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Antioxidant activity of anacardic acids

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In honor of Professor Koji Nakanishi's eightieth birthday

Abstract

Anacardic acids, 6-pentadec(en)ylsalicylic acids isolated from the cashew *Anacardium occidentale* L. (Anacardiaceae) nut and apple, were found to possess preventive antioxidant activity while salicylic acid did not show this activity. These anacardic acids prevent generation of superoxide radicals by inhibiting xanthine oxidase (EC 1.1.3.22, Grade IV) without radical scavenging activity. Notably, the inhibition kinetics of anacardic acids do not follow hyperbolic dependence of enzyme inhibition on inhibitor contents (Michaelis–Menten equation) but follow the Hill equation instead. Anacardic acid ($C_{15:1}$) inhibited the soybean lipoxygenase-1 (EC 1.13.11.12, Type 1) catalyzed oxidation of linoleic acid with an IC_{50} of 6.8 μM. The inhibition is a slow and reversible reaction without residual enzyme activity. The inhibition kinetics indicate that anacardic acid ($C_{15:1}$) is a competitive inhibitor and the inhibition constant, K_I, was 2.8 μM. Anacardic acids act as antioxidants in a variety ways, including inhibition of various prooxidant enzymes involved in the production of the reactive oxygen species and chelate divalent metal ions such as Fe^{2+} or Cu^{2+}, but do not quench reactive oxygen species. The C_{15}-alkenyl side chain is largely associated with the activity.

Keywords: Antioxidant activity; Anacardic acids; Xanthine oxidase; Inhibition kinetics; Soybean lipoxygenase-1

1. Introduction

In recent years the cashew, *Anacardium occidentale* L. (Anacardiaceae) apple, has increased in value, especially in the countries where it is grown, such as Brazil. There is no doubt that the nut (true fruit) is the most important product of the cashew tree. However, this tree also yields the pear-shaped "apple" (pseudo fruit) to which the nut is attached. A number of processes have now been developed for converting the cashew apple into various products, such as juice, jam, syrup, chutney and beverage. Cashew apple juice is, in fact, one of the most popular juices in Brazil today. Anacardic acids, 6[8′(*Z*), 11′(*Z*),14′-pentadecatrienyl]salicylic acid ($C_{15:3}$) (**1**), 6[8′(*Z*),11′(*Z*)-pentadecadienyl]salicylic acid ($C_{15:2}$) (**2**), and 6[8′(*Z*)-pentadecenyl]salicylic acid ($C_{15:1}$) (**3**), were previously characterized from the cashew apple and their diverse biological activities have been described. The reports include their potent antibacterial activity against Gram-positive bacteria (Kubo, Ochi, Vieira, & Komatsu, 1993), moderate cytotoxic activity against several tumor cell lines (Itokawa et al., 1989; Kubo et al., 1993), and tyrosinase (Kubo, Kinst-Hori, & Yokokawa, 1994), lipoxygenase (Shobha, Ramadoss, & Ravindranath, 1994) and prostaglandin endoperoxidase synthase (Grazzini et al., 1991) inhibitory activities.

The oxidation of unsaturated fatty acids in biological membranes leads to a decrease in the membrane fluidity (Dobrestova, Borschevskaya, Petrov, & Vladimirov, 1977) and disruption of membrane structure and function (Machlin & Bendich, 1987; Slater, Cheeseman, Davies, Proudfoot, & Xin, 1987). Cellular damage, due to lipid peroxidation, is associated with carcinogenesis (Yagi, 1987) and other diseases (Garewal, 1997). Inhibition of membrane peroxidation has been shown to have a protective effect in the initiation and promotion of certain cancers (Rousseau,

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Davison, & Dunn, 1992). The past experimental studies have provided compelling evidence that antioxidants play an important role in reducing the risk of cancer. However, previous studies have usually emphasized the scavenging activity when using antioxidant additives in food and lack comprehensiveness. Discovery of new, safe and effective antioxidants is of considerable interest in preventive medicine. Antioxidants isolated from regularly consumed foods and beverages, such as the cashew apple and its processed products, may be superior to non-natural products. Therefore, our investigation has been further extended to test antioxidation activity of anacardic acids. Since anacardic acids are derivatives of salicylic acid (Machlin & Bendich, 1987) with a nonisoprenoid alk(en)yl side chain, their activities were compared with that of salicylic acid.

2. Materials and methods

2.1. Chemicals

Anacardic acids (**1**–**3**) and the corresponding cardanols (**4**–**6**) used for the assay were previously isolated from cashew nut shell oil. Their repurification by recycle HPLC (R-HPLC) was achieved using an ODS C_{18} column (Kubo, Komatsu, & Ochi, 1986). Salicylic acid (**7**), linoleic acid, BHT, EDTA, thiobarbituric acid (TBA), 1,1-diphenyl-2-*p*-picrylhydrazyl (DPPH), 2,2′-azo-*bis*(2-amidinopropane) dihydrochloride (AAPH), ADP, bovine serum albumin and nitroblue tetrazolium were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Assay of autoxidation

Oxidation of linoleic acid was measured by the modified method described previously (Haraguchi, Hashimoto, & Yagi, 1992). Different amounts of samples dissolved in 30 μl EtOH were added to a reaction mixture in a screw cap vial. Each reaction mixture consisted of 0.57 ml of 2.51% linoleic acid in EtOH and 2.25 ml of 40 mM phosphate buffer (pH 7.0). The vial was placed in an oven at 40 °C. After 5 days of incubation, a 0.1 ml aliquot of the mixture was diluted with 9.7 ml of 75% EtOH, which was followed by adding 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500 nm was measured.

2.3. Radical-scavenging activity on DPPH

First, 1 ml of 100 mM acetate buffer (pH 5.5), 1.87 ml of ethanol and 0.1 ml of ethanolic solution of 3 mM of DPPH were put into a test tube. Then, 0.03 ml of the sample solution (dissolved in DMSO) was added to the test tube and incubated at 25 °C for 20 min. The absorbance at 517 nm (DPPH, $\varepsilon = 8.32 \times 10^3\ M^{-1}\ cm^{-1}$) was recorded. As control, 0.03 ml of DMSO was added to the test tube. From decrease of the absorbance, scavenging activity was calculated and expressed as scavenged DPPH molecules per molecule.

2.4. Assay of superoxide anion generated by xanthine oxidase

The xanthine oxidase (EC 1.1.3.22, Grade IV) used for the bioassay was purchased from Sigma Chemical Co. Superoxide anion was generated enzymatically by the xanthine oxidase system. The reaction mixture consisted of 2.70 ml of 40 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10.0), 0.06 ml of 10 mM xanthine, 0.03 ml of 0.5% bovine serum albumin, 0.03 ml of 2.5 mM nitroblue tetrazolium and 0.06 ml of sample solution (dissolved in DMSO). To the mixture at 25 °C, 0.12 ml of xanthine oxidase (0.04 units) was added, and the absorbance at 560 nm was recorded for 60 s (by formation of blue formazan) (Toda, Kumura, & Ohnishi, 1991). A control experiment was carried out by replacing sample solution with the same amount of DMSO.

2.5. Assay of uric acid generated by xanthine oxidase

The reaction mixture consisted of 2.76 ml of 40 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10.0), 0.06 ml of 10 mM xanthine and 0.06 ml of sample solution (dissolved in DMSO). The reaction was started by the addition of 0.12 ml of xanthine oxidase (0.04 U), and the absorbance at 293 nm was recorded for 60 s.

2.6. Lipoxygenase assay

The soybean lipoxygenase-1 (EC 1.13.11.12, Type 1) used for the bioassay was purchased from Sigma Chemical Co. Throughout the experiment, linoleic acid was used as a substrate. In the current spectrophotometric experiment, the enzyme activity of soybean lipoxygenase-1was monitored at 25 °C by Spectra MAX plus spectrophotometer (Molecular device, Sunnyvale, CA). The enzyme assay was performed as previously reported (Kemal, Louis-Flamberg, Krupinski-Olsen, & Shorter, 1987) with slight modification. In general, 5 μl of an ethanolic inhibitor solution was mixed with 15 μl of 3 mM stock solution of linoleic acid and 2.97 ml of 0.1 M sodium borate buffer (pH 9.0) in a quartz cuvet. Then, 10 μl of 0.1 M sodium borate buffer solution (pH 9.0) of lipoxygenase (0.52 μM) were added. The resultant solution was mixed well and the linear increase of absorbance at 234 nm, which expresses the formation of conjugated diene hydroperoxide (13-HPOD, $\varepsilon = 2.50 \times 10^4\ M^{-1}\ cm^{-1}$), was measured continuously. The lag period shown on lipoxygenase reaction (Ruddat, Whitman, Holman, & Bernasconi, 2003) was excluded for the determination of initial rates. The stock solution of linoleic acid was prepared with Tween-20 and sodium borate buffer at pH 9.0 and then, total Tween-20 content in the final assay was adjusted to below 0.002%. For preincubation experiments, enzyme was incubated with various

concentrations of compounds in 0.1 M sodium borate buffer (pH 9.0) at 25 °C. At timed intervals, reactions were started by addition of 15 μM linoleic acid.

2.7. Data analysis and curve fitting

The assay was conducted in triplicate. The data analysis was performed by using Sigma Plot 2000 (SPSS Inc, Chicago, IL). The IC_{50}s were obtained by fitting experimental data to the logistic curve by Langmuir isotherm as follows (Copeland, 2000).

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

Inhibition mode was analyzed with enzyme kinetics module 1.0 (SPSS Inc) equipped with Sigma Plot 2000.

3. Results

The anacardic acid, 6[8′(*Z*),11′(*Z*),14′-pentadecatrienyl]salicylic acid (**1**) (see Fig. 1 for structures) was selected for the present study as a model, because this particular anacardic acid was available in quantity from our previous study (Kubo et al., 1986). This anacardic acid is referred to as anacardic acid ($C_{15:3}$) hereafter for simplicity. Since anacardic acids are derivatives of salicylic acid (Machlin & Bendich, 1987) with a nonisoprenoid alk(en)yl side chain, their activities were compared with that of salicylic acid (**7**). Availability of cardanol, 3[8′(*Z*),11′(*Z*),14′-pentadecatrienyl]phenol, referred to as cardanol ($C_{15:3}$) (**4**), an artifact of the corresponding anacardic acid ($C_{15:3}$) by heating treatment, obtained from the same source, is an additional benefit for comparison.

Lipid peroxidation is known to be one of the reactions set into motion as a consequence of the formation of free radicals in cells and tissues. Membrane lipids are abundant in unsaturated fatty acids. Linoleic acid is especially the target of lipid peroxidation. Effect of anacardic acid

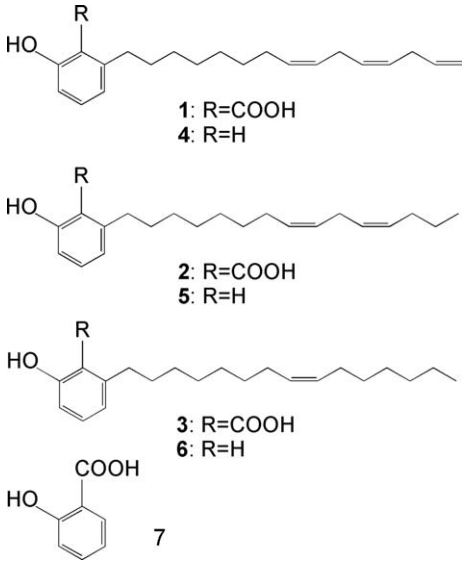

Fig. 1. Chemical structures of anacardic acids and related compounds.

($C_{15:3}$) and salicylic acid on autoxidation of linoleic acid were first tested by the ferric thiocyanate method, as previously described (Osawa & Namiki, 1981). In a control reaction, the production of lipid peroxide increased almost linearly during 8 days of incubation. α-Tocopherol, also known as vitamin E, inhibited linoleic acid peroxidation by almost 50% at 30 mg/ml. However, neither anacardic acid ($C_{15:3}$) nor salicylic acid inhibited this oxidation at the same concentration. The negative result of anacardic acid ($C_{15:3}$) can explain a structural feature in which the electron donating alkenyl group is located at the *meta*-position to the hydroxyl group so that it does not stabilize the phenoxy radicals (Cuvelier, Richard, & Berset, 1992). In connection, salicylic acid does not even possess any alkyl group. On the other hand, cardanol ($C_{15:3}$) inhibited linoleic acid peroxidation by about 30% at 30 mg/ml, but this inhibitory activity is still less than that of α-tocopherol. The result observed indicates that anacardic acids are unlikely to act as radical scavengers because they do not have the ability to donate a hydrogen atom to the peroxy radical derived from the autooxidizing fatty acids. Further evidence for this conclusion was obtained by a more direct experiment for the radical-scavenging activity, that can be measured as decolorizing activity following the trapping of the unpaired electron of DPPH. None of the anacardic acids (**1**–**3**) exhibited notable radical-scavenging activity (0.01 ± 0.02 of scavenged DPPH molecule per anacardic acid molecule). Keeping the above results in mind, further study was investigated.

The human body is known to produce free radicals during the course of its normal metabolism. Free radicals are even required for several normal biochemical processes. For example, the phagocyte cells involved in the body's natural immune defences generate free radicals in the process of destroying microbial pathogens. If free radicals are produced during normal cellular metabolism in sufficient amounts to overcome the normally efficient protective mechanisms, metabolic and cellular disturbances will occur in a variety of ways. Evidence is accumulating that extracellular free radicals are also produced in vivo by several oxidative enzymes in the human body, other than in phagocytes. For example, xanthine oxidase (EC 1.1.3.22), a molybdenum-containing enzyme, produces superoxide anion ($O_2^{\bullet -}$) radical as a normal product (Fong, McCay, Poyer, Steele, & Misra, 1973). The one-electron reduction products of O_2, superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxy radical ($HO^{\bullet}$) from ($O_2^{\bullet -}$), participate in the initiation of lipid peroxidation (Comporti, 1993). In addition to xanthine oxidase, superoxide is produced during mitochondrial respiration (Halliwell & Gutteridge, 1990a) and by NADPH oxidase (Pagano et al., 1995), cyclooxygenase and lipoxygenase (Kukreja, Kontos, Hess, & Ellis, 1986), nitric oxidase synthetase (NOS) (Cosentino, Patton, D'Uscio, & Werner, 1998) and cytochtome P450 (Fleming et al., 2001). The effects of anacardic acids on the generation of superoxide anion by xanthine oxidase were tested and the result is shown in Fig. 2. In the control,

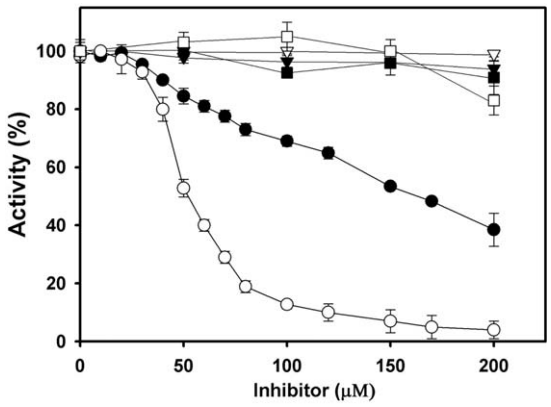

Fig. 2. Inhibition of superoxide anion and uric acid by xanthine oxidase with anacardic acid ($C_{15:3}$) and salicylic acid. Reaction rates by xanthine oxidase were measured at 200 μM xanthine in the presence of 0–200 μM anacardic acid, cardanol and salicylic acid. ○, Superoxide anion generation rates and ●, uric acid generation rates in the presence of anacardic acid ($C_{15:3}$). ▽, Superoxide anion generation rates and ▼, uric acid generation rates in the presence of salicylic acid. □, Superoxide anion generation rates and ■, uric acid generation rates in the presence of cardanol.

superoxide anion, generated by the enzyme, reduces yellow nitroblue tetrazolium to blue formazan. Hence, superoxide anion can be detected by measuring the absorbance of formazan produced at 560 nm. At the concentration of 30 μg/ml, anacardic acid ($C_{15:3}$) (88 μM), inhibited this formazan formation by 82 ± 4%. Interestingly, salicylic acid did not show any observable inhibitory activity up to 138 μg/ml (1.0 mM) and 7 ± 3% inhibition at 276 μg/ml, indicating that the C_{15}-alkenyl side chain is associated with this inhibitory activity. Cardanol did not show this inhibitory activity up to 0.2 mM, indicating that the structure of 2-carboxylphenol (salicylic acid) is also necessary. As the concentrations of anacardic acid ($C_{15:3}$) increased, the remaining enzyme activity was rapidly decreased. Notably, this inhibition mechanism do not follow hyperbolic inhibition by anacardic acid concentration (Michaelis–Menten equation), but follows the Hill equation (Beckmann, Henry, Ulphani, & Lee, 1998) instead. The shape of the inhibition curve of xanthine oxidase by anacardic acid ($C_{15:3}$) is sigmoidal (*S*-shaped) ($IC_{50} = 51.3 \pm 1.5$ μM) as shown in Fig. 3. This inhibition occurred over a very narrow range of anacardic acid ($C_{15:3}$) concentration (0.04–0.14 mM), which is much less than a usual simple equilibrium that would be occurred over a 100-fold concentration range. This indicates that only tight binding of inhibitor, but the curve of inhibition rate followed a Hill equation with a slope factor of 4.2 ± 0.5. This suggests that anacardic acid ($C_{15:3}$) binds cooperative binding to xanthine oxidase (Bray, 1963). It should be noted, however, that a common naturally-occurring antioxidant, α-tocopherol, is less effective in scavenging superoxide anion generated by xanthine oxidase and the IC_{50} is 220 ± 20 μM (Masuoka & Kubo, 2004).

It appears that the antioxidant activity of anacardic acids is not due to radical-scavenging but inhibiting of

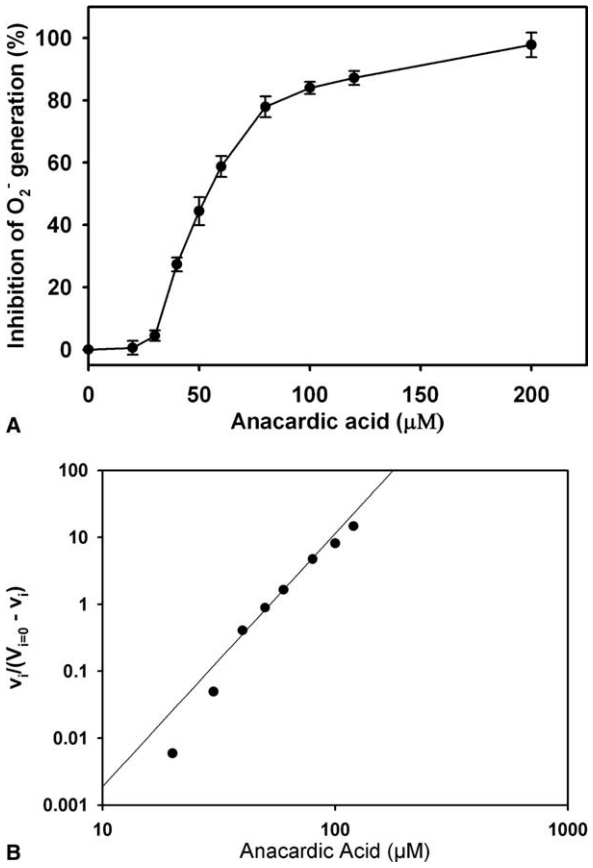

Fig. 3. Inhibited rates of superoxide anion generation by anacardic acid ($C_{15:3}$) and the Hill plot analysis. (A) Inhibited rates of superoxide anion generation were calculated from those of superoxide anion generation by xanthine oxidase in the presence of 0–200 μM anacardic acid ($C_{15:3}$) at 200 μM. xanthine. (B) The rates were plotted according to the Hill equation.

the enzyme activity. In order to verify this conclusion, formation of uric acid was measured because xanthine oxidase is known to convert xanthine to uric acid. This enzyme-catalyzed reaction proceeds via transfer of an oxygen atom to xanthine from the molybdenum centre. The inhibition mechanism also does not follow hyperbolic inhibition by anacardic acid concentration (Fig. 2), but follows the Hill equation instead. The shape of the inhibition curve of xanthine oxidase by anacardic acid ($C_{15:3}$) is sigmoidal ($IC_{50} = 162 \pm 10$ μM). The curve of inhibition rate followed the Hill equation with a slope factor of 1.7 ± 0.2. This result confirmed that anacardic acid ($C_{15:3}$) attaches by cooperative binding to xanthine oxidase and this affects the uric acid formation less than the superoxide anion formation. Interestingly, salicylic acid did not inhibit the enzyme up to 200 μM (27.6 μg/ml) but cooperatively inhibit at higher concentration ($IC_{50} = 580 \pm 28$ μM). The result obtained indicates that the alkyl side chain plays an important role in eliciting the activity. However, the hydrophobic interaction alone is not enough to elicit the xanthine oxidase inhibitory activity, since cardanol ($C_{15:3}$), which possesses the same side chain as anacardic acid ($C_{15:3}$), did not show any inhibitory activity.

Lipoxygenase (EC 1.13.11.12) is a non heme iron enzyme that catalyzes the dioxygenation of polyunsaturated fatty acids containing a 1(*Z*),4(*Z*)-pentadiene system, such as linoleic acid and arachidonic acid, into their 1-hydroperoxy-2(*E*),4(*Z*)-pentadiene products (Shibata & Axelrod, 1995). In this connection, lipoxygenases are of importance, since they may generate peroxides in human low-density lipoproteins (LDL) in vivo and facilitate the development of arteriosclerosis, a process in which lipid peroxidation appears to be intimately involved (Cornicelli & Trivedi, 1999; Kris-Etherton & Keen, 2002). Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction (Witting, 1980). On the other hand, it is also well-known that lipid peroxidation 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 flavours, as well as potentially toxic end-products. In our preliminarily assay, we became aware that anacardic acid ($C_{15:3}$) and anacardic acid ($C_{15:2}$) were oxidized as substrates at lower concentrations (<40 μM) because both possess a 1(*Z*),4(*Z*)-pentadiene system in their C_{15}-alkenyl side chain. Hence, the inhibition kinetics were emphasized with anacardic acid ($C_{15:1}$), although both anacardic acid ($C_{15:3}$) and anacardic acid ($C_{15:2}$) inhibited the oxidation of linoleic acid catalyzed by soybean lipoxygenase-1 (EC 1.13.11.12, Type 1) at higher concentration (>40 μM).

The oxidation of linoleic acid, catalyzed by soybean lipoxygenase-1, follows Michaelis–Menten kinetics. The kinetic parameters for this oxidase, obtained from a Dixon plot, show that K_m is equal to 11.7 μM and V_m is equal to 4.8 μmol/min. The estimated value of K_m obtained with a spectrophotometric method is in good agreement with the previously reported value (Berry, Debat, & Larreta-Garde, 1997; Schilstra, Veldink, Verhagen, & Vliegenthart, 1992). The kinetic and inhibition constants obtained are listed in Table 1. As illustrated in Fig. 4, the inhibition kinetics analyzed by Dixon plots followed the Michaelis–Menten equation, since increasing anacardic acid ($C_{15:1}$) concenterate resulted in a family of linear lines with different slopes. The equilibrium constant for inhibitor binding, K_I, was obtained from the plot. The inhibition kinetics analyzed by Lineweaver–Burk plots confirmed that anacardic acid ($C_{15:1}$) is a competitive inhibitor (data not illustrated). A similar result was also obtained by monitoring oxygen consumption and the results are listed in Table 1. The estimated value of K_m is approximately 4-fold higher than that obtained with a spectrophotometric method. This is in good agreement with the previously reported observations (Berry et al., 1997).

Table 1
Kinetics and inhibition constants of anacardic acid ($C_{15:1}$) for soybean lipoxygenase-1

	Inhibition	
	Increase of A_{234}	O_2 consumption
IC_{50}	6.8 μM	31.5 μM
K_m	11.7 μM	43 μM
V_m	4.8 μmol/min	6.5 μmol/min
Inhibition	Reversible	Reversible
Inhibition type	Competitive	Competitive
K_I	2.8 μM	14.2 μM

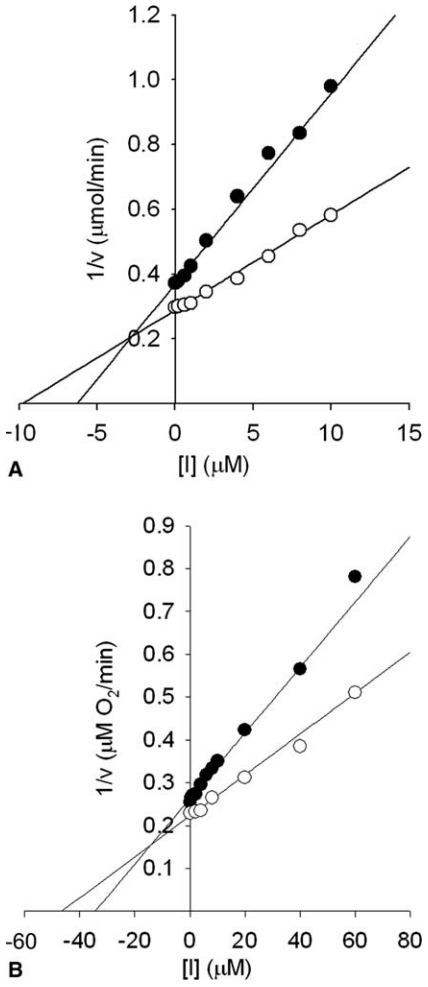

Fig. 4. Dixon plots of 13-HPOD generation and oxygen consumption by soybean lipoxygenase-1 in the presence of anacardic acid ($C_{15:1}$) in borate buffer (pH 9.0) at 25 °C. (A) Plots of 13-HPOD generation (increase of A_{234} nm). ●, At 15 μM linoleic acid substrate, ○, at 30 μM linoleic acid. K_m is equal to 11.7 μM, K_I is equal to 2.8 μM, and V_m is equal to 4.8 μmol/min. (B) Plots of oxygen consumption. ●, At 50 μM linoleic acid, ○, at 80 μM linoleic acid. K_m is equal to 43 μM, K_I is equal to 14.2 μM, and V_m is equal to 6.5 μmol/min.

Salicylic acid (Machlin & Bendich, 1987) did not inhibit soybean lipoxygenase-1 up to 200 μM, suggesting that a pentadecenyl side chain is essential to elicit the activity. However, the pentadecenyl side chain alone is not enough to elicit the activity because cardanol ($C_{15:1}$), which possesses the same side chain as anacardic acid ($C_{15:1}$), acted neither as a substrate nor an inhibitor.

As far as the present cell-free experiment using soybean lipoxygenase-1 is concerned, the inhibition kinetics observed do not exceed 5 min. However, much longer observation is needed from a practical point of view. The time course of oxidation of linoleic acid catalyzed by soybean lipoxygenase-1 in the presence of different anacardic acid

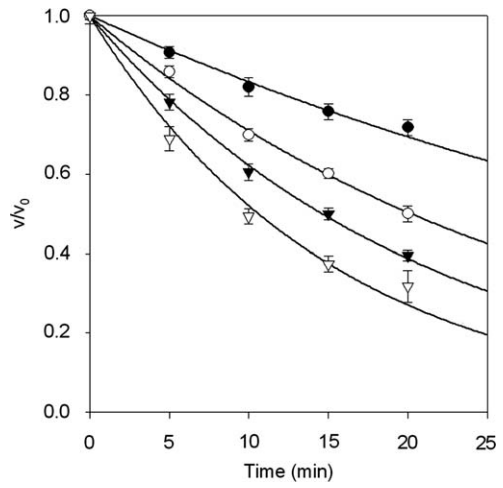

Fig. 5. Time dependence of the fractional velocities for the catalysis of linoleic acid soybean lipoxygenase-1 in the presence of several concentrations of anacardic acid ($C_{15:1}$). Conditions were: 0.1 M sodium borate buffer, pH 9.0, linoleic acid 30 μM, 0.188 μg/ml soybean lipoxygenase-1. ●, Velocities in the presence of 0.8 μM anacardic acid ($C_{15:1}$) ○, 2 μM anacardic acid, ▼, 4 μM anacardic acid, ▽, 6 μM anacardic acid.

($C_{15:1}$) concentrations is shown in Fig. 5. At each concentration of anacardic acid ($C_{15:1}$), the rate slowly decreased with increasing time until a straight line was reached parallel with the *x*-axis, indicating that the enzyme activity was lost.

4. Discussion

Oxidative degradation of polyunsaturated fatty acids occurs in two sequential steps of initiation and propagation (Svingen, Buege, O'Neal, & Aust, 1979). Therefore, antioxidative materials, acting in living systems, are classified as preventive antioxidants and chain-breaking ones (Halliwell & Gutteridge, 1990b). In view of the present investigation, it appears that antioxidant activity of anacardic acids is not due to radical-scavenging but preventing. They may be advantageous for suppressing the formation of free radicals and active oxygen species as a first line of defence. Safety is a primary consideration for antioxidants in food products. In connection with this, the radical-scavenging antioxidant traps an active radical and the antioxidant-derived radical is formed. The fate of this newly formed radical is important in determining the total potency of the antioxidant. For example, several inhibitors of lipid peroxidation have the potential to accelerate free radical damage to other biomolecules (Halliwell, Murcia, Chirico, & Aruoma, 1995). Because of this Janus-like property, scavenging antioxidants are also known as a double-edged sword. The data so far obtained indicate the advantage of anacardic acids as preventive antioxidants. In addition, the fact that anacardic acids are known in the cashew apple and nut that have been continuously consumed by people for many years should be a further considerable advantage.

Anacardic acids were previously reported to have high selectivity toward transition metal ions, especially Fe^{2+} and Cu^{2+} (Nagabhushana, Shobha, & Ravindranath, 1995). The ability of the high selectivity (of chelation toward Fe^{2+} and Cu^{2+}) of anacardic acids should be of considerable advantage as antioxidants (Arora, Nair, & Strasburg, 1998). Transition metal ions are well known as powerful promoters of free radical damage in both the human body (Halliwell & Gutteridge, 1989; Henle & Linn, 1997) and foods (Aruoma & Halliwell, 1991). For example, anacardic acids may prevent cell damage induced by H_2O_2 since this can be converted to the more reactive oxygen species, hydroxy radicals, in the presence of these metal ions (Lodovici, Guglielmi, Meoni, & Dolara, 2001). Salicylic acid does not have this high selectivity of chelation, so the alk(en)yl side chain in anacardic acids is also related to the high selectivity toward transition metal ions. It appears that anacardic acids act as antioxidants in a variety ways, including inhibition of various prooxidant enzymes involved in the production of the reactive oxygen species and they chelate divalent metal ions, such as Fe^{2+} or Cu^{2+}, but do not quench reactive oxygen species.

An antioxidant is, by general definition, any substance capable of preventing oxidation. Deleterious free radical-mediated oxidations occur in aerobic organism as a result of normal oxygen metabolism. Iron, especially ferrous iron (Fe^{2+}), is able to trigger oxidations by reducing, as well as by decomposing, previously-formed peroxides. Hence, an antioxidant that protects against iron toxicity is a substance that can: (a) chelate ferrous iron and prevent the reaction with oxygen or peroxides, (b) chelate iron and maintain it in a redox state that makes iron unable to reduce molecular oxygen, and (c) trap already formed radicals, which is a putative action of any substance that can scavenge free radicals in biological systems, regardless of their origination from iron-dependent reactions or not (Fraga & Oteiza, 2002).

The preventive antioxidant activity of anacardic acids largely comes from their ability to inhibit various oxidative enzymes. It should be noted, however, that these oxidases produce free radicals in the human body as normal products. Hence, anacardic acids, or their metabolites, need to reach the sites where the enzymes are located in living systems and need to regulate the enzyme activity to prevent the generation of only unnecessary radicals. For instance, xanthine oxidase occurs almost exclusively in the liver and small intestinal mucosa in mammals. It is not clear whether anacardic acids or their metabolites can reach the site and regulate this cellular enzyme activity. If anacardic acids act as highly effective xanthine oxidase inhibitors in the human body, they can be toxic since this oxidase is a normal enzyme involved in purine metabolism. Paradoxically, xanthine oxidase inhibitors are useful for treating some diseases, such as gout and urate calculus, by regulating uric acid formation. In any case, it appears that anacardic acids have antioxidant activity by inhibiting oxidation related enzymes and these 6-alk(en)ylsalicylic acids are contained in quantities in the cashew nut and apple. However, their role as antioxidants in the human body is unknown

when orally ingested, but there are several possibilities. The ingested anacardic acids (a) are absorbed into the system through the intestinal tract and delivered to the places where antioxidants are needed, and prevent the generation of unnecessary radicals, (b) are absorbed but metabolized to inactive forms or are not delivered to the right places, or (c) are not absorbed and are excreted. The relevance of the in vitro experiments in simplified systems to in vivo protection from oxidative damage should be carefully considered. The results obtained indicate that further evaluation is needed not only from one aspect, but from a whole and dynamic perspective.

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