Antioxidant Activity of Corn Bran Cell-Wall Fragments in the LDL Oxidation System

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The antioxidant activity of feruloyl arabinose, a model substance of cereal cell-wall fragments, in the LDL autoxidation system by $CuSO_4$ and its absorption were studied. Ferulic acid added as free acid showed little suppressive effect on LDL oxidation. However, ferulic acid sugar esters showed a positive effect. This fact indicated that affinity of LDL particle and free or bound ferulic acid is important for the suppressive effect. The HPLC analysis showed that in rats administered feruloyl arabinose, two peaks were detected with retention times lower than those of feruloyl arabinose and ferulic acid, which appeared upon β-glucuronidase/sulfatase treatment. The study showed that the absorbed form of FAA could be a more polar compound, possibly a conjugated form.

Keywords: *Antioxidant; low-density lipoprotein; cereal cell wall; ferulic acid sugar ester*

INTRODUCTION

Main cereals such as rice, wheat, barley, and corn belong to the family Gramineae and have similar cell-wall composition (Shibuya, 1984; Mueller-Harvey et al., 1986; Onyeneho and Hettiarachchy, 1992; Kato and Nevins, 1985; Ishii et al., 1990; Ishii and Hiroi, 1990). Since cereal cell walls are a good source of dietary fiber, meeting one-half of the daily requirement of 20 g of dietary fiber can be achieved by the regular consumptions of cereals (Ayano, 1992; Ayano et al., 1993; Resources Council, 1982). In the previous paper, we reported that antioxidants in Japanese sake were ferulic acid sugar esters which are cell-wall fragments of rice granule (Ohta et al., 1992) and that the antioxidative compounds were solubilized from insoluble dietary fiber, refined corn bran (RCB), by enzymatic hydrolysis (Ohta et al., 1994). Antioxidative activities (AOAs) of 5-*O*-feruloyl-L-arabinofuranose (FAA) and *O*-(5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX), determined by in vitro assays, were slightly stronger than that of ferulic acid. AOAs of the high molecular fraction were stronger than those of ferulic acid (FA), FAA, and FAXX. Diferulic acid was an active constituent among the alkaline hydrolysate of the high molecular weight fraction. AOAs of ferulic acid sugar esters, FAA and FAXX, were stronger than that of free ferulic acid in the microsomal lipid peroxidation system.

Studies by other workers have shown that phenolic compounds present in tea leaves and wine flavonoids inhibit oxidation of LDL, and hence it is expected that diseases caused by cholesterol oxidation may be protected by them (de Whalley et al., 1990; Frankel et al., 1993, 1995; Kondo et al., 1994; Laranjinha et al., 1992, 1994; Maxwell et al., 1994). Cell-wall fragments are also expected to have antioxidant activity against LDL because they have antioxidant activity against lipids.

Hence, in this study, (1) antioxidant activity of ferulic acid arabinoxylane ester in the $CuSO_4$-induced LDL autoxidation system (2) and absorption of ferulic acid arabinoxylane ester from the digestive tract into the vascular system were studied.

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MATERIALS AND METHODS

Preparation of Cell-Wall Fragment. Refined corn bran was hydrolyzed by 30 mM oxalate, and the hydrolysate was fractionated on Sephadex LH-20 (column dimensions, 15 mm i.d. × 200 cm, solvent: 50% methanol; flow rate, 5 mL/10 min). Absorbance (at 320 nm) of each fraction was measured. Figure 1 shows the result of gel filtration. Fraction A is ferulic acid arabinoxylane ester (high molecular weight fraction, FAXn), and fraction B (FAA) is the low molecular weight fraction which was identified as ferulic acid arabinose ester by NMR analysis (Ohta et al., 1994). These fractions were used as model substances of cereal cell-wall fragments. Structures of these substances are illustrated in Figure 2.

Preparation of LDL. The EDTA-containing stock solution human LDL (15−30 mg LDL/mL) was purchased from Sigma and stored at 4 °C in the dark in a nitrogen atmosphere until use. Protein was determined by the method of Lowry et al. (1951). Before oxidation experiments the LDL solution was dialyzed in a 100-fold volume of 0.01 M phosphate buffer, pH 7.4, 0.16 M NaCl, 0.1 mg/mL chloramphenicol, which was made oxygen-free by vacuum degassing followed by purging with nitrogen (Esterbauer et al., 1989). The buffer was changed four times. This EDTA-free LDL stock solution was used for all oxidation studies. The stock solution was not stored longer than 24 h at 4 °C.

Oxidation Experiments. The EDTA-free LDL stock solution was diluted with oxygen-saturated 0.01 M phosphate buffer, pH 7.4, 0.16 M NaCl, and the oxidation was initiated by the addition of a freshly prepared aqueous 20 μM copper sulfate solution. For conjugated dienes we measured absorption at 234 nm of a dilute preparation of LDL containing 100 μg of LDL protein/mL for 2 h at 37 °C. In the first experiment, 10, 20, and 40 μM FAA were added to study the inhibitory effect. In the second experiment, FA, FAA, and FAXn were added. And in the third experiment, BHT, α-tocopherol, and FAA were added.

Animals and Diet. Male Wistar rats (7 weeks old), weighing 140−160 g and purchased from CLEA Japan (Tokyo, Japan), were acclimatized for 3 days in a room with controlled temperature (22 ± 2 °C). The rats were fed on a commercial diet (type CE-2, CLEA Japan) ad libitum with free access to tap water during the experimental period. All of the rats were then fasted for 12 h before oral administration of 12.2 mg of FAA dissolved in 2 mL of water. Blood was drawn from the heart with a heparinized syringe under pentobarbital anes-

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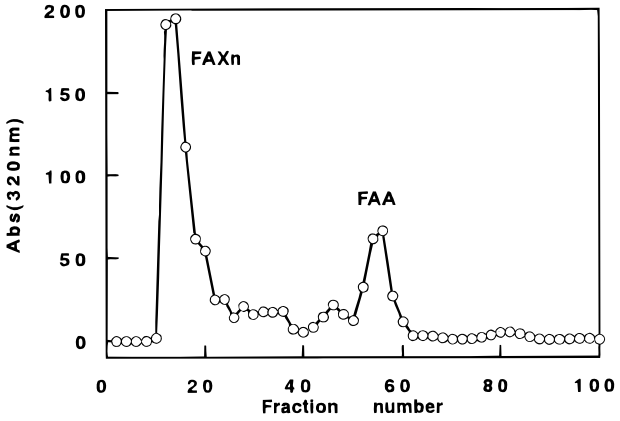

Figure 1. Gel filtration chromatogram of RCB hydrolysate. FAXn, feruloyl arbinoxylane (high molecular weight fraction); FAA, feruloyl arabinose. Refined corn bran hydrolysate was fractionated on Sephadex LH-20 (column dimensions, 15 mm i.d. × 200 cm; solvent, 50% methanol; flow rate, 5 mL/10 min). Absorbance (320 nm) of each fraction was measured.

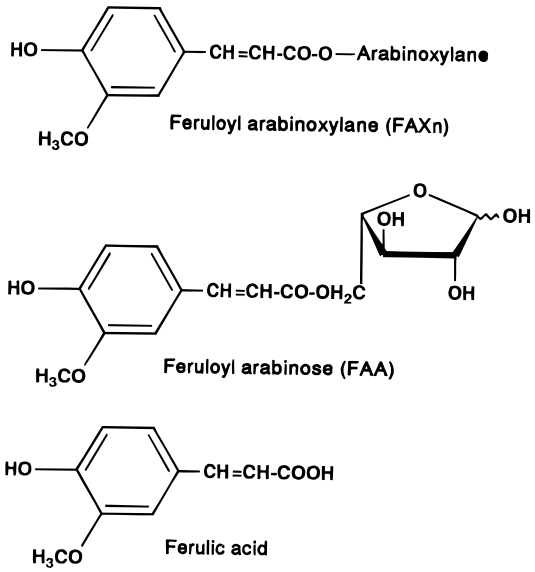

Figure 2. Chemical structure of ferulic acid and its derivatives.

thesia (Nembutal, Dainabot Co., Osaka, Japan) 15, 30, 60, 120, and 240 min after dosing, respectively. Plasma was obtained by centrifuging the blood at 4 °C and 2000*g* for 10 min (Unno et al., 1995). The plasma (0.5 mL) was mixed with trichloroacetic acid (TCA) to a final concentration of 7.5% (w/v) and ultrasonicated at 20 kHz for 30 s. The phenolic compounds were determined by HPLC and detected at 280 nm, using anisic acid as the internal standard. Under the present experimental conditions, most of the phenolic compounds were detected as ferulic acid because the conjugated form is destroyed by the TCA and/or the ultrasonication.

Further Study for Occurrence of Ferulic Acid Derivatives in Plasma. The plasma from rats fed the feruloyl arabinose as well as the plasma from control group were extracted by 4 vol of ethanolic HCl (50 mM) (Manach et al., 1995). Supernatants were neutralized and evaporated under vacuum. A volume of acetate buffer (0.2 M, pH 4.5) equivalent to the initial volume of plasma was added to the dry residues. Solutions were treated for 10 min at 37 °C in the presence or absence of 5×10^5 units of β-glucuronidase/L (EC 3.2.1.31) plus 2.5×10^4 units of sulfatase/L (Sigma Chemical G0876 TypeH-2, crude solution from *Helix pomatia*; β-glucuronidase activity, approximately 100 000 units/mL at pH 5.0; sulfatase activity, contains up to 5000 units/mL). The phenolic compounds were identified by HPLC with detection at 320 nm. Under these exprimental conditions, the conjugated form can be detected.

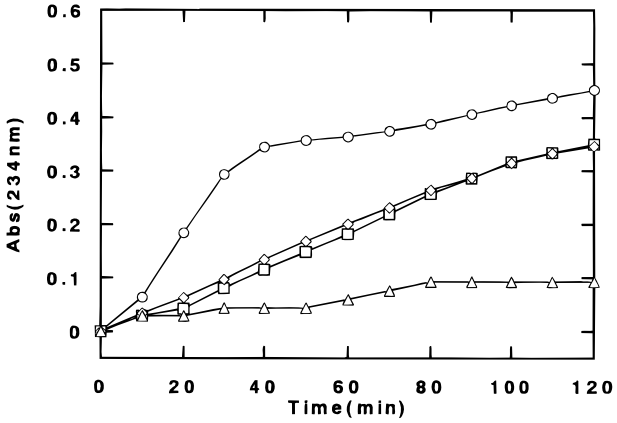

Figure 3. Antioxidant activity of FAA in the LDL oxidation system. ○, Control; ◇, 10 μM FAA; □, 20 μM FAA; △, 40 μM FAA. The EDTA-free LDL stock solution was diluted with oxygen-saturated 0.01 M phosphate buffer, pH 7.4, 0.16 M NaCl, and the oxidation was initiated by the addition of a freshly prepared aqueous 20 μM copper sulfate solution. For conjugated dienes we measured absorption at 234 nm of dilute preparation of LDL containing 100 μg of LDL protein/mL for 2 h at 37 °C.

Table 1. Antioxidant Activity of FAA Compared with Ferulic Acid and FAXn in the LDL Oxidation System[a]

	control	FA	FAXn	FAA
maximal concentration (nmol of dienes/mg of LDL)	180.9	155.6	154.0	85.9
maximal rate (nmol of dienes/min·mg of LDL)	4.9	3.1	2.8	1.1

[a] Calculations were based on the molar absorptivity of conjugated lipid hydroperoxides ($\epsilon_{234} = 29\,500\ M^{-1}\ cm^{-1}$) (Esterbauer et al., 1989) and our experimental conditions (0.10 mg of LDL/mL). Retention of induction time was not observed.

HPLC Analysis. The mixture was centrifuged at 4 °C and 2000*g* for 10 min, and 5 mL of the resulting supernatant was applied to HPLC (pump, L-6200 intelligent pump, Hitachi Co., Ltd., Tokyo, Japan) after filtration through a 0.45 mm membrane. The conditions for HPLC were as follows: column, μBondapak C18 column (Waters), reversed-phase type (3.9 mm i.d. × 300 mm) with a guard; elution, eluted with a linear gradient of acetonitrile (0−50%) buffered with 50 mM sodium acetate buffer (pH 4.0); flow rate, 1.0 mL/min; detector, L-4200 UV−vis detector (Hitachi); absorbance, 320 or 280 nm.

RESULTS

Antioxidant Activity of FAA. The inhibitory effect of FAA on LDL oxidation was studied at the concentrations of 0, 10, 20, and 40 μM FAA. LDL oxidation was detected by measuring the absorbance at 234 nm. Oxidation of LDL was inhibited by FAA (Figure 3). The inhibition effect by 10 μM FAA was as strong as that of 20 μM FAA, whereas treatment with 40 μM FAA showed the strongest inhibition.

Difference in the Antioxidant Activity between Ferulic Acid, FAA, and FAXn. Table 1 shows the antioxidant activity of ferulic acid, FAA, and FAXn in a LDL oxidation system. Antioxidant activity of FAA was stronger than that of ferulic acid and FAXn. Antioxidant activity of ferulic acid sugar esters such as FAA and FAXn were stronger than free ferulic acid. Low molecular weight ester has stronger activity than high molecular weight ester. This fact indicated that the affinity of the LDL particle and free or bound ferulic acid is important in suppressive effect. Retention of induction time was not observed.

Comparison of Antioxidant Activity of FAA and a Well-Known Antioxidant. Figure 4 shows the

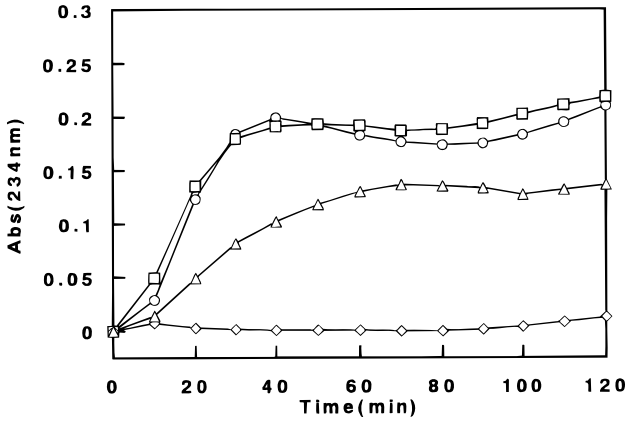

Figure 4. Antioxidant activity of FAA compared with well-known antioxidants in the LDL oxidation system. ○, Control; ◇, 20 μM BHA; □, 20 μM α-tocopherol; △, 20 μM FAA. The EDTA-free LDL stock solution was diluted with oxygen-saturated 0.01 M phosphate buffer, pH 7.4, 0.16 M NaCl, and the oxidation was initiated by the addition of a freshly prepared aqueous 20 μM copper sulfate solution. For conjugated dienes we measured absorption at 234 nm of dilute preparation of LDL containing 100 μg of LDL protein/mL for 2 h at 37 °C.

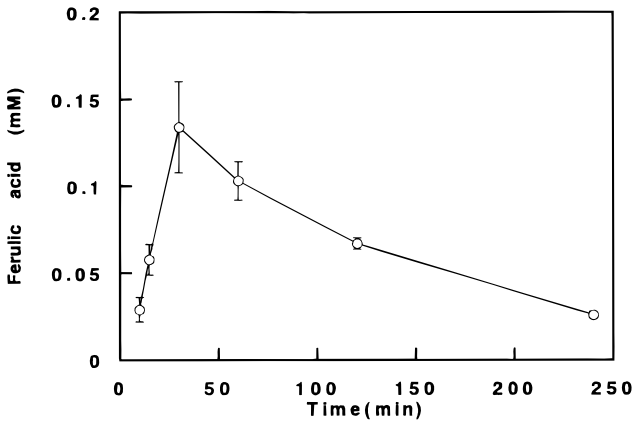

Figure 5. Time course of ferulic acid concentration in the plasma after oral administration of FAA. All the rats were fasted for 12 h before oral administration with 12.2 mg of FAA dissolved in 2 mL of water. Blood was drawn from the heart with a heparinized syringe under pentobarbital anesthesia (Nembutal, Dainabot Co., Osaka, Japan) 15, 30, 60, 120, and 240 min after dosing, respectively. Plasma was obtained by centrifuging the blood at 4 °C and 2000*g* for 10 min (Unno et al., 1995). The plasma (0.5 mL) was mixed with trichloroacetic acid (TCA) to a final concentration of 7.5% (w/v) and ultrasonicated at 20 kHz for 30 s. The phenolic compounds were determined by HPLC and detected at 280 nm, using anisic acid as the internal standard. Circle and error bar, mean average ± standard error ($n = 4$).

antioxidant activity of FAA, α-tocopherol, and BHT in LDL oxidation system. α-Tocopherol showed no antioxidant activity in this LDL oxidation system. The antioxidant activity of FAA was stronger than that of α-tocopherol, and that of BHT was stronger than that of FAA. Retention of induction time was not observed. Addition of 20 μM FAA did not prolong the lag phase but did influence the rate of propagation, from 3.2 to 1.2 nmol of dienes/(min·mg of protein), under the present experimental conditions. Addition of 20 μM BHT drastically affected the duration of the lag phase (>400 min). α-Tocopherol (at 20 μM) was significantly less potent than FAA (even when present at 10 μM). The addition of 20 μM FAA depressed the rate of lipid peroxidation during the propagation phase [1.2 nmol of dienes/(min·mg of protein) vs 3.2 under control conditions]. The conjugated diene could not be detected (lag time > 450 min) in the presence of 20 μM BHT.

Time Course of the FAA Concentration in Some Plasma Samples. We then measured the time course of the FAA concentration in some plasma samples obtained 15, 30, 60, 120, and 240 min after oral administration of 12.2 mg of FAA. As indicated in Figure 5, the ferulic acid concentration in the rat plasma began to increase rapidly and reached its highest level about half an hour after injection. Within 4 h after the administration, ferulic acid had disappeared from the plasma. No FA derivative was detected in the plasma from the control group (unadministered group). This led us to presume that ferulic acid taken up in the plasma was diminished by decomposition or metabolism after its distribution to the tissues. 37.5 μmol (12.2 mg) of FAA was orally administered to rat. On the other hand, the maximum concentration of FA in plasma was 0.134 mM (43.7 μg/mL). 3.6% of administered FAA occurred in plasma 30 min after administration, when blood volume/body weight is 5% and rats of ca. 200 g body weight were used for this study. In conclusion, the absorption of ferulic acid into the circulation system of rats was verified.

Treatment by β-Glucuronidase/Sulfatase. To verify that the circulating form was a conjugated derivative, plasma from rats fed 12.2 mg of FAA was treated by β-glucuronidase/sulfatase before the HPLC procedure. This treatment led to the decrease of the peaks eluted at 12.0 and 12.7 min and to the emergence of two new peaks (Figure 6). The first peak that eluted at 15.4 min was FAA, and the second peak that eluted at 16.6 min was FA. Orally administered FAA existed as the conjugated form of FAA (25%) and FA (75%) in the circulation system.

DISCUSSION

Because potent antioxidant properties have been ascribed to flavonoids (Frankel et al., 1993, 1995), we decided to test their effect on lipid peroxidation. The investigation was conducted using human LDL, and the formation of conjugated dienes was induced by the addition of 20 μM Cu^{2+} either under control conditions or in the presence of feruloyl arabinose.

Oxidation of human plasma LDL induced by $CuSO_4$ was inhibited by the cell-wall fragment FAA. α-Tocopherol is reported to be a pro-oxidant in low concentration and an antioxidant in high concentration. Then concentration dependency may not be detected in the medium concentration. FAA, a more polar and hydrophilic substance than FA, showed stronger effect than free ferulic acid and FAXn. Affinity of LDL particle and ferulic acid ester is important to show the antioxidant activity in LDL oxidation system. The synthetic antioxidant BHT has a stronger activity than FAA, and FAA showed stronger activity than α-tocopherol. The effect of the α-tocopherol on the lag phase appears to be much less pronounced than ferulic acid sugar ester. This is probably due to the experimental conditions and does not reflect the effectiveness of endogenous α-tocopherol contained in native LDL. It is likely that the greater part of the added lipophilic antioxidants was not incorporated in the LDL particle but remained suspended in the aqueous buffer (Esterbauer et al., 1989).

The oxidative modification of LDL is thought to be an important factor in the pathogenesis of atherosclerosis, and there is great interest in the possibility that diets rich in antioxidants may retard the develop-

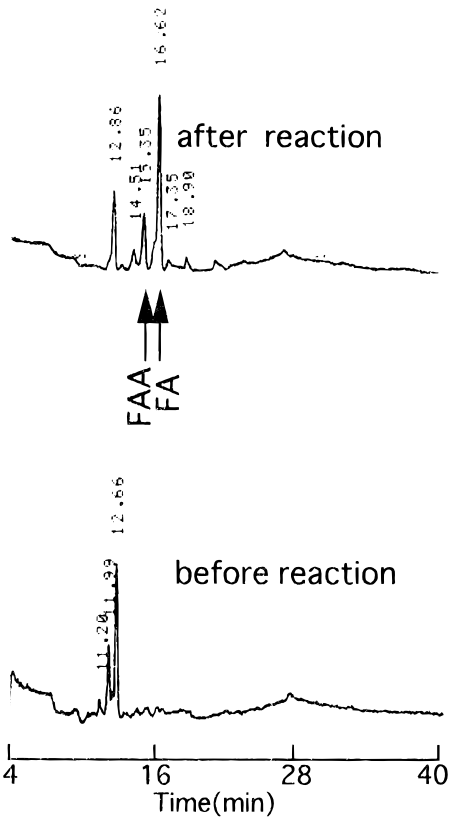

Figure 6. Phenolic substances before and after β-glucuronidase/sulfatase reaction. FAA, feruloyl arabinose; FA, ferulic acid. The plasma from rats fed the feruloyl arabinose was extracted with 4 vol of ethanolic HCl (50 mM) (Manach et al., 1995). Supernatants were neutralized and evaporated under vacuum. A volume of acetate buffer (0.2 M, pH 4.5) equivalent to the initial volume of plasma was added to the dry residues. Solutions were treated for 10 min at 37 °C in the presence or absence of 5×10^5 units of β-glucuronidase/L (EC 3.2.1.31) plus 2.5×10^4 units of sulfatase/L. The phenolic compounds were identified by HPLC with detection at 320 nm.

ment of atherosclerotic complications such as coronary heart disease (CHD). The potent antioxidant activity of phenolic substances in red wine, in particular, has been proposed as an explanation for the "French paradox" (the apparent incompatibility of a high fat diet with low incidence of CHD). Red wine antioxidants protect LDL from oxidation in vitro (Maxwell et al., 1994), and an increase in antioxidant activity has been demonstrated in vivo (Kondo et al., 1994). It has been clearly shown that the oxidation of LDL is a lipid peroxidation process in which the polyunsaturated fatty acids of LDL are successively degraded to a variety of products. These lipid peroxidation products and their interactions with the apoB are likely the cause for the altered functional properties of LDL.

Ferulic acid sugar ester did not prolong the induction period in LDL peroxidation, but decreased the maximal rate and maximal concentration of LDL oxidation system. The antioxidant mechanism of ferulic acid sugar ester in the LDL peroxidation system may be different from that of flavonoid which prolong the induction period in LDL peroxidation, but give no effect on maximal rate and maximal concentration. Because the mucosa of the large intestine and the liver enzymes of the host (Hackett, 1986) have the capacity to alter the number and the position of hydroxyl groups and to produce conjugated derivatives in the liver, we examined whether intact FAA appeared in plasma. The HPLC analysis showed that in rats fed an FAA diet two peaks were detected with retention times lower (12.0 and 12.7 min) than those of FAA (15.4 min) and ferulic acid (16.6 min). This suggests that the peak corresponds to a more polar compound, possibly a conjugated form. No FA derivatives were detected in plasma from the control rats. In conclusion, absorption of FAA into the circulation system of rats was verified. Manach et al. (1995) reported that the comparison of the ileal concentration of rutin or quercetin to that of a nonabsorbable marker of intestinal transit (polyethylene glycol) indicated that >95% of ingested flavonoids were still present in the ileum. The fecal excretion of rutin was negligible in rats fed a rutin diet. These facts indicated that rutin is hydrolyzed by glycosidase and absorbed as quercetin in cecum. FAA may be absorbed in the cecum in the same manner.

It has been revealed that the AOA of FAA which has not only hydrophobic ferulic acid moiety but also hydrophilic sugar moiety is stronger than FA in the LDL oxidation system. The conjugated forms of ferulic acid derivatives which are to be isolated may have stronger AOA depending on their hydrophobicity and hydrophilicity.

Recently, ferulic acid esterases from fungus such as *Aspergillus* and from anearobic microorganism in the rumen have been isolated. These enzymes degrade cereal cell wall with xylanase and arabinofurnaosidase synergistically (Borneman et al., 1992; Faulds and Williamson, 1991, 1994; Ferreira et al., 1993). The cell-wall of cereal plants in the family Gramineae (Smith and Hardey, 1983; Kato et al., 1983; Kato and Navins, 1985; Mueller-Harvey et al., 1986) are made from arabinoxylane which is modified by esterified phenolic acids such as ferulic acid. The pectins of sugar beet (Rombouts and Thibault, 1986) and spinach (Fry, 1982) are modified by ferulic acid, too. In order to degrade the cell wall of Garamineae plant, these phenolic acids should first be removed by ferulic acid esterase (Borneman et al., 1986; Chesson et al., 1982; Jung and Sahlu, 1986). It has been reported that several microorganisms produce such esterase (Faulds and Williamson, 1991, 1993a,b; Bomeman et al., 1991, 1992; Ferreira et al., 1993; Castanares et al., 1992; Tenkanen et al., 1991). These facts indicate that hemicellulase and/or cellulase may activate potential function which occurs in Gramineae cell wall. Enterobacteria such as *Lactobacillus* and/or *Bifidobacterium* may have such enzymes.

In this study FAA obtained from refined corn bran by oxalate hydrolysis was used as a model substance of cell-wall fragment. We verified (1) antioxidant activity of FAA in the LDL autoxidation system by $CuSO_4$ and (2) absorption of FAA from digestive tract to circulation system as the conjugated forms of FAA and FA.

LITERATURE CITED

Ayano, Y. Dietary fiber in cereals: Nutritional and physiological aspect. *Nippon Eiyo, Shokuryo Gakkaishi* **1992**, *45*, 209−219 (in Japanese).

Ayano, Y.; Sanada, H.; Egashira, Y.; Ohtsuka, M.; Cheng, C. C.; Manabe, A.; Shigematsu, K. Contents of dietary fiber in cereal and its processed foods. *Nippon Eiyo, Shokuryo Gakkaishi* **1993**, *46*, 244−247 (in Japanese).

Borneman, W. S.; Akin, D. E.; Van Eseltine, W. P. Effect of phenolic monomers on ruminant bacteria. *Appl. Environ. Microbiol.* **1986**, *52*, 1331−1339.

Borneman, W. S.; Ljungdahl, L. G.; Hartley, R. D.; Akin, D. E. Isolation and characterization of *p*-coumaroyl esterases from the anaerobic fungus *Neocallimastix* strain MC-2. *Appl. Environ. Microbiol.* **1991**, *57*, 2337−2344.

Borneman, W. S.; Ljungdahl, L. G.; Hartley, R. D.; Akin, D. E. Purification and partial characterization of two feruloyl esterases from the anaerobic fungus *Neocallimastix* strain MC-2. *Appl. Environ. Microbiol.* **1992**, *58*, 3762−3766.

Castanares, A.; McCrae, S. I.; Wood, T. M. Purification of properties of a feruloyl/*p*-coumaroyl esterase from the fungus *Penicillium pinophilum*. *Enzyme Microb. Technol.* **1992**, *14*, 875−884.

de Whalley, C. V.; Rankin, S. M.; Hoult, J. R. S.; Jessup, W.; Leake, D. S. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Chem. Pharmacol.* **1990**, *39*, 1743−1750.

Esterbauer, H.; Striegl, G.; Pohl, H.; Rotheneder, M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radical Res. Commun.* **1989**, *6*, 67−75.

Faulds, C. B.; Williamson, G. The purification and characterization of 4-hydroxy-3-methoxycinnamic (ferulic) acid esterase from *Streptomyces olivochromogenes*. *J. Gen. Microbiol.* **1991**, *137*, 2339−2345.

Faulds, C. B.; Williamson, G. Release of ferulic acid from plant polysaccharides by ferulic acid esterase from *Streptomyces olivochromogenes*. *Carbohydr. Polym.* **1993a**, *21*, 153−155.

Faulds, C. B.; Williamson, G. Ferulic acid esterase from *Aspergillus niger*: Purification and partial characterization of two forms from a commercial source of pectinase. *Biotechnol. Appl. Biochem.* **1993b**, *17*, 349−359.

Faulds, C. B.; Williamson, G. Purification and characterization of a ferulic acid esterase (FAE-III) from *Aspergillus niger*: Specificity for the phenolic moiety and binding to microcrystalline cellulose. *Microbiology* **1994**, *140*, 779−787.

Ferreira, L. M. A.; Wood, T. M.; Williamson, G.; Faulds, C.; Hazlewood, G. P.; Black, G. W.; Gilbert, H. J. A modular esterase from *Pseudomonas fluorescens* subsp. cellulosa contains a non-catalytic cellulose-binding domain. *Biochem. J.* **1993**, *294*, 349−355.

Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

Fry, S. C. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annu. Rev. Plant Physiol.* **1986**, *37*, 165−186.

Hacket, A. M. 1986, The metabolism of flavonoid compounds in mammals. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure−Activity Relationship*; Cody, V., Middleton, E., Jr., Harborn, J. B., Eds.; Alan R. Liss: New York, 1986; pp 177−194.

Ishii, T.; Hiroi, T. Isolation and characterization of feruloylated arabinoxylane oligosaccharides from bamboo shoot cell-walls. *Carbohydr. Res.* **1990**, *196*, 175−183.

Ishii, T.; Hiroi, T.; Thomas, J. R. Feruloylated xyloglucan and *p*-coumaroyl arabinoxylaneoligosaccharides from bamboo shoot cell-walls. *Phytochemistry* **1990**, *29*, 1999−2003.

Kato, Y.; Nevins, D. J. Isolation and identification of *O*-(5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose as a component of Zea shoot cell walls. *Carbohydr. Res.* **1985**, *137*, 139−150.

Kato, Y.; Azuma, J.; Koshijima, T. A new feruloylated trisaccharide. *Chem. Lett.* **1983**, 137−140.

Kondo, K.; Matsumoto, A.; Kurata, H.; Tanahashi, H.; Koda, H.; Amachi, T.; Itakura, H. Inhibition of oxidation of low-density lipoprotein with red wine. *Lancet* **1994**, *344*, 1152.

Laranjinha, J. A. N.; Almeida, L. M.; Madeira, V. M. C. Lipid peroxidation and its inhibition in low density lipoproteins: Quenching of *cis*-parinaric acid fluorescence. *Arch. Biochem. Biophys.* **1992**, *297*, 147−154.

Laranjinha, J. A. N.; Almeida, L. M.; Madeira, V. M. C. Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation. *Biochem. Pharmacol.* **1994**, *48*, 487−494.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Manach, C.; Morand, C.; Texier, O.; Favier, M.-L.; Agollo, G.; Demigne, C.; Regerat, F.; Remesy, C. Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. *J. Nutr.* **1995**, *125*, 1911−1922.

Maxwell, S.; Cruickshank, A.; Thorpe, G. Red wine and antioxidant activity in serum. *Lancet* **1994**, *344*, 193−194.

Mueller-Harvey, I.; Hartley, R. D. Linkage of *p*-coumaroyl and feruloyl groups to cell-wall polysaccharides of barley straw. *Carbohydr. Res.* **1986**, *148*, 71−85.

Ohta, T.; Takashita, H.; Todoroki, K.; Iwano, K.; Ohba, T. Antioxidative substance in Sake. *J. Brew. Soc. Jpn.* **1992**, *87*, 922−926 (in Japanese).

Ohta, T.; Yamasaki, S.; Egashira, Y.; Sanada, H. Antioxidative activity of corn bran hemicellulose fragments. *J. Agric. Food Chem.* **1994**, *42*, 653−656.

Onyeneho, S. N.; Hettiarachchy, N. S. Antioxidative activity of durum wheat bran. *J. Agric. Food Chem.* **1992**, *40*, 1496−1500.

Resources Council, Science and Technology Agency, Japan. *Standard Table of Food Composition in Japan*, 4th revised ed.; Science and Technology Agency: Tokyo, Japan, 1982; pp 38−59.

Rombouts, F. M.; Thibault, J.-F. Feruloylated pectic substances from sugar-beet pulp. *Carbohydr. Res.* **1986**, *154*, 177−187.

Shibuya, N. Phenolic acids and their carbohydrate esters in rice endosperm cell walls. *Phytochemistry* **1984**, *23*, 2233−2237.

Smith, M. M.; Hartley, R. D. Occurrence and nature of ferulic acid substitution of cell-wall polysaccharides in graminaceous plants. *Carbohydr. Res.* **1983**, *118*, 65−80.

Tenkanen, M.; Schuseil, J.; Puls, J.; Poutanen, K. Production, purification and characterization of an esterase liberating phenolic acids from lignocellulosics. *J. Biotechnol.* **1991**, *18*, 69−84.

Unno, T.; Takeo, T. Absorption of (−)-epigallocatechin gallate into the circulation system of rats (Note). *Biosci. Biotechnol. Biochem.* **1995**, *59*, 1558−1559.

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