enzyme first quickly and reversibly binds with dodecyl gallate and then undergoes a slow interaction of dodecyl group with the hydrophobic portion near the active site. In the case of octyl gallate (**4**), conversely k_6 is greater than k_5, and hence, it was observed as a competitive inhibitor. The effect of dodecyl gallate on the soybean lipoxygenase-1 catalyzed oxidation of linoleic acid is similar to octyl gallate in many aspects but different to some extents.

Most slow-binding enzyme inhibitors act as competitive inhibitors, binding at the enzyme active site (*20*, *21*), although it is possible for them to interact with the enzyme by competitive, noncompetitive, or uncompetitive inhibition patterns. To distinguish the mode of inhibition of a time-dependent inhibitor, it is convenient to analyze the effect of various substrate concentrations on k_{obs} at fixed inhibitor concentration. A competitive-type inhibitor will display a decrease of k_{obs} with increasing substrate concentration. In contrast, with uncompetitive inhibitors the value of k_{obs} will increase with increasing substrate concentration, while k_{obs} is independent of substrate concentration for noncompetitive-type inhibition (*14*). **Figure 4** also illustrates typical progress curves of time-dependent inhibition of dodecyl gallate (1.0 μM) when the enzymatic reaction is initiated by the addition of lipoxygenase-1 (1.3 nM), but various concentrations of linoleic acids (20, 30, 40, 50, and 60 μM). The k_{obs} values were determined by fitting data to eq 2. As a result, dodecyl gallate is a competitive inhibitor because k_{obs} decreased with increasing substrate concentration (**Figure 4** inset).

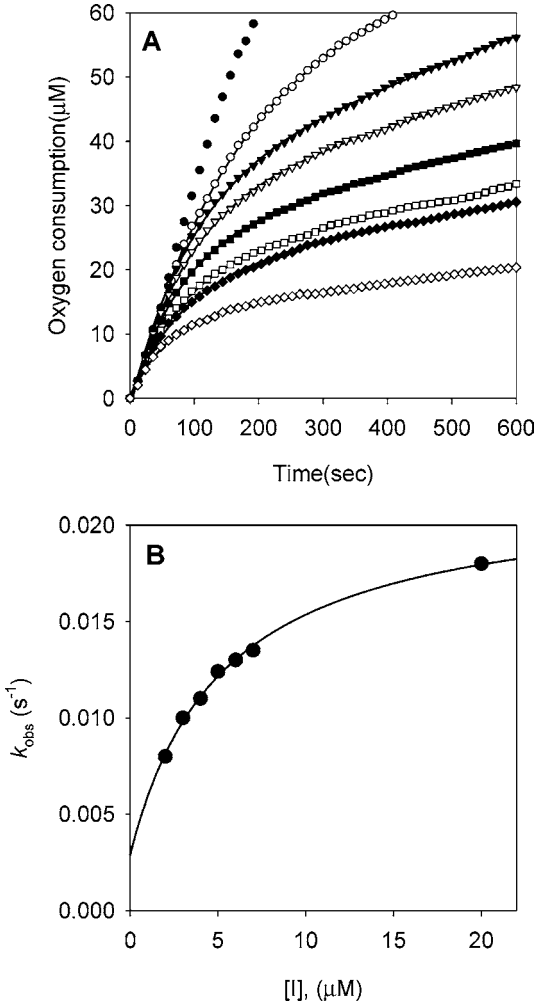


Figure 5. Time-dependent O_2 uptake inhibition of soybean lipoxygenase-1 in the presence of dodecyl gallate. (A) Conditions were as follows: 80 μM linoleic acid, 4.25 nM lipoxygenase-1, and concentrations of dodecyl gallate for curves from top to bottom were 0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 20 μM. The k_{obs} values at each inhibitor concentration were determined by fitting the data to eq 2. (B) Dependence of the values for k_{obs} on the concentration of dodecyl gallate. The k_{obs} values, determined in panel A, were fitted to eq 3.

The progress curves of oxygen consumption show that dodecyl gallate also inhibited soybean lipoxygenase-1 by a slow-binding inhibition mechanism, **Figure 5A**. The k_{obs} values for the dodecyl gallate inhibition of lipoxygenase-1 at different concentrations of dodecyl gallate were determined by fitting data to the slow-binding equation (eq 2). The k_{obs} values were plotted as a function of dodecyl gallate concentration. The results indicated that dodecyl gallate inhibits soybean lipoxygenase-1 by slow enzyme isomerization. This was evidenced by the observation that the k_{obs} values exhibited a hyperbolic dependence on the inhibitor concentration as shown in **Figure 5B**. Thus, analysis of data according to eq 2 yielded the following values: $k_5 = 0.019\ s^{-1}$, $k_6 = 0.00289\ s^{-1}$, and $K_i^{app} = 5.26\ \mu M$.

Subsequently, the inhibition mode of soybean lipoxygenase-1 by dodecyl gallate was investigated by using a Clark-type oxygen electrode. **Figure 6** also illustrates typical progress curves of time-dependent oxygen consumption inhibition of dodecyl gallate (3.0 μM) when the enzymatic reaction is initiated by the addition of lipoxygenase-1 (4.25 nM), but various concentrations of linoleic acids (40, 50, 60, 70, 80, 90, and 100 μM). The k_{obs} values were determined by fitting data to eq 2.

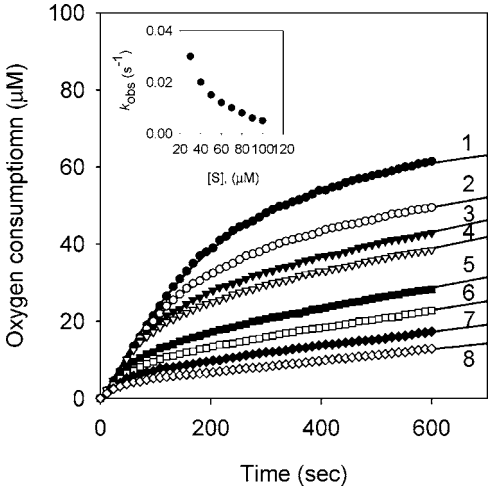


Figure 6. Progress curves for the competitive behavior of dodecyl gallate. Conditions were as follows: 3.0 μM dodecyl gallate, 4.25 nM lipoxygenase-1, and concentrations of linoleic acid for curves 1–8 were 100, 90, 80, 70, 60, 50, 40, and 30 μM. (Inset) Dependence of the values for k_{obs} on the concentration of linoleic acid.

Table 1. Kinetics and Inhibition Constants of Dodecyl Gallate

	detector	
	UV_{234}	O_2
IC_{50}	0.07 μM	0.89 μM
k_5 (s^{-1})	9.6×10^{-3}	0.019
k_6 (s^{-1})	3.7×10^{-5}	2.89×10^{-3}
K_i^{app} (μM)	0.55	5.26
k_5/k_6	259.4	6.6
inhibition type	competitive	competitive

As a result, dodecyl gallate is a competitive inhibitor because k_{obs} decreased with increasing substrate concentration (**Figure 6** inset). The kinetic and inhibition constants obtained are listed in **Table 1**.

Neither gallic acid (**3**) nor dodecanol (**5**) inhibited soybean lipoxygenase-1 up to 200 μM, indicating that a certain balance between specific head structure and alkyl tail portion is needed to elicit this inhibitory activity.

DISCUSSION

Since gallic acid did not inhibit the soybean lipoxygenase-1 catalyzed oxidation of linoleic acid up to 200 μM (*17*), the dodecyl group is essential for eliciting this specific activity. In connection with this, L-ascorbic acid and α-tocopherol (also known as vitamins C and E, respectively) did not inhibit this enzyme up to 300 μM. However, the dodecyl group alone is not enough to elicit the activity because dodecyl alcohol acted neither as a substrate nor an inhibitor. The enzyme first quickly binds dodecyl gallate and then undergoes a slow interaction with the hydrophobic domain near the active site. As long as the appropriate head portion exists, the hydrophobic portion is flexible, although the length and volume of the hydrophobic portion are also associated with the activity. In a previous report, both menthyl gallate (**6**) and bornyl gallate (**7**) were found to inhibit the soybean lipoxygenase-1 catalyzed oxidation of linoleic acid (*17*), indicating that the hydrophobic moiety is flexible for eliciting this inhibitory activity. On the other hand, lipoxygenases are non-heme iron-containing enzymes and alkyl gallates are known to have iron-binding properties. Based on the data obtained, it may not be illogical to assume that galloyl moiety first quickly binds with the active site as a chelator and

then dodecyl group undergoes a slow interaction with the hydrophobic domain surrounding near the active site. Our previous paper reported that the catechole moiety is one of the essential head portions to elicit the lipoxygenase inhibitory activity (*17*). For example, luteolin (**8**), a common flavone bearing a catechole moiety in the B-ring, is known to have this specific activity (*22*). The precise structural feature of the tail portion still remains largely unknown.

Safety is a primary consideration for additives in food. After consumption, dodecyl gallate is likely hydrolyzed (*23*), at least in part, to gallic acid and dodecyl (lauryl) alcohol and both are common in edible plants. For example, the pecan nuts contain gallic acid as a predominant phenolic acid constituting approximately 78% of the total phenolic acid (*24*). This phenolic acid is also a frequent constituent of hydrolyzable tannins in many plants. Gallic acid is known to chelate transition metal ions which are powerful promoters of free radical damage in the human body (*25*). More specifically, gallic acid may prevent cell damage induced by hydrogen peroxide (H_2O_2) since this can be converted to the more reactive oxygen species, hydroxyl radicals, in the presence of these metal ions. In connection with this, the inhibition of iron absorption in vivo was positively correlated with the presence of a galloyl group but not a catechol group (*26*). The nitric oxide ($NO^{\bullet}$), a free radical species produced by several mammalian cell types, plays a role in regulation and function. $NO^{\bullet}$ toxicity is for the greater part mediated by peroxynitrite ($ONOO^-$), formed in the reaction of $NO^{\bullet}$ with $O_2^{\bullet -}$, (*27*). Gallic acid was previously reported to act as a potent peroxynitrite scavenger (*28*) and to show antiproliferative activity against melanocyte cell lines (*29*). Sulfonation is one of the major phase II conjugative reactions involved in the biotransformation of various endogenous compounds, drugs, and xenobiotics as well as in steroid biosynthesis, catecholamine metabolism, and thyroid hormone homeostasis (*30*). Gallic acid enhanced the activity of phenolsulfotransferase and exhibited antioxidant activity as determined by the oxygen radical absorbance capacity assay and Torox equivalent antioxidant capacity assay (*31*). Gallic acid was recently described to activate microsomal glutathione *S*-transferase through oxidative modification of the enzyme (*32*). Despite these benefits biological significance of gallic acid in living systems is still largely unknown. For instance, it is not clear if gallic acid is absorbed into the system through the intestinal tract and delivered to the places where biologically active gallic acid is needed.

LITERATURE CITED

(1) Shibata, D.; Axelrod, B. Plant lipoxygenases. *J. Lipid Mediators Signalling* **1995**, *12*, 213−228.

(2) Grechkin, A. Recent developments in biochemistry of the plant lipoxygenase pathway. *Prog. Lipid Res.* **1998**, *37*, 317−352.

(3) Gray, J. I.; Pearson, A. M. Rancidity and warmed-over flavor. *Adv. Meat Res.* **1987**, *3*, 221−269.

(4) Faustman, C.; Cassens, R. G.; Schaefer, D. M.; Buege, D. R.; Williams, S. N.; Scheller, K. K. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *J. Food Sci.* **1989**, *54*, 858−862.

(5) Monahan, F. J.; Gray, J. I.; Booren, A. M.; Miller, E. R.; Buckley, D. J.; Morrissey, P. A.; Gomaa, E. A. Influence of dietary treatment on cholesterol oxidation in pork. *J. Agric. Food Chem.* **1992**, *40*, 1310−1315.

(6) Tsuda, T.; Ohshima, K.; Kawakishi, S.; Osawa, T. Antioxidative pigments isolated from the seeds of *Phaseolus vulgaris L. J. Agric. Food Chem.* **1994**, *42*, 248−251.

(7) Nihei, K.; Nihei, A.; Kubo, I. Molecular design of multifunctional food additives: Antioxidative antifungal agents. *J. Agric. Food Chem.* **2004**, *52*, 5011−5020.

(8) Kubo, I.; Xiao, P.; Nihei, K.; Fujita, K.; Yamagiwa, Y.; Kamikawa, T. Molecular design of antifungal agents. *J. Agric. Food Chem.* **2002**, *50*, 3992−3998.

(9) Aruoma, O. I.; Murcia, A.; Butler, J.; Halliwell, B. Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *J. Agric. Food Chem.* **1993**, *41*, 1880−1885.

(10) Kubo, I.; Xiao, P.; Fujita, K. Anti-MRSA activity of akyl gallates. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 113−116.

(11) Gibian, M. J.; Galaway, R. A. Steady-state kinetics lipoxygenase oxygenation of unsaturated fatty acid. *Biochemistry* **1976**, *15*, 4209−4214.

(12) Copeland, R. A. *Enzyme: A practical introduction to structure, mechanism, and data analysis*; Wiley-VCH: New York, 2000; pp 266−304.

(13) Rickert, K. W.; Klinman, J. P. Nature of hydrogen transfer in soybean lipoxygenase 1: separation of primary and secondary isotope effects. *Biochemistry* **1999**, *38*, 12218−12228.

(14) Ruddat, V. C.; Whitman, S.; Holman, T. R.; Bernasconi, C. F. Stopped-flow kinetics investigations of the activation of soybean lipoxygenase-1 and the influence of inhibitors on the allosteric site. *Biochemistry* **2003**, *42*, 4172−4178.

(15) Frieden, C. Kinetic aspects of regulation of metabolic processes. The hysteretic enzyme concept. *J. Biol. Chem.* **1970**, *245*, 5788−5799.

(16) Gardner, H. W. Recent investigations into the lipoxygenase pathway of plants. *Biochim. Biophys. Acta* **1991**, *1084*, 221−239.

(17) Ha, T. J; Nihei, K.; Kubo, I. Lipoxygenase inhibitory activity of octyl gallate. *J. Agric. Food Chem.* **2004**, *52*, 3177−3181.

(18) Berry, H.; Debat, H.; Larreta-Garde, V. Excess substrate inhibition of soybean lipoxygenase-1 is mainly oxygen-dependent. *FEBS Lett.* **1997**, *408*, 324−326.

(19) Glickman, M.; Klinman, J. P. Lipoxygenase reaction mechanism: demonstration that hydrogen abstraction from substrate precedes dioxygen binding during catalytic tureover. *Biochemistry* **1996**, *35*, 12882−12892.

(20) Morrison, J. F. The slow binding and slow, tight binding inhibition of enzyme catalysed reaction. *Trends Biochem. Sci.* **1982**, *7*, 102−107.

(21) Morrison, J. F.; Walsh, C. T. The behaviours and significance of slow binding enzyme inhibitors. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1988**, *61*, 201−302.

(22) Sadik, C. D.; Sies, H.; Schewe, T. Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action. *Biochem. Pharmacol.* **2003**, *65*, 773−781.

(23) Van der Heijden, C. A.; Janssen, P. J. C. M.; Strik, J. J. T. W. A. Toxicology of gallates: A review and evaluation. *Food Chem. Toxicol.* **1986**, *24*, 1067−1070.

(24) Senter, S. D.; Horvat, R. J.; Forbus, W. R., Jr. Relation between phenolic acid content and stability of pecan in accelerated storage. *J. Food Sci.* **1980**, *45*, 1380−1382.

(25) Henle, E. S.; Linn, S. Formation, prevention, and repair of DNA damage by iro/hydrogen peroxide. *J. Biol. Chem.* **1997**, *272*, 19095−19098.

(26) Brune, M.; Rossander, L.; Hallberg, L. Iron absorption and phenolic compounds: importance of different phenolic structures. *Eur. J. Clin. Nutr.* **1989**, *43*, 547−557.

(27) Rubbo, H.; Darley-Usmar, V.; Freeman, B. A. Nitric oxide regulation of tissue free radical injury. *Chem. Res. Toxicol.* **1996**, *9*, 809−820.

(28) Heijnen, C. G. M.; Haenen, G. R. M. M.; Vekemans, J. A. J. M.; Bast, A. Peroxynitrite scavenging of flavonoids: structure activity relationship. *Environ. Toxicol. Pharmacol.* **2001**, *10*, 199−206.

(29) Yáñez, J.; Vicente, V.; Alcaraz, M.; Castillo, J.; Benavente-García, O.; Canteras, M.; Teruel, J. A. L. Cytotoxicity antiproliferative activities of several phenolic compounds against three melanocytes cell lines: Relationship between structure and activity. *Nutr. Cancer* **2004**, *49*, 191−199.

(30) Brix, L. A.; Nicoll, R.; Zhu, X.; McManus, M. E. Structural and functional characterization of human sulfotransferases. *Chem. Biol. Interact.* **1998**, *109*, 123−127.
(31) Yeh, C. T.; Yen, G. C. Effects of phenolic acids on human phenolsulfotransferases in relation to their antioxioxidant activity. *J. Agric. Food Chem.* **2003**, *51*, 1474−1479.
(32) Shinno, E.; Shimoji, M.; Imaizumi, N.; Kinoshita, S.; Sunakawa, H.; Aniya, Y. Activation of rat liver microsomal glutathione *S*-transferase by gallic acid. *Life Sci.* **2005**, *78*, 99−106.

Received for review June 12, 2006. Revised manuscript received October 25, 2006. Accepted November 3, 2006.

JF0616457